D. MONOGRAPHS

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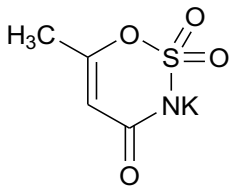
General requirements

Food additives appearing in the Monographs shall meet the specifications and standards specified in the corresponding individual monographs. Notwithstanding compliance with the specifications and standards provided in the Monographs, food additives that have been produced with the use of organisms obtained with recombinant DNA technology shall not be distributed or used in Japan unless they have been proven safe through assessment specified by the Minister of Health, Labour and Welfare and listed as safe. Enzymes that have been proven safe through the assessment on recombinant DNA risk are exempted from the application of the requirements for the sources provided in the definitions of the corresponding enzymes.

Acesulfame Potassium

Acesulfame K

アセスルファムカリウム



$C_4H_4KNO_4S$ Mol. Wt. 201.24

Potassium 6-methyl-4-oxo-4*H*-1,2,3-oxathiazin-3-ide 2,2-dioxide [55589-62-3]

Content Acesulfame Potassium, when dried, contains not less than 99.0% of acesulfame potassium ($C_4H_4KNO_4S$).

Description Acesulfame Potassium occurs as a white crystalline powder. It is odorless and has a strong sweet taste.

Identification

(1) Dissolve 10 mg of Acesulfame Potassium in 1000 mL of water. The solution exhibits an absorption maximum at a wavelength of 225–229 nm.

(2) Acesulfame Potassium responds to all the tests for Potassium Salt in the Qualitative Tests.

(3) To 0.2 g of Acesulfame Potassium, add 2 mL of diluted acetic acid (3 in 10) and 2 mL of water to dissolve. Add a few drops of sodium hexanitrocobaltate(III) TS to the solution. A yellow precipitate is formed.

pH 5.5–7.5 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution　Colorless and clear (1.0 g, water 5.0 mL).

(2) Lead　Not more than 1 μg/g as Pb (4.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Fluoride　Not more than 3.0 μg/g as F.

Test Solution　Weigh 2.00 g of Acesulfame Potassium, transfer it into a beaker, add 10 mL of water, and mix for a while. Add 20 mL of diluted hydrochloric acid (1 in 20) gradually, and dissolve. Heat the solution, boil for 1 minute, transfer into a polyethylene beaker, and immediately cool with ice. Add 10 mL of a solution of disodium dihydrogen ethylenediaminetetraacetate dihydrate (1 in 40) and 15 mL of a solution of trisodium citrate dihydrate (1 in 4), and mix. Adjust the pH of the solution to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5). Transfer this solution into a 100-mL volumetric flask, and dilute with water to volume. Place about 50 mL of the solution in a polyethylene beaker, and use as the test solution.

Control Stock Solution　Weigh 2.210 g of sodium fluoride, previously dried at 110˚C for 2 hours, transfer it into a polyethylene beaker, add 200 mL of water, and dissolve while stirring. Transfer this solution into a 1000-mL volumetric flask, dilute with water to volume, and then transfer into a polyethylene bottle.

Control Solution　Prepare fresh before use. Transfer 3.0 mL of the stock solution into a 1000-mL volumetric flask, and add water to make 1000 mL. Transfer exactly 2 mL of the solution into a polyethylene beaker, add 10 mL of a solution of disodium dihydrogen ethylenediaminetetraacetate dihydrate (1 in 40) and 15 mL of a solution of trisodium citrate dihydrate (1 in 4), and mix. Adjust the pH of the solution to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5). Transfer the solution into a 100-mL volumetric flask, and add water to make 100 mL. Place about 50 mL of the solution into a polyethylene beaker, and use it as the control solution.

Procedure　Measure the electric potentials of both solutions, using a potentiometer connected to a fluorine ion indicator electrode and a silver-silver chloride reference electrode. The electric potential of the test solution is not lower than that of the control solution.

(5) UV active components (Organic impurities)　Not more than 20 μg/g as acesulfame potassium.

Test solution　Weigh accurately about 1 g of Acesulfame Potassium, and dissolve it in water to make exactly 100 mL.

Control Solution　Dilute the test solution with water by 50,000-fold.

Procedure　Analyze 20 μL each of the test solution and the control solution by liquid chromatography under the conditions given below. Continue the chromatography for 3 times the retention time of the main peak of the test solution. The total area of all the peaks, other than the main peak, of the the test solution does not exceed the area of the

main peak of the control solution.

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 227 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 3- to 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 3:2 mixture of tetrabutyl ammonium hydrogen sulfate TS (0.01 mol/L)/acetonitrile.

Flow rate: 1 mL/min.

The column should be capable of separating the peaks of acesulfame potassium and ethyl *p*-hydroxybenzoate when the following test is conducted: Weigh separately 10 mg of Acesulfame Potassium and "Ethyl *p*-Hydroxybenzoate," dissolve together in water to make a mixture, and add water to make 1000 mL. Analyze a 20-μL portion of this solution by liquid chromatography under the above conditions.

Loss on Drying Not more than 1.0% (105°C, 2 hours).

Assay Weigh accurately about 0.15g of Acesulfame Potassium, previously dried, dissolve it in 50 mL of acetic acid, and titrate with 0.1 mol/L perchloric acid solution. The endpoint is usually confirmed by a potentiometer. When crystal violet–acetic acid TS is used as the indicator (2 drops), the endpoint is when the color of the solution changes from dark blue to green and then the color becomes green that persists for at least 30 seconds. Separately, perform a blank test.

Each mL of 0.1 mol/L perchloric acid solution = 20.12 mg of $C_4H_4KNO_4S$

Acetaldehyde

Ethanal

アセトアルデヒド

H_3C-CHO

C_2H_4O Mol. Wt. 44.05

Acetaldehyde [75-07-0]

Content Acetaldehyde contains not less than 98.0% of acetaldehyde (C_2H_4O).

Description Acetaldehyde is a colorless, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Acetaldehyde as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the

Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive index n_D^{20}: 1.330–1.364.

Purity Acid value Not more than 5.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (3). For sample injection, use a microsyringe that is previously cooled at 5°C at least for 30 minutes.

Storing standard Store in a well-filled, hermetic container under inert gas at 5°C or lower.

Acetic Acid

酢酸

Content Acetic Acid contains 29.0–31.0% of acetic acid ($C_2H_4O_2$ = 60.05).

Description Acetic Acid is a colorless, clear liquid having a characteristic pungent odor.

Identification

(1) Acetic Acid is acidic.

(2) Acetic Acid responds to all the tests for Acetate in the Qualitative Tests.

Purity

(1) Lead Not more than 0.5 μg/g as Pb (8.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Readily oxidizable substances Measure 20 mL of Acetic Acid, and add 0.30 mL of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 30 minutes.

(4) Residue on evaporation Not more than 0.010%.

Weigh 20.0 g of Acetic Acid, evaporate, and dry at 100°C for 2 hours, and weigh the residue.

Assay Weigh accurately about 3 g of Acetic Acid, and add 15 mL of water. Titrate with 1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide = 60.05 mg of $C_2H_4O_2$

Glacial Acetic Acid

Acetic Acid, Glacial

氷酢酸

$H_3C-COOH$

$C_2H_4O_2$ Mol. Wt. 60.05

Acetic acid [64-19-7]

Content Glacial Acetic Acid contains not less than 99.0% of acetic acid ($C_2H_4O_2$).

Description Glacial Acetic Acid occurs as colorless or white crystalline lumps or as a colorless, clear liquid. It has a characteristic pungent odor.

Identification

(1) A solution of Glacial Acetic Acid (1 in 4) is acidic.

(2) A solution of Glacial Acetic Acid (1 in 4) responds to all the tests for Acetate in the Qualitative Tests.

Congealing Point Not less than 14.5°C.

Purity

(1) Lead Not more than 0.5 μg/g as Pb (8.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Readily oxidizable substances Weigh 2.0 g of Glacial Acetic Acid, dissolve it in 10 mL of water, and add 0.10 mL of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 30 minutes.

(4) Residue on evaporation Not more than 0.010%.

Weigh 20.0 g of Glacial Acetic Acid, evaporate, dry at 100°C for 2 hours, and weigh the residue.

Assay Weigh accurately about 1 g of Glacial Acetic Acid, and add 40 mL of water. Titrate with 1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide = 60.05 mg of $C_2H_4O_2$

α-Acetolactate Decarboxylase

α-アセトラクタートデカルボキシラーゼ

Definition α-Acetolactate Decarboxylase includes enzymes that decarboxylate α-

acetolactate. It is derived from the culture of bacteria (limited to *Bacillus licheniformis, Bacillus subtilis,* and species from the genus *Serratia*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description α-Acetolactate Decarboxylase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification α-Acetolactate Decarboxylase complies with the α-Acetolactate Decarboxylase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

α-Acetolactate Decarboxylase Activity Test Perform the test using the method given below. If the activity test cannot be performed by the given method, appropriate replacement of the sample, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 0.50 g of α-Acetolactate Decarboxylase, add MES buffer (0.05 mol/L, pH 6.0, containing sodium chloride) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Add 0.1 mL of ethyl 2-acetoxy-2-methylacetoacetate to 6.0 mL of sodium hydroxide TS (0.5 mol/L), shake the mixture for 20 minutes at room temperature, and add about 40 mL of MES buffer (0.05 mol/L, pH 6.0, containing sodium chlorite). Adjust its pH to 6.0 with 0.5 mol/L hydrochloric acid. Add the MES buffer to make 50 mL. Prepare fresh before use.

Test Solution Equilibrate 0.040 mL of the substrate solution at 30°C for 8 minutes,

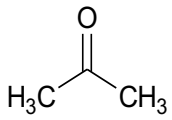
add 0.040 mL of the sample solution, equilibrated at 30°C, and allow the mixture to incubate at 30°C for 11 minutes. Add 0.080 mL of naphthol–creatine TS immediately, and allow to stand for 4 minutes.

Control Solution Prepare a control solution in the same manner as for the test solution using MES buffer (0.05 mol/L, pH 6.0, containing sodium chlorite), equilibrated at 30°C, instead of the sample solution.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 510 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Acetone

アセトン



C_3H_6O Mol. Wt. 58.08

Propan-2-one [67-64-1]

Content Acetone contains not less than 99.0% of acetone (C_3H_6O).

Description Acetone is a colorless, clear, and volatile liquid having a characteristic odor.

Identification To 1 mL of a solution of Acetone (1 in 200), add 1 mL of sodium hydroxide solution (1 in 25), warm in hot water, and add 3 drops of iodine TS. A yellow precipitate is immediately formed.

Specific Gravity d_{20}^{20}: 0.790–0.795.

Boiling Point 55.5–57.0°C (Method 1).

Purity

(1) Readily oxidizable substances Measure 30 mL of Acetone, and add 0.10 mL of 0.02 mol/L potassium permanganate. The pink color does not disappear within 15 minutes.

(2) Phenol Measure 3.0 mL of Acetone, transfer into a crucible, and evaporate to dryness at about 60°C. Add 3 drops of a solution of sodium nitrite in sulfuric acid (1 in 50), allow to stand for 2–3 minutes, and carefully add 3 mL of sodium hydroxide solution (2 in 25). No color develops.

(3) Residue on evaporation Not more than 0.0016% (w/v).

Measure 125 mL of Acetone, evaporate carefully, dry at 105°C for 2 hours, and weigh

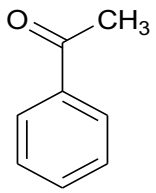
the residue.

Assay Weigh accurately about 1 g of Acetone, transfer it into a flask containing 20 mL of water, and add water to make exactly 1000 mL. Measure exactly 10 mL of this solution, transfer it into a ground-glass stoppered flask, add 25 mL of sodium hydroxide solution (1 in 25), and allow to stand for 5 minutes. Add exactly 25 mL of 0.05 mol/L iodine, stopper, allow to stand in a cool, dark place for 10 minutes, and add 30 mL of diluted sulfuric acid (3 in 100). Titrate with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner.

Each mL of 0.05 mol/L iodine = 0.9680 mg of C_3H_6O

Acetophenone

アセトフェノン



C_8H_8O Mol. Wt. 120.15

1-Phenylethanone [98-86-2]

Content Acetophenone contains not less than 98.0% of acetophenone (C_8H_8O).

Description Acetophenone occurs as white crystalline lumps or as a colorless to light yellow, clear liquid. It has a characteristic odor.

Identification Determine the absorption spectrum of Acetophenone as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.530–1.535.

Specific Gravity d_{25}^{25}: 1.022–1.028.

Assay With a solution (1 in 10) of Acetophenone in ethanol (95), proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Acetylated Distarch Adipate

アセチル化アジピン酸架橋デンプン

Definition Acetylated Distarch Adipate is obtained by esterifying starch with acetic anhydride and adipic anhydride.

Description Acetylated Distarch Adipate occurs as a white to off-white powder, or as flakes or granules. It has a faint odor.

Identification

(1) Add a few drops of iodine TS to a suspension of Acetylated Distarch Adipate (1 in 20). A dark blue to red color develops.

(2) Suspend 2.5 g of Acetylated Distarch Adipate by adding 10 mL of diluted hydrochloric acid (1 in 10) and 70 mL of water, and heat under reflux for about 3 hours. After cooling, add 0.5 mL of the resulting suspension to 5 mL of boiling Fehling's TS. A red precipitate is formed.

(3) Add 10 mL of sodium carbonate TS to 0.5 g of Acetylated Distarch Adipate, boil for 5 minutes, and add 10 mL of 10% sulfuric acid TS. An odor of acetic acid is emitted.

Purity

(1) Adipate groups Not more than 0.135%.

(i) *Test Solution for the Determination of Total Adipic Acid* Weigh accurately about 1 g of Acetylated Distarch Adipate into an Erlenmeyer flask with a stopper, and add 50 mL of water and exactly 1 mL of the internal standard solution. Shake the mixture well to disperse the starch, add 50 mL of sodium hydroxide solution (4 in 25), and shake well for 5 minutes. Place the flask into a water bath of room temperature, and add 20 mL of hydrochloric acid cautiously. After cooling, transfer the contents in the flask into a separating funnel, and wash the inside of the flask with a little amount of water. Add washings to the funnel. Extract three times with 100 mL of ethyl acetate each time. Collect the ethyl acetate layers in a flask, add 20 g of sodium sulfate, allow to stand for 10 minutes with occasional shaking, and filter. Wash the flask and the residue on the filter paper twice with 50 mL of ethyl acetate, and combine the washings with the filtrate. Evaporate the ethyl acetate under a reduced pressure of 6.7 kPa at a temperature below 40°C. Remove the remaining ethyl acetate completely by nitrogen stream. The evaporation of ethyl acetate should be effected as quickly as possible. Successively add 2 mL of pyridine and 1 mL of *N,O*-bis-(trimethylsilyl)trifluoracetamide to the residue, stopper, and dissolve it. Allow the solution to stand for 1 hour, transfer 2 mL of it into a glass vial, and immediately stopper tightly.

Internal Standard Solution Dissolve 0.10 g of glutaric acid, weighed exactly, in water to make exactly 100 mL.

(ii) *Test Solution for Determination of Free Adipic Acid* Weigh accurately about 5 g of Acetylated Distarch Adipate into an Erlenmeyer flask with a stopper, and add 100 mL of water and exactly 1 mL of the internal standard solution. Shake well for 1 hour, and filter through a membrane filter (0.45 μm pore size). To the filtrate, add 1 mL of

hydrochloric acid (in the case of pregelatinized starch or water-soluble starch, directly add 1 mL of hydrochloric acid to the resulting suspension without filtering), and transfer into a separating funnel. Then proceed as directed for the test solution for the determination of total adipic acid, beginning with "～and wash the inside of the flask with a little amount of water into the funnel."

(iii) *Standard Solutions* Dissolve 0.10 g of adipic acid in 90 mL of warm water, cool to room temperature, and add water to make exactly 100 mL. Place exactly 1 mL, 5 mL, 10 mL, and 20 mL of this solution in four separate 50-mL volumetric flasks, and dilute to volume with water. Use these solutions as the standard stock solutions. Weigh 1.0 g of unmodified starch (the same botanical origin as the test substance) into each of four Erlenmeyer flasks with a stopper, and add 50 mL of water and exactly 1 mL of the internal standard solution to each. Then add 5 mL of the standard stock solutions, respectively, to the separate flasks. Shake them well to disperse the starch, add 50 mL of sodium hydroxide solution (4 in 25), and shake for 5 minutes. Place the flasks in a water bath of room temperature, and add cautiously 20 mL of hydrochloric acid. Cool, and separately transfer the contents of the flasks into separating funnels. Then prepare four standard solutions as directed for the test solution for the determination of total adipic acid, beginning with "～and wash the inside of the flask with a little amount of water into the funnel."

(iv) *Procedure* Analyze 1 μL each of the prepared solutions—the test solution for the determination of total adipic acid, the test solution for the determination of free adipic acid, and the standard solutions—by gas chromatography using the operating conditions below. Prepare a calibration curve from the peak area ratios of adipic acid to glutaric acid for the standard solutions and the amounts of adipic acid in the standard solutions. Obtain the peak area ratio of adipic acid to glutaric acid for each of the two different test solutions, and calculate the amount of adipic acid in each test solution from the calibration curve. Determine the content of adipate groups from the following formula:

$$\text{Content (\%) of adipate groups} = \left(\frac{C_T}{M_T} - \frac{C_F}{M_F}\right) \times 100$$

C_T: amount of the adipic acid in the test solution for the determination of total adipic acid (g),

C_F: amount of the adipic acid in the test solution for the determination of free adipic acid (g),

M_T: dry basis weight of the sample in the test solution for the determination of total adipic acid (g),

M_F: dry basis weight of the sample in the test solution for the determination of free adipic acid (g).

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 15 m length) coated

with a 0.25 μm thick layer of a mixture of 50% diphenyl/50% dimethylpolysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 120°C for 5 minute, and raise at 5°C/minute to 150°C.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium or nitrogen.

Flow rate: Adjust the retention times of adipic acid and glutaric acid to about 8 minutes and about 5 minutes, respectively.

Injection method: Split.

Split ratio: 1:30.

(2) Acetyl groups Not more than 2.5%.

Test Solution Weigh accurately about 5 g of Acetylated Distarch Adipate into an Erlenmeyer flask with a stopper, and add 50 mL of water to suspend (100 mL of water in the case of pregelatinized starch or water-soluble starch). Add a few drops of phenolphthalein TS, and add dropwise sodium hydroxide solution (1 in 250) until a pale pink color develops. Add exactly 25 mL of 0.45 mol/L sodium hydroxide, close the flask with the stopper, and agitate for 30 minutes. Remove the stopper, and wash the ground-glass joints and internal surfaces with a little amount of water into the flask.

Procedure Titrate the excess sodium hydroxide with 0.2 mol/L hydrochloric acid, and record the volume consumed as S (mL). The endpoint is when the pale pink color disappears. Separately, conduct a blank test by titrating 25 mL of 0.45 mol/L sodium hydroxide with 0.2 mol/L hydrochloric acid, and record the volume consumed as B (mL). Determine the content of acetyl groups by the following formula:

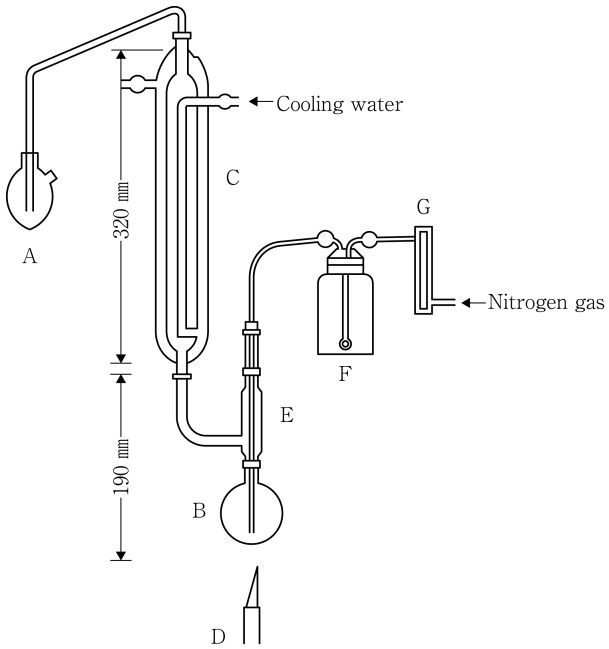
$$\text{Content (\%) of acetyl groups } (CH_3CO-) = \frac{(B - S) \times 0.2 \times 0.043}{\text{Dry-basis weight (g) of the sample}} \times 100$$

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Sulfur dioxide Not more than 50 μg/g.

(i) Apparatus Use the apparatus as illustrated in the figure.



A: 50-mL pear shaped flask

B: 100-mL round bottom flask

C: Double condenser

D: Micro burner

E: Glass capillary

F: Scrubber

G: Flow rate meter

(ii) Method

Test Solution Assemble the apparatus, and connect flask A containing 20 mL of sodium hydroxideTS (0.1 mol/L). Attach flask B containing 20 mL of water, 1 mL of dimedone TS, 1 mL of sodium azide (1 in 100), 2 mL of ethanol (99.5), 2 drops of silicone resin, and 10 mL of diluted phosphoric acid (3 in 10). Pass nitrogen gas through flow rate meter G at 0.5–0.6 L/minute for 5 minutes. Partially detach flask B, promptly put exactly 2.0 g of Acetylated Distarch Adipate into it, and connect again. Adjust the height of burner D so that the top of the flame comes to the bottom of flask B, and heat flask B for about 10 minutes, passing through nitrogen gas at 0.5–0.6 L/minute. Remove flask A, and use the solution obtained in the flask as the test solution.

Standards Solutions Weigh exactly 0.1625 g of sodium hydrogen sulfite, and dissolve it in sodium hydroxide TS (0.1 mol/L) to make 100 mL. Measure exactly 1 mL of this solution, and add sodium hydroxide TS (0.1 mol/L) to make exactly 500 mL. Prepare 6 graduated test tubes (or volumetric flasks) containing exactly 0 mL, 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL of the resulting solution. To each, except for the test tube containing 5 mL of the solution, add sodium hydroxide TS (0.1 mol/L) to make exactly 5 mL. Use the 6 solutions obtained as standards solution for the calibration curve.

Procedure Measure exactly two 5-mL potions of the test solution, and add 0.1 mL of water to one and 0.1 mL of diluted hydrogen peroxide (1 in 100) to the other. Refer to the former as Solution A and the latter as Solution B. To each of Solutions A and B, add exactly 1 mL of pararosaniline–formaldehyde TS, shake well, and allow to stand at room temperature for 15 minutes. Measure the absorbance (A_A and A_B) of both solutions against sodium hydroxide TS (0.1 mol/L) at 580 nm, and determine the difference of the values ($A_A - A_B$).

Prepare a calibration as follows: Add 0.1 mL of water to each of the standard solutions, and proceed as directed for the absorbance measurement for the test solution, beginning with “add 1 mL of pararosaniline–formaldehyde TS” under the Procedure. Measure the

absorbance of each solution, and prepare a calibration curve.

Determine sulfur dioxide concentration (μg/mL) in the test solution from the calibration curve and the absorbance difference ($A_A - A_B$), and calculate the content (μg/g) by the following formula.

$$\text{Content (μg/g) of sulfur dioxide} = \frac{\text{Sulfur dioxide concentration in the test solution (μg/mL)} \times 20}{\text{Dry basis weight (g) of the sample}}$$

Loss on Drying Not more than 21.0% (not more than 13.3kPa, 120°C, 4 hours).

Acetylated Distarch Phosphate

アセチル化リン酸架橋デンプン

[68130-14-3]

Definition Acetylated Distarch Phosphate is obtained by esterifying starch with sodium trimetaphosphate or phosphorus oxychloride, and acetic anhydride or vinyl acetate.

Description Acetylated Distarch Phosphate occurs as a white to off-white powder, or as flakes or granules. It has a faint odor.

Identification

(1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

(3) Proceed as directed in Identification (3) for Acetylated Distarch Adipate.

Purity

(1) Acetyl groups Not more than 2.5%.

Proceed as directed in Purity (2) for Acetylated Distarch Adipate.

(2) Vinyl acetate Not more than 0.1 μg/g.

This specification does not apply to pregelatinized starch.

Test Solution Weigh an amount of Acetylated Distarch Phosphate equivalent to 5.0 g on the dried basis in a 20-mL vial (a vial designed for headspace gas chromatography) containing a stirring bar. Add exactly 5 mL of water, stopper tightly, and stir for 20 minutes.

Standard Solution Weigh 0.10 g of vinyl acetate into a 100-mL volumetric flask containing an appropriate volume of water, dissolve it by adding water, and make up to volume with water. Measure exactly 1 mL of this solution, and dilute to exactly 100 mL with water. Then dilute exactly 1 mL of the resulting solution with water to make a

standard stock solution of exactly 100 mL. Place exactly 5 mL of the standard stock solution into a 20-mL vial (designed for headspace gas chromatography) containing a stirring bar and an amount of unmodified starch (with the same botanical origin as the test substance) equivalent to 5 g on the dried basis, and stopper tightly. Stir for 20 minutes.

Procedure Analyze the test solution and the standard solution by headspace gas chromatography using conditions given below. The peak area of vinyl acetate for the test solution does not exceed that for the standard solution.

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 10 m length) coated with a 3 μm thick layer of styrene divinylbenzene polymer.

Column temperature: A constant temperature of about 90–110°C.

Injection port temperature: 200°C.

Detector temperature: 250°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the peak of vinyl acetate appears about 9–11 minutes after injection.

Injection method: Split.

Split ratio: 1:10

Headspace sampler

Equilibrium temperature in the vial: 70°C.

Equilibrium time in the vial: 30 minutes.

(3) Phosphorous Not more than 0.14% as P.

Test Solution Weigh accurately about 10 g of Acetylated Distarch Phosphate into an evaporating dish, and evenly sprinkle 10 mL of zinc acetate TS to the sample. Carefully evaporate to dryness on a hot plate, and raise its temperature to carbonize the sample. Ignite it in a muffle furnace at 550°C for 1–2 hours until the ash is free from carbon, and cool. Add 15 mL of water, and wash the inner surfaces with 5 mL of diluted nitric acid (1 in 3). Heat to boiling, cool, and transfer the mixture into a 200-mL volumetric flask. Wash the dish three times with three 20-mL portions of water, adding the washings to the flask, and add water to make 200 mL. Transfer an exactly measured aliquot (V mL) of this solution, containing phosphorous (P) not exceeding 1.5 mg, into a 100-mL volumetric flask, and add 10 mL of diluted nitric acid (1 in 3), 10 mL of vanadic acid TS, and 10 mL of hexaammonium heptamolybdate TS for modified starch, mixing thoroughly after each addition. Dilute the mixture to exactly 100 mL with water, and allow to stand for 10 minutes.

Standard Solutions Measure exactly 10 mL of Phosphate Standard Solution, and add water to make exactly 100 mL. Place 5 mL, 10 mL, and 15 mL of this solution in

three separate 100-mL volumetric flasks. To each, add 10 mL of diluted nitric acid (1 in 3), 10 mL of vanadic acid TS, and 10 mL of hexaammonium heptamolybdate TS for modified starch, mixing thoroughly after each addition. Dilute the mixture to exactly 100 mL with water, and allow to stand for 10 minutes.

Procedure Measure the absorbance of each of the test solution and the standard solutions at 460 nm, using the reference prepared as follows: To a 100-mL volumetric flask, add 10 mL of diluted nitric acid (1 in 3), 10 mL of vanadic TS, and 10 mL of hexaammonium heptamolybdate TS for modified starch, mixing thoroughly after each addition, dilute the mixture to exactly 100 mL with water, and allow to stand for 10 minutes.

Determine the concentration of phosphorous in the test solution from the calibration curve, and calculate the content by the following formula.

$$\text{Content (\%) of phosphorus (P)} = \frac{\text{Phosphorus concerntration (mg/mL) in the test solution} \times 2000}{\text{V} \times \text{Dry basis weight (g) of the sample}}$$

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Methd).

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(6) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (Not more than 13.3 kPa, 120°C, 4 hours).

Acetylated Oxidized Starch

アセチル化酸化デンプン

[68187-08-6]

Definition Acetylated Oxidized Starch is obtained through treatment of starch with sodium hypochlorite, followed by esterification with acetic anhydride.

Description Acetylated Oxidized Starch occurs as a white to off-white powder, or as flakes or granules. It has a faint odor.

Identification

(1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

(3) Proceed as directed in Identification (3) for Acetylated Distarch Adipate.

(4) Carboxyl groups Suspend 50 mg of Acetylated Oxidized Starch in 25 mL of a solution of methylene blue (1 in 100), and allow to stand for 5–10 minutes with occasional shaking. Remove the supernatant by decantation, wash the precipitate with water, and examine using an optical microscope. Dark blue starch granules are observed.

In the case of pregelatinized starch, suspend 50 mg of Acetylated Oxidized Starch in 25 mL of a solution (1 in 100) of methylene blue in methanol, and allow to stand overnight. Remove the supernatant by decantation, wash the precipitate with methanol, and examine using an optical microscope. Dark blue fragments of starch granules are observed.

Purity

(1) Acetyl groups Not more than 2.5%.

Proceed as directed in Purity (2) for Acetylated Distarch Adipate.

(2) Carboxyl groups Not more than 1.3%.

If necessary, previously prepare a sample by grinding Acetylated Oxidized Starch with care to prevent moisture, sieving through an 850-μm standard sieve, and mixing well. Weigh 3.00 g of Acetylated Oxidized Starch as is or prepared Acetylated Oxidized Starch into a beaker, add 25 mL of hydrochloric acid (1 in 120), allow to stand for 30 minutes with occasional shaking, and filter with suction. Wash down the residue in the beaker into the filter with the aid of water, and wash the residue on the filter with water until the washings are free of chloride. Transfer the residue into a beaker, add 300 mL of water to suspend, and heat in a water bath with stirring to gelatinize, and heat an additional 15 minutes. Put the beaker out of the water bath, and while the solution is hot, titrate with 0.1 mol/L sodium hydroxide using 3 drops of phenolphthalein TS as the indicator. Record the volume of sodium hydroxide consumed for titration as S (mL). Separately, weigh the equal amount of the sample into a beaker, add 10 mL of water to suspend, and stir for 30 minutes. Filter the suspension with suction, wash down the residue in the beaker into the filter with the aid of water, and wash the residue on the filter paper with 200 mL of water. Suspend the residue in 300 mL of water, proceed as directed for the test above, and record the volume consumed as B (mL). In the case of pregelatinized starch, use 25 mL of a 9 in 1000 solution of hydrochloric acid in 80% (vol) ethanol instead of 25 mL of hydrochloric acid (1 in 120) and 10 mL of 80% (vol) ethanol instead of 10 mL of water. Also the water used for washing the residue should be replaced with 80% (vol) ethanol in that case. Use a filter holder for suction filtration, if necessary.

Obtain the content of carboxyl groups by the formula.

$$\text{Content (\%) of the carboxyl group } (-\text{COOH}) = \frac{(\text{S} - \text{B}) \times 0.45}{\text{Dry basis weight (g) of the sample}}$$

If the sample is originated from of potato starch, the correction should be made as directed below since native phosphate groups present in potato starch increase the titre obtained in this method.

Correction Determine the phosphorous content (%) according to Purity (3) for

Acetylated Distarch Phosphate, calculate the deduction (%) by the following formula, and deduce the percentage from the content of carboxyl groups.

$$\text{Deduction (\%)} = \frac{2 \times 45.02 \times P}{30.97}$$

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (Not more than 13.3 kPa, 120°C, 4 hours).

Acid Clay

酸性白土

Definition Acid Clay is obtained by purifying montmorillonite clay. It consists mainly of hydrous aluminum silicate.

Description Acid Clay occurs as a grayish-white to yellow-brown powder or as granules.

Identification

(1) Mix 1.0 g of Acid Clay with 3.0 g of sodium carbonate and 0.4 g of boric acid, and heat the mixture in a platinum or nickel crucible until it melts completely. Cool, and then add hydrochloric acid until no effervescence is observed. Add an additional 10 mL of hydrochloric acid, heat on a water bath until the mixture becomes gelatinous, cool, and then filter. The filtrate obtained responds to all the tests for Aluminum Salt as directed in the Qualitative Tests.

(2) To a 100-mL measuring cylinder containing 100 mL of water, add 2.0 g of Acid Clay in small portions, and allow to stand for 24 hours. The precipitate formed is not more than 15 mL.

pH 4.0–10.0.

Test Solution Weigh 10.0 g of Acid Clay into an appropriate container, add 100 mL of water, and heat on a water bath for 2 hours with occasional shaking while replenishing the evaporated water. After cooling, filter by suction through a 47-mm diameter membrane filter (0.45-μm pore size). If the filtrate is turbid, repeat the suction filtration through the same filter. Wash the container and the residue on the filter with water, combine the washings with the filtrate, and add water to make 100 mL.

Purity

(1) Water-soluble substances Not more than 0.50%.

Evaporate 50 mL of the test solution prepared for the pH test to dryness. Dry the residue at 110°C for 2 hours, and weigh.

(2) Lead Not more than 40 μg/g as Pb (0.10 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Acid Clay, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while occasionally stirring. Centrifuge the mixture to allow to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, and combine the washings with the filtrate, and allow to cool.

(3) Arsenic Not more than 3 μg/g as As (1.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Acid Clay, add 20 mL of diluted hydrochloric acid (1 in 25) and 50 mL of water, shake well, boil it gently for 30 minutes, and allow to cool. Filter it, wash the residue with water, and combine the washings with the filtrate. Add water to make 100 mL. Evaporate 50 mL of this solution to 5 mL on a water bath.

Loss on Ignition Not more than 35.0% (at 110°C for 3 hours, then at 550°C for 3 hours).

Acid Phosphatase

酸性ホスファターゼ

Definition Acid phosphatase includes enzymes that degrade phosphate monoesters. It is derived from the culture of filamentous fungi (limited to *Aspergillus niger*, *Aspergillus oryzae*) or bacteria (limited to *Escherichia coli*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Acid phosphatase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Acid phosphatase complies with the Acid phosphatase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method). If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits　Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid　Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture　Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Acid phosphatase Activity Test　Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution　Weigh 0.50 g of Acid phosphatase, add water to dissolve it or disperse it uniformly, and make 500 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 0.186 g of disodium *p*-nitrophenylphosphate hexahydrate, and dissolve it in acetic acid–sodium hydroxide buffer (0.2 mol/L) at pH 4.5 to make 50 mL. Prepare fresh before use.

Test solution　Equilibrate 0.5 mL of the substrate solution at 37°C for 5 minutes, add 0.5 mL of the sample solution, and shake immediately. Incubate the mixture at 37°C for 10 minutes, add 4 mL of sodium carbonate TS (0.25 mol/L), and immediately shake.

Control Solution　Equilibrate 0.5 mL of the substrate solution at 37°C for 10 minutes, add 4 mL of sodium carbonate TS (0.25 mol/L), and immediately shake. Then add 0.5 mL of the sample solution.

Procedure　Measure the absorbance of the test solution and the control solution at a wavelength of 405 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Actinidin

アクチニジン

Definition　Actinidin is an enzyme that decomposes protein. It is derived from the fruits of the kiwi plant *Actinidia chinensis* Planch. It is a protease. It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Actinidin occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Actinidin complies with the Actinidin Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Actinidin Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 0.50 g of Actinidin, add water or the diluent prepared in Enzyme Activity Determination for the monograph Papain to dissolve it or disperse it uniformly, and make 200 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same diluent to the resulting solution. Allow the resultig solution to stand in ice water for one hour and use as the sample solution. If the sample is difficult to dissolve or disperse uniformly, irradiate it with ultrasonic waves for 10 minutes while cooling ice water.

Procedure Proceed as directed in the Procedure for Papain. Use trichloroacetic acid solution (9 in 500) instead of trichloroacetic acid TS. Measure the absorbance (A_T & A_b). A_T is larger than A_b.

Activated Acid Clay

活性白土

Definition Activated Acid Clay is obtained by treating acid clay with sulfuric acid. Its

principal constituent is hydrous aluminum silicate.

Description Activated Acid Clay occurs as a whitish to gray powder or as granules.

Identification Mix 1.0 g of Activated Acid Clay with 3.0 g of sodium carbonate and 0.4 g of boric acid, and heat the mixture in a platinum or nickel crucible until it melts completely. Cool, and add hydrochloric acid until no effervescence is observed. Add an additional 10 mL of hydrochloric acid, and heat on a water bath until the mixture becomes gelatinous, cool, and filter. The filtrate obtained responds to all the tests for Aluminum Salt as directed in the Qualitative Tests.

pH 2.0–6.0.

Test Solution Weigh 10.0 g of Activated Acid Clay, add 100 mL of water, and heat on a water bath for 2 hours with occasional shaking while replenishing the evaporated water. After cooling, filter by suction through a 47-mm diameter membrane filter (0.45-μm pore size). If the filtrate is turbid, repeat the suction filtration through the same filter. Wash the container and the residue on the filter with water, combine the washings with the filtrate, and add water to make 100 mL.

Purity

(1) Water-soluble substances Not more than 1.6%.

Measure exactly 50 mL of the test solution prepared for the pH test, and evaporate to dryness. Dry the residue at 110°C for 2 hours, and weigh.

(2) Lead Not more than 40 μg/g as Pb (0.10 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Activated Acid, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while occasionally stirring. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and allow to cool.

(3) Arsenic Not more than 3 μg/g as As (1.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B) .

Test Solution To the specified amount of Activated Acid Clay, add 20 mL of diluted hydrochloric acid (1 in 25) and 50 mL of water, shake well, boil gently for 30 minutes, cool, and filter. Wash the residue with water, combine the washings with the filtrate, and add water to make 100 mL. Evaporate 50 mL of the resulting solution on a water bath to 5 mL.

Loss on Ignition Not more than 35.0% (at 110°C for 3 hours, then at 550°C for 3 hours).

Active Carbon

活性炭

Description Active Carbon occurs as a black powder, or as granules or a fibrous substance. It is odorless and tasteless.

Identification If the sample is a powder, use it as is. If it is in a granular or fibrous form, completely grind into a powder before the tests.

(1) Weigh about 0.1 g of Active Carbon, add 10 mL of 0.001% (w/v) methylene blue TS and 2 drops of diluted hydrochloric acid (1 in 4), shake well, and filter through a dry filter paper for quantitative analysis (5C). The solution is colorless.

(2) Weigh about 0.5 g of powdered Active Carbon into a test tube. When heated over a direct flame while supplying air to the test tube mouth, it burns without flames. When the gas evolved is passed through calcium hydroxide TS, white turbidity is formed.

Purity If the sample is a powder, use it as is. If it is in a granular or fibrous form, completely grind into a powder before the tests. Weigh 4.0 g of Active Carbon, previously dried at 110–120°C for 3 hours, add 180 mL of water containing 0.1 mL of diluted nitric acid (1 in 100), and heat for about 10 minutes, keeping boiling slowly. Cool, add water to make 200 mL, and filter through a dry filter paper for quantitative analysis (5C). Discard about 30 mL of the initial filtrate, and use the subsequent filtrate (Solution A) for tests (1), (2), (3), and (5) below.

(1) Chloride Not more than 0.53% as Cl.

Test Solution 1.0 mL of Solution A.

Control Solution 0.30 mL of 0.01 mol/L hydrochloric acid.

(2) Sulfate Not more than 0.48% as SO_4.

Test Solution 2.5 mL of Solution A.

Control Solution 0.50 mL of 0.005 mol/L sulfuric acid.

(3) Zinc Not more than 0.10% as Zn.

Test Solution Measure 2.0 mL of Solution A, and add water containing 0.1 mL of nitric acid (1 in 100) to make 200 mL.

Control Solution Measure 4.0 mL of Zinc Standard Solution, and add water containing 0.1 mL of nitric acid (1 in 100) to make 200 mL.

Procedure Proceed with the test solution and control solution under the conditions below, as directed under Atomic Absorption Spectrophotometry. The absorbance of the test solution does not exceed that of the control solution.

Operating Conditions

Light source: Zinc hollow cathode lamp.

Wavelength: 213.9 nm.

Supporting gas: Air.

Combustible gas: Acetylene or hydrogen.

(4) Lead Not more than 5 μg/g as Pb (0.80 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Active Carbon, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and allow to cool.

(5) Arsenic Not more than 3 μg/g as As (Method 2, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Measure 25mL of Solution A, and evaporate on a water bath to dryness. Use the residue as the test sample.

Acylase

アシラーゼ

Definition Acylase includes enzymes that hydrolyze *N*-acyl-L-amino acid to produce L-amino acids. It is derived from the culture of filamentous fungi (limited to *Aspergillus ochraceus* and *Aspergillus melleus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Acylase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Acylase complies with the Acylase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Acylase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 0.50 g of Acylase, add water or phosphate buffer (0.02 mol/L) at pH 8.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, 10,000-fold, or 100,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Use either of the following preparation methods.

A. Weigh 0.96 g of *N*-acetyl-DL-methionine, and dissolve it by adding 20 mL of water and 5 mL of sodium hydroxide TS (1 mol/L), adjust its pH to 8.0 with hydrochloric acid TS (0.1 mol/L), and add water to make 50 mL.

B. Weigh 1.23 g of *N*-acetyl-DL-tryptophane, dissolve it by adding 10 mL of water and 10 mL of sodium hydroxide TS (1 mol/L), adjust its pH to 8.0 with hydrochloric acid TS (0.1 mol/L), and add water to make 50 mL.

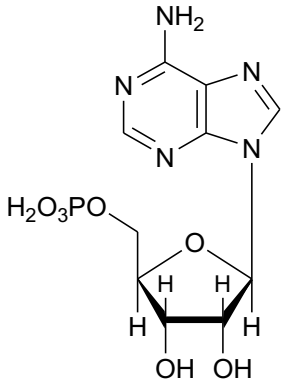
Test Solution To 1 mL of the sample solution, add 2 mL of sodium barbital–hydrochloric acid buffer (0.1 mol/L) at pH 8.0 and 1 mL of cobalt(II) chloride TS (0.5 mmol/L), equilibrate the mixture at 37°C for 5 minutes, add 1 mL of the substrate solution, and shake. Incubate the mixture at 37°C for 30 minutes. Heat 1 mL of the solution immediately in a water bath for 3 minutes, and cool.

Control Solution Proceed as directed for the test solution. Immediately after the addition of the substrate solution, heat 1 mL of the resulting solution in a water bath for 3 minutes, and cool.

Procedure To each of the test solution and control solution, add 2 mL of ninhydrin–2-methoxyethanol–citrate buffer TS and 0.1 mL of tin(II) chloride TS, heat in a water bath for 20 minutes, and cool. Add 10 mL of a 1:1 mixture of 1-propanol/water to each, and shake, and measure the absorbance of them at a wavelength of 570 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

5′-Adenylic Acid

5′-アデニル酸



$C_{10}H_{14}N_5O_7P$ Mol. Wt. 347.22

Adenosine 5′-monophosphoric acid [61-19-8]

Definition 5′-Adenylic Acid is obtained by enzymatic hydrolysis of nucleic acids that are water-extracted from the cells of yeasts (*Candida utilis* only), followed by isolation. It consists of 5′-adenylic acid.

Content 5′-Adenylic Acid, when calculated on the dried basis, contains 98.0–102.0% of 5′-adenylic acid ($C_{10}H_{14}N_5O_7P$).

Description 5′-Adenylic Acid occurs as colorless or white crystals or as a white crystalline powder.

Identification

(1) Dissolve 10 mg of 5′-Adenylic Acid in 1000 mL of diluted hydrochloric acid (1 in 1000). The solution obtained exhibits an absorption maximum at a wavelength of 255–259 nm.

(2) Dissolve 0.25 g of 5′-Adenylic Acid in 1 mL of sodium hydroxide TS (1 mol/L), and add 5 mL of water. To the resulting solution, add 2 mL of magnesia TS. No precipitate is formed. Add 7 mL of nitric acid, and boil for 10 minutes. It responds to test (2) for Phosphate in the Quantitative Tests.

Purity

(1) Clarity of solution Colorless and almost clear.

Test Solution Weigh 0.50 g of 5′-Adenylic Acid, dissolve it in 2 mL of sodium hydroxide TS (1 mol/L), and add water to make 10 mL.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard

Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of 5′-Adenylic Acid in 5 mL of diluted hydrochloric acid (1 in 4).

(4) Absorbance ratio Weigh 10 mg of 5′-Adenylic Acid, and dissolve it in diluted hydrochloric acid (1 in 1000) to make 1000 mL. When the absorbance values of the solution at 250 nm, 260 nm, and 280 nm are expressed as A_1, A_2, and A_3, respectively, A_1/A_2 is 0.82–0.88, and A_3/A_2 is 0.19–0.23.

(5) Other nucleic acid degradation products

Test Solution Weigh 0.10 g of 5′-Adenylic Acid, dissolve it in 0.5 mL of sodium hydroxide TS (1 mol/L), and add water to make 20 mL.

Procedure Analyze a 1-μL portion of the test solution by thin-layer chromatography using a 6:5:2 mixture of 1-propanol/ammonia TS/acetone as the developing solvent. No control solution is used. Use a thin-layer plate coated with fluorescent silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. Examine under ultraviolet light (around 250 nm) in a dark place. Only one spot is observed.

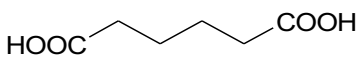
Loss on Drying Not less than 6.0% (120°C, 4 hours).

Assay Weigh accurately about 0.2 g of 5′-Adenylic Acid, dissolve it in 1 mL of sodium hydroxide TS (1 mol/L), and add water to make exactly 200 mL. To exactly 2 mL of this solution, add diluted hydrochloric acid (1 in 1000) to make exactly 200 mL. Measure the absorbance (A) of the solution at 257 nm, and determine the content using the formula:

$$\text{Content (\%) of 5′-adenylic acid } (C_{10}H_{14}N_5O_7P) = \frac{0.2 \times 2.315 \times A}{\text{Drybasis weight (g) of the sample}} \times 100$$

Adipic Acid

アジピン酸



$C_6H_{10}O_4$ Mol. Wt. 146.14

Hexanedioic acid [124-04-9]

Content Adipic Acid contains not less than 99.6% of adipic acid ($C_6H_{10}O_4$).

Description Adipic Acid occurs as white crystals or crystalline powder. It is odorless and has an acid taste.

Identification

(1) To 5 mL of a solution of Adipic Acid (1 in 20), add ammonia TS to adjust the pH to

about 7, and add 2–3 drops of a solution of iron(III) chloride hexahydrate (1 in 10). A brown precipitate is formed.

(2) Transfer 50 mg of Adipic Acid into a test tube, add 50 mg of resorcinol and 1 mL of sulfuric acid, and shake. Heat at 130°C for 10 minutes. Add, dropwise, sodium hydroxide solution (3 in 10) while cooling to make alkaline. Add water to make 10 mL. A red-purple color develops.

Melting Point 151.5–154°C.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

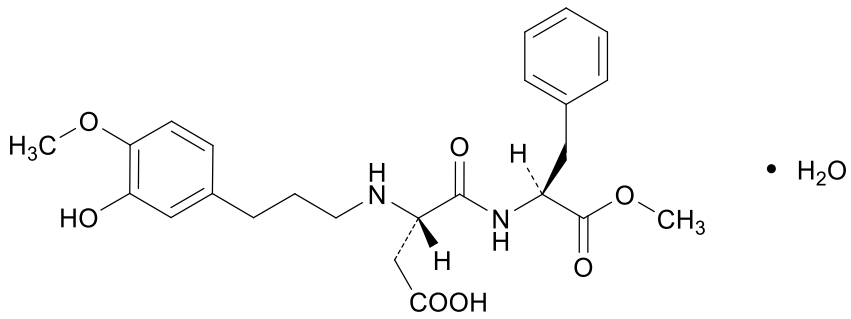
Water Content Not more than 0.20% (1 g, Direct Titration).

Assay Weigh accurately about 1.5 g of Adipic Acid, and dissolve it in 75 mL of freshly boiled and cooled water. Titrate with 0.5 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each mL of 0.5 mol/L sodium hydroxide = 36.54 mg of $C_6H_{10}O_4$

Advantame

アドバンテーム



$C_{24}H_{30}N_2O_7 \cdot H_2O$ Mol. Wt. 476.52

Methyl *N*-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L-α-aspartyl-L-phenylalaninate monohydrate [714229-20-6]

Content Advantame, when calculated on the anhydrous basis, contains 97.0–102.0% of advantame ($C_{24}H_{30}N_2O_7$ = 458.50).

Description Advantame is a white to yellowish-white powder.

Identification Determine the infrared absorption spectrum of Advantame as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference

Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Rotation $[\alpha]_D^{20}$: –39 to –46° (0.2 g, ethanol (99.5), 100 mL, on the anhydrous basis).

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Advantame-acid Not more than 1.0%.

Test Solution Weigh accurately about 0.1 g of Advantame, and add a 7:3 mixture of water/acetonitrile to dissolve it and make exactly 100 mL.

Standard Solution Weigh accuretaly about 0.1 g of advantame-acid, and add a 7:3 mixture of water/ acetonitrile to dissolve it and make exactly 100 mL. Measure exactely 2 mL of this solution, and add a 7:3 mixture of water/acetonitrile to make exactly 20 mL. Measure exactely 2 mL of the second solution, and add a 7:3 mixture of water/acetonitrile to make exactly 20 mL.

Procedure Analyze 20-μL potions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of advantame-acid for the test solution and the standard solution, and determine its amount by the formula:

$$\text{Amount (\%) of advantame} - \text{acid} = \frac{M}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S}$$

M = weight (g) of advantame-acid taken

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength 210 nm).

Column: A stainless steel tube (about 4.6 mm internal diameter and about 25 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of around 50°C.

Mobile phase

A: Dissolve 13.6 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.8 with phosphoric acid. To 900 mL of this solution, add 100 mL of acetonitrile.

B: Dissolve 13.6 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.8 with phosphoric acid. To 400 mL of this solution, add 600 mL of acetonitrile.

Concentration gradient (A/B): Maintain at 85/15 for 30 minutes, and run a linear

gradient from 85/15 to 75/25 in 25 minutes, then 75/25 to 0/100 in 20 minutes, and maintain at 0/100 for 15 minutes.

Flow rate: 1.0 mL/minute.

(3) Related substances other than advantame-acid Not more than 1.5%.

Test Solution and Standard Solution Use the test solution and the standard solution prepatred in Purity (2). Analyze 20-μL potions of these solutions by liquid chromatography using the operating conditions given below. Continues the chromatography for about three times the retention time of advantame-acid. Measure the sum (A_{sum}) of peak areas of all components, other than advantame and advantame-acid, in the test solution. Also measure the peak area (A_S) of advantame-acid in the standard solution. Determine the amount of the related substances other than advantame-acid by the formula:

$$\text{Amount (\%) of related substances} = \frac{M}{\text{Weight (g) of the sample}} \times \frac{A_{sum}}{A_S}$$

M = weight (g) of advantame-acid taken.

Operating Conditions Follow the operating conditions specified in Purity (3).

Water Content Not more than 5.0% (0.1g, Volumetric Titration, Direct Titration).

Residue on Ignition Not more than 0.2% (550°C, 3 hours)

Assay

Test Solution Weigh accurately about 40 mg of Advantame, and add a 7:3 mixture of water/acetonitrile to dissolve it and make exactely 50 mL. To exactly measured 10 mL of this solution, add exactly 5 mL of the internal standard and a 7:3 mixture of water/acetonitrile to make exactly 50 mL.

Standard Solution Weigh accurately about 40 mg of advantame for assay, and proceed as directed for the test solution.

Internal Standard To 40 mg of benzoic acid, exactly weighed, add a 7:3 mixture of water/acetonitrile to make 50 mL.

Procedure Analyze 20-μL potions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Determine the peak area ratios (Q_T and Q_S) of advantame to benzoic acid for both solutions to calculate the Content of advantame by the formula:

$$\text{Content (\%) of advantame } (C_{24}H_{30}N_2O_7) = \frac{\text{Anhydrous based weight (g) of advantame for assay}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength 280 nm).

Column: A stainless steel tube (about 4.6 mm internal diameter and about 25 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of around 40°C.

Mobile phase

A: Dissolve 13.6 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.8 with phosphoric acid. To 750 mL of this solution, add 250 mL of acetonitrile.

B: Dissolve13.6 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 2.8 with phosphoric acid. To 500 mL of this solution, add 500 mL of acetonitrile.

Concentration gradient (A/B): Maintain at 100/0 for 20 minutes, and run a linear gradient from 100/0 to 0/100 in 5 minutes, then maintain at 0/100 for 5 minutes.

Flow rate: 1.0 mL/minute.

Agarase

アガラーゼ

Definition Agarase includes enzymes that hydrolyze β-1, 4- or β-1, 3-galactosidic linkages in agar. It is derived from the culture of basidiomycetes (limited to species of the genus *Coriolus*) or bacteria (limited to species of the genera *Bacillus* and *Pseudomonas*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Agarase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Agarase complies with the Agarase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Agarase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 1.0 g of Agarase, add phosphate buffer (0.01 mol/L) at pH 7.0 or water to dissolve it or disperse it uniformly, and make 10 mL. As necessary, prepare a 10-fold or 100-fold dilution by adding the same buffer or water to the resulting solution.

Substrate Solution Weigh 1.0 g of agar, previously dried under reduced pressure at 80°C for 5 hours, add it to about 70 mL of phosphate buffer (0.01 mol/L) at pH 7.0, and dissolve by boiling. Cool the solution to 40°C, and keep it warming at 40°C. To this solution, add phosphate buffer (0.01 mol/L) at pH 7.0, previously warmed to 40°C, to make 100 mL. Prepare fresh before use. Keep the prepared substrate solution at 40°C.

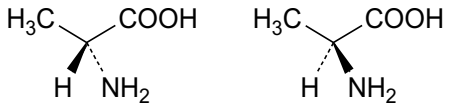
Test Solution To 0.25 mL of the substrate solution, equilibrated at 40°C, add 0.25 mL of the sample solution, equilibrated at 40°C, shake immediately. Incubate the mixture at 40°C for 10 minutes. To this solution, add 1.5 mL of 3,5-dinitro salicylate–phenol TS (reagent for the agarase activity test), shake immediately, and heat in a water bath for 5 minutes. After cooling, add 5 mL of water, agitate, and centrifuge at 3000 rpm for 10 minutes to deposit the gel. Use the supernatant as the test solution.

Control Solution To 0.25 mL of the sample solution, equilibrated at 40°C, add 1.5 mL of 3,5-dinitro salicylate–phenol TS (reagent for the agarase activity test) and 0.25 mL of the substrate solution, and shake. Heat the mixture in a water bath for 5 minutes, and cool it. Proceed as directed for the test solution.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 540 nm. The absorbance value of the test solution is higher than that of the control solution.

DL-Alanine

DL-アラニン



$C_3H_7NO_2$ Mol. Wt. 89.09

(2*RS*)-2-Aminopropanoic acid [302-72-7]

Content DL-Alanine, when calculated on the dried basis, contains 98.5–102.0% of DL-alanine ($C_3H_7NO_2$).

Description DL-Alanine occurs as a colorless to white crystalline powder having a sweet taste.

Identification Determine the absorption spectrum of DL-Alanine as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH 5.5–7.0 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, Water 10 mL).

(2) Chloride Not more than 0.021% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL.)

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

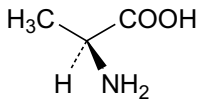
Residue on Ignition Not more than 0.2%.

Assay Weigh accurately about 0.2 g of DL-Alanine, dissolve it in 3 mL of formic acid, add 50 mL of acetic acid, and titrate with 0.1 mol/L perchloric acid. The endpoint is usually confirmed potentiometrically. When crystal violet–acetic acid TS (1 mL) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, make any necessary correction, and calculate on the dried basis.

Each mL of 0.1 mol/L perchloric acid = 8.909 mg of $C_3H_7NO_2$

L-Alanine

L-アラニン



$C_3H_7NO_2$ Mol. Wt. 89.09

(2*S*)-2-Aminopropanoic acid [56-41-7]

Content L-Alanine, when calculated on the dried basis, contains 98.0–102.0% of L-alanine ($C_3H_7NO_2$).

Description L-Alanine occurs as white crystals or crystalline powder. It is odorless, and has a sweetish taste.

Identification

(1) To 5 mL of a solution of L-Alanine (1 in 1000), add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A bluish purple color develops.

(2) Dissolve 0.2 g of L-Alanine in 10 mL of diluted sulfuric acid (1 in 20), add 0.1 g of potassium permanganate, and heat to boil. An odor of acetaldehyde develops.

Specific Rotation $[\alpha]_D^{20}$: +13.5 to +15.5° (10 g, hydrochloric acid TS (6 mol/L), 100 mL, on the dried basis).

pH 5.7–6.7 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.10% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

Residue on Ignition Not more than 0.2%.

Assay Weigh accurately about 0.2 g of L-Alanine, and proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 8.909 mg of $C_3H_7NO_2$

L-Alanine Solution

L-アラニン液

Content L-Alanine Solution contains not more than 15% of L-alanine ($C_3H_7NO_2$ = 89.09) and contains 95.0–110.0% of the labeled content of L-alanine.

Description L-Alanine Solution is a colorless liquid. It is odorless or has a very slight characteristic odor. It has a sweetish taste.

Identification

(1) To 5 mL of diluted L-Alanine Solution (1 in 200), add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A bluish purple color develops.

(2) To 5 g of L-Alanine Solution, add 50 mL of diluted hydrochloric acid (1 in 2), and mix. It shows dextrorotatory.

Purity

(1) Lead Not more than 2 μg/g of L-alanine ($C_3H_7NO_2$) as Pb (Amount equivalent to 2.0 g of L-alanine ($C_3H_7NO_2$), Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g of L-alanine ($C_3H_7NO_2$) as As (Amount equivalent to 0.50 g of L-alanine ($C_3H_7NO_2$), Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Add 5 mL of water to the specified amount of L-Alanine Solution, and heat if necessary.

Residue on Ignition Not more than 0.20% per L-alanine ($C_3H_7NO_2$).

Assay Weigh accurately an amount of L-Alanine Solution equivalent to about 0.2 g of L-alanine ($C_3H_7NO_2$), and proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 8.909 mg of $C_3H_7NO_2$

Alginate Lyase

アルギン酸リアーゼ

Definition Alginate Lyase includes enzymes that remove alginate. It is derived from the culture of bacteria (limited to *Alteromonas macleodii, Flavobacterium multivorum, Flavobacterium* sp., and species of the genera *Pseudomonas* and *Xanthomonas*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Alginate Lyase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Alginate Lyase complies with the Alginate Lyase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Alginate Lyase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 0.50 g of Alginate Lyase, add water or potassium phosphate–sodium hydroxide buffer (0.1 mol/L) at pH 6.3 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer or water to the resulting solution.

Substrate Solution To 0.10 g of sodium alginate, add 50 mL of potassium phosphate–sodium hydroxide buffer (0.2 mol/L) at pH 5.8 and 20 mL of water, and dissolve it by stirring over night. Adjust its pH to 6.3 with sodium hydroxide TS (2 mol/L), and add water to make 100 mL.

Test Solution Equilibrate 4.5 mL of the substrate solution at 37°C for 5 minutes, add 0.15 mL of the sample solution, and shake immediately. Incubate the mixture at 37°C for 30 minutes, add 4.65 mL of sodium hydroxide TS (0.1 mol/L), and shake immediately.

Control Solution Equilibrate 4.5 mL of the substrate solution at 37°C for 5 minutes, add 4.65 mL of sodium hydroxide TS (0.1 mol/L) and 0.15 mL of the sample solution, and shake immediately. Incubate this solution at 37°C for 30 minutes.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 235 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Alginic Acid

アルギン酸

[9005-32-7]

Content Alginic Acid, when calculated on the dried basis, contains 91.0–104.5% of alginic acid.

Description Alginic Acid occurs in white to light yellow filamentous, granular, or powdered form. It has a slight characteristic odor.

Identification Prepare a test solution by dissolving 0.25 g of Alginic Acid in 50 mL of sodium hydroxide TS (1 mol/L). To 10 mL of the test solution, add 2 mL of a solution of calcium chloride dihydrate (1 in 40). A gelatinous precipitate is formed. To 10 mL of the test solution, add 5 mL of ammonium sulfate saturated solution. No precipitate is formed.

Specific Rotation $[\alpha]_D^{20}$: – 80 to – 180° (0.5 g, sodium hydroxide TS (1 mol/L), 100 mL, on the dried basis).

pH 2.0–3.4 (3% suspension),

Purity

(1) Sulfate Not more than 0.96% as SO_4.

Test Solution Weigh 0.10 g of Alginic Acid in a flask, dissolve it in 20 mL of sodium hydroxide TS (1 mol/L), and neutralize with diluted hydrochloric acid (1 in 4). Next, add 1 mL of hydrochloric acid, shake well, heat in a water bath for several minutes, cool, and filter. Wash the flask three times with 10 mL of water each time, filter the washings through the same filter paper, combine the filtrates, and add water to make 50 mL. Measure 10 mL of this solution, and add water to make 50 mL.

Control Solution To 0.40 mL of 0.005 mol/L sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(2) Phosphate Weigh 0.10 g of Alginic Acid, dissolve it 20 mL of sodium hydroxide TS (1 mol/L), and neutralize with diluted nitric acid (1 in 4), and make it uniformly. Cool, add 5 mL of diluted nitric acid (1 in 4) and 20 mL of Ammonium Molybdate TS, and warm. No yellow precipitate is formed.

(3) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 15.0% (105°C, 4 hours).

Residue on Ignition Not more than 10.0% (calculated on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Coliforms: Negative per test.

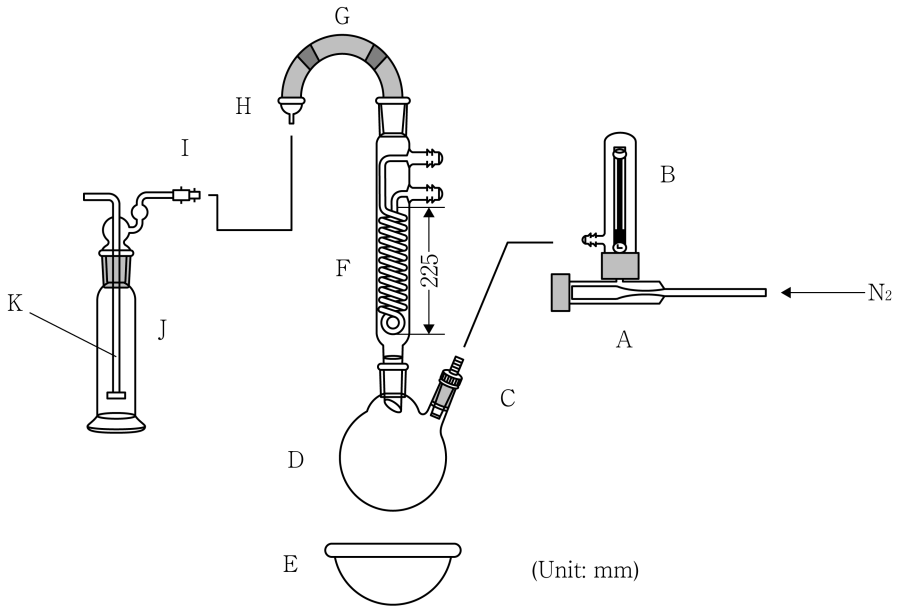
Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 2 for total plate count and the enumeration of yeasts and molds. Previously add sodium hydroxide solution to the diluent to be used.

Pre-enrichment Culture Prepare as directed in Method 2 for the coliform test. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 5 g of the sample with 500 mL of lactose broth to disperse completely, and incubate it at 35 ± 1°C for 24 ± 2 hours.

Assay

(1) Apparatus Use the apparatus as illustrated in the figure.



A: Capillary valve

B: Flow meter

C: Plastic fitting (which is connected to B by a halogenated vinyl plastic tube)

D: Reaction flask

E: Heating mantle

F: 225-mm Coil reflux condenser

G: U-shaped trap (containing two 25-g bands of sandy zinc, being bounded and separated by three 7-cm plugs of glass wool)

H: Adapter (which is connected to I by a halogenated vinyl plastic tube)

I: Connector

J: Gas washing bottle

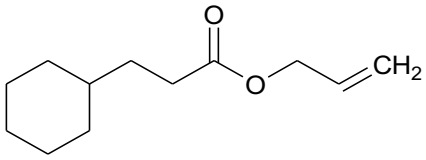
K: Inlet tube (which extends to the bottom of the gas washing bottle and terminates in a fritted disk with a coarse porosity)

(2) Procedure Weigh accurately about 0.25 g of Alginic Acid into reaction flask D, add 50 mL of diluted hydrochloric acid (1 in 120) and several boiling chips, and connect the flask to reflux condenser F. Moisten the joint part with a small amount of phosphoric acid. Connect the nitrogen line to the sidearm of the flask, and adjust the flow of cooling water to about 2 L/minute. Maintain the flow of nitrogen through the apparatus at 90–100 mL/minute. Heat the sample on heating mantle E to boiling, and boil gently for 2 minutes. Lower the mantle, and allow to cool for 10 minutes. Connect the empty gas washing bottle assembly, J, with adapter H, and sweep the system with nitrogen at a rate of 90–100 mL/minute for 5 minutes to purge the air in bottle J. Reduce the nitrogen flow to 60–65 mL/minute, and add 10 drops of 1-butanol and exactly 25 mL of 0.25 mol/L sodium hydroxide. Then add 50 mL of water into bottle J, rinsing down the inside of bottle J and inlet tube K, and replace the cap. Detach connector rubber fitting C from the sidearm, add 46 mL of hydrochloric acid through the sidearm. Reattach the nitrogen line, raise the heating mantle, and heat the reaction mixture in flask D to boiling. After 3 hours of boiling, lower heating mantle E, increase the nitrogen flow to 90–100 mL/minute, and allow to cool for 10 minutes. Disconnect and disassemble the washing bottle, wash inlet tube K with water, and collect the washings in the gas washing bottle. Use nitrogen to gently force all water out of inlet tube K, collecting the washings in bottle J. To bottle J, add (1 in 10) and a magnetic stirring bar. Tightly stopper, stir gently for 1 minute, and allow to stand for 5 minutes. Add 3 drops of phenolphthalein TS, titrate with 0.1 mol/L hydrochloric acid. Separately perform a blank test.

Each mL of 0.25 mol/L sodium hydroxide = 25.00 mg of alginic acid

Allyl Cyclohexylpropionate

シクロヘキシルプロピオン酸アリル



$C_{12}H_{20}O_2$ Mol. Wt. 196.29

Allyl 3-cyclohexylpropionate [2705-87-5]

Content Allyl Cyclohexylpropionate contains not less than 98.0% of allyl cyclohexylpropionate ($C_{12}H_{20}O_2$).

Description Allyl Cyclohexylpropionate is a colorless to light yellow, clear liquid having

a characteristic odor.

Identification Determine the absorption spectrum of Allyl Cyclohexylpropionate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.457–1.462.

Specific Gravity d_{25}^{25}: 0.945–0.950.

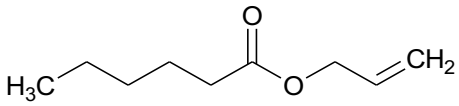
Purity Acid value Not more than 5.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Allyl Hexanoate

Allyl Caproate

ヘキサン酸アリル



$C_9H_{16}O_2$ Mol. Wt. 156.22

Prop-2-en-1-yl hexanoate [123-68-2]

Content Allyl Hexanoate contains not less than 98.0% of allyl hexanoate ($C_9H_{16}O_2$).

Description Allyl Hexanoate is a colorless to light yellow, clear liquid having a pineapple-like odor.

Identification Determine the absorption spectrum of Allyl Hexanoate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.422 –1.426.

Specific Gravity d_{25}^{25}: 0.884–0.890.

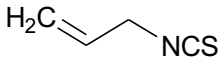
Purity Acid value Not more than 1.0. (Flavoring Substances Tests)

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Allyl Isothiocyanate

Volatile Oil of Mustard

イソチオシアン酸アリル



C_4H_5NS Mol. Wt. 99.15

Allyl isothiocyanate [57-06-7]

Content Allyl Isothiocyanate contains not less than 97.0% of allyl isothiocyanate (C_4H_5NS).

Description Allyl Isothiocyanate is a colorless to light yellow, clear liquid having a strong and irritating mustard-like odor.

Identification Determine the absorption spectrum of Allyl Isothiocyanate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.528–1.532.

Specific Gravity d_{20}^{20}: 1.018–1.024.

Purity Phenols and thiocyanate compounds

Measure 1.0 mL of Allyl Isothiocyanate, dissolve it in 5 mL of ethanol (95), and add 1 drop of a solution of iron(III) chloride hexahydrate (1 in 10). Neither red nor blue color develops.

Assay Weigh accurately about 3 g of Allyl Isothiocyanate, and dissolve it in ethanol (95) to make exactly 100 mL. Measure exactly 5 mL of this solution, add 5 mL of ammonia TS, and add exactly 50 mL of 0.1 mol/L silver nitrate. Heat under a reflux condenser in a water bath for 1 hour. Cool, add water to make exactly 100 mL, and filter through a dry filter paper. Discard about 10 mL of the initial filtrate, then measure exactly 50 mL of the subsequent filtrate, add 5 mL of nitric acid and 2 mL of ammonium iron(III) sulfate–sulfuric acid TS, and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate. Perform a blank test in the same manner.

Each mL of 0.1 mol/L silver nitrate = 4.958 mg of C_4H_5NS

Aluminium Ammonium Sulfate

(Aluminum Ammonium Sulfate)

Crystal: Ammonium Alum
Dried: Burnt Ammonium Alum

硫酸アルミニウムアンモニウム

$AlNH_4(SO_4)_2 \cdot nH_2O$ (n = 12, 10, 4, 3, 2, or 0)　　Mol. Wt. dodecahydrate　453.33

anhydrous　237.15

Aluminum ammonium sulfate dodecahydrate　[7784-26-1]

Aluminum ammonium sulfate decahydrate

Aluminum ammonium sulfate tetrahydrate

Aluminum ammonium sulfate trihydrate

Aluminum ammonium sulfate dihydrate

Aluminum ammonium sulfate　[7784-25-0]

Definition　Aluminum Ammonium Sulfate occurs in crystalline form, called Aluminum Ammonium Sulfate, and in dried form, called Aluminum Ammonium Sulfate (dried).

Content　Aluminum Ammonium Sulfate, when dried at 200°C for 4 hours, contains not less than 96.5% of aluminum ammonium sulfate ($AlNH_4(SO_4)_2$).

Description　Aluminum Ammonium Sulfate occurs as colorless to white crystals, powder, flakes, granules, or lumps. It is odorless and has an astringent taste.

Identification　A solution of Aluminum Ammonium Sulfate (1 in 20) responds to all the tests for Aluminum Salt and for Ammonium Salt, and responds to tests (1) and (3) for Sulfate in the Qualitative Tests.

Purity

(1) Clarity of solution

Crystal: Clarity of solution　Colorless and almost clear (1.0 g, water 10 mL).

(2) Lead　Not more than 3 μg/g as Pb (2.0 g of the sample previously powdered and dried at 200°C for 4 hours, Method 5, Control Solution: Lead Standard Solution 6.0 mL, Flame Method).

Sample Solution　To the specified amount of Aluminum Ammonium Sulfate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution.

If the sample does not dissolve completely, evaporate the solution to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(3) Iron　Not more than 0.019% as Fe (52 mg of the sample previously powdered and

dried at 200°C for 4 hours, Method 1, Control Solution: Iron Standard Solution 1.0 mL).

(4) Arsenic　Not more than 3 μg/g as As (0.50 g of the powdered sample previously dried at 200°C for 4 hours, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Water-insoluble substances

Dried: Water-insoluble substances　Not more than 2.0%.

Weigh 2.0 g of Aluminum Ammonium Sulfate, and add 200 mL of water of about 80°C. Heat in a water bath for 10 minutes while stirring. Cool, and filter through a glass filter (1G4), previously dried at 105°C for 30 minutes, cooled, and accurately weighed. Wash the insoluble residue with 100 mL of water, and dry at 105°C for 2 hours together with the glass filter, weigh, and obtain the insoluble substances.

Assay　Weigh accurately about 0.8 g (as powder) of Aluminum Ammonium Sulfate, previously dried at 200°C for 4 hours, add 100 mL of water, dissolve by heating in a water bath while shaking, and filter. Wash the residue with water, combine the washings with the filtrate, and add water to make exactly 200 mL. Measure exactly 25 mL of this solution, and proceed as directed in the Assay for Aluminum Potassium Sulfate.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.371 mg of $AlNH_4(SO_4)_2$

Aluminium Potassium Sulfate

(Aluminum Potassium Sulfate)

Crystal: Alum or Potassium Alum
Dried: Burnt Alum

硫酸アルミニウムカリウム

$AlK(SO_4)_2 \cdot nH_2O$ (n = 12, 10, 6, 3, 2, or 0)　　Mol. Wt. dodecahydrate　474.39

anhydrous　258.21

Aluminum potassium sulfate dodecahydrate　[7784-24-9]

Aluminum potassium sulfate decahydrate

Aluminum potassium sulfate hexahydrate

Aluminum potassium sulfate trihydrate

Aluminum potassium sulfate dihydrate

Aluminum potassium sulfate　[10043-67-1]

Definition　Aluminum Potassium Sulfate occurs in crystalline form, called Aluminum Potassium Sulfate, and in dried form, called Aluminum Potassium Sulfate (dried).

Content Aluminum Potassium Sulfate, when dried at 200°C for 4 hours, contains not less than 96.5% of aluminum potassium sulfate ($AlK(SO_4)_2$).

Description Aluminum Potassium Sulfate occurs as colorless to white crystals, powder, flakes, granules, or lumps. It is odorless and has an astringent taste.

Identification A solution of Aluminum Potassium Sulfate (1 in 20) responds to all the tests for Aluminum Salt, to test (1) for Potassium Salt, and to tests (1) and (3) for Sulfate in the Qualitative Tests.

Purity

(1) Clarity of solution

Crystal: Clarity of solution Colorless and almost clear (1,0 g, water 10 mL).

(2) Lead Not more than 5 μg/g as Pb (0.80 g of the sample previously powdered and dried at 200°C for 4 hours, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Aluminum Potassium Sulfate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate the solution to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(3) Iron Not more than 0.019% as Fe (54 mg of the sample previously powdered and dried at 200°C for 4 hours, Method 1, Control Solution: Iron Standard Solution 1.0 mL).

(4) Arsenic Not more than 3 μg/g as As (0.50 g of the sample previously powdered and dried at 200°C for 4 hours, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Water-insoluble substances

Dried: Water-insoluble substances Not more than 2.0% .

Add 200 mL of water of about 80°C to 2.0 g of Aluminum Potassium Sulfate, heat in a water bath for 10 minutes, and allow to cool. Filter it through a glass filter (1G4), previously dried at 105°C for 30 minutes, cooled, and weighed accurately. Wash the residue on the filter with 100 mL of water, and dry the glass filter containing the residue at at 105°C for 2 hours. Weigh the filter, and calculate the water-insoluble substances.

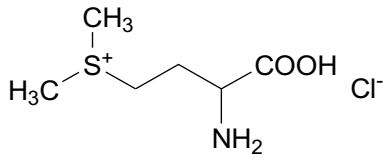
Assay Weigh accurately about 0.8 g (as powder) of Aluminum Potassium Sulfate, previously dried at 200°C for 4 hours, add 100 mL of water, dissolve by heating in a water bath while shaking, and filter. Wash the residue thoroughly with water, combine the washings with filtrate, and add water to make exactly 200 mL. Measure exactly 25 mL of this solution, add exactly 50 mL of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate, and heat to boiling. After cooling, add 7 mL of a solution of sodium acetate trihydrate (2 in 15) and 85 mL of ethanol (99.5), and titrate the excess disodium dihydrogen ethylenediaminetetraacetate with 0.01 mol/L zinc acetate (indicator: 3 drops of xylenol orange TS) until the yellow color of the solution changes to

red.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.582 mg of $AlK(SO_4)_2$

(3-Amino-3-carboxypropyl)dimethylsulfonium Chloride

(3-アミノ-3-カルボキシプロピル)ジメチルスルホニウム塩化物



$C_6H_{14}ClNO_2S$ Mol. Wt. 199.70

(3-Amino-3-carboxypropyl)dimethylsulfonium Chloride [3493-12-7]

Content (3-Amino-3-carboxypropyl)dimethylsulfonium Chloride, when dried, contains not less than 98.0% of (3-amino-3-carboxypropyl)dimethylsulfonium chloride ($C_6H_{14}ClNO_2S$).

Description (3-Amino-3-carboxypropyl)dimethylsulfonium Chloride occurs as white crystals or powder having a characteristic odor.

Identification Dry (3-Amino-3-carboxypropyl)dimethylsulfonium Chloride in a vacuum desiccator for 3 hours, and determine the infrared absorption spectrum as directed in the Paste Method under Infrared Spectrophotometry using optical plates made from sodium chloride. Compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 138–143°C (decomposition).

Assay Weigh accurately about 0.3 g of (3-Amino-3-carboxypropyl)dimethylsulfonium Chloride, previously dried in vacuum desiccator for 3 hours. Dissolve it by adding 70 mL of water and 1 mL of 0.1 mol/L hydrochloric acid, and titrate with 0.1 mol/L potassium hydroxide. Usually, a potentiometer is used to confirm the endpoint. Determine the content using the volume of 0.1 mol/L potassium hydroxide consumed between the first and second inflection points.

Each mL of 0.1 mol/L potassium hydroxide = 19.970 mg of $C_6H_{14}ClNO_2S$

Aminopeptidase

アミノペプチダーゼ

Definition Aminopeptidase includes enzymes that degrade proteins and peptides from the amino terminus. It is derived from the culture of filamentous fungi (limited to

Aspergillus oryzae and *Rhizopus oryzae*), yeasts (limited to *Pseudozyma hubeiensis*), actinomycetes (limited to *Streptomyces avermitilis, Streptomyces cinnamoneus*, *Streptomyces griseus*, *Streptomyces thermoviolaceus*, and *Streptomyces violaceoruber*), or bacteria (limited to *Aeromonas caviae*, *Bacillus licheniformis*, *Lactobacillus casei*, and *Lactococcus lactis*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Aminopeptidase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Aminopeptidase complies with the Aminopeptidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* and Method 2 for the *Salmonella* tests.

Aminopeptidase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of Aminopeptidase, add acetate buffer (0.2 mol/L) at pH 4.0 or water to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer or water to the resulting solution.

Substrate Solution Weigh 55 mg of L-glutamyl-L-tyrosyl-L-glutamic acid, dissolve it in water to make 50 mL.

Test Solution Transfer 1 mL of the substrate solution into a test tube, equilibrate

at 37°C for 5 minutes, add 0.2 mL of the sample solution, and shake. Cover the mouth of the test tube with a glass bead, incubate it at 37°C for 60 minutes, heat in a water bath for 5 minutes, and cool. To 0.1 mL of this solution, add 3 mL of *o*-phthalaldehyde TS (for peptidase activity test), and allow the mixture to stand at room temperature for 5 minutes.

Control Solution Transfer 1 mL of the substrate solution into a test tube, equilibrate at 37°C for 5 minutes, add 0.2 mL of the sample solution, and shake. Cover the mouth of the test tube with a glass bead, immediately heat in a water bath for 5 minutes, and cool. Measure 0.1 mL of this solution, and proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 340 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 0.50 g of Aminopeptidase, add water, zinc chloride TS, or phosphate buffer (0.01 mol/L) at pH 7.0 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water, the same TS, or the same buffer to the resulting solution.

Substrate Solution Weigh 59 mg of L-leucyl-*p*-nitroanilide hydrochloride salt or L-proline *p*-nitroanilide trifluoroacetate salt, and dissolve it in phosphate buffer (0.05 mol/L) at pH 7.0, phosphate buffer (0.01 mol/L) at pH 7.0, Tris buffer (0.1 mol/L) at pH 8.3, or Tris buffer (0.1 mol/L, pH 8.0, containing calcium chloride) to make 100 mL.

Test Solution Equilibrate 4 mL of the substrate solution at 37°C for 5 minutes, add 0.1 mL of the sample solution, and shake. Incubate the mixture at the same temperature for 10 or 30 minutes, and cool.

Control Solution Using water instead of the sample solution, proceed as directed for the test.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 405 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 3

Sample Solution Weigh 0.50 g of Aminopeptidase, add water, potassium phosphate (0.005 mol/L) at pH 7.0, or potassium phosphate (0.005 mol/L, pH 7.0, containing zinc sulfate) to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution　Weigh 30 mg of L-leucyl-glycyl-glycine or L-alanyl-prolyl- glycine, and dissolve it in potassium phosphate buffer (0.05 mol/L) at pH 7.0 to make 50 mL. Dilute this solution with potassium phosphate buffer (0.05 mol/L) at pH 7.0 to 10 times its original volume. Prepare fresh before use.

Test Solution　Transfer 1 mL of the substrate solution into a test tube with a stopper, equilibrate it at 37°C for 5 minutes, add 0.1 mL of the sample solution, and mix. Incubate the mixture at 37°C 60 minutes, heat in a water bath for 5 minutes, and cool to room temperature. To this solution, add 2 mL of ninhydrin–2-methoxyethanol–citrate buffer TS and 0.1 mL of tin(II) chloride TS, plug the test tube with the stopper, heat in a water bath for 20 minutes, and cool. Add 10 mL of 1-propanol (1 in 2), shake.

Control Solution　Transfer 0.1 mL of the sample solution into a test tube with stopper, heat it in a water bath for 5 minutes, and cool. Add 1 mL of the substrate solution, mix, incubate at 37°C for 5 minutes, and cool to room temperature. To this solution, add 2 mL of ninhydrin–2-methoxyethanol–citrate buffer TS and 0.1 mL of tin(II) chloride TS, plug the test tube with the stopper, heat in a water bath for 20 minutes, and cool. Add 10 mL of 1-propanol (1 in 2), and shake.

Procedure　Measure the absorbance of the test solution and the control solution at a wavelength of 570 nm within 5–30 minutes after they are prepared. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Ammonia

アンモニア

NH_3　　Mol. Wt. 17.03

Ammonia　[7664-41-7]

Description　Ammonia is a colorless gas having a characteristic odor.

Identification

(1) Bring a glass rod wetted with hydrochloric acid close to Ammonia. White fumes are evolved.

(2) Ammonia changes the color of a litmus paper (red) wetted with water to blue.

Purity　Perform the following tests, using a test solution prepared by saturating water at 20°C with Ammonia.

(1) Sulfur compounds　Add 5 mL of silver nitrate–ammonia TS to 5 mL of the test solution. Heat at 60°C for 5 minutes while shaking well in a dark place. No brown color develops.

(2) Readily oxidizable substances　Add 7 mL of water to 3.0 mL of the test solution.

Pour gradually 30 mL of diluted sulfuric acid (1 in 20), shake, and add 0.10 mL of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear.

Ammonium Alginate

アルギン酸アンモニウム

Ammonium alginate [9005-34-9]

Content Ammonium Alginate, when calculated on the dried basis, contains 88.7–103.6% of ammonium alginate.

Description Ammonium Alginate occurs in white to light yellowish-brown, filamentous, granular, or powdered form.

Identification

(1) Prepare a test solution as follows: To 0.5 g of Ammonium Alginate, add 50 mL of water while stirring, warm the mixture at 60–70°C for 20 minutes with occasional shaking to make it homogenous, and cool.

(i) To 5 mL of the test solution, add 1 mL of a solution of calcium chloride dihydrate (3 in 40). A gelatinous precipitate is formed immediately.

(ii) To 1 mL of the test solution, add 1 mL of a saturated solution of ammonium sulfate. No precipitate is formed.

(2) Ammonium Alginate responds to the test for Ammonium Salt in the Qualitative Tests.

Purity

(1) Water-insoluble substances Not more than 2.0% (on the dried basis).

Weigh accurately about 2 g of Ammonium Alginate in a 2 L Erlenmeyer flask, add 800 mL of water, neutralize with sodium hydroxide TS (1 mol/L), and then add an additional 3 mL of sodium hydroxide TS (1 mol/L). Add 40 mL of hydrogen peroxide, cover the flask, and boil for 1 hour with frequent stirring. Filter while hot through a glass filter with a glass fiber filter paper with suction. The filter and filter paper should be previously dried at 105°C for about 1 hour, cooled in a desiccator, and accurately weighed. If filtration is slow due to the high viscosity of the sample solution, boil again until the viscosity is sufficiently reduced to permit filtration. Wash the filter with the filter paper thoroughly with hot water, dry them at 105°C for 1 hour, cool, and weigh accurately. Calculate as the percentage of the dry weight.

(2) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL Apparatus B).

Loss on Drying Not more than 15.0% (105°C, 4 hour).

Residue on Ignition Not more than 7.0% (3 g, 800°C, 15 minutes, on the dried basis).

Microbial Limit Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Coliforms: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 2 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture Prepare as directed in Method 2 for the coliform test. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 5 g of the sample with 500 mL of lactose broth to disperse completely, and incubate it at 35 ± 1°C for 24 ± 2 hours.

Assay Proceed as directed in the Assay for Alginic Acid.

Each mL of 0.25 mol/L sodium hydroxide = 27.12 mg of ammonium alginate

Ammonium Bicarbonate

Ammonium Hydrogen Carbonate

炭酸水素アンモニウム

NH_4HCO_3 Mol. Wt. 79.06

Ammonium hydrogencarbonate [1066-33-7]

Content Ammonium Bicarbonate contains 20.0–30.0% of ammonia (NH_3 = 17.03).

Description Ammonium Bicarbonate occurs as white or translucent crystals, crystalline powder, or lumps having an odor of ammonia.

Identification Ammonium Bicarbonate responds to all the tests for Ammonium Salt and for Bicarbonate in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear (2.0 g, water 20 mL).

(2) Chloride Not more than 0.004% as Cl (2.0 g, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Ammonium Bicarbonate, add 20 mL of

diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.01% (10 g).

Assay Proceed as directed in the Assay for Ammonium Carbonate.

Each mL of 0.1 mol/L hydrochloric acid = 1.703 mg of NH_3

Ammonium Carbonate

炭酸アンモニウム

Content Ammonium Carbonate contains not less than 30.0% of ammonia (NH_3 = 17.03).

Description Ammonium Carbonate occurs as white or translucent crystals, crystalline powder, or lumps having an odor of ammonia.

Identification Ammonium Carbonate responds to the test for Ammonium Salt and test (1) for Carbonate in the Qualitative Tests. Add magnesium sulfate TS (0.5 mol/L) to a solution of Ammonium Carbonate (1 in 20), and heat. A precipitate is produced.

Purity

(1) Clarity of solution Almost clear (2.0 g, water 20 mL).

(2) Chloride Not more than 0.004% as Cl (2.0 g, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Ammonium Carbonate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.01% (10 g).

Assay Weigh accurately a ground-glass stoppered flask containing about 30 mL of water, add about 2.5 g of Ammonium Carbonate, and weigh the flask with sample accurately. Transfer into a 250-mL volumetric flask, and add water to make exactly 250 mL. Measure exactly 25 mL of this solution, and gradually add exactly 50 mL of 0.1 mol/L hydrochloric acid. Titrate the excess hydrochloric acid with 0.1 mol/L sodium hydroxide (indicator: 4–5 drops of bromophenol blue TS).

Each mL of 0.1 mol/L hydrochloric acid = 1.703 mg of NH_3

Ammonium Chloride

塩化アンモニウム

NH_4Cl Mol. Wt. 53.49

Ammonium chloride [12125-02-9]

Content Ammonium Chloride, when dried, contains not less than 99.0% of ammonium chloride (NH_4Cl).

Description Ammonium Chloride occurs as a white crystalline powder or as crystalline lumps. It has a salty and cool taste.

Identification Ammonium Chloride responds to all the tests for Ammonium Salt and for Chloride in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear (2.0 g, water 20 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Ammonium Chloride, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool.

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 2.0% (4 hours).

Residue on Ignition Not more than 0.5%.

Assay Weigh accurately about 3 g of Ammonium Chloride, previously dried, and dissolve it in water to make exactly 250 mL. Measure exactly 25 mL of this solution into a flask, and add 10 mL of sodium hydroxide solution (2 in 5). Immediately fit the flask to a distillation apparatus connected to a receiver containing exactly 40 mL of 0.1 mol/L sulfuric acid. Heat to distill the ammonia into sulfuric acid. Titrate the excess sulfuric acid in the receiver with 0.2 mol/L sodium hydroxide (indicator: 3 drops of methyl red TS).

Each mL of 0.1mol/L sulfuric acid = 10.70 mg of NH_4Cl

Ammonium Dihydrogen Phosphate

Ammonium Phosphate, Monobasic
Primary Ammonium Phosphate

リン酸二水素アンモニウム

$NH_4H_2PO_4$ Mol. Wt. 115.03

Ammonium dihydrogenphosphate [7722-76-1]

Content Ammonium Dihydrogen Phosphate contains 96.0–102.0% of ammonium dihydrogen phosphate ($NH_4H_2PO_4$).

Description Ammonium Dihydrogen Phosphate occurs as colorless to white crystals or as a white crystalline powder.

Identification Ammonium Dihydrogen Phosphate responds to all the tests for Ammonium Salt and for Phosphate in the Qualitative Tests.

pH 4.1–5.0 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.035% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.50 mL).

(3) Sulfate Not more than 0.038% as SO_4 (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(4) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Ammonium Dihydrogen Phosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

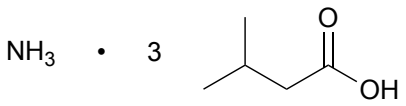
(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay Weigh accurately about 3 g of Ammonium Dihydrogen Phosphate, dissolve it in 30 mL of water, add 5 g of sodium chloride, and shake well. Keep the solution at about 15°C, and titrate with 1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide = 115.0 mg of $NH_4H_2PO_4$

Ammonium Isovalerate

アンモニウムイソバレレート



$C_{15}H_{33}NO_6$ Mol. Wt. 323.43

Ammonia-isovaleric acid (1/3) [1449430-58-3]

Content Ammonium Isovalerate contains 97.0–102.0% of ammonium isovalerate ($C_{15}H_{33}NO_6$), when dried.

Description Ammonium Isovalerate occurs as deliquescent, colorless crystals or as a white crystalline powder. It has a characteristic odor.

Identification Determine the infrared absorption spectrum of Ammonium Isovalerate as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity Melting point 65–68°C

Assay Weigh accurately about 0.2 g of Ammonium Isovalerate, dried for 24 hours in a desiccator, and dissolve it in 50 mL of water. Titrate the resulting solution with 0.1 mol/L potassium hydroxide. Use a potentiometer to confirm the endpoint. The endpoint is the first inflection point.

Each mL of 0.1 mol/L potassium hydroxide = 16.17mg of $C_{15}H_{33}NO_6$

Ammonium Persulfate

過硫酸アンモニウム

$(NH_4)_2S_2O_8$ Mol. Wt. 228.20

Diammonium peroxodisulfate [7727-54-0]

Content Ammonium Persulfate contains not less than 95.0% of ammonium persulfate ($(NH_4)_2S_2O_8$).

Description Ammonium Persulfate occurs as colorless crystals or as a white crystalline powder.

Identification

(1) To 0.5 g of Ammonium Persulfate, add 5 mL of sodium hydroxide solution (1 in 25), and heat. A gas with an odor of ammonia is evolved. This gas changes the color of a litmus paper (red) wetted with water to blue.

(2) To 5 mL of diluted sulfuric acid (1 in 20), add 2–3 drops of a solution of

manganese(II) sulfate pentahydrate (1 in 100), then add 1 drop of silver nitrate solution (1 in 50) and 0.2 g of Ammonium Persulfate, and warm. A pink color develops.

Purity

(1) Clarity of solution　Colorless and almost clear (1.0 g, water 10 mL).

(2) Lead　Not more than 2 μg/g as Pb (2.0 g, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution　Heat the specified amount of Ammonium Persulfate until white fumes no longer are evolved. To the residue, add 1 mL of hydrochloric acid and 5 drops of nitric acid, and evaporate to dryness. To the residue, add 5 mL of diluted hydrochloric acid (1 in 4), and evaporate to dryness. After cooling, add diluted nitric acid (1 in 100) to make exactly 10 mL.

Control Solution　Add diluted nitric acid (1 in 100) to the specific amount of Lead Standard Solution to make exactly 100 mL.

(3) Arsenic　Not more than 3 μg/g as As (1.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B

Test Solution　Dissolve the specified amount of Ammonium Persulfate in 10 mL of water, add 1 mL of sulfuric acid and 10 mL of sulfurous acid solu, evaporate to about 2 mL, and add water to make 10 mL. Use 5 mL of this solution as the test solution.

Residue on Ignition　Not more than 0.2%.

Assay　Weigh accurately about 1.5 g of Ammonium Persulfate, dissolve it in water to make exactly 250 mL. Measure exactly 50 mL of this solution, and add exactly 40 mL of 0.1 mol/L ammonium iron(II) sulfate.　Add 5 mL of phosphoric acid, and titrate the excess ammonium iron(II) sulfate with 0.02 mol/L potassium permanganate. Perform a blank test in the same manner.

Each mL of 0.1 mol/L ammonium iron(II) sulfate = 11.41 mg of $(NH_4)_2S_2O_8$

Ammonium Sulfate

硫酸アンモニウム

$(NH_4)_2SO_4$　　Mol. Wt. 132.14

Ammonium sulfate　[7783-20-2]

Content　Ammonium Sulfate contains not less than 99.0% of ammonium sulfate ($(NH_4)_2SO_4$).

Description　Ammonium Sulfate occurs as colorless crystals or white lumps.

Identification　Ammonium Sulfate responds to all the tests for Ammonium Salt and for Sulfate in the Qualitative Tests.

Purity

(1) Clarity of solution　Colorless and almost clear (1.0 g, water 20 mL).

(2) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution　To the specified amount of Ammonium Sulfate, add 20 mL of diluted hydrochloric acid (1 in 4), boil gently for 15 minutes with a watch glass covering it, and allow to cool.

(3) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition　Not more than 0.25%.

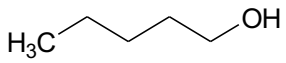
Assay　Weigh accurately about 3 g of Ammonium Sulfate, and dissolve it in water to make exactly 250 mL. Measure exactly 25 mL of this solution, add 10 mL of sodium hydroxide solution (2 in 5), and immediately connect to a distilling apparatus equipped with a receiver, connected to a spray trap and a condenser, containing exactly 40 mL of 0.1 mol/L sulfuric acid. Heat to distill ammonia into the sulfuric acid, and titrate the excess sulfuric acid with 0.2 mol/L sodium hydroxide (indicator: 3 drops of methyl red TS).

Each mL of 0.1 mol/L sulfuric acid = 13.21 mg of $(NH_4)_2SO_4$

Amyl Alcohol

1-Amyl Alcohol
Pentan-1-ol

アミルアルコール



$C_5H_{12}O$　Mol. Wt. 88.15

Pentan-1-ol　[71-41-0]

Content　Amyl Alcohol contains not less than 98.0% of amyl alcohol ($C_5H_{12}O$).

Description　Amyl Alcohol is a colorless to light yellow, clear liquid having a characteristic odor.

Identification　Determine the absorption spectrum of Amyl Alcohol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index　n_D^{20}: 1.407–1.412.

Specific Gravity d_{25}^{25}: 0.810–0.816.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2).

α-Amylase

α-アミラーゼ

Definition α-Amylase includes enzymes that hydrolyze α-1,4-glucosidic linkages in amylaceous polysaccharides, such as starch, and transform them into lower molecule compounds. It is derived from malts or the culture of filamentous fungi (limited to *Aspergillus aureus, Aspergillus foetidus, Aspergillus niger,* and *Aspergillus oryzae*), actinomycetes (limited to *Saccharomonospora viridis, Streptomyces avermitilis, Streptomyces griseus, Streptomyces thermoviolaceus, Streptomyces violaceoruber,* and *Thermomonospora viridis*), or bacteria (limited to *Alcaligenes latus*, *Bacillus amyloliquefaciens*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Cellulosimicrobium cellulans*, *Microbacterium imperiale*, *Paenibacillus alginolyticus*, *Sulfolobus solfataricus*, and species of the genus *Arthrobacter*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description α-Amylase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification α-Amylase complies with the α-Amylase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli*

test and Method 2 for the *Salmonella* test.

The requirement of total plate count can be disregarded if unsterilized α-Amylase is used for captive consumption in the manufacture of food that will be sterilized or pasteurized before the completion of the final pro

duct.

α-Amylase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of α-Amylase, add water or α-amylase sample diluent to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same diluent to the resulting solution.

Substrate Solution Weigh 1.0 g of potato starch, previously dried at105°C for 2 hours, and add 20 mL of water. To this, add 5 mL of sodium hydroxide TS (2 mol/L) gradually while shaking to make the mixture paste. Heat it for 3 minutes in a water bath while stirring, and add 25 mL of water. After cooling, neutralize the mixture with hydrochloric acid TS (2 mol/L) and hydrochloric acid TS (0.1 mol/L). Add 10 mL of buffer for the α-amylase activity test and water to make 100 mL. Prepare fresh before use.

Test Solution Equilibrate 10 mL of the substrate solution at 37°C for 10 minutes, add 1 mL of the sample solution, and mix. Incubate the mixture at 37°C for 10 minutes. To 1 mL of this solution, add 10 mL of hydrochloric acid TS (0.1 mol/L) or diluted sulfuric acid (1 in 1800), and shake immediately. To 0.5 mL of the resulting solution, add 10 mL of iodine–potassium iodide TS (0.2 mmol/L), and mix.

Control Solution Proceed as directed for the test solution using water instead of the sample solution.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 660 nm. The absorbance value of the test solution is lower than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 0.50 g of α-Amylase, add water or α-amylase sample diluent to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same diluent to the resulting solution.

Substrate Suspension Weigh 10.0 g of potato starch, previously dried at105°C for 2 hours, add 10 mL of buffer for the α-amylase activity test and water to make100 mL. Prepare fresh before use.

Test Solution Transfer 10 mL of the substrate suspension into a test tube, add 1 mL of the sample solution, plug the tube with a rubber stopper, and agitate the mixture to suspend the starch uniformly. Immediately remove the stopper, and heat in a water bath while agitating vigorously to gelatinize the starch. Immediately warm this solution at 65°C for 15 minutes.

Control Solution Transfer 10 mL of substrate suspension in a test tube, add 1 mL of the diluent used to dilute the sample instead of the sample solution, proceed as directed for the test solution.

Procedure Tilt the mouths of the test tubes 45 degree downward from the horizontal line. The fluidity of the test solution is higher than that of the control solution.

Method 3

Sample Solution Weigh 0.50 g of α-Amylase, add water or α-amylase sample diluent to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same diluent to the resulting solution.

Substrate Solution Weigh non-reducing-end blocked *p*-nitrophenyl-α-D maltoheptoside–enzyme, and dissolve it in 10 mL of the α-amylase sample diluent.

Test Solution Add 0.4 mL of the substrate solution to 0.05 mL of the sample solution, equilibrated at 37°C for 2 minutes, and mix them immediately. Incubate the mixture at the same temperature for 5 minutes. Add 0.5 mL of boric acid–sodium hydroxide buffer (0.2 mol/L) at pH 10.2, and shake well.

Control Solution Proceed as directed for the test solution using the diluent used to dilute the sample instead of the sample solution.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 410 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 4

Sample Solution Weigh 0.50 g of α-Amylase, add water or α-amylase sample diluent to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same diluent to the resulting solution.

Substrate Solution Add 20 mL of water to 2.0 g of soluble starch, and gradually add the mixture to about 50 mL of boiling water while stirring well. Boil it for about 2 minutes while stirring, and cool. Add 5 mL of acetic acid–sodium hydroxide buffer (2 mol/L) at pH 4.6 and water to make 100 mL. Prepare fresh before use.

Test Solution Equilibrate 10 mL of the substrate solution at 30°C for 15 minutes, add 5 mL of the sample solution, and shake immediately. Incubate the mixture again at 30°C for 20 minutes. Add 1 mL of this solution to 5 mL of iodine–potassium iodide TS (for α-amylase activity test), and shake immediately.

Control Solution　Proceed as directed for the test solution using the diluent used to dilute the sample instead of the sample solution.

Procedure　Transfer the test solution and the control solution immediately into separate color comparator tubes (square tubes). Examine the brightness and depth of color for both solutions using the standard color disks.　The test solution is brighter in color than the control solution .

Method 5

Sample Solution　Weigh 0.50 g of α-Amylase, add water or α-amylase sample diluent to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same diluent used to dissolve α-Amylase.

Substrate Solution　Weigh 1.0 g of maltotriose, dissolve it in citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.0, and make 50 mL. Prepare fresh before use.

Test Solution　Equilibrate 0.5 mL of the substrate solution at 37°C for 10 minutes, add 0.5 mL of the sample solution, equilibrated at 37°C, and shake immediately. Incubate the mixture at 37°C for 30 minutes. Add 1 mL of sodium hydroxide TS (0.12 mol/L), and shake well. To this solution, add 3 mL of TS for D-glucose determination (containing hexokinase), shake well, and allow the mixture to stand at room temperature for 30 minutes.

Control Solution　To 0.5 mL of the sample solution, add 1 mL of sodium hydroxide TS (0.12 mol/L), and shake well. Add 0.5 mL of the substrate solution, and shake well. To this solution, add 3 mL of TS for D-glucose determination (containing hexokinase), shake well, and allow the mixture to stand at room temperature for 30 minutes. Proceed as directed for the test solution.

Procedure　Determine the absorbance of the test solution and the control solution at a wavelength of 340 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

β-Amylase

β–アミラーゼ

Definition　β-Amylase includes enzymes that act on starch, dextrin, and glycogen to produce maltose. It is derived from malts, the seeds of cereal grains, the seeds of legumes, the tuberous roots, tubers, or rhizophores of potatoes, or the culture of filamentous fungi (limited to *Aspergillus oryzae*), actinomycetes (limited to species from the genus *Streptomyces*), or bacteria (limited to *Bacillus amyloliquefaciens, Bacillus flexus, Bacillus polymyxa,* and *Bacillus subtilis*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description β-Amylase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification β-Amylase complies with the β-Amylase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the Escherichia *coli* test and Method 2 for the *Salmonella* test.

β-Amylase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of β-Amylase, add water, water cooled by ice, or β-amylase sample diluent to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water, the cold water, or the same diluent to the resulting solution.

Substrate Solution When potato starch is used as the substrate: Weigh 1.0 g of potato starch, previously dried at105°C for 2 hours, and add 20 mL of water. To this, add 5 mL of sodium hydroxide TS (2 mol/L) gradually while shaking to make a paste. Heat it for 3 minutes in a water bath while stirring, and add 25 mL of water. After cooling, neutralize it with hydrochloric acid TS (2 mol/L) and hydrochloric acid TS (0.1 mol/L). Add 10 mL of buffer for the β-amylase activity test and water to make 100 mL. Prepare fresh before use.

When soluble starch is used as the substrate: Suspend 1.0 g of soluble starch in a small amount of water. Add this suspension gradually to about 50 mL of boiling water while stirring, and boil for 5 minutes after it starts to boil. After cooling, add 10 mL of buffer for the β-amylase activity test and water to make 100 mL. Prepare fresh before use.

Test Solution Equilibrate 10 mL of the substrate solution at 37°C for 10 minutes, add 1 mL of the sample solution, and shake immediately. Incubate the mixture at the same temperature for 10 or 30 minutes. To this, add 4 mL of Fehling's TS, shake lightly, and heat in a water bath for 15 minutes. Cool the mixture to a temperature of 25°C or below not exceeding 25°C, and add 2 mL of potassium iodide TS (for β-amylase/invertase activity test) and 2 mL of diluted sulfuric acid (1 in 6).

Control Solution Proceed as directed for the test solution using 10 mL of water instead of the substrate solution.

Procedure Titrate the liberated iodine in the test solution and the control solution with sodium thiosulfate TS (0.05 mol/L). The amount of sodium thiosulfate TS consumed by the test solution is less than that of sodium thiosulfate TS consumed by the control solution. The endpoint is when the blue color produced by the addition of 1 to 2 drops of soluble starch TS near the endpoint disappears.

Method 2

Sample Solution Weigh 0.50 g of β-Amylase, add water, ice cold water, or β-amylase sample diluent to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water, the cold water, or the same diluent to the resulting solution.

Substrate Solution Suspend 20.0 g of soluble starch in a small amount of water. Add this suspension gradually to about 750 mL of boiling water, and boil for 2 minutes after it starts boiling again. After cooling, add 20 mL of buffer for the β-amylase activity test and water to make 1000 mL. Prepare fresh before use

Test Solution Equilibrate 200 mL of the substrate solution at 20°C for 30 minutes, add 10 mL of the sample solution, mix immediately, and allow the mixture to incubate at 20°C for 30 minutes. Add 20 mL of sodium hydroxide TS (0.5 mol/L) and water to make 250 mL. To 5 mL of this solution, add 10 mL of potassium hexacyanoferrate(III) (0.05 mol/L), shake lightly, and heat the mixture in a water bath for 20 minutes. Cool it to a temperature of 25°C or below, and add 25 mL of acetic acid–potassium chloride–zinc sulfate TS and 1 mL of 50% (w/v) potassium iodide TS.

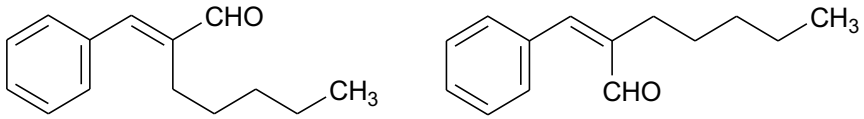
Control Solution Add 10 mL of the sample solution to 20 mL of sodium hydroxide TS (0.5 mol/L), mix, and then add 200 mL of the substrate solution and water to make 250 mL. Measure 5 mL of this solution, and proceed as directed for the test solution.

Procedure Titrate the liberated iodine in the test solution and the control solution with sodium thiosulfate TS (0.05 mol/L). The amount of sodium thiosulfate TS consumed by the test solution is less than that of sodium thiosulfate TS consumed by the control solution. The endpoint is when the blue color produced by the addition of 1 to 2 drops of soluble starch TS near the endpoint disappears.

α-Amylcinnamaldehyde

α-Amylcinnamic Aldehyde
Alpha-Pentylcinnamaldehyde

α-アミルシンナムアルデヒド



$C_{14}H_{18}O$ Mol. Wt. 202.29

2-(Phenylmethylene)heptanal [122-40-7]

Content α-Amylcinnamaldehyde contains not less than 97.0% of α-amylcinnamaldehyde ($C_{14}H_{18}O$).

Description α-Amylcinnamaldehyde is a light yellow to yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of α-Amylcinnamaldehyde as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.554–1.562.

Specific Gravity d_{25}^{25}: 0.962–0.969.

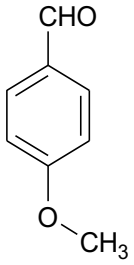
Purity Acid value Not more than 5.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Anisaldehyde

_p_-Methoxybenzaldehyde
4-Methoxybenzaldehyde

アニスアルデヒド



$C_8H_8O_2$ Mol. Wt. 136.15

4-Methoxybenzaldehyde [123-11-5]

Content Anisaldehyde contains not less than 97.0% of anisaldehyde ($C_8H_8O_2$).

Description Anisaldehyde is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Anisaldehyde as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.570–1.574.

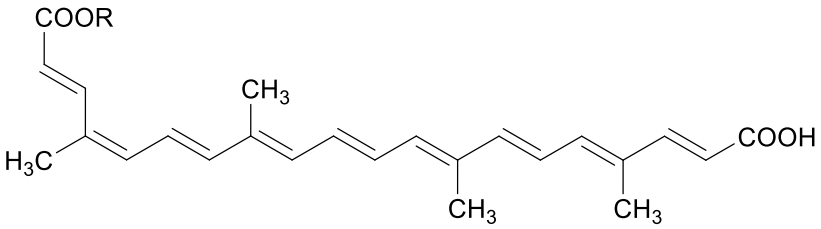
Specific Gravity d_{25}^{25}: 1.119–1.127.

Purity Acid value Not more than 6.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Annatto Extract

アナトー色素



Bixin: R = CH_3

Norbixin: R = H

Definition Annatto Extract is obtained from the pericarp of the seeds of the annatto tree *Bixa orellana* L. There are two types: one that consists mainly of bixin and one that consists mainly of norbixin. They are called bixin and norbixin, respectively. Annatto Extract may contain dextrin, lactose, or edible fat or oil.

Bixin

ビキシン

$C_{25}H_{30}O_4$ Mol. Wt. 394.50

(2*E*,4E,6*E*,8*E*,10*E*,12*E*,14*E*,16*Z*,18*E*)-20-methoxy-4,8,13,17-tetramethyl-20-oxoicosa-2,4,6,8,10,12,14,16,18-nonaenoic acid [6983-79-5]

Content (Color Value) Bixin contains the equivalent of not less than 25% of bixin ($C_{25}H_{30}O_4$) or the Color Value ($E_{1cm}^{10\%}$) is not less than 7725, and the content (Color Value) is in the range of 90–120% of the labeled value.

Description Bixin occurs as a red-brown to dark brown powder, as lumps, or as a paste or liquid. It has a slight characteristic odor.

Identification

(1) Weigh an amount of Bixin equivalent to 40 mg of bixin with a bixin content of 25%, add 50 mL of water, and shake. It is practically insoluble.

(2) Prepare a test solution in the following manner: Weigh an amount of Bixin equivalent to 20 mg of norbixin with a bixin content of 25%, dissolve it in 25 mL of *N,N*-dimethyl formamide, and centrifuge or filter if necessary. To 5 mL of this solution, add *N,N*-dimethyl formamide to make 25 mL, and 25 mL of acetonitrile.

Prepare a standard solution in the following manner: Dissolve 10 mg of Bixin in 25

mL of *N,N*-dimethyl formamide. To 5 mL of this solution, add *N,N*-dimethyl formamide to make 25 mL, and add 25 mL of acetonitrile.

Analyze 10 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. The retention time of the main peak of the test solution corresponds to that of the norbixin peak of the standard solution.

Operating Conditions

Detector: Visible spectrophotometer (determination wavelength: 460 nm)

Column: A stainless steel tube (4–5 mm internal diameter and 15–30 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 35°C.

Mobile phase: A 13:7 mixture of acetonitrile/acetic acid (1 in 50).

Flow rate: A constant rate of 1.0–1.5 mL/minute.

(3) A solution of Bixin in acetone exhibits absorption maxima at wavelengths of 452–460 nm and 482–490 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B) .

(3) Mercury Not more than 1.0 μg/g as Hg.

Proceed as directed in Purity (3) for Norbixin.

Assay (Color Value Determination) Proceed as directed under Color Value Determination, using the operating conditions given below. Determine the color value or the bixin content by dividing the color value by 309. Prepare a test solution as follows: Dissolve an appropriate amount of Bixin, accurately weighed, in 10 mL of tetrahydrofuran, and add acetone to make exactly 100 mL. To exactly 1 mL of this solution, add acetone to make exactly 100 mL.

Operating Conditions

Solvent: Acetone.

Wavelength: Maximum absorption wavelength of 482–490 nm.

Norbixin

ノルビキシン

$C_{24}H_{28}O_4$ Mol. Wt. 380.48

(2*E*,4*Z*,6*E*,8*E*,10*E*,12*E*,14*E*,16*E*,18*E*)-4,8,13,17-tetramethylicosa-2,4,6,8,10,12,14,16,18-nonaenedioic acid [626-76-6]

Content (Color Value) Norbixin contains the equivalent of not less than 15% of norbixin ($C_{24}H_{28}O_4$) or the Color Value ($E_{1cm}^{10\%}$) is not less than 4305, and the content (Color Value) is in the range of 90–120% of the labeled value.

Description Norbixin occurs as a red-brown to dark brown powder, as lumps, or as a paste or liquid. It has a slight characteristic odor.

Identification

(1) Weigh an amount of Norbixin equivalent to 0.1 g of norbixin with a norbixin content of 15%, add 50 mL of water, and shake. It is practically insoluble.

(2) Prepare a test solution in the following manner: Weigh an amount of Norbixin equivalent to 10 mg of norbixin with a norbixin content of 15%, dissolve it in 25 mL of *N,N*-dimethyl formamide, and centrifuge or filter if necessary. Add 25 mL of acetonitrile.

Prepare standard solutions in the following manner: Separately dissolve 10 mg each of Norbixin and Bixin in 25 mL of *N,N*-dimethyl formamide. To 5 mL of each solution, add *N,N*-dimethyl formamide to make 25 mL, and add 25 mL of acetonitrile to each.

Analyze 10 μL each of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Continue the chromatography until the peak of bixin is completely eluted. The retention time of the main peak of the test solution corresponds to that of the norbixin peak of the standard solution.

Operating Conditions

Detector: Visible spectrophotometer (determination wavelength: 460 nm)

Column: A stainless steel tube (4–5 mm internal diameter and 15–30 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 35°C.

Mobile phase: A 13:7 mixture of acetonitrile/diluted acetic acid (1 in 50).

Flow rate: A constant rate of 1.0–1.5 mL/minute.

(3) A solution of Norbixin in potassium hydroxide solution (1 in 200) exhibits absorption maxima at wavelengths of 448–456 nm and 476–484 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Mercury Not more than 1.0 μg/g as Hg.

Test Solution To 1.0 g of Norboxin, add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat gently under a reflux condenser for 5 hours. If the solution is not clear, cool and heat again with an additional 5 mL of nitric acid. Add 5 mL of nitric acid repeatedly if necessary. After cooling, add 10 mL of water and 1.5 g of potassium permanganate, and heat on a water bath. If a purple color does not develop, add potassium permanganate again, and heat again. Repeat this procedure until a purple color develops. After cooling, add hydroxylammonium chloride solution (1 in 5) until the purple color disappears, and add water to make exactly 150 mL.

Control Solution To exactly 10 mL of Mercury Standard Solution, add 5 mL of sulfuric acid and 5 mL of nitric acid, and proceed as directed for the test solution.

Procedure Analyze the test solution and the control solution as directed under Cold-vapor Atomic Absorption Spectrophotometry. Place appropriate portions of these solutions into separate water testing vials, and add 10 mL of tin(II) chloride–hydrochloric acid TS. Immediately connect them to the atomic absorption spectrophotometer, circulate the air hermetically by pumping operation, and measure the absorbance using the operating conditions given below. The absorbance value of the test solution is not higher than that of the control solution.

Operating Conditions

Light Source: Mercury hollow cathode lamp.

Wavelength: 253.7 nm.

Carrier gas: Air.

Assay (Color Value Determination) Proceed as directed under Color Value Determination using the operating conditions given below. Determine the color value or the norbixin content by dividing the color value by 287.

Operating Conditions

Solvent: Potassium hydroxide solution (1 in 200).

Wavelength: Maximum absorption wavelength of 476–484 nm.

Annatto, Water-soluble

水溶性アナトー

Definition Annatto, Water-soluble is prepared from the red pericarp of seeds of the annatto tree *Bixa orellana* L. by hydrolysis. The coloring principle is the potassium or sodium salt of norbixin.

Content Annatto, Water-soluble contains the equivalent of 95–120% of the labeled content of norbixin ($C_{24}H_{28}O_4$ = 380.48).

Description Annatto, Water-soluble occurs as a red-brown to brown powder, as lumps, or as a liquid or paste. It has a slight characteristic odor.

Identification

(1) Dissolve 1 g of Annatto, Water-soluble in 40 mL of water, add 4 mL of diluted sulfuric acid (1 in 20), shake, and filter. Wash the residue on the filter paper three times with 40 mL of water each time.

(i) Dissolve a little portion of the residue in sodium hydroxide solution (1 in 2500). The solution exhibits absorption maxima at wavelengths of approximately 452–456 nm and 480–484 nm.

(ii) Dissolve a little portion of the residue in 10 ml of ethanol (95). Apply one drop of the solution on a filter paper, and air-dry. Drip 2–3 drops of sodium nitrite solution (1 in 20) and then 2–3 drops of sulfuric acid TS (0.5 mol/L) on it. The yellow color on the filter paper disappears.

(2) Dissolve about 10 mg of the residue obtained in (1) in 25 mL of *N*-*N*-dimethylformamide, centrifuge or filter the solution if necessary, and add 25 mL of acetonitrile. Use this solution as the test solution. Anlyze 10 μL of the test solution by liquid chromatography using the operating conditions given below. The peak of the main pigment is found around the retention time of norbixin (5–10 minutes)

Operating Conditions

Detector: Visible spectrophotometer (wavelength: 460 nm).

Column: A stainless steel tube (4–5 mm internal diameter and 15–30 cm length).

Column packing material: 5-μm octadecylsilanized silica for liquid chromatography.

Column temperature: 35°C.

Mobile phase: A 13 : 7 mixture of acetonitrile/diluted acetic acid (1 in 50).

Flow rate: A constant rate of 1.0–1.5 mL/minute.

Purity

(1) Free alkali Dissolve 10 g of Annatto, Water-soluble in 100 mL of water, shake, add 8 ml of hydrochloric acid TS (1 mol/L), stir thoroughly, and allow to stand for 30 minutes. The pH of the filtrate is not more than 7.0.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay

Test Solution Weigh accurately an appropriate amount of Annatto, Water-soluble so that the absorbance comes to a range of 0.3–0.7. Dissolve it in potassium hydroxide solution (1 in 200) to make exactly 100 mL. Measure exactly 1 mL of the solution, and add potassium hydroxide solution (1 in 200) to make exactly 100 mL.

Procedure Measure the absorbance (A) of the test solution at the maximum between 476–484 nm against potassium hydroxide solution (1 in 200), and calculate the content of norbixin by the formula:

$$\text{Content (\%) of norbixin } (C_{24}H_{28}O_4) = \frac{A}{2870} \times \frac{100}{\text{Weight (g) of the sample}} \times 100$$

Anthocyanase

アントシアナーゼ

Definition Anthocyanase includes enzymes that hydrolyze the glucoside or galactoside group in anthocyanin. It is derived from malts, the seeds of cereal grains, or the culture of filamentous fungi (limited to *Aspergillus oryzae*, *Aspergillus niger*, and *Penicillium decumbens*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Anthocyanase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Anthocyanase complies with the Anthocyanase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits　Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid　Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture　Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Anthocyanase Activity Test　Perform the test using either of the methods given below. If the identification test cannot be performed by either method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

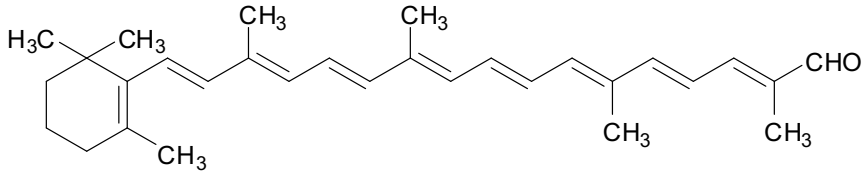
Proceed as directed in Method 2 of the β-Glucosidase Activity Test for β-Glucosidase.

Method 2

Proceed as directed in Method 3 of the β-Galactosidase Activity Test for β-Galactosidase.

β-Apo-8′-carotenal

β-アポ-8′-カロテナール



$C_{30}H_{40}O$　Mol. Wt. 416.64

(2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*,16*E*)-2,6,11,15-tetramethyl-17-(2,6,6-trimethylcyclohex-1-en-1-yl)heptadeca-2,4,6,8,10,12,14,16-octaenal　[1107-26-2]

Content β-Apo-8′-carotenal contains not less than 96.0% of β-apo-8′-carotenal ($C_{30}H_{40}O$).

Description β-Apo-8′-carotenal occurs as dark violet crystals or crystalline powder having a metallic luster.

Identification

(1) A 1 in 20,000 solution of β-Apo-8′-carotenal in acetone produces an orange color.

To 5 mL of this solution, add 1 mL of sodium nitrite solution (1 in 20) and 1 mL of 0.5 mol/L sulfuric acid. The solution is immediately decolored.

(2) The test solution prepared in the Assay below exhibits absorption maxima at wavelengths of around 461 nm and 488 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Absorbance ratio Measure the absorbance values (A_1 and A_2) of the test solution prepared in Assay at wavelengths of 461 nm and 488 nm. A_2/A_1 is 0.80–0.84.

(4) Subsidiary colors Not more than 3%.

Test Solution To 10 mg of β-Apo-8′-carotenal, add tetrahydrofuran with BHT to dissolve it, and make 100 mL. To 1 mL of this solution, add ethanol (95) to make 10 mL.

Procedure Analyze 10-μL portions of the test solution by liquid chromatography using the operating conditions below. Continue the chromatography for about 6 times the retention time of the main peak. Normalize the sum of the peak areas of all components in the test solution to 100. Consider all peaks other than the main peak to be derived from subsidiary colors and determine the peak area percentage.

Operating Conditions

Detector: Visible spectrophotometer (wavelength 463 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μL hexadecylamidopropylsilanized silica gel for liquid chromatography.

Column temperature: 30°C.

Mobile phase: To 20 mL of a 1 in 400 solution of butylated hydroxytoluene in 2-propanol, add 0.2 mL of *N*-ethyl-*N*-(1-methylethyl)propane-2-amine, 25 mL of ammonium acetate solution (1 in 500), 455 mL of acetonitrile, and 450 mL of methanol. Dilute with methanol to make 1000 mL. Prepare fresh before use.

Flow rate: Adjust the retention time of β-apo-8′-carotenal to about 7–9 minutes.

Residue on Ignition Not more than 0.10%.

Assay

Test Solution Weigh accurately about 40 mg of β-Apo-8′-carotenal, add 10 mL of chloroform to dissolve it, and add cyclohexane to make exactly 50 mL. To 5 mL of this solution, exactly measured, add cyclohexane to make exactly 100 mL. To 5 mL of the second solution, add cyclohexane to make exactly 100 mL.

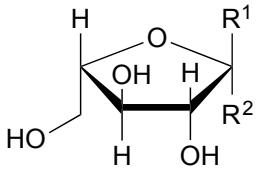
Procedure Measure the absorbance (A) of the test solution at the maximum at about 461 nm against cyclohexane. Calculate the content by the formula:

$$\text{Content (\%) of β-apo-8′-carotenal } (C_{30}H_{40}O) = \frac{200}{\text{Weight (g) of the sample}} \times \frac{A}{2640} \times 100$$

Storage Standards Store in a hermetic, light-resistant container under inert gas.

L-Arabinose

L-アラビノース



β-L-Arabinose: R^1 = H, R^2 = OH

α-L-Arabinose: R^1 = OH, R^2 = H

$C_5H_{10}O_5$ Mol. Wt. 150.13

L-Arabinofuranose [87-72-9]

Definition L-Arabinose is obtained by hydrolysis and isolation from the polysaccharides (mainly arabinan) of gum arabic, gum ghatti, corn fibers, or sugar beet pulps. It consists of L-arabinose ($C_5H_{10}O_5$).

Content L-Arabinose, when dried, contains not less than 95.0% of L-arabinose ($C_5H_{10}O_5$).

Description L-Arabinose occurs as colorless or white crystals or as a white to light yellowish-white crystalline powder. It is odorless and has a sweet taste.

Identification

(1) Add 2–3 drops of a solution of L-Arabinose (1 in 20) to 5 mL of boiled Fehling's TS. A red precipitate is formed.

(2) Dissolve 1 g of L-Arabinose in 3 mL of water, add 3 mL of a 5:2 mixture of diluted hydrochloric acid (1 in 4)/diphenylamine solution in ethanol (95) (1 in 40), and heat on a water bath for 5 minutes. A yellow to light orange color is produced.

Specific Rotation $[\alpha]_D^{20}$: Not less than + 95° (2 g, water, 50 mL, on the dried basis).

Before determination, allow the resulting sample solution to stand for 24 hours at room temperature.

Purity

(1) Clarity of solution Colorless and almost clear (4.0 g, water 20 mL).

(2) Free acids Dissolve 1.0 g of L-Arabinose in 10 mL of freshly boiled and cooled water. Add 1 drop of phenolphthalein TS, and then add 1 drop of 0.2 mol/L sodium

hydroxide solution. A red color develops.

(3) Sulfate Not more than 0.005% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.10 mL).

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (0.50g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 1.0% (105°C, 3 hours).

Residue on Ignition Not more than 0.2% (5 g, 600°C, 8 hours).

Assay

Test Solution and Standard Solution Weigh accurately about 2 g each of the test sample and L-arabinose for assay, previously dried. To each, add exactly 10 mL of a 4:1 mixture of water/propylene glycol, and then add water to make exactly 50 mL.

Procedure Analyze 10 μL each of the test solution and standard solution by liquid chromatography according to the operating conditions given below. Measure the peak areas of L-arabinose and propylene glycol for the test solution and the standards solution, and determine the peak area ratios (Q_T and Q_S) of L-arabinose to propylene glycol for each solution. Calculate the content by the formula:

$$\text{Content (\%) of L-arabinose } (C_5H_{10}O_5) = \frac{\text{Weight (g) of L-arabinose for assay}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (4–8 mm internal diameter and 20–35 cm length).

Column packing material: 7- to 11-μm strongly acidic cation-exchange resin for liquid chromatography.

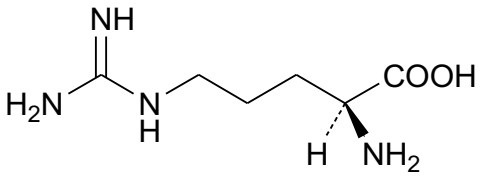
Column temperature: A constant temperature of 60–70°C.

Mobile phase: Water.

Flow rate: Adjust the retention time of L-arabinose to 10–15 minutes.

L-Arginine

L-アルギニン



$C_6H_{14}N_4O_2$ Mol. Wt. 174.20

(2*S*)-2-Amino-5-guanidinopentanoic acid [74-79-3]

Content L-Arginine, when calculated on the dried basis, contains 98.0–120.0% of L-arginine($C_6H_{14}N_4O_2$).

Description L-Arginine occurs as white crystals or crystalline powder. It has a characteristic odor and has a characteristic taste.

Identification

(1) To 5 mL of a solution of L-Arginine (1 in 1000), add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A bluish purple color develops.

(2) A solution of L-Arginine is alkaline.

Specific Rotation $[\alpha]_D^{20}$: +25.0 to +27.9° (8 g, hydrochloric acid (6 mol/L), 100 mL, on the dried basis).

pH 10.5–12.5 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.1% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.02 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 1.0% (105°C, 3 hours).

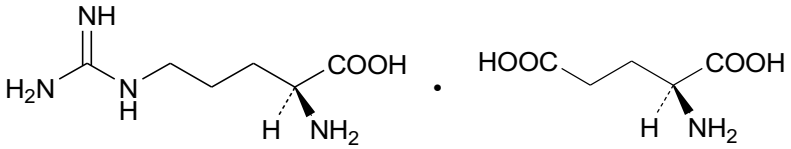
Residue on Ignition Not more than 0.2%.

Assay Weigh accurately about 0.2 g of L-Arginine, and proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 8.710 mg of $C_6H_{14}N_4O_2$

L-Arginine L-Glutamate

L-アルギニン L-グルタミン酸塩



$C_{11}H_{23}N_5O_6$ Mol. Wt. 321.33

(2*S*)-2-Amino-5-guanidinopentanoic acid mono[(2*S*)-2-Aminopentanedioate]

[4320-30-3]

Content L-Arginine L-Glutamate, when calculated on the anhydrous basis, contains 98.0–102.0% of L-arginine L-glutamate ($C_{11}H_{23}N_5O_6$).

Description L-Arginine L-Glutamate occurs as a white powder. It is odorless or has a slight odor and has a characteristic taste.

Identification

(1) To 5 mL of a solution of L-Arginine L-Glutamate (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) Use a solution of L-Arginine L-Glutamate (1 in 500) as the test solution. Add water to 0.1 g of L-arginine hydrochloride and 0.1 g of sodium L-glutamate monohydrate to make 100 mL, and use this solution as the control solution. Analyze 5 μL each of the test solution and the control solution by paper chromatography using a 5:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. Use a No. 2 filter paper for chromatography, and stop the development when the developing solvent ascends to a point about 30 cm above the starting line. Air-dry the filter paper, dry at 100°C for 20 minutes, and spray with a solution (1 in 50) of ninhydrin in acetone. Heat it at 100°C for 5 minutes to develop a color, and observe in daylight. Two spots corresponding to the spots from the control solution are observed.

Specific Rotation $[\alpha]_D^{20}$: +28.0 to +30.0° (4 g, hydrochloric acid (6 mol/L), 50 mL, on the anhydrous basis).

pH 6.0–7.5 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.041% as Cl (0.30 g, Control Solution: 0.01 mol/L hydrochloric acid 0.35 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Water Content Not more than 15.4% (0.3 g, Volumetric Titration, Back Titration).

Residue on Ignition Not more than 0.3%.

Assay Proceed as directed in the Assay for DL-Alanine, and calculate on the anhydrous base.

Each mL of 0.1 mol/L perchloric acid = 10.71 mg of $C_{11}H_{23}N_5O_6$

Argon

アルゴン

Ar

Mol. Wt. 39.95

Argon

[7440-37-1]

Definition Argon is a substance produced using the air liquefaction separation method.

Content Argon contains not less than 99.0% (vol) of argon (Ar).

Description Argon is a colorless gas having no odor.

Identification

(1) When a burning wood chip with a flame is placed in a test tube containing Argon, the flame goes off.

(2) Introduce Argon into a 1-mL gas measuring tube for gas chromatography, and analyze it by gas chromatography using the operation conditions given in Purity (ii) below. The retention time of the main peak corresponds to that of the main peak obtained when the reagent argon is analyzed in the same manner.

Purity Oxygen and Nitrogen Not more than 1.0% (vol) as the total amount.

(i) Oxygen Measure oxygen in Argon using a yellow phosphor luminescent oxygen analyzer and determine the amount (% (vol)). If the amount of oxygen exceeds the measurement range of the analyzer, exactly dilute the sample gas with oxygen-free nitrogen, analyze oxygen, and determine the amount.

(ii) Nitrogen Introduce Argon into a 1.0-mL gas measuring tube for gas chromatography at constant flow rate of 50–150 mL/min, analyze it using the operating conditions below, and measure the peak area (A_T) of nitrogen. Separately, prepare a constant amount of a uniform gas mixture with the nitrogen concentration of about 0.5% (vol) by mixing a constant amount of nitrogen, measured exactly, with the carrier gas given below. Use this as the standard gas mixture. Introduce the standard gas mixture into a gas measuring tube with same amount at the same flow rate, proceed as directed for Argon, and measure the peak area (A_S) of nitrogen in the gas mixture. Determine the amount (% (vol)) of nitrogen in Argon by the formula:

$$\text{Amount (\% (vol)) of nitrogen } (N_2) = V_S \times \frac{A_T}{A_S}$$

V_S = Amount (% (vol)) of nitrogen in the standard gas mixture

Operating Conditions
Detector: Thermal conductivity detector
Column: A stainless steel tube (about 3 mm internal diameter and about 3 m length)
Column packing material: 180–250 μm zeolite for gas chromatography
Column temperature: A constant temperature of 50–150°C
Carrier gas: Hydrogen or helium
Flow rate: A constant rate of 20–40 mL/min
Injection: Loop injection using a gas measuring tube

(iii) Total amount Determine the total amount of oxygen and nitrogen from the amount (% (vol)) of oxygen obtained in (i) and the amount (% (vol)) of nitrogen obtained in (ii) by the formula:

$$\text{Total amount (\% (vol)) of oxygen and nitrogen (\% (vol))} = V_O + V_N$$

V_o= Amount (% (vol)) of oxygen obtained in (i)

V_N= Amount (% (vol)) of nitrogen obtained in (ii)

Water Content Not more than 0.05% (vol)

Measure using a capacitance moisture meter to determine the amount (% (vol)) of the water content from the obtained value.

Assay Calculate the content of argon from the total amount of oxygen and nitrogen obtained in Purity (iii), and the water content by the formula:

$$\text{Content of argon (\% (vol))} = 100 - V_{ON} - V_W$$

V_{ON} = Total amount (% (vol)) of oxygen and nitrogen

V_W = Amount (% (vol)) of water content

Ascorbate Oxidase

アスコルビン酸オキシダーゼ

Definition Ascorbate Oxidase includes enzymes that oxidize L-ascorbic acid. It is derived from cucurbitaceous plants (limited to the genera *Cucurbita*, *Cucumis*, *Luffa*, *Sechium*, and *Trichosanthes*), the cabbage *Brassica oleracea* L., the spinach *Spinacia oleracea* L., or the culture of filamentous fungi (limited to *Eupenicillium brefeldianum* and *Trichoderma lignorum*) or actinomycetes (limited to *Streptomyces cinnamoneus* and *Streptomyces violaceoruber*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Ascorbate Oxidase occurs as white to dark brown or gray to light green granules, powder, or paste, or as a colorless to dark brown or light blue-green to green liquid. It is odorless or has a characteristic odor.

Identification Ascorbate Oxidase complies with the Ascorbate Oxidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Ascorbate Oxidase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 0.50 g of Ascorbate Oxidase, add water, disodium hydrogen phosphate TS (0.01 mol/L, containing albumin), or disodium hydrogen phosphate TS (0.2 mol/L, containing albumin) to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same

diluent to the resulting solution.

Substrate Solution Weigh 88 mg of L(+)-ascorbic acid, and dissolve it in disodium dihydrogen ethylenediaminetetraacetate–hydrochloric acid TS (0.001 mol/L) to make 50 mL. Dilute this solution with potassium dihydrogen phosphate TS (0.2 mol/L, containing disodium dihydrogen ethylenediaminetetraacetate) to 10 times its original volume. Prepare fresh before use.

Test Solution To 0.5 mL of the substrate solution, add 0.5 mL of disodium hydrogen phosphate TS (0.01 mol/L), and allow the mixture to equilibrate at 30°C for 5 minutes. Add 0.1 mL of the sample solution, and shake immediately, allow the mixture to incubate at 30°C for 5 minutes, add 3 mL of hydrochloric acid TS (0.2 mol/L), and mix.

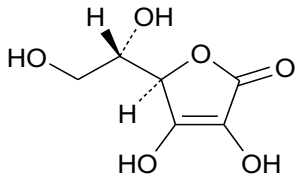
Control Solution To 0.5 mL of the substrate solution, add 0.5 mL of disodium hydrogen phosphate TS (0.01 mol/L) and 3 mL of hydrochloric acid TS (0.2 mol/L), mix, add 0.1 mL of the sample solution, shake, and allow the mixture to incubate at 30°C for 5 minutes.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 245 nm. The absorbance value of the test solution is lower than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

L-Ascorbic Acid

Vitamin C

L-アスコルビン酸



$C_6H_8O_6$ Mol. Wt. 176.12

(5*R*)-5-[(1*S*)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one [50-81-7]

Content L-Ascorbic Acid, when dried, contains not less than 99.0% of L-ascorbic acid ($C_6H_8O_6$).

Description L-Ascorbic Acid occurs as white to yellowish-white crystals or crystalline powder. It is odorless and has an acid taste.

Identification

(1) Dissolve 0.1 g of L-Ascorbic Acid in 100 mL of metaphosphoric acid solution (1 in

50), and add iodine TS dropwise to 5 mL of the solution until a slightly yellowish color develops. Add 1 drop each of a solution of copper(II) sulfate pentahydrate (1 in 1000) and pyrrole, and warm in a water bath at 50–60°C for 5 minutes. A blue to blue-green color develops.

(2) To 10 mL of a solution of L-Ascorbic Acid (1 in 100), add 1–2 drops of 2,6-dichloroindophenol sodium salt TS. A blue color develops and disappears immediately.

Specific Rotation $[\alpha]_D^{20}$: +20.5 to +21.5° (1 g, freshly boiled and cooled water, 10 mL, on the dried basis).

Melting point 187–192°C.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arseni Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.4% (reduced pressure, 3 hours).

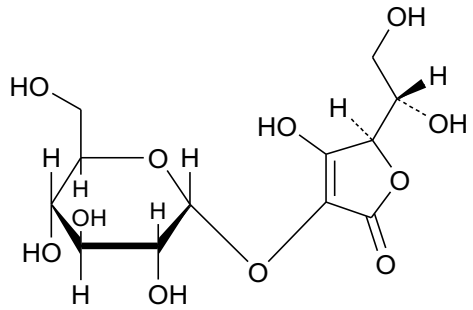
Residue on Ignition Not more than 0.1% .

Assay Weigh accurately about 0.2 g of L-Ascorbic Acid, previously dried, and dissolve it in 50 mL of metaphosphoric acid solution (1 in 50). Titrate with 0.05 mol/L iodine (indicator: starch TS).

Each mL of 0.05 mol/L iodine = 8.806 mg of $C_6H_8O_6$

L-Ascorbic Acid 2-Glucoside

L-アスコルビン酸 2-グルコシド



$C_{12}H_{18}O_{11}$ Mol. Wt. 338.26

(5*R*)-5-[(1*S*)-1,2-Dihydroxyethyl]-4-hydroxy-2-oxo-2,5-dihydrofuran-3-yl α-D-glucopyranoside [129499-78-1]

Content L-Ascorbic Acid 2-Glucoside contains not less than 98.0% of L-ascorbic acid 2-glucoside ($C_{12}H_{18}O_{11}$) when calculated on the dried basis.

Description L-Ascorbic Acid 2-Glucoside occurs as a while to yellowish-white powder or crystalline powder. It is odorless and has an acid taste.

Identification Determine the absorption spectrum of L-Ascorbic Acid 2-Glucoside as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Rotation $[\alpha]_D^{20}$: +186.0 to +188.0° (5 g, water, 100 mL, on the dried basis).

Melting Point 158–163°C

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 0.8 μg/g as As (2.5g, Method 3, Standard Color: Arsenic Standard Solution 4.0 mL, Apparatus B).

Loss on Drying Not more than 1.0% (105ºC, 2 hours).

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.5 g each of the sample and L-ascorbic acid 2-glucoside for assay, and separately dissolve them in water. To each solution, add exactly 10 mL of 5% (w/v) glycerol solution, as the internal standard solution, and water to make exactly 50 mL. Use these solutions as the test solution and the standard solution, respectively. Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Calculate the peak area ratio of L-ascorbic acid 2-glucoside to glycerol for each solution, and express as Q_T for the test solution and Q_S for the standard solution. Calculate the content by the formula:

$$\text{Content (\%) of L-ascorbic acid 2-glucoside } (C_{12}H_{18}O_{11}) = \frac{\text{Dry-basis weight (g) of L-ascorbic acid 2-glucoside for assay}}{\text{Dry-basis weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (4–8 mm internal diameter and 20–50 cm length).

Column packing material: Strongly acidic cation-exchange resin for liquid chromatography.

Column temperature: 35°C.

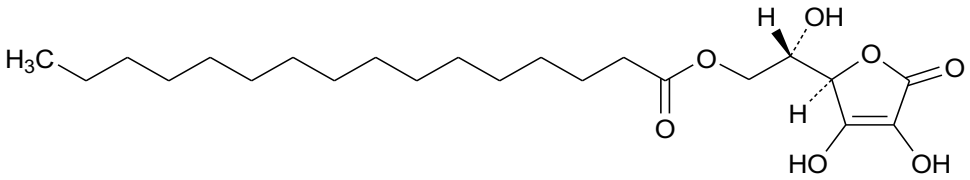
Mobile phase: Nitric acid (1 in 10,000).

Flow rate: Adjust the retention time of L-ascorbic acid 2-glucoside to about 10 minutes.

L-Ascorbyl Palmitate

Vitamin C Palmitate

L-アスコルビン酸パルミチン酸エステル



$C_{22}H_{38}O_7$ Mol. Wt. 414.53

(2*S*)-2[(2*R*)-3,4-Dihydroxy-5-oxo-2,5-dihydrofuran-2-yl]-2-hydroxyethyl hexadecanoate [137-66-6]

Content L-Ascorbyl Palmitate contains not less than 95.0% of L-ascorbyl palmitate ($C_{22}H_{38}O_7$).

Description L-Ascorbyl Palmitate occurs as a white or yellowish-white powder.

Identification

(1) To 0.1 g of L-Ascorbyl Palmitate, add 100 mL of sodium lauryl sulfate–propylene glycol TS, and dissolve by warming. Cool, and add iodine TS dropwise to 5 mL of this solution until a slight yellow color develops. Add 1 drop each of a solution of copper(II) sulfate pentahydrate (1 in 1000) and pyrrole, and warm at 50–60°C for 5 minutes. A blue to blue-green color develops.

(2) To 10 mL of a solution (1 in 100) of L-Ascorbyl Palmitate in ethanol (95), add 1 or 2 drops of 2,6-dichloroindophenol sodium salt TS. The color of the solution changes to blue and disappears immediately.

Specific Rotation $[\alpha]_D^{20}$: +21 to +24° (10 g, methanol, 100 mL).

Melting Point 107–117°C.

Purity

(1) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.2 g of L-Ascorbyl Palmitate, add 30 mL of ethanol (95), dissolve by warming if necessary. Add 15 mL of metaphosphoric acid solution (1 in 5) and 10 mL of diluted sulfuric acid (1 in 2). Add exactly 10 mL of potassium iodate TS, shake well, and allow to stand for 10 minutes in a dark place. Add 10 mL of potassium iodide TS and 100 mL of water, and allow to stand for 5 minutes in a dark place. Titrate

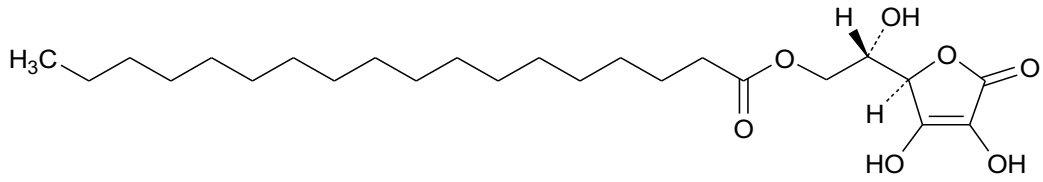
the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 10 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner.

Each mL of 0.1 mol/L sodium thiosulfate = 20.73 mg of $C_{22}H_{38}O_7$

L-Ascorbyl Stearate

Vitamin C Stearate

L-アスコルビン酸ステアリン酸エステル



$C_{24}H_{42}O_7$ Mol. Wt. 442.59

(2*S*)-2[(2*R*)-3,4-Dihydroxy-5-oxo-2,5-dihydrofuran-2-yl]-2-hydroxyethyl octadecanoate [25395-66-8]

Content L-Ascorbyl Stearate contains not less than 95.0% of L-ascorbyl stearate ($C_{24}H_{42}O_7$).

Description L-Ascorbyl Stearate occurs as a white to yellowish-white powder.

Identification

(1) To 0.1 g of L-Ascorbyl Stearate, add 100 mL of sodium lauryl sulfate–propylene glycol TS, and dissolve by warming. Cool, and add iodine TS dropwise to 5 mL of this solution until a slightly yellow color develops. Add 1 drop each of a solution of copper(II) sulfate pentahydrate (1 in 1000) and pyrrole, and warm at 50–60°C for 5 minutes. A blue to blue-green color develops.

(2) To 10 mL of a solution (1 in 100) of L-Ascorbyl Stearate in ethanol (95), add 1–2 drops of 2,6-dichloroindophenol sodium salt TS. A blue color develops, and then disappears immediately.

Melting Point 114–119°C.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 m, Apparatus B).

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.2 g of L-Ascorbyl Stearate, add 30 mL of ethanol (95), and dissolve by warming if necessary. Add 15 mL of metaphosphoric acid solution (1 in 5) and 10 mL of diluted sulfuric acid (1 in 2). Add exactly 10 mL of potassium iodate TS, shake well, and allow to stand for 10 minutes in a dark place. Add 10 mL of potassium iodide TS and 100 mL of water, and allow to stand for 5 minutes in a dark place. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 10 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner.

Each mL of 0.1 mol/L sodium thiosulfate = 22.13 mg of $C_{24}H_{42}O_7$

Asparaginase

アスパラギナーゼ

Definition Asparaginase is an enzyme that hydrolyzes asparagine into aspartic acid and ammonia. It is derived from the filamentous fungi (limited to *A. niger* ASP-72 and *A. oryzae* NZYM-SP), in which asparaginase productivity is improved by amplifying the asparaginase gene intrinsically occurring in filamentous fungi (limited to *Aspergillus. niger* and *Aspergillus. oryzae*). There are two types of Asparaginase: *A. niger* ASP-72-derived and *A. oryzae* NZYM-SP-derived products. It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. Also, it may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity

Asparaginase (*A. niger* ASP-72-derived)

Enzyme Activity Asparaginase has an enzyme activity of not less than 2375 units per gram.

Description Asparaginase occurs as a clear, yellow to brown liquid or as pale gray or slightly yellowish white granules.

Identification When tested by the enzyme activity determination, Asparaginase shows activity.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid　Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture　Prepare as directed in Method 3 for the *Escherichia. coli* test and Method 2 for the *Salmonella* test.

Enzyme Activity Determination

(i) Substrate Solution　Weigh 1.50 g of L-asparagine monohydrate, add citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.0, and dissolve it completely by stirring. Add the citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.0 to make exactly 100 mL. Prepare fresh before use.

(ii) Sample Solution　Weigh accurately about 2.5 g of Asparaginase, dissolve in 20 mL of citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.0, and then add the citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.0 again to make exactly 25 mL. Dilute this solution with the same citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.0 to prepare a solution containing 6 units/mL.

(iii) Control Stock Solution　Weigh an amount of *A. niger*-derived asparaginase for enzyme activity determination equivalent to 4000 units, dissolve in 20 mL of citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.0, and then add the citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.0 again to make exactly 25 mL. Dilute this solution with the citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.0 to prepare a solution containing 6 units/mL.

(iv) Ammonium Sulfate Standard Solutions　Weigh accurately about 3.9 g of ammonium sulfate, add 40 mL of citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.0, and shake for 15 minutes. Again add the citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.0 to make 50 mL. Dilute the resulting solution with the citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.0 4, 6, 10, 30, and 60 times, respectively, to prepare five standard solutions.

(v) Procedure

Test Solution and Control Solution　Place 2.0 mL portions of the substrate solution into two separate test tubes, and warm them at 37°C for 10 minutes. Add 0.100 mL of the sample solution to one test tube and 0.100 mL of the control stock solution to the other, and stir. Warm them at 37°C exactly for 30 minutes, add 0.400 mL portions of trichloroacetic acid solution (1 in 4) to them, stir, and add 2.5 mL of water to each, and again stir. Take 0.100 mL from each test tube into 4.0 mL of water separately, add 0.850 mL of phenol–nitroprusside TS (basic) to each, and stir. Add 0.850 mL of sodium hypochlorite–sodium hydroxide TS for *A. niger*-derived asparaginase activity determination to each, and allow them to stand at 37°C for 10 minutes. Use them as the test solution and the control solution, respectively.

Reference Solutions　Place 2.0 mL portions of the substrate solution into two separate test tubes, and add 0.400 mL portions of trichloroacetic acid solution (1 in 4) to them, and stir. Add 0.100 mL of the sample solution to one test tube and 0.100 mL of the

control stock solution to the other, stir, and warm them at 37°C for 30 minutes. Add 2.5 mL of water to each, and stir again. Take 0.100 mL from each test tube into 4.00 mL of water separately, add 0.850 mL of phenol-nitroprusside TS (basic) to each, and stir. Add 0.850 mL of sodium hypochlorite–sodium hydroxide TS for *A. niger*-derived asparaginase activity determination to each, allow them stand at 37°C for 10 minutes. Use them as the reference solutions for the test solution and the control solution, respectively.

Calibration Curve Place 2.0 mL portions of the substrate solution into five separate test tubes, and warm them at 37°C for 10 minutes. To each test tube, add a 0.100 mL portion of each of the ammonium sulfate standard solutions with different concentrations, instead of the sample solution, and proceed as directed for the test solution. Measure the absorbance of them against water at 600 nm. Prepare a calibration curve using the absorbance values obtained and the ammonium sulfate concentrations in the ammonium sulfate standard solutions.

Determination Measure the absorbance (A_T and A_C) of the test solution and the control solution against water at 600 nm. Also measure the absorbance (A_{BT} and A_{BC}) of the reference solutions for the test solution and the control solution against water at 600 nm. Measure the slope, a (mL/mg), of the calibration curve. Calculate the enzyme activity of *A. niger* -derived asparaginase for enzyme activity determination used for the preparation of the control solution by the formula given below. When the obtained activity is in the range of 91 to 109% of the labeled value, determine the enzyme activity of the sample by the following formula. One unit of enzyme activity is equivalent to the amount of the enzyme required to liberate 1 μmol of ammonia per minute from L-asparagine when the enzyme activity is determined as directed in the Procedure.

$$\text{Enzyme activity (unit/g)} = \frac{A \times D_f \times 25 \times 2 \times 10^3}{a \times M \times 132.14 \times 30}$$

A = the value obtained by deducting the absorbance (A_{BT} or A_{BC}) of the corresponding reference solution from the absorbance (A_T or A_C) of the test solution or the control solution, whichever is appropriate,

D_f = the dilution factor of the sample solution or the control stock solution,

M = the weight (g) of the sample or *A. niger* -derived asparaginase for enzyme activity determination.

Asparginase (*A. oryzae* NZYM-SP-derived)

Enzyme Activity Asparaginase has an enzyme activity of not less than 3500 units per gram.

Description Asparaginase occurs as a light brown liquid or as white to grayish-white granules.

Identification When tested by the enzyme activity determination, Asparaginase shows

activity.

Purity

(1) Lead Not more than 5 μg/g as Pb.

Weigh 0.8 g of Asparaginase, and proceed as directed in Purity (1) for Asparaginase (*A. niger* ASP-72-derived).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for the total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia. coli* test and Method 2 for the *Salmonella* test.

Enzyme Activity Determination

(i) Substrate Solution Weigh 0.25 g of L-asparagine monohydrate, add 15 mL of MOPS buffer (0.1 mol/L, pH7.0), and dissolve it completely by stirring. Cover the container to block out light. Refer the resulting solution to as Solution A. To Solution A, add 0.011 g of β-nicotinamide adenine dinucleotide disodium salt *n*-hydrate (reduced form), 0.063 g of disodium 2-ketoglutarate *n*-hydrate, and an appropriate amount of L-glutamic acid dehydrogenase (bovine liver-derived) equivalent to not less than 1680 units, and stir well to dissolve them. Add MOPS buffer (0.1 mol/L, pH7.0) to make exactly 25 mL. Prepare fresh before use.

(ii) Sample Solution Weigh accurately about 1.0 g of Asparaginase, and dissolve it in acetate buffer (0.1 mol/L, pH5.0, containing polyoxyethylene(23) lauryl ether) to make exactly 100 mL. Dilute the resulting solution with the acetate buffer to prepare a solution containing 0.6 units/mL.

(iii) Standard Stock Solutions Weigh an amount of *A. oryzae*-derived aparaginase for enzyme activity determination equivalent to 775 units, dissolve it in acetate buffer (0.1 mol/L, pH5.0, containing polyoxyethylene(23) lauryl ether) to make exactly 100 mL. Dilute this solution with the acetate buffer 8, 10, 15, 20, and 30 time to prepare five solutions containing 0.9688, 0.7750, 0.5167, 0.3875, and 0.2583 units/mL, respectively.

(iv) Procedure

Test Solution Transfer 4.6 mL of the substrate solution into a test tube, and warm at 37.0 ± 0.5°C for 8 minutes. Add 0.400 mL the sample solution, shake, and warm at 37.0 ± 0.5°C for 90 seconds.

Standard Solutions Transfer 4.6 mL portions of the substrate solution into five separate test tubes, and warm at 37.0 ± 0.5°C for 8 minutes. To each test tube, add 0.400 mL of each of the standard stock solutions with the different concentrations,

instead of the sample solution, and proceed as directed for the test solution.

Calibration Curve Measure the absorbance of the standard solutions against water at 340 nm and prepare the calibration curve from the absorbance values obtained and the enzyme activity in 1 mL (unit/mL) of each standard stock solution.

Determination Measure the absorbance, A, of the test solution against water at 340 nm. Determine the enzyme activity, U (unit/mL), of the sample solution from absorbance A and the calibration curve. Then calculate the enzyme activity of the sample by the following formula. One unit of enzyme activity is equivalent to the amount of the enzyme required to liberate 1 μmol of ammonia per minute from L-asparagine when the enzyme activity is determined as directed in the procedure.

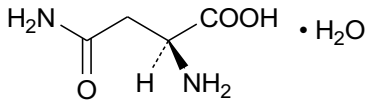
$$\text{Enzyme activity (unit/g)} = \frac{\text{U} \times \text{D} \times 100}{\text{Weight (g) of the sample}}$$

U = the enzyme activity of the sample solution,

D = the dilution factor of the sample solution.

L-Asparagine

L-アスパラギン



$C_4H_8N_2O_3 \cdot H_2O$ Mol. Wt. 150.13

(2*S*)-2-Amino-3-carbamoylpropanoic acid monohydrate [5794-13-8]

Content L-Asparagine, when calculated on the dried basis, contains 98.0–102.0% of L-asparagine ($C_4H_8N_2O_3$ = 132.12).

Description L-Asparagine occurs as white crystals or crystalline powder. It is odorless, and has a sweetish taste.

Identification

(1) To 5 mL of a solution of L-Asparagine (1 in 1000), add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A purple color develops.

(2) To 0.1g of L-Asparagine, add 5 mL of sodium hydroxide solution (1 in 10), and heat in a water bath. The generated gas (NH_3) changes litmus paper (red) moistened with water to blue.

Specific Rotation $[\alpha]_D^{20}$: +33.0 to +36.5° (10 g, hydrochloric acid (6 mol/L), 100 mL, on the dried basis).

pH 3.5–5.5 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution　Colorless and clear (1.0 g, water 50 mL).

(2) Chloride　Not more than 0.1% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　11.5–12.5% (130°C, 3 hours).

Residue on Ignition　Not more than 0.1%

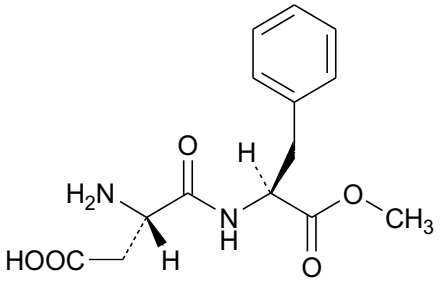
Assay　Weigh accurately about 0.3 g of L-Asparagine, dissolve it in 3 mL of formic acid, add 50 mL of acetic acid, and titrate with 0.1 mol/L perchloric acid. The endpoint is usually confirmed by a potentiometer. When an indicator (1 mL of crystal violet–acetic acid TS) is used, titrate until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, make any necessary correction, and calculate on the dried basis.

Each mL of 0.1 mol/L perchloric acid = 13.21 mg of $C_4H_8N_2O_3$

Aspartame

L-α-Aspartyl-L-phenylalanine Methyl Ester

アスパルテーム



$C_{14}H_{18}N_2O_5$　Mol. Wt. 294.30

Methyl L-α-aspartyl-L-phenylalaninate　[22839-47-0]

Content　Aspartame, when calculated on the dried basis, contains 98.0–102.0% of aspartame ($C_{14}H_{18}N_2O_5$).

Description　Aspartame occurs as a white crystalline powder or as granules. It is odorless and has a strong sweet taste.

Identification

(1) Determine the absorption spectrum of Aspartame as directed in the Paste Method under the Infrared Spectrophotometry. It exhibits absorption bands at about 3300 cm^{-1}, 1737 cm^{-1}, 1666 cm^{-1}, 1379 cm^{-1}, 1227 cm^{-1}, and 699 cm^{-1}.

(2) To 5 mL of a solution of Aspartame (1 in 1000), add 1 mL of ninhydrin solution (1 in 50), and heat on a water bath for 3 minutes. A blue-purple color develops.

Specific Rotation $[\alpha]_D^{20}$: +14.5 to +16.5° (2 g, formic acid (15 mol/L), 50 mL, on the dried basis). Measure within 30 minutes.

pH 4.5–6.0 (1.0 g, water 125 mL).

Purity

(1) Clarity of solution Colorless and clear (0.20 g, hydrochloric acid (1 in 60) 20 mL).

(2) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) 5-Benzyl-3,6-dioxo-2-piperazineacetic acid Not more than 1.5% as 5-benzyl-3,6-dioxo-2-piperazineacetic acid.

Test Solution Dissolve 0.10 g of Aspartame in 20 mL of an 1: 9 mixture of methanol/water.

Control Solution Dissolve 25 mg of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in 10 mL of methanol, and add water to make 100 mL. To 15 mL of this solution, add a 1:9 mixture of methanol/water to make 50 mL.

Procedure Analyze 20 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. The peak area of the 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the test solution does not exceed that of the 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the control solution.

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 210 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 250 mm length).

Column packing material: 10-μm octylsilanized silica gel for liquid chromatography

Mobile phase: A solution prepared by dissolving 5.6 g of potassium dihydrogen phosphate in 820 mL of water and adding 180 mL of methanol.

Flow rate: 2 mL/minute.

(5) Other optical isomers Not more than 0.04% as L-α-aspartyl-D-phenylalanine methyl ester.

Test Solution Weigh 0.50 g of Aspartame, and dissolve it in citrate buffer (pH 2.2)

to make 100 mL.

Control Solution Measure 10 mL of a solution of L-α-aspartyl-D-phenylalanine methyl ester (1 in 50,000), and add citrate buffer (pH 2.2) to make 100 mL.

Procedure Analyze equal volumes of the test solution and the control solution by liquid chromatography using the operating conditions given below. The peak height of the L-α-aspartyl-D-phenylalanine methyl ester of the test solution does not exceed the peak height of the L-α- aspartyl-D-phenylalanine methyl ester of the control solution.

Operating Conditions

Detector: Visible spectrophotometer (wavelength: 570 nm).

Column: A glass tube (9 mm internal diameter and 55 cm length).

Column packing material: 17-μm strongly acidic cation-exchange resin for gas chromatography.

Column temperature: 55°C.

Mobile phase: Citrate buffer (pH 5.28).

Flow rate: 1 mL/min.

Reaction coil: Teflon tube 0.5 mm in internal diameter and 29 m in length.

Reaction chamber temperature: 100°C.

Flow rate of ninhydrin−2-methoxyethanol TS: 0.5 mL/min.

Injection amount of the test solution and control solution: A constant amount of 50–500 μL.

Loss on Drying Not more than 4.5% (105°C, 4 hours).

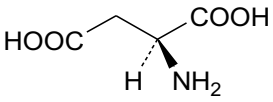
Residue on Ignition Not more than 0.2%.

Assay Weigh accurately 0.3 g of Aspartame, dissolve it in 3 mL of formic acid, add 50 mL of acetic acid, and immediately titrate with 0.1 mol/L perchloric acid. For confirmation of endpoint, use potentiometer. When 0.5 mL of *p*-naphtholbenzein TS is used as the indicator, the endpoint is when the brown color of the solution changes to green. Perform a blank test in the same manner to make any necessary correction. Calculate on the dried basis.

Each mL of 0.1 mol/L perchloric acid = 29.43 mg of $C_{14}H_{18}N_2O_5$

L-Aspartic Acid

L-アスパラギン酸



$C_4H_7NO_4$ Mol. Wt. 133.10

(2*S*)-2-Aminobutanedioic acid [56-84-8]

Content L-Aspartic Acid, when calculated on the dried basis, contains 98.0–102.0% of L-aspartic acid ($C_4H_7NO_4$).

Description L-Aspartic Acid occurs as white crystals or crystalline powder. It is odorless, and has a sour taste.

Identification

(1) To 5 mL of a 1 in 1000 solution of L-Aspartic Acid, add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A bluish purple color develops.

(2) To 5 mL of a 1 in 25 solution of L-Aspartic Acid in hydrochloric acid TS (1 mol/L), add 1 mL of sodium nitrite solution (1 in 10). The solution effervesces, evolving a colorless gas.

Specific Rotation $[\alpha]_D^{20}$: +24.0 to +26.0° (8 g, hydrochloric acid TS (6 mol/L), 100 mL, on the dried basis).

pH 2.5–3.5 (A saturated solution).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, hydrochloric acid TS (1 mol/L) 20 mL).

(2) Chloride Not more than 0.1% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

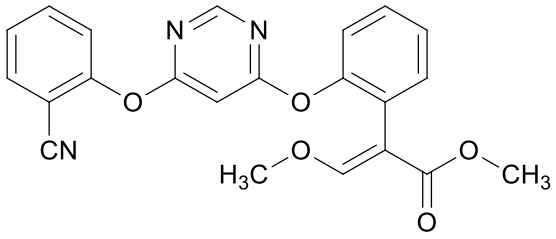
Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.3 g of L-Aspartic Acid, dissolve it in 6 mL of formic acid, and proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 13.31 mg of $C_4H_7NO_4$

Azoxystrobin

アゾキシストロビン



$C_{22}H_{17}N_3O_5$ Mol. Wt. 403.39

Methyl (*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate [131860-33-8]

Content Azoxystrobin contains not less than 95.0% of azoxystrobin ($C_{22}H_{17}N_3O_5$).

Description Azoxystrobin occurs as a white to yellow-red, odorless powder.

Identification Determine the infrared absorption spectrum of Azoxystrobin as directed in the Paste Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2230 cm^{-1}, 1625 cm^{-1}, 1587 cm^{-1}, 1201 cm^{-1}, 1155 cm^{-1}, 840 cm^{-1}.

Melting Point 114–119°C.

Purity Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Water Content Not more than 0.50% (2 g, Volumetric Titration, Direct Titration).

Assay Weigh accurately about 50 mg each of Azoxystrobin and azoxystrobin for assay. Dissolve them separately in acetonitrile to make exactly 100 mL of each. Use them as test solution and standard solution, respectively. Analyze 10 μl each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of azoxystrobin for the test solution and the standard solution. Calculate the azoxystrobin content by the formula:

$$\text{Content (\%)of azoxystrobin } (C_{22}H_{17}N_3O_5) = \frac{\text{Weight (g) of azoxystrobin for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 260 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 15 cm length).

Column packing material: 5-μm octadecyl silanized silica gel for liquidchromatography.

Column temperature: 40°C.

Mobile phase: An 11:9 mixture of water/acetonitrile.

Flow rate: Adjust the retention time of azoxystrobin to about 15 minutes.

Bacillus Natto Gum

納豆菌ガム

Definition Bacillus Natto Gum is obtained from the culture fluid of *Bacillus subtilis* and consists mainly of polyglutamic acid.

Content Bacillus Natto Gum, when dried, contains not less than 70.0% of polyglutamic acid.

Description Bacillus Natto Gum occurs as a white to light brown, hygroscopic powder, or as lumps or granules. It has little or no odor.

Identification

(1) Place 5 mL of a solution (1 in 200) of Bacillus Natto Gum in a test tube with a stopper, add 5 mL of hydrochloric acid, and stopper tightly. Hydrolyze the mixture at 110°C for 24 hours. Cool, and add sodium hydroxide solution (6 in 25) to make it weakly acidic. To 5 mL of this solution, add 1 mL of ninhydrin TS, and heat in a water bath for 5 minutes. A purple color develops.

(2) Add 1 g of Bacillus Natto Gum to 50 mL of water, and stir for 30 minutes. The solution is clear.

(3) Add 1 g of Bacillus Natto Gum to 10 mL of hydrochloric acid, and stir for 30 minutes. A turbidity or precipitate is produced.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 15.0% (reduced pressure, 40°C, 24 hours).

Residue on Ignition Not more than 43.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 1 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture Prepare as directed in Method 1, respectively, for the *Escerichia coli* test and the *Salmonella* test.

Assay

Test Solution Weigh accurately about 0.1 g of Bacillus Natto Gum, previously dried, and dissolve it in water to make 10 mL. Place exactly 5 mL of this solution in a pressure-resistant test tube, add exactly 5 mL of hydrochloric acid, seal tightly, and hydrolyze at 110 °C for 24 hours. After cooling, measure exactly 1 mL of the resulting solution, and add water to make exactly 200 mL.

Standard Solution Weigh accurately about 0.1 g L-glutamic acid for assay, previously dried, add 1 mL of diluted hydrochloric acid (1 in 6) and 20 mL of water to dissolve, and add water to make exactly 100 mL. Measure exactly 5 mL of this solution, and add water to make exactly 200 mL.

Procedure Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) for the test solution and the standard solution, and calculate the content by the formula:

$$\text{Content (\%) of polyglutamic acid} = \frac{\text{Weight (g) of L-glutamic acid for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 0.8775 \times 100$$

Operating Conditions

Detector: Visible-range spectrophotometer (wavelength: 570 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 6 cm length).

Column packing material: Strongly acidic cation exchange resin for liquid chromatography.

Column temperature: A constant temperature of about 55°C.

Chemical reaction bath temperature: A constant temperature of about 135°C.

Mobile phase: Buffer for bacillus natto gum (pH 3.3).

Reaction reagent: Ninhydrin TS for bacillus natto gum assay.

Mobile phase flow rate: Adjust the retention time of glutamic acid to about 7 minutes.

Reaction reagent flow rate: 0.35 mL/minute.

Baking Powder

合成膨張剤

Single Baking Powder

一剤式合成膨張剤

Description Single Baking Powder occurs as a white to gray-white powder or as brittle lumps of clustered powder.

pH 5.0–8.5.

Test Solution Weigh 1.0 g of Single Baking Powder, add 50 mL of water, heat in a water bath until effervescence ceases, and cool.

Purity

(1) Nitric acid-insoluble substances Not more than 2.0%.

Weigh 5.0 g of Single Baking Powder, add 30 mL of water, and shake for 3 minutes. Filter with a filter paper to separate the insoluble substances, and wash the residue on the filter paper thoroughly with water filled with carbon dioxide. Bore a small hole at the bottom of the filter paper, wash the residue into a beaker with 40 mL of diluted nitric acid (1 in 10), and boil for 1 minute. Cool, filter through a filter paper for quantitative analysis (5B), and wash it with water until the washings are no loner acidic. Place the residue with the filter paper in a ceramic crucible, previously weighed. Then ignite the crucible at about 550°C to constant weight, and weigh the mass.

(2) Heavy metals Measure a small amount of Single Baking Powder, and heat. If the sample is carbonized, proceed as directed in (i); if it is not carbonizd, proceed as directed in (ii).

(i) Not more than 40 μg/g as Pb (0.50 g, Method 2, Control Solution: Lead Standard Solution (for heavy metals limit test) 2.0 mL).

(ii) Not more than 40 μg/g as Pb.

Test solution Weigh 2.0 g of Single Baking Powder, add 5 mL of nitric acid, and heat on a water bath for 15 minutes. Cool, add 5 mL of water, and filter. Wash the residue on the filter paper with 5 mL of water, combine the washings with the filtrate, add 2 drops of phenolphthalein TS, and then add sodium hydroxide solution (1 in 10) until the color of the solution changes to a slightly pink color. Add 5 mL of diluted hydrochloric acid (1 in 4). Adjust the pH to 2.5–3.5 with ammonia TS, add 8 mL of diluted acetic acid (1 in 20) and water to make 100 mL, measure 25 mL of this solution, add water to make 50 mL.

Control Solution To 2.0 mL of Lead Standard Solution (for heavy metals limit test), add 2 mL of diluted acetic acid (1 in 20) and water to make 50 mL.

(3) Arsenic Take a small amount of Single Baking Powder and heat it. If the sample

is carbonized, proceed as directed in (i); if it is not carbonized, proceed as directed in (ii).

(i) Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(ii) Not more than 3 μg/g as As (5.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of Single Baking Powder into a 100-mL flask, add 10 mL of water, heat until effervescence ceases, and neutralize with diluted hydrochloric acid (1 in 4) or sodium hydroxide solution (1 in 25). When ammonia solution or ammonia TS is used to neutralize the solution, adjust the pH to 2.5–3.5. Add 5 mL of hydrochloric acid, and heat in a water bath for 30 minutes. Cool, and add water to make 25 mL. Measure 5 mL of this solution, add 10 mL of sulfurous acid solution, and evaporate to about 2 mL, and add water to make 10 mL. Use 5 mL of the second solution as the test solution.

(4) Gas evolution Measure the volume of the gas evolved. The volume is not less than 70 mL.

Duplex Baking Powder

二剤式合成膨張剤

Prepare a sample by mixing the two substances in the proportion indicated, and proceed as directed under Single Baking Powder.

Ammonia Type Baking Powder

アンモニア系合成膨張剤

Proceed as directed under Single Baking Powder. The pH is 6.0–9.0. Use water as the replacement solution for the determination of the gas volume specified in Purity (4).

Bees Wax

ミツロウ

Definition Bees Wax is obtained from the honeycombs of bees *Apis* spp. and consists mainly of myricyl palmitate.

Description Bees Wax occurs as a white to yellowish-white or yellow to light brown solid having a characteristic odor of honey .

Identification To 1 g of Bees Wax, add 50 mL of 2-propanol, and dissolve by warming to 65°C in a water bath. Add 5 mL of lukewarm water while stirring. White flocculent

substances are formed.

Melting Point 60–67°C.

Saponification Value 77–103 (Fats and Related Substances Tests).

Purity

(1) Acid value 5–24 (Fats and Related Substances Tests).

Weigh accurately about 3 g of Bees Wax, dissolve it in 80 mL of a 5:3 mixture of ethanol (95)/xylene, and use the solution obtained as the test solution. Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests. When turbidity is produced while cool, titrate while warming.

(2) Peroxide value Not more than 5.

Weigh accurately about 5 g of Bees Wax, transfer it into a 200 mL ground-glass stoppered Erlenmeyer flask. Add 30 mL of a 3:2 mixture of acetic acid/chloroform, stopper, heat in warm water, and dissolve with gentle shaking. Cool, replace the air in the flask with nitrogen. While passing nitrogen through, add exactly 1 mL of Potassium Iodide TS into the flask. Then stop the passage of the nitrogen, stopper immediately, shake for 1 minute, and allow to stand for 5 minutes in a dark place. Add 30 mL of water to this solution, stopper again, and shake vigorously. Titrate with 0.01 mol/L Sodium Thiosulfate (indicator: 1–3mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Calculate the content by the following formula. Perform the blank test in the same manner, and make any necessary correction.

$$\text{Peroxide value} = \frac{\text{Volume (mL) of 0.01 mol/L sodium thiosulfate consumed}}{\text{Weight (g) of the sample}} \times 10$$

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Fats, Japan wax, rosin and soap To 1 g of Bees Wax, add 35 mL of sodium hydroxide solution (1 in 7). Heat for 30 minutes on a water bath with occasional shaking while replenishing the evaporated water. Cool, and filter the solution. Acidify the filtrate with hydrochloric acid. No precipitate is formed.

Residue on Ignition Not more than 0.1%.

Beet Red

ビートレッド

Definition Beet Red is obtained from the roots of the beet *Beta vulgaris* L. and consists

mainly of isobetanine and betanine. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Beet Red is not less than 15 and is in the range of 90–110% of the labeled value.

Description Beet Red occurs as a red-purple to dark purple powder, as lumps, or as a paste or liquid. It has a slight, characteristic odor.

Identification

(1) Weigh an amount of Beet Red equivalent to 1 g of beet red with a Color Value 15, and dissolve it in 50 mL of acetate buffer (pH 5.4). A red-purple color develops.

(2) To 5 mL of the solution obtained in Identification (1), add 1 mL of sodium hydroxide solution. The color changes to yellow.

(3) A solution of Beet Red in acetate buffer (pH 5.4) exhibits an absorption maximum at a wavelength of 525–540 nm.

(4) Weigh an amount of Beet Red equivalent to 1 g of beet red with a Color Value 15, dissolve it in 5 mL of water, add 20 mL of methanol, and mix. Centrifuge the solution at 3000 rpm for 10 minutes, and use the supernatant as the test solution. Analyze an 8-μL portion of the test solution by thin-layer chromatography using a 4:3:2 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with microcrystalline cellulose for thin-layer chromatography as the solid support and then dried at 60–80°C for 20 minutes. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. A purple spot is observed at an R_f value of about 0.3–0.5. Place the plate in a container filled with ammonia vapor, and allow to stand for at least 30 minutes. The purple color of the spot changes to light gray to dark brown.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Nitrate Not more than 0.27% as NO_3 per Color Value 15.

Test Solution Weigh accurately about 0.1 g of Beet Red, and dissolve it in water to make exactly 100 mL.

Standard Solution Measure exactly 0.2 mL, 1 mL, 10 mL, and 50 mL of Nitrate Ion Standard Stock Solution, and add water to each to make exactly 100 mL.

Analyze 20 μL each of the test solution, the standard solutions, and the Standard Stock Solution by ion chromatography. Measure the peak height or peak area of nitrate ion for each of the standard solutions and the Standard Stock Solution, and prepare a calibration curve. Measure the peak height or peak area of nitrate ion for the test solution, and obtain the amount using the calibration curve.

Operating Conditions

Detector: Electric conductivity detector.

Column: A stainless steel tube (4.6–6.0 mm internal diameter and 5–10 cm length).

Column packing material: Porous anion exchange resin.

Guard column: Used a column with the same internal diameter that is packed with the same packing material as for the column above.

Temperature: 40°C.

Eluent: A solution (pH 4.0) prepared by dissolving 0.42 g of phthalic acid and 0.29 g of 2-amino-2-hydroxymethyl-1,3-propandiol in 1000 mL of water.

Flow rate: 1.5 mL/min.

Color Value Determination Proceed as directed under Color Value Determination, using the conditions below.

Operating Conditions

Solvent: Acetate buffer (pH 5.4).

Wavelength: Maximum absorption wavelength of 525–540 nm.

Bentonite

ベントナイト

Definition Bentonite is obtained by drying bentonite mined from mineral deposits. It consists mainly of hydrous aluminum silicate.

Description Bentonite occurs as a white to light yellow-brown powder or as flakes. When moistened, it produces an odor like soil or clay.

Identification

(1) To 0.5 g of Bentonite, add 3 mL of diluted sulfuric acid (1 in 3), and heat until white fumes are evolved. After cooling, add 20 mL of water, and filter. To 5 mL of the filtrate, add 3 mL of ammonia TS. A white gelatinous precipitate is formed, and when alizarin red S solution (1 in 1000) is added, the precipitate turns to red.

(2) Wash the residue obtained by filtration in Identification (1) with water, add 2 mL of methylene blue solution (1 in 10,000), and again wash with water. The residue turns blue.

(3) Mix 6.0 g of Bentonite with 0.3 g of magnesium oxide. Add the mixture in installments to a 500-mL stoppered measuring cylinder containing 200 mL of water, and shake for 1 hour. Transfer 100 mL of the suspension to a 100-mL measuring cylinder, and allow to stand for 24 hours. The solution separates into two layers. The upper clear solution is not more than 2 mL.

pH 8.5–10.5 (2% suspension).

Purity

(1) Lead Not more than 40 μg/g as Pb (0.10 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Bentonite, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

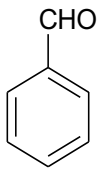
(2) Arsenic Not more than 3 μg/g as As (2.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Bentonite, add 12 mL of diluted hydrochloric acid (1 in 10) and 8 mL of water, boil for 30 minutes while replenishing the lost water, and evaporate to dryness. Dry the residue at 100°C for 1 hour. To the residue, add 20 mL of diluted hydrochloric acid (1 in 10), boil gently for 5 minutes, and filter the supernatant. To the residue, add 10 mL of diluted hydrochloric acid (1 in 10), boil gently for 5 minutes, and filter the supernatant through the filter. Combine the filtrates, and add water to make 100 mL. Use 25 mL of the resulting solution.

Loss on Drying Not more than 12.0% (105°C, 2 hours).

Benzaldehyde

ベンズアルデヒド



C_7H_6O Mol. Wt. 106.12

Benzaldehyde [100-52-7]

Content Benzaldehyde contains not less than 98.0% of benzaldehyde (C_7H_6O).

Description Benzaldehyde is a colorless, clear liquid having an almond-like odor.

Identification Determine the absorption spectrum of Benzaldehyde as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.544–1.547.

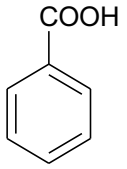
Specific Gravity d_{25}^{25}: 1.040–1.047.

Purity Acid value Not more than 5.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Benzoic Acid

安息香酸



$C_7H_6O_2$ Mol. Wt. 122.12

Benzenecarboxylic acid [65-85-0]

Content Benzoic Acid, when dried, contains not less than 99.5% of benzoic acid ($C_7H_6O_2$).

Description Benzoic Acid occurs as white laminar crystals or needles. It is odorless or has a slight odor of benzaldehyde.

Identification Dissolve 1 g of Benzoic Acid in 20 mL of sodium hydroxide solution (1 in 25). The solution responds to test (2) for Benzoate in the Qualitative Tests.

Melting Point 121–123°C.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Readily oxidizable substances Add 1.5 mL of sulfuric acid to 100 mL of water, and then add 0.02 mol/L potassium permanganate dropwise while boiling until the pink color persists for 30 seconds. Weigh 1.0 g of Benzoic Acid, and dissolve it in the solution. Titrate with 0.02 mol/L potassium permanganate at about 70°C until the pink color persists for 15 seconds. The amount is not more than 0.5 mL.

(4) Chlorinated compounds Not more than 0.014% as Cl.

Test Solution Weigh 0.50 g of Benzoic Acid and 0.7 g of calcium carbonate, and transfer them into a porcelain crucible. Add a small amount of water, mix, dry at 100°C, and heat at about 600°C for 10 minutes. Cool, add 20 mL of diluted nitric acid (1 in 10) to dissolve the residue, and filter. Wash the insoluble substances with about 15 mL of water, combine the washings with the filtrate, and add water to make 50 mL.

Control Solution Weigh 0.7 g of calcium carbonate, dissolve it in 20 mL of diluted nitric acid (1 in 10), filter if necessary, and add 0.20 mL of 0.01 mol/L hydrochloric acid and water to make 50 mL.

Procedure Add 0.5 mL of silver nitrate solution (1 in 50) to both solutions, shake well, and allow to stand for 5 minutes. The test solution is not more turbid than the control solution.

(5) Phthalic acid Not more than 50 μg/g.

Test Solution Weigh 1.0 g of Benzoic Acid, dissolve it in 20 mL of methanol, and add diluted acetic acid (1 in 100) to make exactly 50 mL.

Control Solution Weigh 10 mg of phthalic acid, dissolve it in 30 mL of methanol, and add diluted acetic acid (1 in 100) to make exactly 100 mL. Measure 1.0 mL of this solution, and add a 3:2 mixture of diluted acetic acid (1 in 100)/methanol to make exactly 100 mL.

Procedure Measure 20 μL each of the test solution and the control solution, and proceed with the operating conditions given below, as directed under Liquid Chromatography. The peak height of the phthalic acid of the test solution does not exceed that of the control solution.

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 228 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 7-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 7:3 mixture of diluted acetic acid (1 in 100)/methanol.

Flow rate: 1 mL/minute.

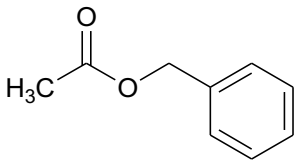
Loss on Drying Not more than 0.5% (3 hours).

Assay Weigh accurately about 0.25 g of Benzoic Acid, previously dried, dissolve it in 25 mL of 50% (vol) ethanol neutralized with 0.1 mol/L sodium hydroxide, and titrate with 0.1 mol/L sodium hydroxide (indicator: 3 drops of phenol red TS).

Each mL of 0.1 mol/L sodium hydroxide = 12.21 mg of $C_7H_6O_2$

Benzyl Acetate

酢酸ベンジル



$C_9H_{10}O_2$ Mol. Wt. 150.17

Phenylmethyl acetate [140-11-4]

Content Benzyl Acetate contains not less than 98.0% of benzyl acetate ($C_9H_{10}O_2$).

Description Benzyl Acetate is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Benzyl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensity of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.500–1.504.

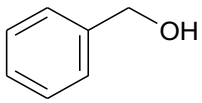
Specific Gravity d_{25}^{25}: 1.049–1.059.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Benzyl Alcohol

ベンジルアルコール



C_7H_8O Mol. Wt. 108.14

Phenylmethanol [100-51-6]

Content Benzyl Alcohol contains not less than 98.0% of benzyl alcohol (C_7H_8O).

Description Benzyl Alcohol is a colorless, clear liquid having a weak characteristic odor.

Identification Determine the absorption spectrum of Benzyl Alcohol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.536–1.541.

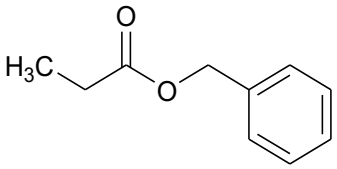
Specific Gravity d_{25}^{25}: 1.040–1.050.

Purity Acid value Not more than 0.5 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Benzyl Propionate

プロピオン酸ベンジル



$C_{10}H_{12}O_2$ Mol. Wt. 164.20

Phenylmethyl propanoate [122-63-4]

Content Benzyl Propionate contains not less than 98.0% of benzyl propionate ($C_{10}H_{12}O_2$).

Description Benzyl Propionate is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Benzyl Propianate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.495–1.500.

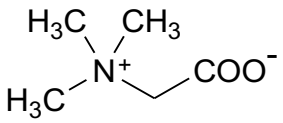
Specific Gravity d_{25}^{25}: 1.028–1.033

Purity Acid value Not more than 1.0. (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Betaine

ベタイン



$C_5H_{11}NO_2$ Mol. Wt. 117.15

2-(*N*,*N*,*N*-Trimethylammonio)acetate [107-43-7]

Definition Betaine is obtained by isolation from molasses from the sugar beet *Beta vulgaris* L. It consists mainly of betaine ($C_5H_{11}NO_2$).

Content Betaine, when dried, contains 98.0–102.0% of betaine ($C_5H_{11}NO_2$).

Description Betaine occurs as white, hygroscopic and deliquescent crystals having a

slight odor. It has sweet and slightly bitter tastes.

Identification Determine the absorption spectrum of Betaine, previously dried, as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH 5.0–7.0 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.005% as Cl (1.0 g, Control Solution: 0.01 mol/L hydrochloric acid 0.15 mL).

(3) Sulfate Not more than 0.01% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.20 mL).

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 3.0% (105°C, 3 hours).

Residue on Ignition Not more than 0.1% (500°C, 3 hours).

Assay *Test Solution* Weigh accurately about 1 g of Betaine, previously dried, and dissolve it in water to make exactly 100 mL.

Standard Solutions Weigh accurately 0.5-g and 1.0-g portions, respectively, of betaine for assay, previously dried, and separately dissolve them in water to make exactly 100 mL of each.

Procedure Analyze 10 μL each of the test solution and the standard solutions by liquid chromatography using the operating conditions given below, and measure the peak areas. Prepare a calibration curve from the peak areas for the standard solutions. Determine the betaine content, using the following formula, from the calibration curve and the peak area for the test solution.

$$\text{Content (\%) of betaine } (C_5H_{11}NO_2) = \frac{\text{Amount (g) of betaine in the test solution}}{\text{Weight (g) of the sample}} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (4 mm internal diameter and 25 cm length).

Column packing material: Strongly acidic cation exchange resin.

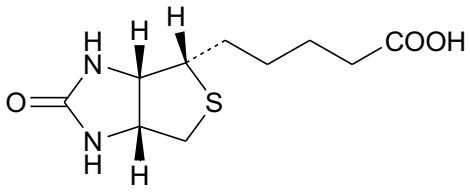
Column temperature: 70°C.

Mobile phase: Water.

Flow rate: Adjust the retention time of betaine to about 9 minuets.

Biotin

ビオチン



$C_{10}H_{16}N_2O_3S$ Mol. Wt. 244.31

5-[(3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl] pentanoic acid [58-85-5]

Content Biotin contains not less than 98.0% biotin ($C_{10}H_{16}N_2O_3S$) when dried.

Description Biotin occurs as white crystals or crystalline powder. It is odorless and tasteless.

Identification

(1) To 5 mL of a solution (1 in 10,000) of Biotin in ethanol (95), add 1 mL of *p*-dimethylaminocinnamaldehyde TS and 3 drops of sulfuric acid, and shake. An orange to red color develops.

(2) Determine the absorption spectrum of Biotin, previously dried, as directed in the Disk Method under Infrared Spectrophotometry. The spectrum exhibits absorption bands at about 3315 cm^{-1}, 1708 cm^{-1}, 1687 cm^{-1}, 1481 cm^{-1}, 1320 cm^{-1}, and 1274 cm^{-1}.

Specific Rotation $[\alpha]_D^{20}$: +89 to +93° (0.4 g, sodium hydroxide TS (0.1 mol/L), 20 mL, on the dried basis).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, 0.5 mol/L sodium hydroxide TS 10 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 2.1 μg/g as As (0.71 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Take the specified amount of Biotin in a Kjeldahl flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, place a small funnel in the mouth of the flask, and

heat carefully until white fumes are evolved. After cooling, add two 2-mL portions of nitric acid, heat, add two to three 2-mL portions of hydrogen peroxide, and heat until the solution is colorless to pale yellow. After cooling, add 2 mL of a saturated solution of ammonium oxalate, heat to evaporate until white fumes are evolved again. After cooling, add water to make 5 mL.

(4) Related substances

Test Solution Dissolve 0.10 g of Biotin, weighed accurately, in ammonia solution (28) (7 in 100) and make exactly 10 mL.

Standard Solution Take exactly 1 mL of the test solution, add ammonia solution (28) (7 in 100) to make exactly 500 mL.

Procedure Analyze 5 μL each of the test solution and the standard solution by thin-layer chromatography, using a 5:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. When the solvent front has ascended to a point 10 cm above the starting line, stop developing. Air-dry the plate, and dry at 105°C for an additional 30 minutes. Spray evenly with a mixture of equal volumes of a solution of *p*-dimethylaminocinnamaldehyde in ethanol (95) (1 in 500) and a solution (1 in 50) of sulfuric acid in ethanol (95). The main red spot from the test solution is observed, and no other spot from the test solution is more intense than the main spot from the standard solution.

Loss on Drying Not more than 0.5% (105°C, 4 hour).

Residue on Ignition Not more than 0.1%.

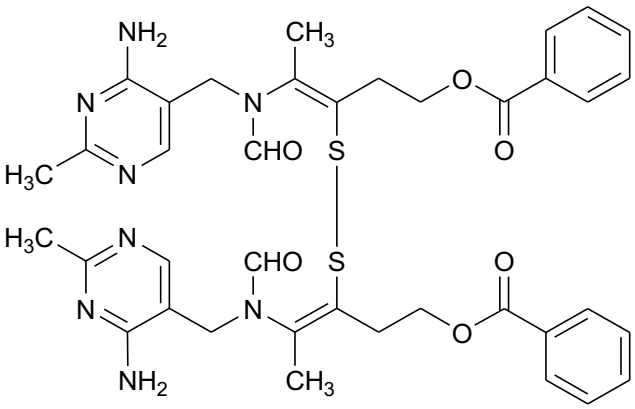
Assay Weigh accurately about 0.25 g of Biotin, previously dried, and add exactly 20 mL of 0.1 mol/L sodium hydroxide solution to dissolve. Titrate the excess sodium hydroxide with 0.1 mol/L hydrochloric acid. Use 2 drops of phenolphthalein as the indicator. Separately, perform a blank test to make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide = 24.43 mg of $C_{10}H_{16}N_2O_3S$

Bisbentiamine

Benzoylthiamine Disulfide

ビスベンチアミン



$C_{38}H_{42}N_8O_6S_2$ Mol. Wt. 770.92

N,N'-(Disulfanediylbis{2-[2-(benzoyloxy)ethyl]-1-methylethene-2,1-diyl})bis{*N*-[(4-amino-2-methylpyrimidin-5-yl)methyl]formamide} [2667-89-2]

Content Bisbentiamine, when dried, contains 98.0–102.0% of bisbentiamine ($C_{38}H_{42}N_8O_6S_2$).

Description Bisbentiamine occurs as white crystals or crystalline powder. It is odorless and has a slightly bitter taste.

Identification

(1) To 50 mg of Bisbentiamine, add 5 mL of methanol, and dissolve by warming. Add 2 mL of a 1:1 mixture of sodium hydroxide solution (3 in 20)/hydroxylammonium chloride solution (3 in 20), and warm in a water bath at 50–60°C for 2 minutes. To the resulting solution, add 0.8 mL of hydrochloric acid and 0.5 mL of a solution of iron(III) chloride hexahydrate (1 in 10), and 8 mL of water. A red-purple color develops.

(2) To 5 mg of Bisbentiamine, add 1 mL of methanol, and dissolve by warming. Add 2 mL of water, 2 mL of a solution of L-cysteine hydrochloride monohydrate (1 in 100), and 1 mL of sodium hydroxide solution (1 in 25), shake, and allow to stand for 5 minutes. To the resulting solution, add 1 mL of a freshly prepared solution of potassium hexacyanoferrate(III) (1 in 10) and 5 mL of 2-methyl-1-propanol, shake vigorously for 2 minutes, and allow to stand. Examine under ultraviolet light. The 2-methyl-1-propanol layer emits a blue-purple fluorescence, which disappears when the solution is made acidic, and reappears when it is made alkaline.

Melting Point 140–145°C (decomposition).

Purity

(1) Clarity of solution Colorless and clear (0.10 g, methanol 20 mL).

(2) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

Loss on Drying Not more than 0.5% (24 hours).

Residue on Ignition Not more than 0.2%.

Assay Weigh accurately about 0.5 g of Bisbentiamine, previously dried, dissolve it in 50 mL of acetic acid, and titrate with 0.1 mol/L perchloric acid (indicator: 1 mL of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 38.55 mg of $C_{38}H_{42}N_8O_6S_2$

Black Currant Color

ブラックカーラント色素

Definition Black Currant Color is obtained from the fruits of the black currant *Ribes nigrum* L. and consists mainly of delphinidin-3-rutinoside. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Black Currant Color is not less than 40 and is in the range of 90–110% of the labeled value.

Description Black Currant Color occurs as a dark red powder, viscous paste, or liquid having a slight, characteristic odor.

Identification

(1) Weigh an amount of Black Currant Color equivalent to 1 g of black currant color with a Color Value 40, dissolve it in 100 mL of citrate buffer (pH 3.0). A red to red-purple color develops.

(2) Add sodium hydroxide solution (1 in 25) to the solution obtained in Identification (1) to make alkaline. The color changes to dark green.

(3) A solution of Black Currant Color in citrate buffer (pH 3.0) exhibits an absorption maximum at a wavelength of 510–520 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Sulfur dioxide Not more than 0.005% per a Color Value of 1.

Proceed as directed in Purity (3) for Grape Skin Extract.

Color Value Determination Proceed as directed under Color Value Determination,

using the conditions below.

Operating Conditions

Solvent: Citrate buffer (pH 3.0).

Wavelength: Maximum absorption wavelength of 510–520nm.

Bone Charcoal

骨炭

Definition Bone Charcoal is obtained by carbonizing and crushing the bones of cattle, *Bos Taurus* Linnaeus. It consists mainly of calcium phosphate and carbon powder.

Description Bone Charcoal occurs as a black powder or as granules. It is odorless and tasteless.

Identification

(1) Weigh about 0.1 g of Bone Charcoal, previously triturated for a granular sample, add 10 mL of 0.001% (w/v) methylene blue TS and 2 drops of diluted hydrochloric acid (1 in 4), shake well, and filter through a dry filter paper for quantitative analysis (5C). The solution obtained is colorless.

(2) Transfer about 0.5 g of Bone Charcoal, previously triturated for a granular sample, into a test tube, and heat by direct fire while supplying air from the tube mouth. The sample burns without a flame. When the generated gas is passed through calcium hydroxide TS, a white turbidity is produced.

(3) To 0.1 g of Bone Charcoal, previously incinerated, add 10 mL of diluted hydrochloric acid (1 in 7), and dissolve by warming. Add 2.5 mL of ammonia TS while shaking, and then add 5 mL of a solution of ammonium oxalate monohydrate (1 in 30). A white precipitate is formed.

(4) To 0.1 g of Bone Charcoal, previously incinerated, add 5 mL of 10% nitric acid TS, and dissolve by warming. Add 2 mL of ammonium molybdate TS. A yellow precipitate is formed.

Purity

Sample Preparation If the sample is in granular form, triturate it before weighing. If it is in powder form, use it as is. Weigh 4.0 g of Bone Charcoal, previously dried at 110–120°C for 3 hours, add 180 mL of water containing 0.1 mL of diluted nitric acid (1 in 100), and heat for about 10 minutes to maintain gentle boiling. After cooling, add 200 mL of water, and filter through a dry filter paper for quantitative analysis (5C). Discard the first 30 mL of the filtrate, and use the subsequent filtrate (Solution A) for the following tests.

(1) Chloride Not more than 0.53% as Cl.

Test Solution Use 1.0 mL of Solution A.

Control Solution　Use 0.30 mL of 0.01 mol/L hydrochloric acid.

(2) Sulfate　Not more than 0.48% as SO_4.

Test Solution　Use 2.5 mL of Solution A.

Control Solution　Use 0.50 mL of 0.005 mol/L sulfuric acid.

(3) Lead　Not more than 5 μg/g as Pb (0.80 g, Method 5, Control solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution　To the specified amount of Bone Charcoal, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge this solution to deposit the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container with 5 mL of hot water, and combine the washings with the filtrate.

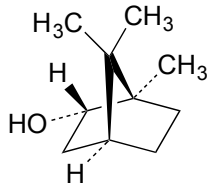
(4) Arsenic　Not more than 3 μg/g as As (Method 2, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

*Test Samp*le　Evaporate 25 mL of Solution A to dryness on a water bath. Use the residue.

d-Borneol

Borneol

d-ボルネオール



$C_{10}H_{18}O$　　Mol. Wt. 154.25

(*1R,2S,4R*)-1,7,7-Trimethylbicyclo [2.2.1] heptan-2-ol　[464-43-7]

Content　*d*-Borneol contains not less than 95.0% of *d*-borneol ($C_{10}H_{18}O$).

Description　*d*-Borneol occurs as white crystals, crystalline powder, or lumps having a Borneo camphor-like odor.

Identification

(1) Grind and mix *d*-Borneol with an equal amount of thymol. It liquefies.

(2) Place about 0.1g of *d*-Borneol in a test tube, heat the bottom of the test tube tilted about 45° in a colorless flame of Bunsen burner for 1 minute. A white sublimate is

produced on the upper place of the test tube.

Specific Rotation $[\alpha]_D^{20}$: +16.0 to +37.0° (2.5 g, ethanol (95), 25 mL).

Melting Point 205–210°C.

Assay Weigh accurately about 1 g of *d*-Borneol into a 200-mL flask with a stopper, and add exactly 5 mL of acetic anhydride–pyridine TS. Equip the flask with a reflux condenser, moisten the ground-glass joint with 2–3 drops of pyridine, and heat in a water bath for 3 hours. After cooling, Rinse the inside of flask by running 10 mL of water through the reflux condenser, and allow to cool to ordinary temperature. Add 10 mL of water, stopper tightly, and shake well. Rinse the joint part of the stopper and the inside of the flask with 5 mL of ethanol (neutralized), and titrate with 0.5 mol/L ethanolic potassium hydroxide (indicator: 10 drops of cresol-red-thymol blue TS). Perform a blank test in the same manner.

Each mL of 0.5 mol/L ethanolic potassium hydroxide = 77.12 mg of $C_{10}H_{18}O$

Bromelain

ブロメライン

Definition Bromelain is a proteolytic enzyme obtained from the fruit and rhizoma of the pineapple *Ananas comosus* (L.) Merr. It may contain lactose or dextrin.

Enzyme Activity Bromelain has an enzyme activity of not less than 500,000 units per gram.

Description Bromelain occurs as a white to light yellow-brown powder. It is odorless or has a slight characteristic odor.

Identification Bromelain presents activity when tested by Enzyme Activity Determination.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If during the preparation of the test solution, the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100), use Method 3 in the Lead Limit Test

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Cyanide Weigh 5.0 g of Bromelain, transfer into a distillation flask, add 2 g of L(+)-tartaric acid and 50 mL of water, and add 1 drop of silicone resin if necessary. Connect the flask with distillation apparatus that is joined to a receiver with a condenser containing 2 mL of sodium hydroxide TS (1 mol/L) and 10 mL of water. Distill until 25 mL of distillate is obtained, and add water to the distillate to make 50 mL. To 25 mL of this solution, add 0.5 mL of iron(II) sulfate TS, 0.5 mL of a solution of iron(III) hydrochloride hexahydrate (9 in 5000), and 1 mL of 10% sulfuric acid TS. No blue color

develops.

Microbial Limits Proceed as directed in the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Enzyme Activity Determination

(i) Test solution Dissolve 5.27 g of L-cysteine hydrochloride monohydrate, 2.23 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate and 23.4 g of sodium chloride in water. Adjust the pH to 4.5 with sodium hydroxide TS (1 mol/L), and add water to make 1000 mL. Use this solution as the diluent.

Weigh accurately about 0.1 g of Bromelain, transfer it into a mortar, add the diluent, and mix. Add the diluent to make exactly 100 mL. Centrifuge this solution if necessary. Dilute the supernatant liquid with the diluent to prepare a solution containing 30 to 50 units per mL.

(ii) Procedure Measure exactly 1 mL of the test solution, transfer into a test tube, and warm for 5 minutes at 37 ± 0.5°C. Add exactly 5 mL of casein TS (pH 7.0), previously warmed to 37 ± 0.5°C, shake immediately and react for exactly 10 minutes at 37 ± 0.5°C. Add exactly 5 mL of trichloroacetic acid TS, and shake. Allow to stand for 40 minutes at 37 ± 0.5°C, and filter through a filter paper for quantitative analysis (5C). Discard the first 3 mL of the filtrate, and measure the absorbance (A_T) of the subsequent filtrate at 275 nm, using water as the reference.

Separately, measure exactly 1 mL of the test solution, add exactly 5 mL of trichloroacetic acid TS, and shake well. Add exactly 5 mL of casein TS (pH 7.0), shake well, allow to stand for 40 minutes at 37 ± 0.5°C. Measure the absorbance (A_0) of this solution, proceeding in the same manner as for the measurement of absorbance A.

Separately, measure the absorbance (A_S and A_{S0}) of Tyrosine Standard Solution and hydrochloric acid TS (0.1 mol/L), respectively, at 275 nm, using water as the reference.

Calculate the enzyme activity by the formula below. One unit of the enzyme activity is the quantity of enzyme that produces amino acids equivalent to 1 μg of tyrosine per minute when the test is performed as directed in the Procedure.

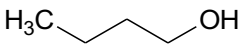
$$\text{The enzyme activity of Bromelain (units/g)} = \frac{(A_T - A_0) \times 50}{A_S - A_{S0}} \times \frac{11}{10} \times \frac{1000}{M}$$

M = weight (mg) of Bromelain in 1 mL of the test solution.

Butanol

Butan-1-ol
Butyl Alcohol

ブタノール



$C_4H_{10}O$ Mol. Wt. 74.12

Butan-1-ol [71-36-3]

Content Butanol contains not less than 99.5% of butanol ($C_4H_{10}O$).

Description Butanol is a colorless, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Butanol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.393–1.404.

Specific Gravity d_{25}^{25}: 0.807–0.809.

Purity

(1) Acid value Not more than 2.0 (Flavoring Substances Tests).

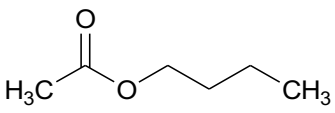
(2) Dibutyl ether Not more than 0.15%.

Perform the test by gas chromatography, according to the direction in the Assay given below. The peak area of dibutyl ether is not more than 0.15% of the total peak area of all peaks. Use operating conditions that can provide complete resolution of the peaks of butanol and dibutyl ether when 1 μL of a solution (3 in 2000) of dibutyl ether in 1-butanol is chromatographed.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests, using operating conditions (2).

Butyl Acetate

酢酸ブチル



$C_6H_{12}O_2$ Mol. Wt. 116.16

Butyl acetate [123-86-4]

Content Butyl Acetate contains not less than 98.0% of butyl acetate ($C_6H_{12}O_2$).

Description Butyl Acetate is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Butyl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.393–1.396.

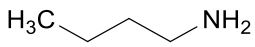
Specific Gravity d_{25}^{25}: 0.877–0.881.

Purity Acid Value Not more than 2.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (2).

Butylamine

ブチルアミン



$C_4H_{11}N$ Mol. Wt. 73.14

Butylamine [109-73-9]

Content Butylamine contains not less than 99.0% of butylamine ($C_4H_{11}N$).

Description Butylamine occurs as a colorless to yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Butylamine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

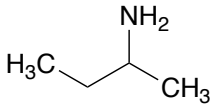
Refractive Index n_D^{20}: 1.398–1.404.

Specific Gravity d_{25}^{25}: 0.732–0.740.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2), except for the column. Use a fused silica tube (0.25–0.53 mm internal diameter and 30–60 m length) coated with a 0.25–1 μm thick layer of dimethyl polysiloxane.

sec-Butylamine

sec-ブチルアミン



$C_4H_{11}N$ Mol. Wt. 73.14

Butan-2-amine [13952-84-6]

Content *sec*-Butylamine contains not less than 95.0% of *sec*-butylamine ($C_4H_{11}N$).

Description *sec*-Butylamine occurs as a colorless to yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of *sec*-Butylamine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive index n_D^{20}: 1.387–1.396

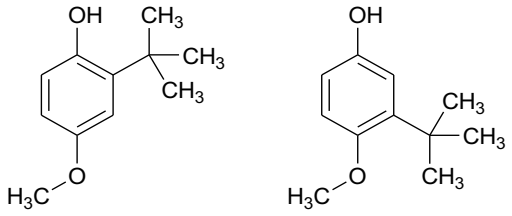
Specific gravity d_{25}^{25}: 0.715–0.724

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operating conditions (2) except for the column. Use a fused silica tube (0.25–0.53 mm internal diameter and 30–60 m length) coated with a 0.25–1 μm thick layer of dimethylpolysiloxane for gas chromatography.

Butylated Hydroxyanisole

BHA

ブチルヒドロキシアニソール



$C_{11}H_{16}O_2$ Mol. Wt. 180.24

Mixture of 2-(1,1-dimethylethyl)-4-methoxyphenol and 3-(1,1-dimethylethyl)-4-

methoxyphenol [25013-16-5]

Description Butylated Hydroxyanisole occurs as colorless or slightly yellow-brown crystals or lumps, or as a white crystalline powder. It has a slight characteristic odor.

Identification

(1) To 2–3 mL of a solution (1 in 100) of Butylated Hydroxyanisole in ethanol (95), add 2–3 drops of a solution of sodium tetraborate decahydrate (1 in 50) and crystals of 2,6-dichloroquinonechloroimide, and shake. A purple-blue color develops.

(2) Proceed as directed in Identification (2) for Butylated Hydroxytoluene.

Melting Point 57–65°C.

Purity

(1) Clarity of solution Colorless and clear (0.50 g, ethanol (95) 10 mL).

(2) Sulfate Not more than 0.019% as SO_4.

Test Solution Weigh 0.50 g of Butylated Hydroxyanisole, dissolve it in 35 mL of acetone, and add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

Control Solution To 0.20 mL of 0.005 mo/L sulfuric acid, add 35 mL of acetone, 1 mL of diluted hydrochloric acid (1 in 4), and water to make 50 mL.

(3) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

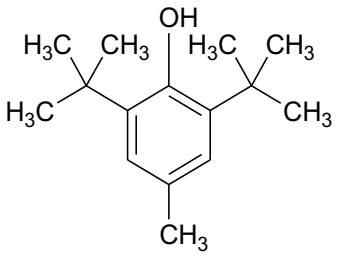
(5) *p*-Hydroxyanisole Weigh 1.0 g of Butylated Hydroxyanisole, dissolve it in 20 mL of a 1:1 mixture of diethyl ether/petroleum benzine, add 10 mL of water and 1 mL of sodium hydroxide solution (1 in 25), shake well, allow to stand, and collect the lower layer. To this solution, add 20 mL of a 1:1 mixture of diethyl ether/petroleum benzine, shake well, allow to stand, collect the lower layer, and add water to make 500 mL. Transfer 1.0 mL of this solution into a Nessler tube, and add 2 mL of sodium hydroxide solution (1 in 25), 5 mL of boric acid solution (3 in 100), and water to make 30 mL. Add 5 mL of 4-amino-antipyrine solution (1 in 1000), shake, add 1 mL of potassium hexacyanoferrate(III) solution (1 in 100), shake again, add water to make 50 mL, and allow to stand for 15 minutes. The color of the solution is not darker than that of a solution prepared by diluting 0.6 mL of Cobalt(II) Chloride CSSS to 50 mL with water.

Residue on Ignition Not more than 0.05%.

Butylated Hydroxytoluene

BHT

ジブチルヒドロキシトルエン



$C_{15}H_{24}O$ Mol. Wt. 220.35

2,6-Bis(1,1-dimethylethyl)-4-methylphenol [128-37-0]

Description Butylated Hydroxytoluene occurs as colorless crystals, as a white crystalline powder, or as white lumps. It is odorless or has a slight characteristic odor.

Identification

(1) To 5 mg of Butylated Hydroxytoluene, add 1–2 drops of a solution (1 in 100) of 5-nitroso-8-hydroxyquinoline in sulfuric acid. It dissolves, producing a yellow color, which changes to red-brown.

(2) To 1 mL of a solution (1 in 30) of Butylated Hydroxytoluene in ethanol (95), add 3–4 drops of a solution of iron(III) chloride hexahydrate (1 in 500). No color develops. To this solution, add 2,2′-dipyridyl crystals. A red color develops. Before the test, perform a blank test for a solution of iron(III) chloride hexahydrate to confirm that no color develops.

Melting Point 69–72°C.

Purity

(1) Clarity of solution Colorless and clear (1.0 g, ethanol (95) 10 mL).

(2) Sulfate Not more than 0.019% as SO_4.

Test Solution Weigh 0.50 g of Butylated Hydroxytoluene, add 30 mL of water, heat in a water bath for 5 minutes with occasional shaking, cool, and filter.

Control Solution Use 0.20 mL of 0.005 mol/L sulfuric acid.

(3) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) *p*-Cresol Not more than 0.10% as *p*-cresol.

Sample Solution Weigh 1.0 g of Butylated Hydroxytoluene, add 10 mL of water and

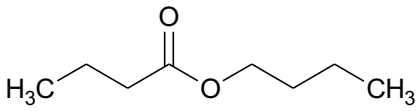
1 mL of ammonia solution (28), heat in a water bath for 3 minutes with occasional shaking. Cool, and filter. Wash the residue on the filter paper with a small amount of water, combine the washings with the filtrate, and add water to make 100 mL.

Procedure Measure 3.0 mL of the sample solution, transfer into a Nessler tube, add 1 mL of a solution (1 in 20) of phosphomolybdic acid *n*-hydrate in ethanol (95) and 0.2 mL of ammonia TS, and shake. Add water to make 50 mL, and allow to stand for 10 minutes. The color of the solution is not darker than that of the solution prepared in the same manner as the test solution, using 3.0 mL of *p*-cresol solution (1 in 100,000).

Residue on Ignition Not more than 0.05%

Butyl Butyrate

酪酸ブチル



$C_8H_{16}O_2$ Mol. Wt. 144.21

Butyl butanoate [109-21-7]

Content Butyl Butyrate contains not less than 98.0% of butyl butyrate ($C_8H_{16}O_2$).

Description Butyl Butyrate is a colorless to light yellow, clear liquid having a fruity odor.

Identification Determine the absorption spectrum of Butyl Butyrate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.405–1.407.

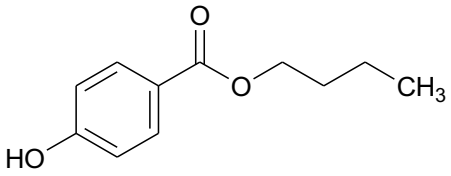
Specific Gravity d_{25}^{25}: 0.867–0.871.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Butyl *p*-Hydroxybenzoate

パラオキシ安息香酸ブチル



$C_{11}H_{14}O_3$ Mol. Wt. 194.23

Butyl 4-hydroxybenzoate [94-26-8]

Content Butyl *p*-Hydroxybenzoate, when dried, contains not less than 99.0% of butyl *p*-hydroxybenzoate ($C_{11}H_{14}O_3$).

Description Butyl *p*-Hydroxybenzoate occurs as colorless crystals or as a white crystalline powder. It is odorless.

Identification

(1) Proceed as directed in Identification for Isobutyl *p*-Hydroxybenzoate.

(2) To 50 mg of Isobutyl *p*-Hydroxybenzoate, add 2 drops of acetic acid and 5 drops of sulfuric acid, and warm for 5 minutes. An odor of butyl acetate is evolved.

Melting Point 69–72°C.

Purity

(1) Free acid Not more than 0.55% as *p*-hydroxybenzoic acid.

Proceed as directed in Purity (1) for Isobutyl *p*-Hydroxybenzoate.

(2) Sulfate Not more than 0.024% as SO_4.

Proceed as directed in Purity (2) for Isobutyl *p*-Hydroxybenzoate.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.5% (5 hours).

Residue on Ignition Not more than 0.1% .

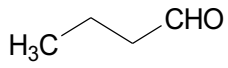
Assay Proceed as directed in Assay for Isobutyl *p*-Hydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide = 194.2 mg of $C_{11}H_{14}O_3$

Butyraldehyde

Butanal

ブチルアルデヒド



C_4H_8O Mol. Wt. 72.11

Butanal [123-72-8]

Content Butyraldehyde contains not less than 98.0% of butyraldehyde (C_4H_8O).

Description Butyraldehyde is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Butyraldehyde, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.377–1.387.

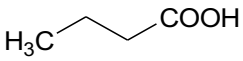
Specific Gravity d_{25}^{25}: 0.797–0.802.

Purity Acid value Not more than 5.0 (Flavoring Substance Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (3).

Butyric Acid

酪酸



$C_4H_8O_2$ Mol. Wt. 88.11

Butanoic acid [107-92-6]

Content Butyric Acid contains not less than 99.0% of butyric acid ($C_4H_8O_2$).

Description Butyric Acid is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Butyric Acid as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.397–1.399.

Specific Gravity d_{25}^{25}: 0.954–0.958.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Cacao Color

カカオ色素

Definition Cacao Color is obtained from the seeds of the cacao tree *Theobroma cacao* L. by fermentation, roasting, extraction with an alkaline solution, and neutralization. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Cacao Color is not less than 50 and is in the range of 90–120% of the labeled value.

Description Cacao Color occurs as a red-brown to black powder, as lumps, or as a paste or liquid. It has a slight, characteristic odor.

Identification

(1) Weigh an amount of Cacao Color equivalent to 0.2 g of cacao color with a Color Value 50, and dissolve it in 100 mL of citrate buffer (pH 7.0). The resulting solution is brown.

(2) Weigh an amount of Cacao Color equivalent to 0.4 g of cacao color with a Color Value 50, and dissolve it in 100 mL of water. To 5 mL of this solution, add 2–3 drops of hydrochloric acid, and allow to stand. A brown to dark brown precipitate is produced.

(3) To 5 mL of the solution prepared in Identification (2), add 2–3 drops of iron(III) chloride hexahydrate solution (1 in 10). The solution immediately turns to dark brown. Then, allow to stand for 30 minutes, and centrifuge at 3000 rpm for 10 minutes. A dark brown precipitate is produced.

(4) Weigh an amount of Cacao Color equivalent to 0.4 g of cacao color with a Color Value 50, and dissolve it in 100 mL of sodium hydroxide solution (1 in 250). To 5 mL of this solution, add 10 mL of diluted hydrochloric acid (9 in 1000) and 0.1 mL of zinc chloride TS (pH 3.0), and stir. Stopper it, and warm at 50°C for 20 minutes. Centrifuge at 3000 rpm for 10 minutes. A yellow-brown to dark brown precipitate is produced.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Mercury Not more than 1.0 μg/g as Hg.

Test Solution To 0.50 g of Cocoa Color, add 10 mL of nitric acid, 5 mL of sulfuric acid, and 2.5 mL of perchloric acid, and heat gently under a reflux condenser to decompose it until a light yellow color is produced. After cooling, add water to make exactly 100 mL.

Control Solution To 5 mL of Mercury Standard Solution, exactly measured, add 10 mL of diluted sulfuric acid (1 in 2), and dilute with water to make exactly 100 mL.

Procedure Add 5 mL of tin(II) chloride–sulfuric acid TS to each of the test solution and the control solution, and analyze as directed under Cold-vapor Atomic Absorption Spectrophotometry (reduction vaporing method) using the operating conditions given below. The absorbance value of the test solution is not higher than that of the control solution.

Operating Conditions

Light Source: Mercury hollow cathode lamp.

Wavelength: 253.7 nm.

Carrier gas: Air.

(4) Acetone Not more than 30 μg/g (in terms of a Color Value 50).

Test Solution Weigh an amount of Cacao Color equivalent to 1.00 g of cacao color with a Color Value 50, place it into a 10-mL volumetric flask, and add water to dissolve. Add exactly 2 mL of the internal standard solution, and dilute with water to make 10 mL. Refer to this solution as the sample solution. Prepare a graphite carbon cartridge (500 mg) by pouring 4 mL of ethanol and 10 mL of water into it, and discarding the effluent. Pour exactly 1 mL of the sample solution, and collect the effluent into a 5-mL of volumetric flask. Pour water into the cartridge until the total volume of effluent reaches 5 mL at a flow rate that does not allow cacao color to elute. Use the effluent obtained as the test solution.

Control Solution To 0.15 g of acetone, add water to make exactly 100 mL. To 1 mL of this solution, exactly measured, add water to make exactly 100 mL. Then, to 2 mL of this solution, exactly measured, add exactly 2 mL of the internal standard solution, and add water to make exactly 50 mL.

Internal Standard Solution To 2.5 g of ethanol (99.5), add water to make 100 mL. To 1 mL of this solution, add water to make 100 mL.

Procedure Analyze 10 μL each of the test solution and the control solution by gas chromatography using the operating conditions given below. The peak area ratio of acetone to ethanol of the test solution does not exceed that for the control solution.

Operating Conditions

Detector: Flame ionization detector.

Column: A glass or stainless steel tube (3–4 mm internal diameter and 2–3 m length).

Column packing material: 180–250 µm of styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Injection port temperature: About 200°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the retention time of acetone to 9–11minutes.

Color Value Determination Proceed as directed under Color Value Determination, using the operating conditions given below.

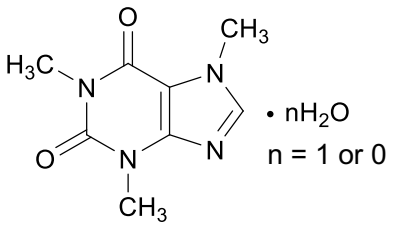
Operating Conditions

Solvent: Citrate buffer (pH 7.0).

Wavelength: 500 nm.

Caffeine (Extract)

カフェイン(抽出物)



$C_8H_{10}N_4O_2 \cdot nH_2O$ (n = 1 or 0) Mol. Wt. monohydrate 212.21

anhydrous 194.19

1,3,7-Trimethyl-1*H*-purine-2,6 (3*H*,7*H*)-dione monohydrate [5743-12-4]

1,3,7-Trimethyl-1*H*-purine-2,6 (3*H*,7*H*)-dione [58-08-2]

Definition Caffeine (Extract) is obtained from the seeds of plants from the genus *Coffea* or leaves of the tea tree *Camellia sinensis* (L.) Kuntze. It consists mainly of caffeine.

Content Caffeine (Extract), when dried, contains not less than 98.5% of caffeine ($C_8H_{10}N_4O_2$).

Description Caffeine (Extract) occurs as white acicular crystals or powder.

Identification

(1) To 2 mL of a solution of Caffeine (Extract) (1 in 500), add a few drops of tannic acid TS. A white precipitate is produced that dissolves by the addition of drops of tannic acid TS.

(2) To 10 mg of Caffeine (Extract), add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, and evaporate on a water bath to dryness. The residue is yellow-red. The residue turns red-purple when put over a flask containing 2–3 drops of ammonia TS, and the color produced disappears when 2–3 drops of sodium hydroxide TS (1 mol/L) are added.

(3) Dissolve 10 mg of Caffeine (Extract) in water to make 50 mL. To 5 mL of this solution, add 3 mL of diluted acetic acid (1 in 100) and 5 mL of diluted pyridine (1 in 10), and mix. To the resulting solution, add 2 mL of sodium hypochlorite TS (1 in 2), and allow to stand for 1 minute. Then, add 2 mL of sodium thiosulfate TS (0.1 mol/L) and 5 mL of sodium hydroxide TS (1 mol/L). A yellow color is produced.

Melting Point 235–238°C (after drying).

Purity

(1) Chloride Not more than 0.01% as Cl.

Test Solution Dissolve 2.0g of Caffeine (Extract) in 80 mL of hot water, immediately cool to 20°C, and add water to make 100 mL. Refer to the resulting solution as the sample solution. To 40 mL of the sample solution, add 6 mL of diluted nitric acid (1 in 10) and water to make 50 mL.

Control Solution Use 0.25 mL of 0.01 mol/L hydrochloric acid.

(2) Sulfate Not more than 0.024% as SO_4

Test Solution To 40 mL of the sample solution prepared in Purity (1), add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

Control Solution Use 0.40 mL of 0.005 mol/L sulfuric acid.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Related substances

Test Solution Dissolve 0.10 g of Caffeine (Extract) in 10 mL of a 1:1 mixture of toluene/ethanol (99.5).

Control Solution Measure exactly 1 mL of the test solution, and add a 1:1 mixture of toluene/ethanol (99.5) to make exactly 10 mL. To exactly 1 mL of this solution, add a 1:1 mixture of toluene/ethanol (99.5) to make exactly 10 mL. To exactly 1 mL of the second solution, add a 1:1 mixture of toluene/ethanol (99.5) to make exactly 10 mL.

Procedure Analyze a 10-μL portion each of the test solution and the control solution by thin-layer chromatography using a 7:3 mixture of toluene/ethanol (99.5) as the

developing solvent. Use a thin-layer plate coated with fluorescent silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. Examine under ultraviolet light (around 254 nm). Any single spot, other than the main spot, from the test solution is not more intense than spot from the control solution.

(6) Readily Carbonizable Substances Use 0.50 g of Caffeine (Extract) as the sample and perform the test using Matching Fluid D.

Loss on Drying Not more than 8.5% (1 g, 80°C, 4 hours).

Residue on Ignition Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.4 g of Caffeine (Extract), previously dried, and dissolve it in 70 mL of a 6:1 mixture of acetic anhydride/acetic acid, and titrate with 0.1 mol/L perchloric acid (indicator: 3 drops of a solution (1 in 100) of crystal violet in acetic acid). The endpoint is when the color of the solution changes from purple through green to yellow. Perform a blank test in the same manner to make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 19.42 mg of $C_8H_{10}N_4O_2$

Calcinated Bone Calcium

骨焼成カルシウム

Definition Calcinated Bone Calcium is obtained by calcinating animal and fish bones. It consists mainly of calcium phosphate.

Content Calcinated Bone Calcium, when dried, contains the equivalent of not less than 95.0–105.0% of tricalcium phosphate ($Ca_3(PO_4)_2$ = 310.18).

Description Calcinated Bone Calcium occurs as a white to grayish-white powder.

Identification

(1) Add 5 mL of 10% nitric acid TS to 0.1 g of Calcinated Bone Calcium, dissolve it by warming, and add 2 mL of ammonium molybdate TS. A yellow precipitate is produced.

(2) To 0.1 g of Calcinated Bone Calcium, add 5 mL of diluted acetic acid (1 in 4), and boil. After cooling, filter it. To the filtrate, add 5 mL of a solution of ammonium oxalate monohydrate (1 in 30). A white precipitate is produced.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 0.50%.

To 5.0 g of Calcinated Bone Calcium, add 100 mL of water, and then add hydrochloric acid dropwise while shaking until the sample no longer dissolves. Boil this solution for 5 minutes, cool, and filter through a filter paper for quantitative analysis (5C). Thoroughly wash the residue on the filter with water until the washings are free from chloride. Ignite

the residue with the filter paper in a crucible, and weigh the residue.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcinated Bone Calcium, add 20 mL of diluted hydrochloric acid (1 in 4), cover with a watch glass, and boil gently for 15 minutes. After cooling, add 30 mL of water. Use the resulting solution as the sample solution. If the sample does not dissolve, evaporate to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Cover with a watch glass, boil gently for 5 minutes, cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 2.0% (200°C, 3 hours).

Assay Weigh accurately about 0.3 g of Calcinated Bone Calcium, previously dried, add 10 mL of diluted hydrochloric acid (1 in 4) to dissolve, and add water to make exactly 200 mL. Use this solution as the test solution. Proceed as directed in Method 2 under Calcium Salt Determination.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.068 mg of $Ca_3(PO_4)_2$

Calcinated Eggshell Calcium

卵殻焼成カルシウム

Definition Calcinated Eggshell Calcium* is obtained by calcinating eggshells. It consists mainly of calcium oxide.

Contain Calcinated Eggshell Calcium, when ignited, contains the equivalent of not less than 95.0% of calcium oxide (CaO = 56.08).

Description Calcinated Eggshell Calcium occurs as a white to grayish-white powder.

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* Calcinated Eggshell Calcium is one of the substances belonging to the "Calcinated Calcium" category. "Calcinated Calcium" is defined in the List of Existing Food Additives as a substance that is obtained by calcinating sea urchin shells, shells, reef corals, whey, bones, or eggshells and that consists mainly of calcium.

Identification

(1) Moisten about 1 g of Calcinated Eggshell Calcium with water. It generates heat. Then add about 5 mL of water. The resulting suspension is alkali.

(2) To 1 g of Calcinated Eggshell Calcium, add 20 mL of water and 10 mL of acetic acid (1 in 3) to dissolve, and neutralize with ammonia TS. The resulting solution responds to the tests for Calcium Salt as described in the Quantitative Tests.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 0.50%.

To 5.0 g of Calcinated Eggshell Calcium, add 100 mL of water, and then add hydrochloric acid dropwise while shaking until the sample no longer dissolves. Boil for 5 minutes, cool, and filter through a filter paper for quantitative analysis (5C). Wash the residue well on the filter with hot water until the washings are free from chloride. Heat the residue with the filter paper gradually to carbonize, ignite the residue at 450–550°C for 3 hours, and weigh the residue.

(2) Carbonate To 2.0 g of Calcinated Eggshell Calcium, add 50 mL of water, shake well, and add 25 mL of hydrochloric acid (1 in 4). It does not bubble vigorously.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcinated Eggshell Calcium, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, add 30 mL of water, use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate this solution to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes with a watch glass covering it, allow to cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Calcinated Eggshell Calcium in 5 mL of diluted hydrochloric acid (1 in 4).

Residue on Ignition Not less than 10.0% (900°C, 30 minutes).

Assay Weigh accurately about 1.5 g of Calcinated Eggshell Calcium, previously ignited, dissolve it in 30 mL of diluted hydrochloric acid (1 in 4), and add water to make exactly 250 mL. Using this solution as the test solution, proceed as directed in Method 1 for Calcium Salt Determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.804 mg of CaO

Calcinated Shell Calcium

貝殻焼成カルシウム

Definition Calcinated Shell Calcium* is obtained by calcinating shells. It consists mainly of calcium oxide.

Contain Calcinated Shell Calcium, when ignited, contains the equivalent of not less than 91.0% of calcium oxide (CaO = 56.08).

Description Calcinated Shell Calcium occurs as white to grayish-white lumps, granules, or powder.

Identification

(1) Suspend 1 g of Calcinated Shell Calcium in 5 mL of water. The resulting liquid is alkali.

(2) To 1 g of Calcinated Shell Calcium, add 20 mL of water and 10 mL of acetic acid (1 in 3) to dissolve, and neutralize with ammonia TS. The resulting solution responds to the tests for Calcium Salt as described in the Quantitative Tests.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 0.50%.

Weigh 5.0 g of Calcinated Shell Calcium, add 100 mL of water, and add hydrochloric acid dropwise while shaking until the sample no longer dissolves. Boil for 5 minutes, cool, and filter through a filter paper for quantitative analysis (No. 5C). Wash the residue well on the filter with hot water until the washings are free of chloride. Heat the residue with the filter paper gradually to carbonize, and ignite the residue at 450–550°C for 3 hours. Weigh the residue.

(2) Carbonate To 1.0 g of Calcinated Shell Calcium, add a little volume of water, crush it, mix well with 50 mL of water, and allow to stand. Decant off the supernatant milky liquid, and add an excess amount of diluted hydrochloric acid (1 in 4) to the residue. It does not bubble vigorously.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcinated Shell Calcium, add 20 mL of diluted hydrochloric acid (1 in 4), treat it ultrasonically, and evaporate on a water bath to dryness. Dissolve the residue by adding 20 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

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* Calcinated Shell Calcium is one of the substances belonging to the "Calcinated Calcium" category. For the definition of Calcinated Calcium, see the footnote for the Calcinated Eggshell Calcium.

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution　Dissolve the specified amount of Calcinated Shell Calcium in 5 mL of diluted hydrochloric acid (1 in 4).

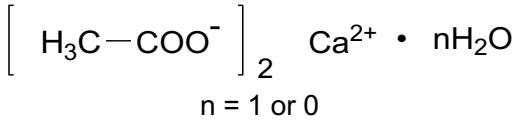
Loss on Ignition　Not less than 10.0% (900ºC, 30 minutes).

Assay Weigh accurately about 1.5 g Calcinated Shell Calcium, previously ignited, add 30 mL of diluted of hydrochloric acid (1 in 4), and dissolve by heating. After cooling, add water to make exactly 250 mL. Using this solution as the test solution, proceed as directed in Method 1 for Calcium Salt Determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.804 mg of CaO

Calcium Acetate

酢酸カルシウム



$C_4H_6CaO_4 \cdot nH_2O$ (n = 1 or 0)　　Mol. Wt. 176.18 (monohydrate)
158.17 (anhydrous)

Calcium acetate monohydrate　[5743-26-0]

Calcium acetate　[62-54-4]

Content Calcium Acetate, when dried, contains not less than 98.0% of calcium acetate ($C_4H_6CaO_4$).

Description Calcium Acetate occurs as white crystals, powder, or granules. It has a slight odor of acetic acid.

Identification　Calcium Acetate responds to all the tests for Calcium Salt and Acetate in the Qualitative Tests.

pH 6.0–9.0 (2.0 g, water 20 mL).

Purity

(1) Water-insoluble substances　Not more than 0.30 %.

Weigh accurately a crucible-type glass filter (1G4), previously dried at 105ºC for 30 minutes and cooled in a desiccators. Weigh accurately about 10 g of Calcium Acetate, add 100 mL of warm water, and agitate. Filter it through the glass filter, and wash the residue on the filter with 30 mL of water. Dry the filter containing the residue at 105 ºC

for 2 hours, cool in desiccators, and weigh the filter accurately.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Acetate, add 20 mL of diluted hydrochloric acid (1 in 4), dissolve it by ultrasonic agitation, and evaporate to dryness. Add 20 mL of water to the residue.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Readily oxidizable substances Not more than 1000 μg/g as HCOOH.

Weigh accurately about 5 g of Calcium Acetate, add 100 mL of water to dissolve it, then add 0.5 g of sodium carbonate, and shake. Add exactly 10 mL of 0.02 mol/L potassium permanganate, shake, and heat on a water bath for 15 minutes. After cooling, add 25 mL of diluted sulfuric acid (9 in 100) and 0.3 g of potassium iodide, shake well, and titrate with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears.

Separately, perform a blank test. Calculate the content of readily oxidizable substances (HCOOH) by the formula:

$$\text{Content (μg/g) of readily oxidizable substances} = \frac{(a - b) \times 2301}{\text{Weight (g) of the sample}}$$

a = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the blank test,

b = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the test.

Loss on Drying Not more than 11.0% (200°C, 4 hours)

Assay Weigh accurately about 4 g of Calcium Acetate, previously dried, add 30 mL of diluted hydrochloric acid (1 in 4) to dissolve it, add water to make exactly 250 mL. Using this solution as the test solution, proceed as directed in Method 1 under Calcium Salt Determination.

Each mL of 0.05mol/L disodium dihydrogen ethylenediaminetetraacete = 7.908 mg of $C_4H_6CaO_4$

Calcium Alginate

アルギン酸カルシウム

Calcium alginate [9005-35-0]

Content Calcium Alginate, when calculated on the dried basis, contains 89.6–104.5% of calcium alginate.

Description Calcium Alginate occurs in white to yellowish-white, filamentous, granular, or powdered form.

Identification

(1) To 0.25 g of Calcium Alginate, add 50 mL of a solution of sodium carbonate decahydrate (1 in 400) while stirring. Warm the mixture at 60–70°C for 20 minutes with occasional shaking to make it homogenous, and cool. Use this solution as the test solution. Proceed as directed in Identification (1) for Ammonium Alginate.

(2) Ignite 1 g of Calcium Alginate at 550–600°C for 3 hours. To the residue, add 10 mL of water and 5 mL of acetic acid (1 in 3) to dissolve, and filter if necessary. Boil it, cool, and neutralize with ammonia TS. The solution obtained responds to all the tests for Calcium Salt described in the Qualitative Tests.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Alginate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, and add 30 mL of water. Use this solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes with a watch glass covering it, allow to cool, and add 30 mL of water. Use this solution as the sample solution.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 15.0% (105°C for 4 hours).

Microbial Limit Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Coliforms: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 1 for total plate count and the enumeration of yeasts and molds.

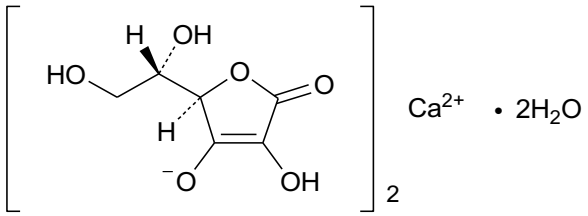
Pre-enrichment Culture Prepare as directed in Method 1, respectively, for the coliform test and the *Salmonella* test.

Assay Proceed as directed in the Assay for Alginic Acid.

Each mL of 0.25 mol/L sodium hydroxide = 27.38 mg of calcium alginate

Calcium L-Ascorbate

L-アスコルビン酸カルシウム



$C_{12}H_{14}CaO_{12}\cdot 2H_2O$ Mol. Wt. 426.34

Monocalcium bis{(2*R*)-2-[(1*S*)-1,2-dihydroxyethyl]-4-hydroxy-5-oxo-2,5-dihydrofuran-3-olate} dihydrate [5743-28-2]

Content Calcium L-Ascorbate contains not less than 98.0% of calcium L-ascorbate ($C_{12}H_{14}CaO_{12}\cdot 2H_2O$).

Description Calcium L-Ascorbate occurs as a white to yellowish-white crystalline powder. It has no or faint odor.

Identification

(1) To 10 mL of a solution (1 in 100) of Calcium L-Ascorbate, add 1 to 2 drops of 2,6-dichloroindophenol sodium salt TS. The solution turns blue and disappears at once.

(2) A solution (1 in 10) of Calcium L-Ascorbate responds to all the tests for Calcium Salts in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{25}$: +95 to +97° (1 g, newly boiled and cooled water 20 mL).

pH 6.0–7.5 (2.0 g, water 20 mL)

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Fluoride Not more than 10.0 μg/g as F.

Test Solution Weigh 1.00 g of Calcium L-Ascorbate into a beaker, and dissolve it in 10 mL of water. Add gradually 20 mL of diluted hydrochloric acid (1 in 10), and boil for 1 minute. Transfer the solution to a polyethylene beaker, and immediately cool by ice. Then add 10 mL of a solution (1 in 40) of disodium dihydrogen

ethylenediaminetetraacetate dihydrate and 15 mL of a solution of trisodium citrate dihydrate (1 in 4), and mix. Adjust the pH to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5), and transfer the solution to a 100-mL volumetric flask, add water to volume. Transfer 50 mL of this solution to a polyethylene beaker.

Control Solution Weigh 2.210 g of sodium fluoride, previously dried at 110°C for 2 hours, into a polyethylene beaker, and dissolve it in 200 mL of water with stirring. Transfer the solution to a 1000-mL polyethylene volumetric flask, and add water to volume. Use this solution as the control stock solution. Store the stock solution in a polyethylene bottle. Prepare the control solution fresh before use. Place 1 mL of the control stock solution, measured exactly, into a 100-mL polyethylene volumetric flask, and add water to volume. Place 1 mL of this solution, exactly measured, into a polyethylene beaker, add 10 mL of a solution (1 in 40) of disodium dihydrogen ethylenediaminetetraacetate dihydrate and 15 mL of a solution of trisodium citrate dihydrate (1 in 4), and mix. Adjust the pH to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5), and transfer the solution to a 100-mL volumetric flask, add water to volume. Transfer 50 mL of the resultant solution to a polyethylene beaker.

Procedure Measure the electrical potential using a potentiometer connected to a fluoride ion indicator electrode and a silver-silver chloride reference electrode. The potential of the test solution is not less than that of the control solution.

Assay Dissolve 0.2 g of Calcium L-Ascorbate, accurately weighed, in 50 mL of metaphosphoric acid solution (1 in 50), and titrate with 0.05 mol/L iodine using 1 mL of starch TS as an indicator.

Each mL of 0.05 mol/L iodine = 10.66 mg of $C_{12}H_{14}CaO_{12}\cdot 2H_2O$

Calcium Carbonate I

炭酸カルシウム

$CaCO_3$ Mol. Wt. 100.09

Calcium carbonate [471-34-1]

Content Calcium Carbonate, when dried, contains 98.0–102.0% of calcium carbonate ($CaCO_3$).

Description Calcium Carbonate occurs as a fine white powder. It is odorless.

Identification To 1 g of Calcium Carbonate, add 10 mL of water and 7 mL of diluted acetic acid (1 in 4). It effervesces and dissolves. When boiled and neutralized with ammonia TS, this solution responds to all the tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 0.20%.

Weigh 5.0 g of Calcium Carbonate, add 10 mL of water, then gradually add 12 mL of hydrochloric acid dropwise while stirring, and add water to make 200 mL. Filter through a filter paper for quantitative analysis (5C), and wash the residue on the filter paper with boiling water until the washings are free of chloride. Heat gradually the residue together with the filter paper to carbonization, incinerate at 450–550°C for 3 hours or more, and weigh.

(2) Free alkali Weigh 3.0 g of Calcium Carbonate, add 30 mL of freshly boiled and cooled water, shake for 3 minutes, and filter the solution. To 20 mL of the filtrate, add 2 drops of phenolphthalein TS. A pink color develops, and it disappears when 0.20 mL of 0.1 mol/L hydrochloric acid is added.

(3) Lead Not more than 3 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 6.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Carbonate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, add 30 mL of water, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes with a watch glass covering it, allow to cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(4) Alkali metals and magnesium Not more than 1.0%.

Weigh 1.0 g of Calcium Carbonate, dissolve it by gradually adding 30 mL of diluted hydrochloric acid (1 in 10), and let the carbon dioxide out by boiling. Cool, neutralize with ammonia TS, add 60 mL of a solution of ammonium oxalate monohydrate (1 in 25), and heat on a water bath for 1 hour. After cooling, add water to make 100 mL, stir thoroughly, and filter. Measure 50 mL of the filtrate, add 0.5 mL of sulfuric acid, evaporate to dryness, ignite to constant weight at 600°C, and weigh.

(5) Barium Not more than 0.030% as Ba.

Test Solution Weigh 1.0 g of Calcium Carbonate, dissolve it in 8 mL of diluted hydrochloric acid (1 in 4), and add water to make 20 mL.

Procedure Add 2 g of sodium acetate trihydrate, 1 mL of diluted acetic acid (1 in 20), and 0.5 mL of potassium chromate solution (1 in 20) to the test solution, and allow to stand for 15 minutes. The solution is not more turbid than a control solution prepared as follows: To 0.30 mL of Barium Standard Solution, add water to make 20 mL, and then treat in the same manner as the test solution.

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of Calcium Carbonate, moisten with 1 mL of water, and dissolve it in 4 mL of hydrochloric acid (1 in 4).

Loss on Drying Not more than 2.0% (200°C, 4 hours).

Assay Weigh accurately about 1 g of Calcium Carbonate, previously dried. Add it gradually to 10 mL of diluted hydrochloric acid (1 in 4) to dissolve, and add water to make exactly 100 mL. Use this solution as the test solution. Proceed as directed in Method 1 under Calcium Salt Determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraactate = 5.004 mg of $CaCO_3$

Calcium Carboxymethylcellulose

Calcium Cellulose Glycolate

カルボキシメチルセルロースカルシウム

[9050-04-8]

Description Calcium Carboxymethylcellulose occurs as a white to light yellow powder or fibrous substance. It is odorless.

Identification

(1) Determine the absorption spectrum of Calcium Carboxymethylcellulose, previously dried as directed in the Disk Method in Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Ignite 1 g of Calcium Carboxymethylcellulose at 550–600°C for 3 hours. To the residue obtained, add 10 mL of water and 5 mL of diluted acetic acid (1 in 3), and filter if necessary. Boil the solution, cool, and neutralize with ammonia TS. The solution responds to all the tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Free alkali Weigh 1.0 g of Calcium Carboxymethylcellulose, add 50 mL of freshly boiled and cooled water, shake well, and then add 2 drops of phenolphthalein TS. No pink color develops.

(2) Chloride Not more than 0.35% as Cl.

Sample Solution Weigh 0.10 g of Calcium Carboxymethylcellulose, add 10 mL of water, stir thoroughly, and add 2 mL of a sodium hydroxide solution (1 in 25). Shake, allow to stand for 10 minutes, and make the solution slightly acidic with diluted nitric acid (1 in 10). Add 0.5 mL of hydrogen peroxide, and heat in a water bath for 30 minutes. After cooling, add water to make 100 mL, and filter through a dry filter paper. Use 20 mL of the filtrate as the sample solution.

Control Solution Use 0.20 mL of 0.01 mol/L hydrochloric acid.

(3) Sulfate　Not more than 0.96% as SO_4.

Sample Solution　Weigh 0.10 g of Calcium Carboxymethylcellulose, add 10 mL of water, stir thoroughly, and add 2 mL of sodium hydroxide solution (1 in 25). Shake, allow to stand for 10 minutes, and make slightly acidic with diluted hydrochloric acid (1 in 4). Add 0.5 mL of hydrogen peroxide, and heat in a water bath for 30 minutes. Use 20 mL of the filtrate as the sample solution.

Control Solution　Use 0.40 mL of 0.005 mol/L sulfuric acid.

(4) Lead　Not more than 2 μg/g (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more than 10.0% (105°C, 3 hours).

Residue on Ignition　10.0–20.0% (dried sample, 1 g).

Calcium Chloride

塩化カルシウム

$CaCl_2 \cdot nH_2O$ (n = 2, 1, 1/2, 1/3, or 0)　　Mol. Wt. dihydrate　147.01
anhydrous　110.98

Calcium chloride dihydrate　[10035-04-8]

Calcium chloride monohydrate

Calcium chloride hemihydrate

Calcium chloride 1/3 hydrate

Calcium chloride　[10043-52-4]

Content　Calcium Chloride contains not less than 70.0% of calcium chloride ($CaCl_2$).

Description　Calcium Chloride occurs as white crystals, powder, flakes, granules, or lumps. It is odorless.

Identification　Calcium Chloride responds to all the tests for Calcium Salt and for Chloride in the Qualitative Tests.

Purity

(1) Clarity of solution　Slightly turbid (1.0 g, water 20 mL).

(2) Free acid and free alkali　Weigh 1.0 g of Calcium Chloride, dissolve it in 20 mL of freshly boiled and cooled water, add 2 drops of phenolphthalein TS, and perform the following test with this solution:

(i) If the solution is colorless, add 2.0 mL of 0.02 mol/L sodium hydroxide. A pink color develops.

(ii) If the solution is pink, add 2.0 mL of 0.02 mol/L hydrochloric acid. The color disappears.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Chloride, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, and use as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes with a watch glass covering it. After cooling, add 30 mL of water, and use as the sample solution.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(4) Alkali metals and magnesium Not more than 5.0%.

Weigh 1.0 g of Calcium Chloride, dissolve it in 50 mL of water, mix with 0.50 g of ammonium chloride, and boil for 1 minute. Quickly add 40 mL of a solution of oxalic acid dihydrate (3 in 50), stir vigorously to form a precipitate, immediately add 2 drops of methyl red TS, and then add ammonia TS dropwise to neutralize it, and cool. Transfer the solution into a 100-mL measuring cylinder, add water to make 100 mL, allow to stand for 4 hours to overnight, and filter the supernatant through a dried filter paper. Measure 50 mL of the filtrate, add 0.5 mL of sulfuric acid, evaporate to dryness, ignite to constant weight, and weigh the residue.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

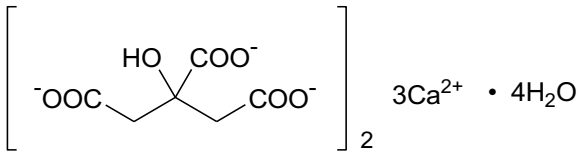
Assay Weigh accurately about 1.5 g of Calcium Chloride, dissolve it in 50 mL of water, and add water to make exactly 100 mL. Perform the test, using this solution as the test solution. Proceed as directed in Method 1 under Calcium Salt Determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 5.549 mg of $CaCl_2$

Calcium Citrate

Tricalcium Citrate

クエン酸カルシウム



$C_{12}H_{10}Ca_3O_{14}\cdot 4H_2O$ Mol. Wt. 570.49

Tricalcium bis(2-hydroxypropane-1,2,3-tricarboxylate) tetrahydrate [5785-44-4]

Content Calcium Citrate, when dried, contains not less than 97.0% of calcium citrate ($C_{12}H_{10}Ca_3O_{14}$ = 498.43).

Description Calcium Citrate occurs as a white powder. It is odorless.

Identification

(1) Ignite Calcium Citrate at 300–400°C for 1 hour. The residue obtained responds to all the tests for Calcium Salt in the Qualitative Tests.

(2) To 0.5 g of Calcium Citrate, add 10 mL of water and 2.5 mL of diluted nitric acid (1 in 10) to dissolve. The solution responds to test (2) for Citrate in the Qualitative Tests.

pH 5.5–8.0 (5% suspension).

Purity

(1) Hydrochloric acid-insoluble substances Not more than 0.060%.

Weigh 5.0 g of Calcium Citrate, add 10 mL of hydrochloric acid and 50 mL of water, and heat on a water bath for 30 minutes. Add water to make 200 mL, and filter through a filter paper for quantitative analysis (5C). Wash the residue on the filter paper with boiling water until the washings are free of chlorides. Heat gradually the residue with the filter paper until they are carbonized, ignite at 450–550°C for 3 hours, and weight the residue.

(2) Chloride Not more than 0.007% as Cl.

Test Solution Weigh 1.0 g of Calcium Citrate, add 10 mL of diluted nitric acid (1 in 10), dissolve while heating, cool, and add water to make 50 mL.

Control Solution To 0.20 mL of 0.01 mol/L hydrochloric acid, add 6 mL of diluted nitric acid (1 in 10) and water to make 50 mL.

(3) Sulfate Not more than 0.024% as SO_4.

Test Solution Weigh 1.0 g of Calcium Citrate, add 10 mL of diluted hydrochloric acid (1 in 4), and dissolve it while heating. Cool, and add water to make 50 mL.

Control Solution To 0.50 mL of 0.005 mol/L sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Citrate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, and add 30 mL of water to use as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes with a watch glass covering it, allow to cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen

citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Calcium Citrate, add 5 mL of diluted hydrochloric acid (1 in 4), and dissolve while heating.

Loss on Drying 10.0–14.0% (150°C, 4 hours).

Assay Weigh accurately about 1 g of Calcium Citrate, previously dried, dissolve it in 10 mL of diluted hydrochloric acid (1 in 4), and add water to make exactly 50 mL. Proceed as directed in Method 1 under Calcium Salt Determination, using this solution as the test solution.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 8.307 mg of $C_{12}H_{10}Ca_3O_{14}$

Calcium Dihydrogen Phosphate

Calcium Phosphate, Monobasic
Monocalcium Phosphate
Primary Calcium Phosphate

リン酸二水素カルシウム

$Ca(H_2PO_4)_2 \cdot nH_2O$ (n = 1 or 0) Mol. Wt. monohydrate 252.07
anhydrous 234.05

Calcium bis(dihydrogenphosphate) monohydrate [10031-30-8]

Calcium bis(dihydrogenphosphate) [7758-23-8]

Content Calcium Dihydrogen Phosphate, when dried, contains 95.0–105.0% of calcium dihydrogen phosphate ($Ca(H_2PO_4)_2$).

Description Calcium Dihydrogen Phosphate occurs as colorless to white crystals or as a white powder.

Identification

(1) Moisten Calcium Dihydrogen Phosphate with silver nitrate solution (1 in 50). A yellow color develops.

(2) To 0.1 g of Calcium Dihydrogen Phosphate, add 20 mL of water, and shake. Filter, and add 5 mL of a solution of ammonium oxalate monohydrate (1 in 30). A white precipitate is formed.

Purity

(1) Clarity of solution Very slightly turbid.

Test Solution Weigh 2.0 g of Calcium Dihydrogen Phosphate, add 18 mL of water and 2.0 mL of hydrochloric acid, and dissolve it by heating in a water bath for 5 minutes.

(2) Free acid and monohydrogen phosphate Weigh 1.0 g of Calcium Dihydrogen Phosphate, add 3 mL of water, and mix well. Add 100 mL of water, stir for 5 minutes to disperse, and add 1 drop of methyl orange TS. A light yellow-red color develops. Add 1.0 mL of 1 mol/L sodium hydroxide. The solution turns light yellow.

(3) Carbonate Weigh 2.0 g of Calcium Dihydrogen Phosphate, add 5 mL of water, and boil. Cool, and add 2 mL of hydrochloric acid. No effervescence occurs.

(4) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Dihydrogen Phosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, add 30 mL of water, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate the solution to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Calcium Dihydrogen Phosphate in 5 mL of diluted hydrochloric acid (1 in 4).

Loss on Drying Not more than 17.0% (180°C, 3 hours).

Assay Weigh accurately about 0.8 g of Calcium Dihydrogen Phosphate, previously dried, dissolve it in 6 mL of diluted hydrochloric acid (1 in 4), and add water to make exactly 200 mL. Proceed as directed in Method 2 under Calcium Salt Determination, using this solution as the test solution.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate = 4.681 mg of $Ca(H_2PO_4)_2$

Calcium Dihydrogen Pyrophosphate

Acid Calcium Pyrophosphate
Calcium Acid Pyrophosphate
Calcium Dihyrogen Diphosphate

ピロリン酸二水素カルシウム

$CaH_2P_2O_7$ Mol. Wt. 216.04

Calcium dihydrogendiphosphate [14866-19-4]

Content Calcium Dihydrogen Pyrophosphate, when dried, contains not less than 90.0% of calcium dihydrogen pyrophosphate ($CaH_2P_2O_7$).

Description Calcium Dihydrogen Pyrophosphate occurs as white crystals or powder.

Identification

(1) To 0.5 g of Calcium Dihydrogen Pyrophosphate, add 10 mL of water, and shake. The resulting solution is acidic.

(2) To 0.2 g of Calcium Dihydrogen Pyrophosphate, add 5 mL of diluted nitric acid (1 in 10), and dissolve it by warming. Then add 2 mL of ammonium molybdate TS, and warm. A yellow precipitate is formed.

(3) To 0.3 g of Calcium Dihydrogen Pyrophosphate, add 9 mL of water and 1 mL of diluted hydrochloric acid (1 in 4), dissolve it by warming, cool, and filter. To the filtrate, add 3 mL of a solution of ammonium oxalate monohydrate (1 in 30). A white precipitate is formed. The precipitate dissolves on the addition of 5 mL of diluted hydrochloric acid (1 in 30).

Purity

(1) Hydrochloric acid-insoluble substances Not more than 0.40%.

Weigh accurately a glass filter (1G4), previously dried at 110°C for 30 minutes and allowed to cool in a desiccator. Weigh 5.0 g of Calcium Dihydrogen Pyrophosphate, add 100 mL of diluted hydrochloric acid (1 in 4), and allow to stand for 1 hour with occasional shaking. Collect the insoluble substances by filtration with the glass filter, wash with 30 mL of water, and dry at 110°C for 2 hours together with the glass filter. Allow to cool in a desiccator, and weigh accurately the glass filter containing the residue.

(2) Orthophosphate Weigh 1.0 g of Calcium Dihydrogen Pyrophosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(3) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Dihydrogen Pyrophosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, and add 30 mL of water. Use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness,

and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes with a watch glass covering it. After cooling, add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Calcium Dihydrogen Pyrophosphate in 5 mL of diluted hydrochloric acid (1 in 4).

Loss on Drying Not more than 5.0% (150°C, 4 hours).

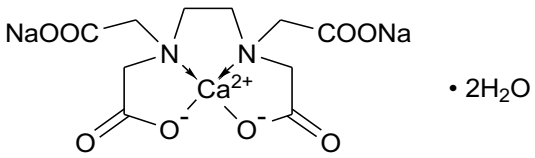
Assay Weigh accurately about 0.7 g of Calcium Dihydrogen Pyrophosphate, previously dried, add 20 mL of diluted hydrochloric acid (1 in 4), and boil. After cooling, add water to make exactly 200 mL, and proceed with the resulting solution, as directed in Method 2 under Calcium Salt Determination.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate = 4.321 mg of $CaH_2P_2O_7$

Calcium Disodium Ethylenediaminetetraacetate

Calcium Disodium EDTA

エチレンジアミン四酢酸カルシウム二ナトリウム



$C_{10}H_{12}CaN_2Na_2O_8 \cdot 2H_2O$ Mol. Wt. 410.30

Disodium (ethylenediaminetetraacetato)calciate(2-) dihydrate [anhydrous 62-33-9]

Content Calcium Disodium Ethylenediaminetetraacetate, when calculated on the anhydrous basis, contains 97.0–102.0% of calcium disodium ethylenediaminetetra-acetate ($C_{10}H_{12}CaN_2Na_2O_8$ = 374.27).

Description Calcium Disodium Ethylenediaminetetraacetate occurs as a white to whitish crystalline powder or as granules. It is odorless and has a slightly salty taste.

Identification

(1) A solution of Calcium Disodium Ethylenediaminetetraacetate (1 in 20) responds

to test (2) for Calcium Salt and to all tests for Sodium Salt in the Qualitative Tests.

(2) Add 50 mg of Calcium Disodium Ethylenediaminetetraacetate to 5 mL of water, previously mixed with 2 drops of ammonium thiocyanate solution (2 in 25) and 2 drops of a solution of iron(III) chloride hexahydrate (1 in 10), and shake. The red color of the solution disappears.

pH 6.5–8.0.

Test Solution Weigh 1.0 g of Calcium Disodium Ethylenediaminetetraacetate, and dissolve it in water to make 15 mL.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Magnesium-chelating substance Weigh 1.0 g of Calcium Disodium Ethylenediaminetetraacetate, dissolve it in 5 mL of water, add 5 mL of ammonium buffer (pH 10.7), and titrate with 0.1 mol/L magnesium acetate (indicator: 5 drops of eriochrome black T TS). The volume consumed is not more than 2.0 mL.

Water Content Not more than 13.0% (0.3 g, Volumetric Titration, Direct Titration).

Assay Weigh accurately about 1 g of Calcium Disodium Ethylenediaminetetraacetate into a 250-mL volumetric flask, and dissolve it in water to make exactly 250 mL. Measure exactly 25 mL of this solution, adjust the pH to about 2 with diluted nitric acid (1 in 10), and titrate with 0.01 mol/L bismuth nitrate (indicator: 3 drops of xylenol orange TS) until a red color develops. Calculate on the anhydrous basis.

Each mL of 0.01 mol/L bismuth nitrate = 3.743 mg of $C_{10}H_{12}CaN_2Na_2O_8$

Calcium Ferrocyanide

Calcium Hexacyanoferrate(II)

フェロシアン化カルシウム

$Ca_2[Fe(CN)_6]\cdot 12H_2O$ Mol. Wt. 508.29

Calcium hexacyanoferrate(II) dodecahydrate [anhydrous 13821-08-4]

Content Calcium Ferrocyanide contains not less than 99.0% of calcium ferrocyanide ($Ca_2[Fe(CN)_6]\cdot 12H_2O$).

Description Calcium Ferrocyanide occurs as yellow crystals or crystalline powder.

Identification

(1) Proceed as directed in Identification (1) for Potassium Ferrocyanide.

(2) Calcium Ferrocyanide responds to all the tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Cyanide Proceed as directed in Purity (1) for Potassium Ferrocyanide.

(2) Ferricyanide Proceed as directed in Purity (2) for Potassium Ferrocyanide.

(3) Lead Not more than 5 μg/g as Pb (0.80 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

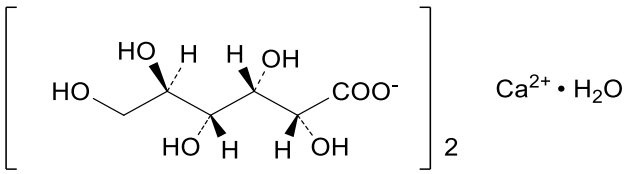
Proceed as directed in Purity (3) for Potassium Ferrocyanide.

Assay Weigh accurately about 1 g of Calcium Ferrocyanide, and dissolve it in 200 mL of water. To this solution, add 10 mL of sulfuric acid, and titrate with 0.02 mol/L potassium permanganate. The endpoint is when the red color of the solution persists for 30 seconds.

Each mL of 0.02 mol/L potassium permanganate = 50.83 mg of $Ca_2[Fe(CN)_6] \cdot 12H_2O$

Calcium Gluconate

グルコン酸カルシウム



$C_{12}H_{22}CaO_{14} \cdot H_2O$ Mol. Wt. 448.39

Monocalcium bis(D-gluconate) monohydrate [anhydrous 99-28-5]

Content Calcium Gluconate, when dried, contains 98.0–104.0% of calcium gluconate ($C_{12}H_{22}CaO_{14} \cdot H_2O$).

Description Calcium Gluconate occurs as a white crystalline powder or granular powder. It is odorless and tasteless.

Identification

(1) To 1 mL of a solution of Calcium Gluconate (1 in 40), add 1 drop of a solution of iron(III) chloride hexahydrate (1 in 10). A dark yellow color develops.

(2) Measure 5 mL of a solution of Calcium Gluconate in warm water (1 in 10), and proceed as directed in Identification (2) for Glucono-δ-Lactone.

(3) A solution of Calcium Gluconate (1 in 40) responds to all the tests for Calcium Salt in the Qualitative Tests.

pH 6.0–8.0 (1.0 g, water 20 mL).

To the specified amount of Calcium Gluconate, add specified volume of water, and dissolve it by warming to 60°C, and cool. Measure the pH of the resulting solution.

Purity

(1) Clarity of solution Almost clear.

Test Solution Weigh 1.0 g of Calcium Gluconate, add 20 mL of water, and dissolve by warming to 60°C.

(2) Chloride Not more than 0.071% (0.30 g, Control Solution: 0.01 mol/L hydrochloric acid 0.60 mL).

(3) Sulfate Not more than 0.048% (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Gluconate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, add 30 mL of water, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes with a watch glass covering it, allow to cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Calcium Gluconate, add 5 mL of water, and dissolve it while warming. Add 5 mL of diluted sulfuric acid (3 in 50) and 1 mL of Bromine TS, and concentrate to 5 mL by heating on a water bath.

(6) Sucrose or reducing sugars Proceed as directed in Purity (6) for Glucono-δ-Lactone.

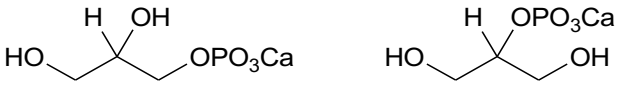
Loss on Drying Not more than 0.5% (80°C, 2 hours).

Assay Weigh accurately about 2.5 g of Calcium Gluconate, previously dried, dissolve it in 25 mL of diluted hydrochloric acid (1 in 4), and add water to make exactly 50 mL. Using this solution as the test solution, proceed as directed in Method 1 under Calcium Salt Determination. In Method 1, to the test solution, add 15 mL of potassium hydroxide solution (1 in 10) instead of 10 mL.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate = 22.42 mg of $C_{12}H_{22}CaO_{14}\cdot H_2O$

Calcium Glycerophosphate

グリセロリン酸カルシウム



$C_3H_7CaO_6P$ Mol. Wt. 210.14

Mixture of monocalcium 2,3-dihydroxylpropanyl phosphate and monocalcium 1,3-dihydroxypropan-2-yl phosphate [27214-00-2]

Content Calcium Glycerophosphate, when calculated on the dried basis, contains not less than 98.0% of calcium glycerophosphate ($C_3H_7CaO_6P$).

Description Calcium Glycerophosphate occurs as a white powder. It is odorless and has a slightly bitter taste.

Identification To 1 g of Calcium Glycerophosphate, add 10 mL of water of 5°C or below, and shake well. Use this solution as the test solution.

(1) Boil the test solution. White crystals are deposited.

(2) To 3 mL of the test solution, add 2–3 drops of lead(II) acetate TS. A white, curd-like precipitate is formed. The precipitate dissolves on the addition of 3 mL of nitric acid.

(3) The test solution responds to all the tests for Calcium Salt and for Glycerophosphate in the Qualitative Tests.

Purity

(1) Clarity of solution Very slightly turbid (1.0 g, water 50 mL).

(2) Ethanol-soluble substance Not more than 1.0%.

Weigh 1.0 g of Calcium Glycerophosphate, add 25 mL of ethanol (99.5), shake, and filter. Evaporate the filtrate on a water bath, dry the residue at 60°C for 1 hour, and weight the mass.

(3) Free alkali Weigh 1.0 g of Calcium Glycerophosphate, dissolve it in 60 mL of water, add 5 drops of phenolphthalein TS, and titrate with 0.05 mol/L sulfuric acid. The consumed volume is not more than 1.5 mL.

(4) Chloride Not more than 0.071% as Cl (0.25 g, Control Solution: 0.01 mol/L hydrochloric acid 0.50 mL).

(5) Sulfate Not more than 0.048% as SO_4 (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(6) Phosphate Not more than 0.040% as PO_4.

Weigh 1.0 g of Calcium Glycerophosphate, dissolve it in 10 mL of diluted nitric acid (1 in 10), add 10 mL of cold ammonium molybdate TS, and allow to stand for 10 minutes. The solution is not more turbid than a control solution prepared as directed below.

Control Solution Weigh 0.192 g of potassium dihydrogen phosphate, dissolve it in

100 mL of water, measure 3.0 mL of this solution, add diluted nitric acid (1 in 10) to make 100 mL. Measure 10 mL of this solution, add 10 mL of cold ammonium molybdate TS, and allow to stand for 10 minutes.

(7) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Glycerophosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, and add 30 mL of water. Use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil the solution gently for 5 minutes with a watch glass covering it, allow it to cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(8) Arsenic Not more than 3 μg/g as As (1.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Calcium Glycerophosphate in 25 mL of water, add 1 mL of sulfuric acid and 10 mL of sulfurous acid solution, evaporate to about 2 mL, and add water to make 10 mL. Use 5 mL of this solution as the test solution.

Loss on Drying Not more than 13.0% (0.5g, 150°C, 4 hours).

Assay Weigh accurately about 1 g of Calcium Glycerophosphate, previously dried, dissolve it in 10 mL of diluted hydrochloric acid (1 in 4), and add water to make exactly 50 mL. Using this solution as the test solution, proceed as directed in Method 1 under Calcium Salt Determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 10.51 mg of $C_3H_7CaO_6P$

Calcium Hydroxide

Slaked Lime

水酸化カルシウム

$Ca(OH)_2$ Mol. Wt. 74.09

Calcium hydroxide [1305-62-0]

Content Calcium Hydroxide contains not less than 95.0% of calcium hydroxide ($Ca(OH)_2$).

Description Calcium Hydroxide occurs as a white powder.

Identification

(1) To Calcium Hydroxide, add 3–4 times as much water as the sample amount. It becomes muddy and alkaline.

(2) To 1 g of Calcium Hydroxide, add 20 mL of water and 6 mL of diluted acetic acid (1 in 3) to dissolve. The solution responds to all the tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 0.50%.

Weigh 2.0 g of Calcium Hydroxide, add 10 mL of hydrochloric acid and 20 mL of water to dissolve, and boil. After cooling, add water to make 200 mL, filter through a filter paper for quantitative analysis (5C), wash the residue on the filter paper with boiling water until the washings are free from chloride. Heat gradually the residue with a filter paper to carbonization, then incinerate at 450–550°C for 3 hours, and weigh the mass.

(2) Carbonate Weigh 2.0 g of Calcium Hydroxide, add 50 mL of water, shake well, and add 25 mL of diluted hydrochloric acid (1 in 4). No remarkable effervescence occurs.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Hydroxide, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, and add 30 mL of water. Use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes with a watch glass covering it, and allow to cool. Add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(4) Alkali metals and magnesium Not more than 6.0%.

Weigh 0.50 g of Calcium Hydroxide, dissolve it in 30 mL of diluted hydrochloric acid (1 in 10), and boil for 1 minute. Quickly add 40 mL of oxalic acid solution (3 in 50), and proceed as directed in Purity (4) for Calcium Chloride.

(5) Barium Not more than 0.030% as Ba.

Test Solution Weigh 1.50 g of Calcium Hydroxide, dissolve it in 15 mL of diluted hydrochloric acid (1 in 4), add water to make 30 mL, and filter. Use 20 mL of the filtrate as the test solution.

Procedure To the test solution, add 2 g of sodium acetate trihydrate, 1 mL of diluted acetic acid (1 in 20), and 0.5 mL of potassium chromate solution (1 in 20), and allow to stand for 15 minutes. The solution is not more turbid than a control solution prepared as follows: To 0.30 mL of Barium Standard Solution, add water to make 20 mL, and then treat in the same manner as the test solution.

(6) Arsenic　Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

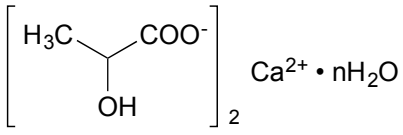
Test Solution　Weigh the specified amount of Calcium Hydroxide, and dissolve it in 5 mL of diluted hydrochloric acid (1 in 4).

Assay　Weigh accurately about 2 g of Calcium Hydroxide, dissolve it in 30 mL of diluted hydrochloric acid (1 in 4), and add water to make exactly 250 mL. Proceed as directed in Method 1 under Calcium Salt Determination, using this solution as the test solution.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 3.705 mg of $Ca(OH)_2$

Calcium Lactate

乳酸カルシウム



(n = 5,3,1, or 0)

$C_6H_{10}CaO_6 \cdot nH_2O$ (n = 5, 3, 1, or 0)　Mol. Wt. pentahydrate　308.29

anhydrous　218.22

Monocalcium bis(2-hydroxypropanoate) pentahydrate　[5743-47-5]

Monocalcium bis(2-hydroxypropanoate) trihydrate　[139061-06-6]

Monocalcium bis(2-hydroxypropanoate) monohydrate

Monocalcium bis(2-hydroxypropanoate)　[814-80-2]

Content　Calcium Lactate, when calculated on the dried basis, contains not less than 97.0% of calcium lactate ($C_6H_{10}CaO_6$).

Description　Calcium Lactate occurs as a white powder or as granules. It is odorless or has a slight, characteristic odor.

Identification　A solution of Calcium Lactate (1 in 20) responds to all the tests for Calcium Salt and for Lactate in the Qualitative Tests.

pH　6.0–8.0.

Measure the pH of a solution prepared as follows: Dissolve 1.0 g of Calcium Lactate in 20 mL of water by heating in a water bath and allow to cool.

Purity

(1) Clarity of solution　Colorless and clear.

Test Solution　Weigh 1.0 g of Calcium Lactate, add 20 mL of water, and dissolve it

by heating in a water bath.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Lactate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, add 30 mL of water, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes with a watch glass covering it, allow to cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(3) Alkali metals and magnesium Not more than 1.0%.

Weigh 1.0 g of Calcium Lactate, dissolve it in about 40 mL of water, add 0.5 g of ammonium chloride, and boil. Add about 20 mL of a solution of ammonium oxalate monohydrate (1 in 25), heat on a water bath for 1 hour, cool, add water to make 100 mL, and filter. Measure 50 mL of the filtrate, add 0.5 mL of sulfuric acid, evaporate to dryness, ignite at 450–550°C to constant weigh, and weigh the residue.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Calcium Lactate by adding 2 mL of water and 3 mL of hydrochloric acid.

(5) Salts of volatile fatty acids Weigh 0.5 g of Calcium Lactate, add 1 mL of sulfuric acid, and heat in a water bath. No butyric acid-like odor is evolved.

Loss on Drying Not more than 30.0% (120°C, 4 hours).

Assay Weigh accurately about 2 g of Calcium Lactate, dissolve it in 20 mL of diluted hydrochloric acid (1 in 4), and add water to make exactly 100 mL. Using this solution as the test solution, proceed as directed in Method 1 under Calcium Salt Determination, and calculate on the dried basis.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 10.91 mg of $C_6H_{10}CaO_6$

Calcium Monohydrogen Phosphate

Calcium Phosphate, Dibasic
Dicalcium Phosphate
Secondary Calcium Phosphate

リン酸一水素カルシウム

$CaHPO_4 \cdot nH_2O$ (n = 2, 1 ½ , 1, ½ , or 0)　　Mol. Wt. dihydrate　172.09

anhydrous　136.06

Calcium hydrogenphosphate dihydrate　[7789-77-7]

Calcium hydrogenphosphate sesquihydrate

Calcium hydrogenphosphate monohydrate

Calcium hydrogenphosphate hemihydrate

Calcium hydrogenphosphate　[7757-93-9]

Content　Calcium Monohydrogen Phosphate, when dried, contains 98.0–103.0% of calcium monohydrogen phosphate ($CaHPO_4$).

Description　Calcium Monohydrogen Phosphate occurs as white crystals or powder.

Identification

(1) Moisten Calcium Monohydrogen Phosphate with silver nitrate solution (1 in 50). A yellow color develops.

(2) To 0.1 g of Calcium Monohydrogen Phosphate, add 5 mL of diluted acetic acid (1 in 4), boil, cool, and filter. To the filtrate, add 5 mL of a solution of ammonium oxalate monohydrate (1 in 30).　A white precipitate is formed.

Purity

(1) Clarity of solution　Very slightly turbid.

Test Solution　Weigh 2.0 g of Calcium Monohydrogen Phosphate, add 16 mL of water and 4.0 mL of hydrochloric acid, and dissolve by heating in a water bath for 5 minutes.

(2) Carbonate　Weigh 2.0 g of Calcium Monohydrogen Phosphate, add 5 mL of water, and boil. Cool, and add 2 mL of hydrochloric acid. No effervescence occurs.

(3) Lead　Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution　To the specified amount of Calcium Monohydrogen Phosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, and add 30 mL of water. Use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate the solution to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes with a watch glass covering it. Allow to cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Calcium Monohydrogen Phosphate in 5 mL of diluted hydrochloric acid (1 in 4).

Loss on Drying Not more than 22.0% (200°C, 3 hours).

Assay Weigh accurately about 0.4 g of Calcium Monohydrogen Phosphate, previously dried, dissolve it in 12 mL of diluted hydrochloric acid (1 in 4), and add water to make exactly 200 mL. Proceed as directed in Method 2 under Calcium Salt Determination, using this solution as the test solution.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.721 mg of $CaHPO_4$

Calcium Oxide

酸化カルシウム

CaO Mol. Wt. 56.08

Calcium oxide [1305-78-8]

Content Calcium Oxide, when ignited, contains not less than 95.0% of calcium oxide (CaO).

Description Calcium Oxide occurs as a white to light gray powder, or as granules or lumps.

Identification

(1) When moistened with water, 1 g of Calcium Oxide generates heat. To this, add 5 mL of water. The resulting suspension is alkaline.

(2) To 1 g of Calcium Oxide, add 20 mL of water, and add acetic acid dropwise to dissolve the precipitate. The resulting solution responds to all the tests for Calcium salts in the Qualitative Tests.

Purity

(1) Hydrochloric acid insoluble substances Not more than 1.0%

Weigh accurately a crucible-type glass filter (1G4), previously dried at 105°C for 30 minutes and cooled in a desiccators. Weigh exactly 5.0 g of Calcium Oxide, and add 100 mL of water. Add hydrochloric acid dropwise while shaking until the sample no longer dissolves, and boil. After cooling, add hydrochloric acid if necessary to make it acidic. Filter it through the glass filter, wash the residue on the filter with water until the

washings are free of chlorides. Dry the glass filter containing the residue at 105 ºC for 1 hour, cool in desiccators, and weigh the filter accurately.

(2) Fluoride Not more than 150 μg/g as F.

Test Solution Weigh 0.10 g of Calcium Oxide into a beaker, and add 10 mL of diluted hydrochloric acid (1 in 10) to dissolve it. Heat it, boil for 1 minute, transfer into a polyethylene beaker, and immediately cool in ice. Add 15 mL of a solution of trisodium citrate dihydrate (1 in 4) and 10 mL of a solution of disodium dihydrogen ethylenediaminetetraacetate dihydrate (1 in 40), and mix it. Then, adjust the pH to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5). Transfer the resulting solution into a 100-mL volumetric flask, and dilute with water to volume. Transfer 50 mL of this solution into a polyethylene beaker.

Control Solution Measure exactly 5 mL of Fluoride Ion Standard Stock Solution into a 1000-mL volumetric flask, and dilute with water to volume. Transfer exactly 3 mL of this solution into a polyethylene beaker, add 15 mL of a solution of trisodium citrate dihydrate (1 in 4) and 10 mL of disodium dihydrogen ethylenediaminetetraacetate dihydrate (1 in 40), and mix it. Then, adjust the pH to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5). Transfer the resulting solution into a 100-mL volumetric flask, and dilute with water to volume. Transfer 50 mL of this solution into a polyethylene beaker.

Procedure Measure the potential of the test solution and the control solution using a potentiometer connected to a reference electrode and a fluoride ion electrode. The potential of the test solution is not less than that of the control solution.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution Weigh the specified amount g of Calcium Acetate, add 20 mL of diluted hydrochloric acid (1 in 4), dissolve it by ultrasonic agitation, and evaporate to dryness, and add 20 mL of water to the residue. Use the resulting solution as the sample solution. In Method 5, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(4) Alkali metals and magnesium Not more than 3.6%.

Weigh about 0.5 g of Calcium Oxide, add 30 mL of water and 15 mL of diluted hydrochloric acid (1 in 4) to dissolve it, and boil for 1 minute. Immediately add 40 mL of oxalic acid dihydrate (3 in 50), agitate, and add 2 drops of methyl red TS, then add ammonia solution until the solution exhibits a yellow color to precipitate the calcium. Heat this solution on a water bath for 1 hour, cool, and add water to make 100 mL. Mix well, and filter. Transfer 50 mL of the filtrate into a platinum crucible, previously ignited at 800°C for 30 minutes, cooled in a desiccator, and weighed accurately. Evaporate with 0.5 mL of sulfuric acid, ignite at 800°C to constant weight, and weigh the crucible to determine the weight of the residue.

(5) Barium Not more than 300 μg /g as Ba.

Test Solution Weigh accurately about 1.0 g of Calcium Oxide, add diluted

hydrochloric acid (1 in 10) to dissolve it, and make exactly 50 mL. Measure 5 mL of this solution, and add diluted nitric acid (1 in 150) to make exactly 100 mL.

Control Solution Measure exactly 1 mL of Barium Standard Solution, and add diluted nitric acid (1 in 150) to make exactly 1000 mL. Measure exactly 30 mL of this solution, and add diluted nitric acid (1 in 150) to make exactly 100 mL.

Procedure Proceed as directed under Inductively Coupled Plasma-Atomic Emission Spectrometry. The emission intensity of the test solution is not more than that of the control solution.

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Calcium Oxide in 8 mL of diluted hydrochloric acid (1 in 4).

Loss on Ignition Not more than 10.0 % (800°C, constant weight).

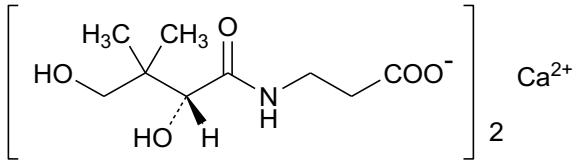
Assay

Weigh accurately about 1.5 g of Calcium Oxide, previously ignited, add 30 mL of diluted hydrochloric acid (1 in 4) to dissolve it, and add water to make exactly 250 mL. Use the resulting solution as the test solution. Proceed as directed under Method 1 in Calcium Determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.804 mg of CaO

Calcium Pantothenate

パントテン酸カルシウム



$C_{18}H_{32}CaN_2O_{10}$ Mol. Wt. 476.53

Monocalcium bis{3-[(2*R*)-2,4-dihydroxy-3,3-dimethylbutanoylamino]propanoate}

[137-08-6]

Content Calcium Pantothenate, when calculated on the dried basis, contains 5.7–6.0% of nitrogen (N = 14.01) and 8.2–8.6% of calcium (Ca = 40.08).

Description Calcium Pantothenate occurs as a white powder. It is odorless and has a slightly bitter taste.

Identification

(1) Dissolve 50 mg of Calcium Pantothenate in 5 mL of sodium hydroxide solution (1 in 25), and add 1 drop of a solution of copper(II) sulfate pentahydrate (1 in 10). A blue-purple color develops.

(2) To 50 mg of Calcium Pantothenate, add 5 mL of sodium hydroxide solution (1 in 25), and boil for 1 minute. Cool, and add 2 mL of diluted hydrochloric acid (1 in 4) and 2 drops of a solution of iron(III) chloride hexahydarate (1 in 10). A dark yellow color develops.

(3) Calcium Pantothenate solution (1 in 20) responds to all the tests for Calcium Salt in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +25.0 to +28.5° (after dried, 1.25 g, water, 25 mL).

pH 7.0–9.0 (2.0 g, water 10 mL).

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Pantothenate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, and add 30 mL of water. Use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, allow to cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Alkaloid Weigh 50 mg of Calcium Pantothenate, dissolve it in 5 mL of water, and add 0.5 mL of ammonium molybdate TS and 0.5 mL of phosphoric acid (1 in 10). No white turbidity appears.

Loss on Drying Not more than 5.0% (105°C, 3 hours).

Assay

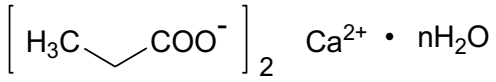
(1) Nitrogen Weigh accurately about 50 mg of Calcium Pantothenate, proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination, and calculate on the dried basis.

(2) Calcium Weigh accurately about 2.5 g of Calcium Pantothenate, dissolve it by adding 5 mL of diluted hydrochloric acid (1 in 4) and 20 mL of water, and then add water to make exactly 50 mL. Proceed as directed in Method 1 under Calcium Salt Determination, using this solution as the test solution, and calculate on the dried basis.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.004 mg of Ca

Calcium Propionate

プロピオン酸カルシウム



(n = 1 or 0)

$C_6H_{10}CaO_4 \cdot nH_2O$ (n = 1 or 0) Mol. Wt. monohydrate 204.23

anhydrous 186.22

Monocalcium dipropanoate monohydrate

Monocalcium dipropanoate [4075-81-4]

Content Calcium Propionate, when dried, contains not less than 98.0% of calcium propionate ($C_6H_{10}CaO_4$).

Description Calcium Propionate occurs as white crystals, powder, or granules. It is odorless or has a slight, characteristic odor.

Identification

(1) To 5 mL of a solution of Calcium Propionate (1 in 10), add 5 mL of diluted sulfuric acid (1 in 10), and heat. A characteristic odor is evolved.

(2) Calcium Propionate responds to all the tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Water-insoluble substances Not more than 0.30%.

Weigh 10.0 g of Calcium Propionate, add 100 mL of water, and allow to stand for 1 hour with occasional shaking. Filter the insoluble substances through a glass filter (1G4), wash with 30 mL of water, and dry at 180°C for 4 hours, and weigh the residue.

(2) Free acid and free alkali Weigh 2.0 g of Calcium Propionate, dissolve it in 20 mL of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS and 0.30 mL of 0.1 mol/L hydrochloric acid. The solution is colorless. On the addition of 0.6 mL of 0.1 mol/L sodium hydroxide, the color of the solution changes to red.

(3) Lead Not more than 5 μg/g as Pb (0.80 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Propionate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, add 30 mL of water, and use the resulting solution as the

sample solution. If the sample does not dissolve completely, evaporate this solution to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, allow to cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 9.5% (120°C, 2 hours).

Assay Weigh accurately about 1 g of Calcium Propionate, previously dried, and dissolve it in water to make exactly 100 mL. Measure exactly 25 mL of this solution, add 75 mL of water and 15 mL of sodium hydroxide solution (1 in 10), and allow to stand for about 1 minute. Add 0.1 g of NN indicator, and immediately titrate with 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate until the red color completely disappears and the solution turns blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 9.311 mg of $C_6H_{10}CaO_4$

Calcium 5′-Ribonucleotide

5′-リボヌクレオチドカルシウム

Definition Calcium 5′-Ribonucleotide is a mixture of calcium 5′-inosinate, calcium 5′-guanylate, calcium 5′-cytidylate, and calcium 5′-uridylate, or a mixture of calcium 5′-inosinate and calcium 5′-guanylate.

Content Calcium 5′-Ribonucleotide, when calculated on the anhydrous basis, contains 97.0–102.0% of calcium 5′-ribonucleotide, of which not less than 95.0% consists of calcium 5′-inosinate and calcium 5′-guanylate.

Description Calcium 5′-Ribonucleotide occurs as white to whitish crystals or powder. It is odorless and has a slight, characteristic taste.

Identification

(1) To 0.1 g of Calcium 5′-Ribonucleotide, add 200 mL of water, and dissolve it by heating in a water bath, and cool. To 1 mL of the resulting solution, add 0.2 mL of orcinol–ethanol TS, then add 3 mL of ammonium Iron(II) sulfate–hydrochloric acid TS, and heat in a water bath for 10 minutes. A green color develops.

(2) Dissolve 0.1 g of Calcium 5′-Ribonucleotide in 200 mL of diluted hydrochloric acid (1 in 4). To 2 mL of the resulting solution, add 0.1 g of zinc powder, and proceed as directed in Identification (2) for Disodium 5′-Ribonucleotide.

(3) To 0.1 g of Calcium 5′-Ribonucleotide, add 500 mL of water, dissolve it by heating

in a water bath, and cool. To 1 mL of the resulting solution, add 1 mL of diluted hydrochloric acid (1 in 4), and heat in a water bath for 10 minutes. Cool, and add 0.5 mL of Folin's TS and 2 mL of sodium carbonate saturated solution. A blue color develops.

(4) To 0.1 g of Calcium 5′-Ribonucleotide, add 5 mL of water and 5 mL of nitric acid, boil gently for 10 minutes. Cool, and neutralize with ammonia solution or ammonia TS. The solution responds to test (2) for Phosphate in the Qualitative Tests.

(5) To 0.1 g of Calcium 5′-Ribonucleotide, add 200 mL of water, dissolve it by heating in a water bath, and cool. The solution responds to all the tests for Calcium Salt in the Qualitative Tests.

pH 7.0–8.0.

Test Solution Weigh 0.10 g of Calcium 5′-Ribonucleotide, add 200 mL of water, dissolve it while heating in a water bath, and cool.

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Calcium 5′-Ribonucleotide in 5 mL of diluted hydrochloric acid (1 in 4).

(3) Water-soluble substances Not more than 16%.

Weigh 1.0 g of Calcium 5′-Ribonucleotide, add 50 mL of water, allow to stand for 10 minutes with occasional shaking, and filter through a dry filter paper for quantitative analysis (5C). Measure 25 mL of the filtrate, and evaporate to dryness. Dry the residue at 105°C for 1 hour, and weigh.

Water Content Not more than 23.0% (0.15 g, Volumetric Titration, Back Titration).

Before titrating, add water determination TS in excess, and stir for 20 minutes.

Assay Calculate the content of calcium 5′-ribonucleotide and the total content of calcium 5′-inosinate ($C_{10}H_{11}CaN_4O_8P$) and calcium 5′-guanylate ($C_{10}H_{12}CaN_5O_8P$) by the formulae from the values of I_{Ca}, G_{Ca}, and P_{Ca} obtained in (1), (2), and (3) below.

$$\text{Content (\%) of calcium 5′-ribonucleotide} = \frac{I_{Ca} + G_{Ca} + P_{Ca}}{100 - \text{Water content (\%)}} \times 100$$

$$\text{Content (\%) of calcium 5′-inosinate } (C_{10}H_{11}CaN_4O_8P) \text{ and calcium 5′-guanylate } (C_{10}H_{12}CaN_5O_8P) = \frac{I_{Ca} + G_{Ca}}{100 - \text{Water content (\%)}} \times 100$$

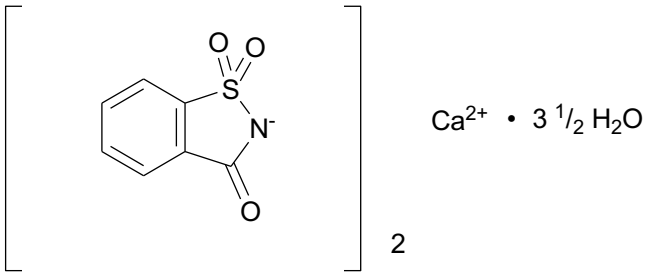
(1) Calcium 5′-inosinate　Weigh accurately about 0.65 g of Calcium 5′-Ribonucleotide, dissolve it in diluted hydrochloric acid (1 in 100) to make exactly 500 mL, and use this solution as the sample solution. Proceed as directed in Assay (1) for Disodium 5′-Ribonucleotide to determine the content of disodium 5′-inosinate ($C_{10}H_{11}N_4Na_2O_8P$). Multiply the content (%) by 0.985 to determine the content I_{Ca} (%) of calcium 5′-inosinate ($C_{10}H_{11}CaN_4O_8P$).

(2) Calcium 5′-guanylate　Measure accurately 1 mL of the sample solution obtained in (1) above, and proceed as directed in Assay (2) for Disodium 5′-Ribonucleotide to determine the content of disodium 5′-guanylate ($C_{10}H_{12}N_5Na_2O_8P$). Multiply the content (%) by 0.986 to determine the content G_{Ca} (%) of calcium 5′-guanylate ($C_{10}H_{12}CaN_5O_8P$).

(3) Calcium 5′-cytidylate and calcium 5′-uridylate　Weigh accurately about 1.5 g of Calcium 5′-Ribonucleotide, dissolve it in 10 mL of diluted hydrochloric acid (1 in 10), add 1 mL of a solution of sodium dihydrogenphosphate dihydrate (3 in 5), adjust the pH to 7.0 with sodium hydroxide solution (1 in 25), and filter. Wash the residue on the filter paper with 10 mL of water, combine the washings with the filtrate, and add water to make exactly 50 mL. Use this solution as the sample solution. Proceed as directed in Assay (3) for Disodium 5′-Ribonucleotide to determine the content of disodium 5′-cytidylate ($C_9H_{12}N_3Na_2O_8P$) and disodium 5′-uridylate ($C_9H_{11}N_2Na_2O_9P$). Multiply the content (%) by 0.984 to obtain the total content P_{Ca} (%) of calcium 5′-cytidylate ($C_9H_{12}CaN_3O_8P$) and calcium 5′-uridylate ($C_9H_{11}CaN_2O_9P$).

Calcium Saccharin

サッカリンカルシウム



$C_{14}H_8CaN_2O_6S_2 \cdot 3½H_2O$　Mol. Wt. 467.48

Calcium bis(3-oxo-3*H*-1,2-benzothiazol-2-ide) 1,1-dioxide hemiheptahydrate [6381-91-5]

Content　Calcium Saccharin, when dried, contains not less than 98.0% of calcium saccharin ($C_{14}H_8CaN_2O_6S_2$).

Description　Calcium Saccharin occurs as white crystals or crystalline powder. It has an extremely sweet taste.

Identification

(1) To 10 mL of a solution of Calcium Saccharin (1 in 10), add 1 mL of hydrochloric

acid, collect the resulting crystalline precipitate by filtration, and wash well with cold water. Dry the precipitate for 2 hours at 105°C, and measure the melting point. It starts melting at 226°C and melts completely at up to 230°C.

(2) Mix 20 mg of Calcium Saccharin with40 mg of resorcinol, add 10 drops of sulfuric acid, and heat for 3 minutes at 200°C. Cool, and add 10 mL of water and 10 mL of sodium hydroxide solution (1 in 25). The resulting solution emits a green fluorescence.

(3) To 0.1 g of Calcium Saccharin, add 5 mL of sodium hydroxide solution (1 in 25), evaporate to dryness while gently heating, and fuse the residue, being careful not to carbonize it. Keep heating until it no longer evolves the odor of ammonia, and cool. To the residue, add about 20 mL of water, make slightly acidic with diluted hydrochloric acid (1 in 10), and filter. Add a drop of a solution of iron(III) chloride hexahydrate (1 in 10) to the filtrate. A purple to red-purple color develops.

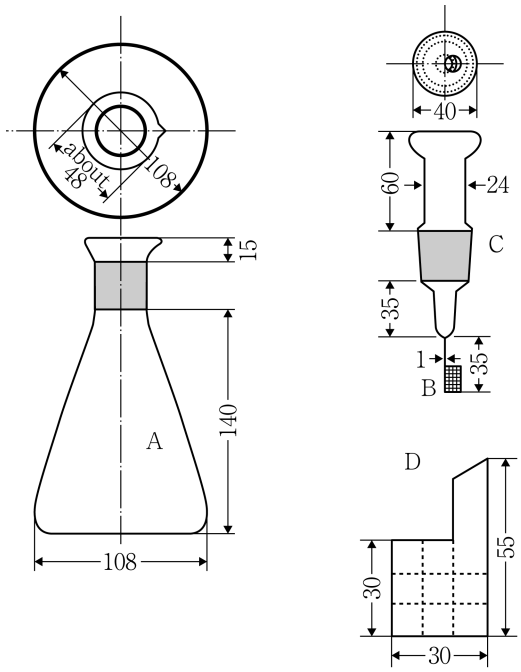
(4) Calcium Saccharin responds all tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Selenium Not more than 30 μg/g as Se.

(i) Apparatus Use the apparatus as illustrated in the figure.



A: Colorless, hard-glass, thick-walled flask with a flared mouth (2 mm in wall thickness and 500-mL in capacity)

B: Platinum basket or platinum gauze cylinder (hung from the bottom of stopper C with a platinum wire)

C: Hard-glass, ground stopper

D: Filter paper (Cut out a filter paper to make the shape given in the figure, and make folding lines as described by dot lines. The long end is used for light-off.)

(ii) Method

Sample Solution Place 50 mg of Calcium Saccharine, previous dried, on the center of the filter paper, and wrap carefully along with the folding lines without spilling. Place the wrapped sample into B (a platinum basket or platinum mesh cylinder), leaving the long end on the outside. Put 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid into flak A. Fill flask A with oxygen, moisten the ground part of stopper C, light off the long end (ignition site), and immediately place it into A. Keep flask A airtight until the combustion is completed. Shake A occasionally until the white fumes inside A completely disappear, then allow it stand for 15–30minutes. Pour 10 mL of water to the upper part of A, carefully remove the stopper, and transfer the solution in A to a beaker. Wash C, B, and the inside wall of A with 20 mL of water, and add the washings to the beaker. Boil the resulting solution gently for 10 minutes. Cool to room temperature, and use the sample solution.

Control Stock Solution Measure exactly 6 mL of Selenium Standard Stock Solution, and add water to make exactly 100 mL. To exactly 5 mL of this solution, add water to make exactly 200 mL. Take exactly 1 mL of the resulting solution into a beaker, and add 50 mL of diluted nitric acid (1 in 60).

Procedure Adjust the pH of each solution to 1.8–2.2 with ammonia solution, and add water to make about 60 mL each. Transfer them into separate separating funnels, wash the beakers with 10 mL of water each, and add the washings to the corresponding separate funnels. To each, add 0.2 g of hydroxylammonium chloride, and dissolve it while gently shaking. To each, add 5 mL of 2,3-diaminonaphthalene TS, shake, and allow to stand for 100 minutes. Add 5.0 mL of cyclohexane, and shake well for 2 minutes. Collect the cyclohexane layer, centrifuge at 3000 rpm for 10 minutes, and use the supernatants as the test solution and the control solution, respectively. Against the reference solution that is prepared in the same manner using 50 mL of diluted nitric acid (1 in 60) instead of the sample solution, measure the absorbance at the maximum at near 378 nm. The absorbance of the test solution is not greater than that of the control solution.

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Benzoic acid and salicylic acid Dissolve 0.5 g of Calcium Saccharine in 10 mL of water, and add 5 drops of acetic acid and 3 drops of a solution of iron(III) chloride hexahydrate (1 in 10). No precipitate is produced, and a violet to red-violet color is not formed.

(5) Toluenesulfonamides Not more than 25 μg/g as the sum of *o*-toluenesulfonamide and *p*-toluenesulfonamide.

Test Solution Dissolve 10.0 g of Calcium Saccharine in 50 mL of water. Extract three times with 30 mL of ethyl acetate each time, combine the ethyl acetate layers, wash with 30 mL of sodium chloride solution (1 in 4), and transfer the ethyl acetate layer to a dry flask. Add about 10 g of sodium sulfate, shake, and filter into an eggplant-shape flask.

Wash the residue on the filter twice with 10 mL of ethyl acetate each time, and add the washings to the flask. Concentrate under reduced pressure to remove the ethyl acetate. To the residue, add exactly 1.0 mL of a solution (1 in 4,000) of caffeine monohydrate in ethyl acetate, shake, and allow to stand for 1 minute. Use the resulting supernatant as the test solution. If necessary, centrifuge to obtain the supernatant.

Standard Solution Dissolve about 25 mg each of *o*-toluenesulfonamide and *p*-toluenesulfonamide, accurately weighed, in ethyl acetate to make exactly 100 mL. Evaporate exactly 1 mL of the resulting solution under reduced pressed to remove the ethyl acetate. Dissolve the residue by adding 1 ml of a solution (1 in 4,000) of caffeine monohydrate in ethyl acetate.

Procedure Analyze 1 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below.

Operating conditions

Detector: Flame ionization detector.

Column: Use a fused silica tube (0.32 mm internal diameter and 30 m length) coated with a 0.25 μm thick layer of a mixture of 5%diphenyl and 95%dimethylpolysiloxane for gas chromatography.

Column temperature: 185°C.

Injection port temperature: 250°C.

Carrier gas: Helium or nitrogen.

Flow rate: Adjust so that the peak of caffeine appears about 10 minutes after injection.

Injection method: Split.

Split ratio: 1:10.

Measure the peak area ratios of *o*-toluenesulfonamide to caffeine and *p*-toluenesulfonamide to caffeine for both test solution and standard solution, and designate the ratio as Q_{T1} and Q_{T2} for the test solution and as Q_{S1} and Q_{S2} for the standard solution, respectively. Determine the amount of toluenesulfonamides by the formula:

$$\text{Amount of toluenesulfonamides (\%)} = \left[\frac{Q_{T1}}{Q_{S1}} \times M_{S1} + \frac{Q_{T2}}{Q_{S2}} \times M_{S2}\right] \times \frac{1}{\text{Weight (g) of the sample}} \times 100$$

M_{S1} = weight (g) of *o*-toluenesulfonamide in 1 mL of the standard solution

M_{S2} = weight (g) of *p*-toluenesulfonamide in 1 mL of the standard solution

(6) Readily carbonizable substances Dissolve 0.20 g of Calcium Saccharine in 5 mL of sulfuric acid for readily carbonizable substances test, and keep at 48–50°C for 10 minutes. The color of the resulting solution is not deeper than that of Matching Fluid A.

Loss on Drying Not more than 15.0% (120°C, 4 hours).

Assay Weigh accurately about 0.3 g of Calcium Saccharine, previously dried, add 40 mL of acetic acid for nonaqueous titration to dissolve it, and titrate with 0.1 mol/L perchloric acid. Use a potentipmeter to confirm the endpoint. Separately, perform a blank test to make correction.

Each mL of 0.1 mol/L perchloric acid = 20.22 mg of $C_{14}H_8CaN_2O_6S_2$

Calcium Silicate

ケイ酸カルシウム

Calcium silicate [1344-95-2]

Definition Calcium Silicate is a compound consisting of silicon dioxide and calcium oxide.

Content Calcium Silicate, when dried, contains 50.0–95.0% of silicon dioxide (SiO_2 = 60.08) and 3.0–35.0% of calcium oxide (CaO = 56.08).

Description Calcium Silicate occurs as a white to grayish-white, hygroscopic fine powder.

Identification

(1) Mix 0.5 g of Calcium Silicate with 0.2 g of sodium carbonate and 2 g of potassium carbonate. Transfer the mixture into a platinum or nickel crucible, and heat until it melts completely. Cool, add 5 mL of water, and allow to stand for about 3 minutes. Heat the bottom of crucible gently to detach the melt, and transfer to a beaker with about 50 mL of water. Add gradually hydrochloric acid until effervescence no longer occurs, then add an additional 10 mL of hydrochloric acid, and evaporate the mixture to dryness on a water bath. Cool, add 20 mL of water, boil, and filter the mixture. Transfer the gelatinous residue on the filter paper into a platinum dish, and add 5 mL of hydrofluoric acid. The precipitate dissolves. Heat and hold in the vapors a glass stirring rod with a drop of water on the tip. The drop becomes turbid.

(2) Neutralize the filtrate obtained in Identification (1) with ammonia TS using 2 drops of methyl red TS as an indicator. Then add 10% hydrochloric acid TS dropwise until the solution is acid. On the addition of a solution of ammonium oxalate monohydrate (7 in 200), a white granule precipitate forms. This precipitate is insoluble in acetic acid but soluble in hydrochloric acid.

pH 8.4–12.5 (5% suspension).

Purity

(1) Lead Not more than 5 μg/g as Pb (5.0 g, Control Solution: Lead Standard Solution 10.0 mL, Flame Method).

Test Solution Weigh the specified amount of Calcium Silicate into a beaker, add 50

mL of diluted hydrochloric acid solution (1 in 4), and stir. Cover the beaker with a watch dish, and boil gently for 15 minutes. Filter the solution by suction through a quantitative filter paper (5C) into a 50-mL volumetric flask. Wash the beaker and the residue on the filter paper with hot water, and add the washings to the flask. Cool, and add diluted hydrochloric acid (1 in 4) to make exactly 50 mL.

Control Solution Measure exactly the specified amount of Lead Standard Solution, add diluted hydrochloric acid (1 in 4) to make 20 mL.

Procedure Measure the absorbance of the test solution and the control solution by Atomic Absorption Spectrophotometry (Flame Type) using the operating conditions given below. The absorbance value of the test solution is not more than that of the control solution.

Operating conditions

Light source: Lead hollow cathode lamp.

Analytical line wavelength: 217 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(2) Arsenic Not more than 3 μg/g as As (Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Use 5 mL of the test solution prepared in Purity (1).

(3) Fluoride Not more than 50 μg/g as F.

Test Solution Weigh exactly 2 g of Calcium Silicate into a polyethylene beaker, add 40 mL of water, and stir for 15 minutes. Transfer the suspension to a 50 mL volumetric flask, and add water to volume. Centrifuge the suspension, transfer exactly 30 mL of the supernatant solution to a polyethylene beaker, and add 15 mL of disodium dihydrogen ethylenediaminetetraacetate–Tris TS.

Control Solution Weigh 2.210 g of sodium fluoride, previously dried at 110°C for 2 hours, into a polyethylene beaker, and dissolve it in 200 mL of water with stirring. Transfer the solution to a 1000-mL polyethylene volumetric flask, and add water to volume. Use this solution as the control stock solution. Store the stock solution in a polyethylene bottle. Prepare the control solution fresh before use. To 2 mL of the control stock solution, measured exactly, add water to make exactly 1000 mL. Take exactly 30 mL of this solution into a polyethylene beaker, and add 15 mL of disodium dihydrogen ethylenediaminetetraacetate–Tris TS.

Procedure Measure the electrical potentials using a potentiometer with a fluorine ion indicator electrode and a silver-silver chloride reference electrode. The potential of the test solution is not less than that of the control solution.

Loss on Drying Not more than 10.0% (105°C, 2 hours).

Loss on Ignition 5.0–14.0% (1000°C, constant weight, dried sample).

Assay

(1) Silicon dioxide Weigh accurately about 0.4 g of Calcium Silicate, dried previously, into a beaker, add 5 mL of water and 10 mL of perchloric acid, and heat until white fumes are evolved. Cover the beaker with a watch dish, and heat for additional 15 minutes. Cool, and add 30 mL of water, and filter the contents through a quantitative filter paper (5C). Wash the residue on the filter paper with 200 mL of hot water, combine the washings with the filtrate, and refer to as Solution A. Transfer the residue with the filter paper into a platinum crucible, heat gently until the filter paper is carbonized. Cool, add a few drops of sulfuric acid, and ignite at 1,300°C to constant weight. Cool in a desiccator, and weigh the crucible with the residue (M g). To the residue, add 5 drops of sulfuric acid and 15 mL of hydrofluoric acid, heat at 1000°C to constant weight, and cool in a desiccator, and weigh the crucible (m g). Obtain the content of silicone dioxide by the formula:

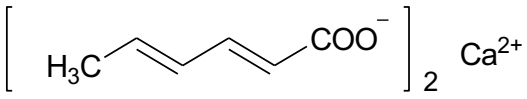
$$\text{Content (\%) of silicone dioxide } (SiO_2) = \frac{\text{M (g)} - \text{m (g)}}{\text{Weight (g) of the sample}} \times 100$$

(2) Calcium oxide Neutralize Solution A obtained in Assay (1) with sodium hydroxide solution (1 in 25), and add 15 mL of sodium hydroxide solution (1 in 25) and 0.3 g of NN indicator, and titrate with 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate. The end point is when the red-purple color of the solution completely disappears and changes to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.804 mg CaO

Calcium Sorbate

ソルビン酸カルシウム



$C_{12}H_{14}CaO_4$ Mol. Wt. 262.32

Monocalcium bis[(2*E*,4*E*)-hexa-2,4-dienoate] [7492-55-9]

Content Calcium Sorbate contains, when dried, contains 98.0–102.0% of calcium sorbate ($C_{12}H_{14}CaO_4$).

Description Calcium Sorbate occurs as a white, fine crystalline powder.

Identification

(1) To 2 mL of a solution of Calcium Sorbate (1 in 200), add 2 drops of bromine TS, and shake. The color of the solution disappears immediately.

(2) Calcium Sorbate responds to test (1) and a solution of Calcium Sorbate (1 in 200) responds to test (2) for Calcium Salt in the Qualitative Tests.

(3) To 100 mL of a solution Calcium Sorbate (1 in 200), add 15 mL of diluted hydrochloric acid (1 in 4). Filter the resulting precipitate with suction, wash well with water, and dry in a vacuum desiccator for 4 hours. Its melting point is 132–135°C.

Purity

(1) Fluoride Not more than 10 μg/g as F.

Test Solution Transfer 1.00 g of Calcium Sorbate into a beaker, add 10 mL of water, and stir for a few minutes. Add 20 mL of diluted hydrochloric acid (1 in 20) gradually to dissolve it. Boil this solution for 1 minute, transfer into a polyethylene beaker, and immediately cool with ice. Add 10 mL of a solution of disodium dihydrogen ethylenediaminetetraacetate dihydrate (1 in 40) and 15 mL of a solution of sodium citrate (1 in 4), and mix. Adjust the pH of the solution to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or a solution of sodium hydroxide (2 in 5). Transfer it into a 100-mL volumetric flask, and dilute with water to volume. Place about 50 mL of this solution in a polyethylene beaker.

Control Solution Weigh 2.210 g of sodium fluoride, previously dried at 110˚C for 2 hours, transfer into a polyethylene beaker, add 200 mL of water, and dissolve it while stirring. Transfer this solution into a 1000-mL volumetric flask, dilute with water to volume, and then store in a polyethylene bottle. Use this as the control stock solution. Prepare a control solution fresh before use. Transfer exactly 5 mL of the control stock solution into a 1000-mL volumetric flask, and dilute to volume with water. Transfer exactly 2.0 mL of this solution into a polyethylene beaker, add 10 mL of a solution of disodium dihydrogen ethylenediaminetetraacetate dihydrate (1 in 40) and 15 mL of a solution of trisodium citrate dihydrate (1 in 4), and mix. Adjust the pH of the solution to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or a solution of sodium hydroxide (2 in 5). Transfer this solution into a 100-mL volumetric flask, and dilute to volume with water. Place about 50 mL of the solution into a polyethylene beaker.

Procedure Measure the electric potentials of both solutions, using a potentiometer with a fluorine-ion indicator electrode and a silver/silver chloride reference electrode. The electric potential of the test solution is not lower than that of the control solution.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 4, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Aldehyde Not more than 0.1% as formaldehyde.

Test Solution Adjust the pH of a solution of Calcium Sorbate (3 in 500) to 4 with diluted hydrochloric acid (1 in 12), and filter. Use exactly 5 mL of the resulting solutionas the test solution.

Control Solution Measure exactly 2.5 mL of formaldehyde solution, and add water to make exactly 1000 mL. To exactly 3 mL of this solution, add water to make exactly

500 mL. Use exactly 5 mL of the resulting solution as the control solution.

Procedure Add 2.5 mL of fuchsin–sodium hydrogen sulfite TS to each of the test solution and control solution, and allow them to stand for 15–30 minutes. The color of the test solution is not darker than that of the control solution.

Loss on Drying Not more than 1.0% (105°C, 3 hours).

Assay Weigh accurately about 0.25 g of Calcium Sorbate, previously dried, add 35 mL of acetic acid and 4 mL of acetic anhydride, and dissolve it while heating at 45–50°C. After cooling, titrate with 0.1 mol/L perchloric acid using 2 drops of a solution (1 in 100) of crystal violet in acetic acid as the indicator until the blue color of the solution changes to green.

Each mL of 0.1 mol/L perchloric acid = 13.12 mg of $C_{12}H_{14}CaO_4$

Calcium Stearate

ステアリン酸カルシウム

Definition Calcium Stearate is a mixture of calcium salts consisting principally of stearic acid and palmitic acid.

Content Calcium Stearate, when calculated on the dried basis, contains 6.4–7.1% of calcium (Ca = 40.08).

Description Calcium Stearate occurs as a white, light, bulky powder. It is odorless or has a slight, characteristic odor.

Identification

(1) To 3.0 g of Calcium Stearate, add 20 mL of diluted hydrochloric acid (1 in 2) and 30 mL of diethyl ether, shake vigorously for 3 minutes, and allow to stand. The separated water layer responds to test (1) for calcium salts in the Qualitative Tests.

(2) Collect the diethyl ether layer obtained in Identification (1), and wash sequentially with a 20-mL potion of 10% hydrochloric acid TS, a 10-mL portion of dilute hydrochloric acid, and a 20-mL potion of water. Evaporate the diethyl ether on a water bath. The melting point of the residue is not lower than 54°C.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Calcium Stearate, add 5 mL of hydrochloric acid (1 in 2) and 20 mL of chloroform, shake vigorously for 3 minutes, allow to stand, and collect the water layer.

(3) Free fatty acids Not more than 3.0% as stearic acid.

Weigh accurately about 2 g of Calcium Stearate into a 100-mL Erlenmeyer flask, and add 50 mL of acetone. Heat under a condenser for 10 minutes in a water bath, and cool. Filter it through two-ply paper filter for quantitative analysis (5C), wash the inside of the flask, the residue, and the filter paper with 50 mL of acetone, and combine the washings with the filtrate. Add 2–3 drops of phenolphthalein TS and 5 mL of water, and titrate with 0.1 mol/L sodium hydroxide. Perform a blank test using a mixture of 100 mL of acetone and 5 mL of water.

Each mL of 0.1 mol/L sodium hydroxide = 28.45 mg $C_{18}H_{36}O_2$

Loss on Drying Not more than 4.0% (105°C, 3 hours).

Assay Weigh accurately about 0.5 g of Calcium Stearate in a crucible. Carefully heat gently at first, and then ignite in an electric furnace at 700°C for 3 hours to incineration. After cooling, add 10 mL of 10% hydrochloric acid TS to the residue, and warm on a water bath for 10 minutes. Transfer the contents of the crucible into a flask with the aid of two 10-mL potions of warm water and one 5-mL potion of warm water. Add sodium hydroxide TS (1 mol/L) until the solution is slightly turbid. Next, add 25 mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate, 10 mL of ammonium buffer (pH 10.7), 4 drops of eriochrome black T TS, and 5 drops of methyl yellow TS. Immediately titrate the excess disodium dihydrogen ethylenediaminetetraacetate with 0.05 mol/L magnesium chloride. The endpoint is when the green color of the solution disappears and a red color is produced. Separately, perform a blank test.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.004 mg of Ca

Calcium Stearoyl Lactylate

Calcium Stearoyl-2-lactylate

ステアロイル乳酸カルシウム

[5793-94-2]

Definition Calcium Stearoyl Lactylate is a mixture of calcium salts of stearoyl lactylic acids and minor proportions of related acids and calcium salts of these related acids. Its principal component is calcium salts of stearoyl lactylic acids.

Description Calcium Stearoyl Lactylate occurs as a white to yellowish powder or solid. It is odorless or has a characteristic odor.

Identification

(1) Ignite 1 g of Calcium Stearoyl Lactylate at 500°C for 1 hour, and dissolve the residue obtained in 5 mL of diluted hydrochloric acid (1 in 4). The solution responds to all the tests for Calcium Salt in the Qualitative Tests.

(2) To 2 g of Calcium Stearoyl Lactylate, add 10 mL of diluted hydrochloric acid (1 in

4), stir thoroughly, heat in a water bath, and filter while hot. Collect the residue on the filter paper, add 30 mL of sodium hydroxide solution (1 in 25), and heat in a water bath at a temperature of not lower than 95°C for 30 minutes while stirring. After cooling, add 20 mL of diluted hydrochloric acid (1 in 4), and extract twice with 30 mL of diethyl ether each time. Combine the diethyl ether extracts, wash with 20 mL of water, then dehydrate with sodium sulfate, and filter. Heat the filtrate on a water bath, remove the diethyl ether by evaporation, and measure the melting point of the residue. The melting point is 54–69°C.

(3) Calcium Stearoyl Lactylate responds to the test for Lactate in the Qualitative Tests.

Purity

(1) Acid value 50–86.

Test Solution Weigh about 0.5 g of powdered Calcium Stearoyl Lactylate, and dissolve it in 20 mL of a 1:1 mixture of ethanol (95)/diethyl ether mixture.

Procedure Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests. The endpoint is when a pink color persists for 20 seconds.

(2) Ester Value 125–164 (Fats and Related Substances Tests).

To determine the ester value, use the acid value obtained in Purity (1). Measure the saponification value as directed in the Saponification Value Test in the Fats and Related Substances Tests, using about 1 g of Calcium Stearoyl Lactylate, weighed accurately. In the saponification value test, be careful not to let the precipitate adhere to the wall of the flask when 3.5% (w/v) potassium hydroxide–ethanol TS is added. Perform the titration while the solution is hot.

(3) Total lactic acid 32–38% as lactic acid ($C_3H_6O_3$).

Test Solution Transfer 0.2 g of Calcium Stearoyl Lactylate, accurately weighed, into a 100-mL flask, add 10 mL of 3.5% (w/v) potassium hydroxide–ethanol TS and 10 mL of water, and heat under a reflux condenser in a water bath for 45 minutes. Wash the flask and condenser with 40 mL of water, add the washings to the flask, and heat until the amount of the solution is not more than one-third of the original volume. Mix with 6 mL of diluted sulfuric acid (1 in 2), add 25 mL of petroleum ether, shake well, transfer the entire amount to a separating funnel, and allow to stand to let it separate into two layers. Transfer the water layer to a 100-mL volumetric flask, wash the petroleum ether layer twice with 20 mL of water each time, adding the washings to the volumetric flask, and then add water to make exactly 100 mL. Measure accurately 1 mL of this solution, add water to make exactly 100 mL.

Calibration Curve Measure exactly 5 mL, 7 mL, and 10 mL of Lithium Lactate Standard Solution, and add water to each to make exactly 100 mL. Transfer exactly 1 mL of each solution into separate stoppered test tubes, measure the absorbance of them in the same manner as for the test solution, and prepare a calibration curve.

Procedure Measure accurately 1 mL of the test solution, place into a stoppered test tube, add 1 drop of a solution of copper(II) sulfate pentahydrate (1 in 8), and mix. Rapidly

add 9 mL of sulfuric acid, place the stopper loosely, heat for exactly 5 minutes in a water bath at 90°C, and immediately cool to 20°C in ice water. Add 0.2 mL of *p*-phenylphenol TS, shake well, and keep in a water bath at 30°C for 30 minutes. During that time, shake the contents two or three times. Heat in a water bath at 90ºC for exactly 90 seconds, immediately cool to room temperature in ice water, allow to stand for 30 minutes, and measure the absorbance at a wavelength of 570 nm. Use a reference solution prepared in the same manner as for the test solution, using 1.0 mL of water instead of the test solution.

Determine the amount (mg) of lactic acid in the test solution from the calibration curve and the absorbance of the test solution, and calculate the content of total lactic acid ($C_3H_6O_3$) by the formula:

$$\text{Amount (\%) of total lactic acid } (C_3H_6O_3) = \frac{\text{Weight (mg) of lactic acid in the test solution}}{\text{Weight (g) of the sample} \times 10} \times 100$$

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B)

Residue on Ignition 14.3–17.7% (800ºC).

Calcium Sulfate

硫酸カルシウム

$CaSO_4 \cdot 2H_2O$ Mol. Wt. 172.17

Calcium sulfate dihydrate [7778-18-9]

Content Calcium Sulfate contains 98.0–105.0% of calcium sulfate ($CaSO_4 \cdot 2H_2O$).

Description Calcium Sulfate occurs as a white crystalline powder.

Identification To 1 g of Calcium Sulfate, add 100 mL of water, shake well, and filter. The filtrate responds to all the tests for Calcium Salt and for Sulfate in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear.

Test Solution Weigh 0.20 g of Calcium Sulfate, add 10 mL of diluted hydrochloric acid (1 in 4), and dissolve by heating.

(2) Free alkali Weigh 0.5 g of Calcium Sulfate, add 100 mL of water, shake, and filter. Measure 10 mL of the filtrate, and add 1 drop of phenolphthalein TS. No pink

color develops.

(3) Chloride Not more than 0.21% as Cl.

Sample Solution Weigh 0.20 g of Calcium Sulfate, add 20 mL of water, shake well, and filter. Use 5 mL of the filtrate as the sample solution.

Control Solution Use 0.30 mL of 0.01 mol/L hydrochloric acid.

(4) Carbonate Weigh 0.5 g of Calcium Sulfate, and add 5 mL of diluted hydrochloric acid (1 in 4). No effervescence occurs.

(5) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Calcium Sulfate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, and add 30 mL of water. Use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate the solution to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool. Add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Method 2, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Ignition 18.0–24.0%.

Assay Weigh accurately about 1 g of Calcium Sulfate, add 40 mL of diluted hydrochloric acid (1 in 4), dissolve by heating on a water bath, and cool. Add water to make exactly 100 mL. Proceed as directed in Method 1 under Calcium Salt Determination, using this solution as the test solution.

Each mL of 0.05 mol/L disodium dihydrogen ethyelendiaminetetraacetate = 8.609 mg of $CaSO_4 \cdot 2H_2O$

Candelilla Wax

カンデリラロウ

Definition Candelilla Wax is obtained from the stems of the candelilla plant *Euphorbia antisyphilitica* Zucc. (*Euphorbia cerifera* Alcocer) and consists mainly of hentriacontane.

Description Candelilla Wax occurs as a light yellow to brown solid having a luster. When heat it, an aroma is evolved.

Identification Determine the absorption spectrum of Candelilla Wax as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference

Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 68–73°C.

Saponification Value 43–65. Weigh accurately about 1 g of Candelilla Wax, add exactly 50 mL of a 5:3 mixture of ethanol (95)/xylene and exactly 25 mL of a solution of 0.5 mol/L ethanolic potassium hydroxide. Heat under a reflux condenser for 1 hour with occasional shaking. Proceed as directed in the Saponification Value Test in the Fats and Related Substances Tests.

Purity

(1) Acid value 12–22.

Weigh accurately about 3 g of Candelilla Wax, dissolve it in 80 mL of a 5:3 mixture of ethanol (95)/xylene, and use the solution obtained as the test solution. Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests. When turbidity is produced while cool, titrate while warming.

(2) Ester Value 31–43 (Fats and Related Substances Tests).

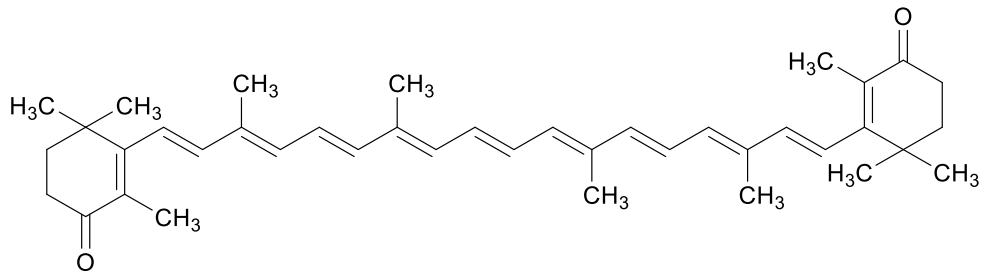
(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.3% .

Canthaxanthin

カンタキサンチン



$C_{40}H_{52}O_2$ Mol. Wt. 564.84

β,β-Carotene-4,4'-dione [514－78－3]

Content Canthaxanthin contains not less than 96.0% of canthaxanthin ($C_{40}H_{52}O_2$).

Description Canthaxanthin occurs as dark-purple crystals or crystalline powder.

Identification

(1) A solution (1 in 25,000) of Canthaxanthin in acetone develops an orange color. To 5 mL of this solution, add 1 mL of sodium nitrite solution (1 in 20) and 1 mL of sulfuric acid TS (0.5 mol/L). The solution is immediately decolored.

(2) A solution (1 in 400,000) of Canthaxanthin in cyclohexane exhibits an absorption maximum at a wavelength of about 470 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method) .

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Subsidiary Colors Not more than 5%.

Test Solution Weigh 20 mg of Canthaxanthin, and dissolve it in 25 mL of dichloromethane.

Procedure Perform thin-layer chromatography. Use a thin-layer plate coated with silica gel for thin-layer chromatography and dried at 110°C for 1 hour. Apply 400 μl of the test solution in an about 3 mm-wide strip on the starting line on the thin-layer plate. Develop using a 95:5 mixture of dichloromethane and diethyl ether as the developing solvent and using no control solution. Stop the development when the solvent front has ascended to a point about 15 cm above the starting line, and air-dry the plate. Scrap the darkest colored part—which contains the main constituent—off the plate, and transfer in a centrifuging tube. Add exactly 40 mL of dichloromethane, and shake for 10 minutes, and centrifuge. Measure exactly 10 mL of the supernatant, and add dichloromethane to make exactly 50 mL. Refer to this solution as Solution A. Similarly, scrap off the other colored part into a centrifuging tube, add exactly 20 mL of dichloromethane, shake for 10 minutes, and centrifuge. Refer to the resulting supernatant as Solution B. Measure the absorbance of Solutions A and B (A_A and A_B) at a wavelength of 485 nm against dichloromethane. Determine the amount of the subsidiary colors by the following formula.

Note: The above procedure should be conducted while avoiding light exposure.

$$\text{Amount (\%)} = \frac{A_B}{A_A \times 10 + A_B} \times 100$$

Loss on Drying Not more than 0.10%.

Assay Weigh accurately about 50 mg of Canthaxanthin, dissolve it in 10 mL of chloroform, and add cyclohexane to make exactly 50 mL. Measure exactly 5 mL of this solution, and add cyclohexane to make exactly 100 mL. To exactly 5 mL of the second solution, add cyclohexane to make exactly 100 mL. Measure the absorbance (A) of the resulting solution at the maximum at about 470 nm.

$$\text{Content (\%) of canthaxanthin } (C_{40}H_{52}O_2) = \frac{200}{\text{Weight (g) of the sample}} \times \frac{A}{2200} \times 100$$

Storage standards Store in a hermetic container, protected from light, under inert gas.

Caramel I (Plain caramel)

カラメル I

[8028-89-5]

Definition Caramel I is produced by heating edible carbohydrates, including starch hydrolysates, molasses, and sugars, with or without acids or alkalis. No ammonium or sulfite compounds are used.

Description Caramel I occurs as a dark brown to black powder, as lumps, or as a paste or liquid. It is odorless or has a slight, characteristic odor. It is tasteless or has a slight, characteristic taste.

Identification

(1) A solution of Caramel I (1 in 100) is light brown to black-brown.

(2) Prepare a solution of Caramel I with an approximate absorbance of 0.5 at a wavelength of 560 nm by transferring an appropriate amount of the sample into a 100-mL volumetric flask. Add hydrochloric acid TS (0.025 mol/L) to make exactly 100 mL. Centrifuge if necessary, and refer to the supernatant as Solution A. To 20 mL of Solution A, add 0.20 g (0.7 meq/g exchange-capacity; the amount of use should be adjusted in proportion to the cellulose-exchange capacity) of weakly basic DEAE–cellulose anion exchanger (–O–C_2H_4–$N(C_2H_5)_2$), shake well, and centrifuge. Collect the supernatant, and refer to as Solution B. Determine the absorbance (A_A and A_B) of Solution A and Solution B in a 1-cm cell at a wavelength of 560 nm, using hydrochloric acid TS (0.025 mol/L) as the reference. $(A_A - A_B) / A_A$ is not more than 0.75.

(3) Weigh 0.20–0.30 g of Caramel I, and add hydrochloric acid TS (0.025 mol/L) to make exactly 100 mL. Centrifuge if necessary, and refer to the supernatant as Solution C. To 40 mL of Solution C, add 2.0 g (0.85 meq/g exchange-capacity, amount of use should be adjusted in proportion to the cellulose-exchange capacity) of strongly acidic phosphorylated cellulose cation exchanger (–O–PO_3H_2), shake well, and centrifuge. Collect the supernatant, and refer to it as Solution D. Determine the absorbance (A_C and A_D) of Solution C and Solution D in a 1-cm cell at a wavelength of 560 nm, using hydrochloric acid TS (0.025 mol/L) as the reference. $(A_C–A_D)/A_C$ is not more than 0.50.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 0.8 μg /g as As (2.5 g, Method 3, Standard Color: Arsenic Standard Solution 4.0 mL, Apparatus B).

(3) Solid content Not less than 55%.

Weigh 30.0 g of sea sand, transfer to a weighing dish, and weigh accurately the total mass (M_s). Weigh accurately 1.5 to 2.0 g (M_c) of Caramel I, transfer to the weighing dish, mix thoroughly with small amount of water, and evaporate to dryness on a water bath. Dry under reduced pressure at 60°C for 5 hours until a constant weight is attained, weigh accurately the mass (M_f), and calculate the solid content by the formula:

$$\text{Solid content (\%)} = [(M_f - M_s) / M_c)] \times 100$$

(4) Total sulfur Not more than 0.3% (on a solid basis).

Place 1 to 3 g of magnesium oxide or 6.4–19.2 g of magnesium nitrate hexahydrate, 1 g of sucrose, and 50 mL of nitric acid in an evaporating dish. Add 5–10 g of Caramel I, and evaporate on a water bath to paste. Place the evaporating dish in an unheated electric muffle (ordinary temperature), and gradually heat (not higher than 525°C) until all nitrogen dioxide fumes are driven off. Cool the evaporating dish, add diluted hydrochloric acid (2 in 5) to dissolve the residue, and neutralize the residue, and then again add 5mL of the same hydrochloric acid. Filter, heat to boiling, and add dropwise 5 mL of a solution of barium chloride dihydrate (1 in 10). Evaporate to 100 mL, allow to stand overnight, filter using filer paper for quantitative analysis (5C), and wash with warm water. Transfer the filter paper and the residues in a previously weighed crucible, ignite to constant weight, and weigh accurately as barium sulfate. Calculate the total sulfur by the formula below, and calculate on the solid basis. Perform a blank test in the same manner.

$$\text{Total sulfur (\%)} = \frac{\text{Weight of barium sulfate (g)} \times 0.1374}{\text{Weight (g) of the sample}} \times 100$$

(5) Total nitrogen Not more than 4.0% (on a solid basis).

Weigh accurately about 1 g of Caramel I, and proceed as directed in the Kjeldahl Method under Nitrogen Determination.

(6) 4-Methylimidazole

Test Solution Weigh accurately an amount of Caramel I equivalent to about 10 g of solids into a 150-mL polypropylene beaker, add 5 mL of sodium hydroxide TS (3.0 mol/L), mix, and adjust the pH to 12 or more. To the beaker, add 20 g of diatomaceous earth for chromatography, and mix the contents until a semidry mixture is obtained. Fill the mixture into a glass tube for chromatography (with a teflon stopcock, about 2 cm internal diameter) whose bottom is filled with the glass wool so that the mixture is about 25 cm high. Wash the polypropylene beaker with ethyl acetate, and pour the contents into the glass tube. When the ethyl acetate reaches the bottom of the glass tube, close the stopcock, and allow to stand for 5 minutes. Open the stopcock, and pour ethyl acetate into the glass tube until the total volume of effluent is about 200 mL. To the effluent collected, add exactly 1 mL of the internal standard solution. Transfer the contents to an

eggplant-shape flask, and evaporate the ethyl acetate at below 35°C. Dissolve the residue by adding acetone, and make exactly 5 mL.

Standard Solution Weigh accurately about 20 mg of 4-methylimidazole, add 20 mL of the internal standard, and add acetone to dissolve it and make 100 mL.

Internal Standard To 50 mg of 2-methylimidazole, add ethyl acetate to dissolve, and make exactly 50 mL.

Procedure Analyze 5 µl each of the test solution and the standard solution by gas chromatography using the operating conditions given below. No peak of 4-methylimidazole is observed for the test solution.

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube (4 mm internal diameter and 1 m length).

Column packing material

Liquid phase: A mixture of 7.5% polyethylene glycol 20M/2% potassium hydroxide of the amount of the solid support.

Solid support: 150- to 160-µm diatomaceous earth for gas chromatography.

Column temperature: 180°C.

Injection port temperature: 200°C.

Carrier gas: Nitrogen.

Flow rate: 50 mL/minute.

Caramel II (Sulfite caramel)

カラメル II

[8028-89-5]

Definition Caramel II is produced by heating edible carbohydrates, including starch hydrolysates, molasses, and sugars, in the presence of sulfite compounds, with or without acids or alkalis. No ammonium compounds are used.

Description Caramel II occurs as a dark brown to black powder, as lumps, or as a paste or liquid. It is odorless or has a slight, characteristic odor. It is tasteless or has a slight, characteristic taste.

Identification

(1) A solution of Caramel II (1 in 100) is light brown to black-brown.

(2) Proceed as directed in Identification (2) for Caramel I. The value is not less than 0.50.

(3) Weigh 0.10 g of Caramel II, add water to make exactly 100 mL, and centrifuge if

necessary. Refer to the supernatant as Solution A. Measure 5 mL of Solution A, add water to make exactly 100 mL, and refer to as Solution B. Determine the absorbance (A_A) of Solution A in a 1-cm cell at a wavelength of 560 nm against water and the absorbance (A_B) of Solution B in a 1-cm cell at a wavelength of 280 nm against water. $A_B \times 20 / A_A$ is not less than 50.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 0.8 μg/g as As (2.5 g, Method 3, Standard Color: Arsenic Standard Solution 4.0 mL, Apparatus B).

(3) Solid content Not less than 65%.

Proceed as directed in Purity (3) for Caramel I.

(4) Total sulfur Not more than 2.5% (on a solid basis).

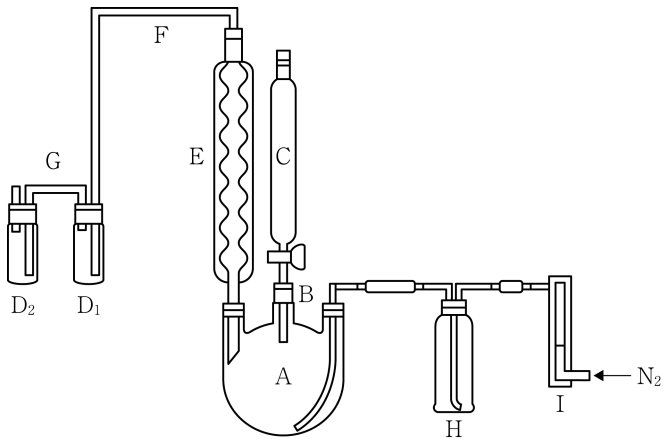
Proceed as directed in Purity (4) for Caramel I.

(5) Total Nitrogen Not more than 0.2% (on a solid basis).

Proceed as directed in Purity (5) for Caramel I.

(6) Sulfur dioxide Not more than 0.2% (on a solid basis).

(i) Apparatus Use the apparatus as illustrated in the figure.



A: Three-neck flask (1L)

B: Stopper (silicon)

C: Separator (100-mL capacity cylindrical separator)

D_1, D_2: Receivers (50-mL capacity centrifuge tubes)

E: Allihn condensor (300-mm diameter)

F, G: Joint tube

H: Glass scrubber (250-mL capacity)

I: Flow meter

(ii) Method　Place 180 mL of water and 25 mL of phosphoric acid (1 in 4) into three-neck flask A. Place 20 mL of the hydrogen peroxide TS in each of receivers D_1 and D_2. Heat flask A while passing through nitrogen gas (from which oxygen has been removed by pyrogallol TS (alkaline)) at a rate of 200 ± 10 mL/minute and controlling the temperature of the mantle heater to make the water droplet from condenser E at 80 to 90 drops/minute. Boil for about three minutes and cool. Weigh accurately about 10 g of Caramel II, and transfer immediately to flask A. While flowing the nitrogen gas as described above at a rate of 200 ± 10 mL/minute, heat flask A to boil gently, and continue to heat for 60 minutes. Then stop supplying water to condenser E, and continue to heat for a while until water droplet appears on the inside of connecting tube F near condenser E and the temperature of the upper part of condenser E reaches 60 to 70°C. Remove receivers D_1 and D_2, wash joint tubes G and F with small amount of water, and transfer the condensed fluid in the receivers to a beaker. Add 2 drops of methyl red TS, then add sodium hydroxide TS (1 mol/L) until the solution changes to yellow. To this solution add 4 drops of hydrochloric acid TS (1 mol/L), boil, and add gradually 2 mL of a solution of barium chloride dihydrate (1 in 6). Heat the solution on the water bath for one hour, cool, and allow to stand overnight. Filter the solution using a filter paper for quantitative analysis (5C), wash the residues on the filter paper with warm water until the washings do not respond to the test of chloride. Dry the residues as well as the filter paper, ignite to constant weight, weigh accurately as the barium sulfate, and calculate according to the formula below. Calculate further on the basis of solid content.

$$\text{Content (\%) of sulfure dioxide } (SO_2) = \frac{\text{Weight (g) of barium sulfate} \times 0.2745}{\text{Weight (g) of the sample}}$$

Caramel III (Ammonia caramel)

カラメル III

[8028-89-5]

Definition　Caramel III is produced by heating edible carbohydrates, including starch hydrolysates, molasses, and sugars, in the presence of ammonium compounds, with or without acids or alkalis. No sulfite compounds are used.

Description　Caramel III occurs as a dark brown to black, powder, as lumps, or as a paste or liquid. It is odorless or has a slight, characteristic odor. It is tasteless or has a slight, characteristic taste.

Identification

(1) A solution of Caramel III (1 in 100) is light brown to black-brown.

(2) Proceed as directed in Identification (2) for Caramel I. The value is not more than 0.50.

(3) Proceed as directed in Identification (3) for Caramel I. The value is not less than 0.50.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 0.8 μg/g as As (2.5 g, Method 3, Standard Color: Arsenic Standard Solution 4.0 mL, Apparatus B).

(3) Solid content Not less than 53%.

Proceed as directed in Purity (3) for Caramel I.

(4) Ammoniacal nitrogen Not more than 0.4% (on a solid basis).

Add 25 mL of 0.05 mol/L sulfuric acid to a 500-mL receiving flask, and connect it to a distillation apparatus consisting of a Kjeldahl flask connecting bulb and a condenser such that the condenser delivery tube is immersed beneath the surface of the acid solution in the receiving flask. Weigh accurately about 2 g of Caramel III, transfer it into an 800-mL Kjeldahl flask, and add 2 g of magnesium oxide, 200 mL of water, and several boiling chips. Shake the flask well to mix the contents, and then quickly connect it to the distillation apparatus. Heat the Kjeldahl flask to boiling, and collect about 100 mL of distillate into the receiving flask. Wash the tip of the delivery tube with 2–3 mL of water, collecting the washings into the receiving flask, then add 4 or 5 drops of methyl red TS, and titrate with 0.1 mol/L sodium hydroxide. Express the volume (mL) of 0.1 mol/L sodium hydroxide consumed as S. Conduct a blank test in the same manner, and express the volume (mL) consumed as B. Calculate the content of ammoniacal nitrogen in the sample using the following formula. Determine the content on the basis of solid content.

$$\text{Content (\%) ammoniacal nitrogen} = \frac{(B - S) \times 0.0014}{\text{Weight (g) of the sample}} \times 100$$

(5) Total sulfur Not more than 0.3% (on a solid basis).

Proceed as directed in Purity (4) for Caramel I.

(6) Total nitrogen Not more than 6.8% (on a solid basis).

Weigh accurately about 0.5 g of Caramel III, and proceed as directed in the Kjeldahl Method under Nitrogen Determination.

(7) 4-Methylimidazole Not more than 0.30 mg/g (on a solid basis).

Prepare a test solution as directed in Purity (6) for Caramel I.

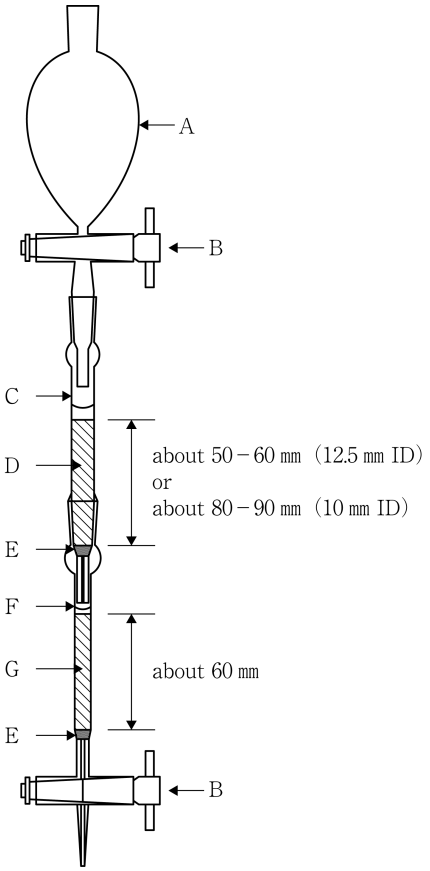
Standard Solutions Weigh accurately about 20 mg, 60 mg, and 0.1 g of 4-methylimidazole, and add exactly 20 mL of the internal standard to each. Dissolve separately by adding acetone, and make exactly 100 mL of each.

Internal Standard　To 0.10 g of 2-methylimidazole, accurately weighed, add ethyl acetate to dissolve, and make exactly 100 mL.

Procedure　Analyze 5 µl each of the test solution and the standard solutions by gas chromatography using operating conditions specified in Purity (6) for Caramel I. Measure the peak area ratios of 4-methylimidazole to 2-methylimidazole for the standard solutions and the concentrations of 4-methylimidazole in the standard solutions, and prepare a calibration curve. Calculate the 4-methylimidazole content from the peak area ratio of 4-methylimidazole to 2-methylimidazole for the test solution and the calibration curve.

(8) 2-Acetyl-4-tetrahydroxybutylimidazole　Not more than 40 µg/g (on a solid basis).

(i) Apparatus　Use a combination column as illustrated below. All joint parts should be made from ground glass.



A: Dropping funnel (100 mL capacity)

B: Teflon stopcock

C: Glass column (12.5 mm internal diameter, 150 mm length including the joint parts; or 10 mm internal diameter, 200 mm length including the joint parts)

D: Weakly acidic cation-exchange resin (fine particle)

E: Cotton stopper

F: Glass column (10 mm internal diameter, 175 mm length including the joint parts)

G: Strongly acidic cation-exchange resin (fine particle)

(ii) Method

Test solution Weigh accurately 0.20–0.25 g of Caramel III, and dissolve it in 3 mL of water. Use this as the sample solution. Quantitatively transfer the sample solution directly to glass column C of the combination column. Wash the column with about 100 mL of water. Disconnect the column, and connect dropping funnel A to glass column F. Next, run hydrochloric acid TS (0.5 mol/L) through column F. Discard the first 10 mL of eluate, and collect the following 35 mL of eluate. Concentrate the solution to dryness at 40°C, 2.0 kPa. Dissolve the syrup residue in 0.25 mL of methanol and add 0.25 mL of 2,4-dinitrophenylhydrazine hydrochloride TS. Transfer the reaction mixture to a septum-capped vial, and allow to stand for 5 hours at room temperature.

Standard Solutions Weigh accurately about 10 mg of 2-acetyl-4-tetrahydroxybutylimidazole-2,4-dinitrophenylhydrazone, and dissolve it in methanol to make exactly 100 mL. Dilute appropriate portions of this solution with methanol, and prepare standard solutions containing 2-acetyl-4-tetrahydroxybutylimidazole-2,4-dinitrophenylhydrazone at 0 μg/mL, 20 μg/mL, 40 μg/mL, 60 μg/mL, 80 μg/mL, and 0.1 mg/mL.

Procedure Analyze 5 μL each of the test solution and the standard solutions by liquid chromatography using the conditions given below. Measure the peak areas of the standard solutions, and prepare a calibration curve. Measure the peak area of the test solution, and then determine the amount of 2-acetyl-4-tetrahydroxybutylimidazole. 0.1 mg/mL of 2-acetyl-4-tetrahydroxybutylimidazole-2,4-dinitrophenylhydrazone is equivalent to 47.58 μg/mL of 2-acetyl-4-tetrahydroxybutylimidazole.

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength 385 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 10-μm octylsilanized silica gel for liquid chromatography.

Column temperature: Room temperature.

Mobile phase: A 1:1 mixture of diluted phosphoric acid (17 in 2500)/methanol.

Flow rate: Adjust the retention time of 2-acetyl-4-tetrahydroxybutylimidazole-2,4-dinitrophenylhydrazone to 6.3 ± 0.1 minutes.

Caramel IV (Sulfite ammonia caramel)

カラメル IV

[8029-89-5]

Definition Caramel IV is produced by heating edible carbohydrates, including starch hydrolysates, molasses, and sugars, in the presence of both sulfite and ammonia compounds, with or without acids or alkalis.

Description Caramel IV occurs as a dark brown to black powder, as lumps, or as a paste or liquid. It is odorless or has a slight, characteristic odor. It is tasteless or has a slight, characteristic taste.

Identification

(1) A solution of Caramel IV (1 in 100) is light brown to black-brown.

(2) Proceed as directed in Identification (2) for Caramel I. The value is not less than 0.50.

(3) Proceed as directed in Identification (3) for Caramel II. The value is not more than 50.

Purity

(1) Lead Not more than 2 μg/g (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 0.8 μg/g as As (2.5 g, Method 3, Standard Color: Arsenic Standard Solution 4.0 mL, Apparatus B).

(3) Solid content Not less than 40%.

Proceed as directed in Purity (3) for Caramel I.

(4) Ammoniacal nitrogen Not more than 2.8% (on a solid basis).

Proceed as directed in Purity (4) for Caramel III.

(5) Total sulfur Not more than 10.0% (on a solid basis).

Proceed as directed in Purity (4) for Caramel I.

(6) Total Nitrogen Not more than 7.5% (on a solid basis).

Proceed as directed in Purity (6) for Caramel III.

(7) Sulfur dioxide Not more than 0.5% (on a solid basis).

Proceed as directed in Purity (6) for Caramel II.

(8) 4-Methylimidazole Not more than 1.0mg/g (on a solid basis).

Proceed as directed in Purity (7) for Caramel III. For the standard solutions, prepare according to the following method: Weigh accurately about 20 mg, 60 mg, 0.1 g, and 0.2 g of 4-methylimidazole, to each add exactly 20 mL of the internal standard prepared according to purity (7) for Caramel III, and separately dissolve by adding acetone to

make exactly 100 mL of each.

Carbon Dioxide

Carbonic Acid Gas

二酸化炭素

CO_2 Mol. Wt. 44.01

Carbon dioxide [124-38-9]

Content Carbon Dioxide contains not less than 99.5% (vol) of carbon dioxide (CO_2).

Description Carbon Dioxide is a colorless gas. It is odorless.

Identification Pass Carbon Dioxide through calcium hydroxide TS. A white precipitate is formed. Collect the precipitate, and add diluted acetic acid (1 in 4). It dissolves with effervescence.

Purity The amounts of Carbon Dioxide to be weighed in the purity tests are expressed as numbers of milliliters at a temperature of 20°C and an atmospheric pressure of 101.3 kPa.

(1) Free acid Transfer 50 mL of freshly boiled and cooled water into a Nessler tube. Insert a gas induction tube (with the internal diameter of about 1 mm) into the Nessler tube, keep the end of the gas induction tube at a position within 2 mm above the bottom of the Nessler tube, pass 1000 mL of Carbon Dioxide through in 15 minutes, and add 0.1 mL of methyl orange TS. The color of solution is not darker than that of a control solution prepared by adding 0.1 mL of methyl orange TS and 50 mL of freshly boiled and cooled water to 1.0 mL of 0.01 mol/L hydrochloric acid.

(2) Hydrogen phosphide, hydrogen sulfide, and reducing organic substances

Transfer 25 mL of silver nitrate–ammonia TS and 3 mL of ammonia TS into a Nessler tube, and pass 1000 mL of Carbon Dioxide through in the same manner as (1) above, protecting from light. No brown color develops.

(3) Carbon monoxide Analyze 5-mL portions of Carbon Dioxide, introduced using a gas measuring tube or injection syringe for gas chromatography, by gas chromatography under the conditions given below. The peak is not observed at the peak position corresponding to carbon monoxide.

Operating Conditions

Detector: Thermal conductivity detector. The height of the peak on the recording paper is not less than 50% of the full scale when 4 mL of hydrogen or helium containing 0.02% (vol) of nitrogen is introduced.

Column: A glass or stainless steel tube (3–4 mm internal diameter and 1–3 m length).

Column packing material: 297- to 500-μm zeolite for gas chromatography.

Column temperature: A constant temperature of around 40°C.

Carrier gas: Hydrogen or helium.

Flow rate: A constant flow rate of 30–80 mL/minute.

Assay For sampling, follow the requirements directed under Purity. Transfer potassium hydroxide solution (1 in 3) into a gas pipet with a suitable capacity. Exactly take an amount not less than 100 mL of Carbon Dioxide into a gas burette with a capacity of not less than 100 mL, previously filled with sodium chloride solution (3 in 10). Transfer it into the gas pipet, and shake well. When the volume of the gas not absorbed is constant, measure the volume and express as V (mL), and calculate the content by the formula:

$$\text{Content (\% (vol)) of carbon dioxide } (CO_2) = \frac{\text{Volume (mL) of the sample} - \text{V (mL)}}{\text{Volume (mL) of the sample}} \times 100$$

Carboxypeptidase

カルボキシペプチダーゼ

Definition Carboxypeptidase includes enzymes that degrade proteins and peptides from the carboxy terminus. It is derived from the seed coats and bran of the wheat plant *Triticum aestivum* L. or the culture of filamentous fungi (limited to species of the genus *Aspergillus*), yeasts (limited to *Pseudozyma hubeiensis* and *Saccharomyces cerevisiae*), or actinomycetes (limited to *Streptomyces avermitilis*, *Streptomyces cinnamoneus*, *Streptomyces griseus*, *Streptomyces thermoviolaceus*, and *Streptomyces violaceoruber*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Carboxypeptidase occurs as white to dark brawn granules, powder, or paste, or as a colorless to dark brawn liquid. It is odorless or has a characteristic odor.

Identification Carboxypeptidase complies with the Carboxypeptidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid　Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture　Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Carboxypeptidase Activity Test　Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution　Weigh 0.50 g of Carboxypeptidase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water to the resulting solution.

Substrate Solution　Weigh 23 mg of *N*-carbobenzoxy-L-glutamyl-L-tyrosine, dissolve it in 5 mL of methanol, add 10 mL of acetic acid–sodium hydroxide buffer (0.5 mol/L) at pH 3.5 and water to make 100 mL.

Test Solution　Transfer 1 mL of the substrate solution into a test tube, equilibrate it at 40°C for 5 minutes, add 0.1 mL of the sample solution, and immediately shake. Cover the mouth of the test tube with a glass bead, incubate at 40°C for 20 minutes, add 0.5 mL of ninhydrin TS, immediately shake, and heat the mixture in a water bath for 15 minutes.　After cooling, add 5 mL of water to the resulting solution, shake, and allow to stand for 5 minutes.

Control Solution　Transfer 1 mL of the substrate solution into a test tube, equilibrate it at 40°C for 5 minutes, add 0.5 mL of ninhydrin TS and 0.1 mL of the sample solution, and immediately shake. Cover the mouth of the test tube with a glass bead, heat in a water bath for 15 minutes, and cool. To this solution, add 5 mL of water, shake, allow it to stand for 5 minutes.

Procedure　Measure the absorbance of the test solution and the control solution at a wavelength of 570 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Carnauba Wax

Brazil Wax

カルナウバロウ

[8015-86-9]

Definition Carnauba Wax is obtained from the leaves of the Brazilian wax palm *Copernicia prunifera* (Mill.) H.E.Moore (*Copernicia cerifera* (Arruda) Mart.) and consists mainly of ceryl hydroxycerotate.

Description Carnauba Wax occurs as a light yellow to light brown, hard, brittle solid having clear fracture faces, and has an aroma.

Identification Determine the absorption spectrum of Carnauba Wax as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 80–86°C.

Saponification value 78–95.

Weigh accurately about 1 g of Carnauba Wax, add exactly 50 mL of a 5:3 mixture of ethanol (95)/xylene and exactly 25 mL of 0.5 mol/L ethanolic potassium hydroxide. Heat under a reflux condenser for 1 hour with occasional shaking.

Proceed as directed in the Saponification Value Test in the Fats and Related Substances Tests.

Purity

(1) Acid value Not more than 10.

Weigh accurately about 1 g of Carnauba Wax, dissolve it in 80 mL of a 5:3 mixture of ethanol (95)/xylene, and use as the test solution. Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests. When turbidity is produced while the solution is cool, titrate while warming.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.25%.

Carob Bean Gum

Locust Bean Gum

カロブビーンガム

Definition Carob Bean Gum is obtained by grinding or dissolving, and precipitating the seed endosperm of the locust tree *Ceratonia siliqua* (L.). It may contain sucrose, glucose, lactose, dextrin, or maltose.

Description Carob Bean Gum occurs as a white to light yellow-brown powder or as granules. It is odorless or has slightly odor.

Identification

(1) To 2 g of Carob Bean Gum, add 4 mL of 2-propanol, and stir vigorously. Add 200 mL of water with vigorous stirring, and continue the stirring until the gum is completely dispersed. A slightly viscous solution is formed. Heat 100 mL of the solution on the water bath for about 10 minutes, and cool to room temperature. The solution is more viscous than before heating.

(2) To 10 mL of the viscous solution obtained finally in Identification (1), add 2 mL of a solution of sodium tetraborate decahydrate (1 in 20), mix, and allow to stand. A gel is formed.

Purity

(1) Protein Not more than 7.0%.

Weigh accurately about 0.2 g of Carob Bean Gum, and proceed as directed in the semi-micro Kjeldahl Method under Nitrogen Determination.

Each mL of 0.005 mol/L sulfuric acid = 0.8754 mg of protein

(2) Acid-insoluble substances Not more than 4.0%.

Proceed as directed in Purity (4) for Semirefined Carrageenan.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Starch Weigh 0.10 g of Carob Bean Gum, add 10 mL of water, and heat to boiling. After cooling, add 2 drops of Iodine TS. No blue color develops.

(6) 2-Propanol Not more than 1.0%.

(i) Apparatus Use the apparatus illustrated in Purity (7) for Semirefined Carrageenan.

(ii) Method

Test Solution Weigh accurately about 2 g of Carob Bean Gum in eggplant-shaped flask A, add 200 mL of water, a few boiling chips, and 1 mL of silicone resin, and stir well. Place exactly 4 mL of the internal standard solution in volumetric flack E, and assemble the apparatus. Moisten the joint parts with water. Distill at a rate of 2 to 3 mL/minute, taking care not to allow bubbles to get in delivery tube C, and collect about 90 mL of distillate. To the distillate, add water to make exactly 100 mL. Use 2-methyl-2-propanol solution (1 in 1000) as the internal standard solution.

Standard Solution Weigh accurately about 0.5 g of 2-propanol, and add water to make exactly 50 mL. Measure exactly 5 mL of this solution, and add water to make exactly 50 mL. Next, measure exactly 20 mL of the second solution and 4 mL of the internal standard solution in a 100-mL volumetric flask, and add water to volume.

Procedure Analyze 2.0 μL each of the test solution and the standard solution by gas chromatography using the operating conditions below. Determine the peak area ratios

(Q_T and Q_S) of 2-propanol to 2-methyl-2-propanol for the test solution and the standard solution, respectively. Obtain the content of 2-propanol by the formula:

$$\text{Content (\%) of 2-propanol} = \frac{\text{Weight (g) of 2-propanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 4$$

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube (3 mm internal diameter and 2 m length).

Column packing material: 180- to 250-μm styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Injection port temperature: A constant temperature of about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention time of 2-propanol to about 10 minutes.

Loss on Drying Not more than 14.0% (105°C, 5 hours).

Ash Not more than 1.2% (800°C, 3–4 hours).

Microbial limit Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 2 for total plate count and the enumeration of yeasts and molds. *Pre-enrichment Culture* For the *Escherichia coli* test, prepare as directed in Method 2. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 5 g of Carob Bean Gum with 500 mL of lactose broth to disperse completely, and incubate at 35 ± 1°C for 24 ± 2 hours.

Carob Germ Color

カロブ色素

Definition Carob Germ Color is obtained by grinding the seed germs of the locust tree *Ceratonia siliqua L.* It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Carob Germ Color is not less than 30 and is in the range of 90–110% of the labeled value.

Description Carob Germ Color occurs as a light yellow to light yellow-brown powder or as granules. It has a slight characteristic odor.

Identification

(1) Weigh an amount of Carob Germ Color equivalent to 0.5 g of carob germ color with a Color Value 30, add 50 mL of 70% (vol) methanol, shake, and centrifuge. The resulting supernatant is light yellow to yellow.

(2) To the supernatant obtained in Identification (1), add sodium hydroxide solution (1 in 20) to make it alkaline. It turns dark yellow.

(3) To the supernatant obtained in Identification (1), add diluted hydrochloric acid (1 in 3) to make it acidic. It becomes colorless.

(4) To 5 mL of the supernatant obtained in Identification (1), add 1 mL of iron(III) chloride hexahydrate solution (1 in 10). It turns to yellow-brown.

(5) Weigh an amount of Carob Germ Color equivalent to 0.1 g of carob germ color with a Color Value 30, add 100 mL of sodium hydroxide solution (1 in 1250), and filter the mixture through a filter paper for quantitative analysis (5C). The resulting solution exhibits an absorption maximum at a wavelength of 385–400 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Starch Weigh the equivalent of 0.10 g of Carob Germ Color with a Color Value 30, add 10 mL of water, and heat to boiling. After cooling, add 2 drops of iodine TS. A blue color does not develop.

Loss on Drying Not more than 12.0% (105°C, 5 hours).

Ash Not more than 8.0%.

Color Value Determination

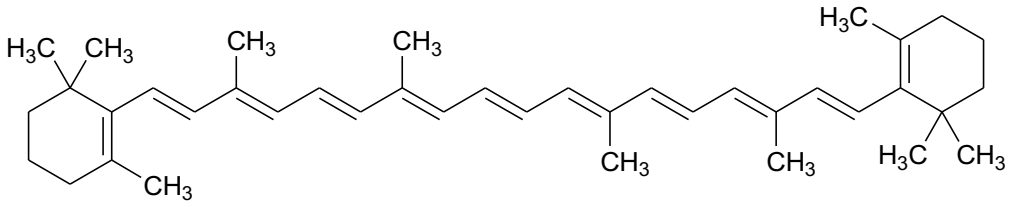
Test Solution Weigh accurately about 0.5 g of Carob Germ Color, and add 70% (vol) methanol to make exactly 50 mL. Sonicate this solution for 10 minutes, and centrifuge at 5000 rpm for 10 minutes. To exactly 5 mL of the supernatant, add sodium hydroxide TS (0.01 mol/L) to make exactly 50mL. If it is turbid, filter through a membrane filter (0.20 μm pore size).

Procedure Measure the absorbance (A) at the maximum at 385–400nm against sodium hydroxide TS (0.01 mol/L) as the reference. Determine the color value by the formula:

$$\text{Color Value} = \frac{\text{A}}{\text{weight (g) of the sample}} \times 50$$

β-Carotene

β-カロテン



$C_{40}H_{56}$ Mol. Wt. 536.87

(1*E*,3*E*,5*E*,7*E*,9*E*,11*E*,13*E*,15*E*,17*E*)-3,7,12,16-Tetramethyl-1,18-bis(2,6,6-trimethyl-cyclohex-1-en-1-yl) octadeca-1,3,5,7,9,11,13,15,17-nonaene [7235-40-7]

Content β-Carotene, when dried, contains not less than 96.0% of β-carotene ($C_{40}H_{56}$).

Description β-Carotene occurs as red-purple to dark red crystals or crystalline powder having a slight, characteristic odor and taste.

Identification

(1) A 1 in 1000 solution of β-Carotene in a 1:1 mixture of acetone/cyclohexane is orange. Dilute this solution with acetone to make a 1 in 25 solution. To 5 mL of the solution obtained, add 1 mL of sodium nitrite solution (1 in 20) and 1 mL of sulfuric acid TS (0.5 mol/L). The color of the solution immediately disappears.

(2) To 0.5 mL of a 1 in 250 solution of β-Carotene in a 1:1 mixture of acetone/cyclohexane, add 1000 mL of cyclohexane. The solution exhibits absorption maxima at wavelengths of 454–456 nm and 482–484 nm.

Melting Point 176–183°C (in a sealed tube under reduced pressure, decomposition).

Purity

(1) Clarity of solution Clear (10 mg, a 1:1 mixture of acetone/cyclohexane 10 mL).

(2) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Absorbance ratio

Test Solution Weigh accurately about 40 mg of β-Carotene, previously dried, dissolve it in 10 mL of a 1:1 mixture of acetone/cyclohexane, and add cyclohexane to make exactly 100 mL. Measure exactly 5 mL of this solution, and add cyclohexane to make exactly 100 mL.

Diluted Test Solution Measure exactly 10 mL of the test solution, add cyclohexane to make exactly 100 mL.

Procedure Measure the absorbance (A_1 and A_2) of the test solution at wavelengths

of 340 nm and 362 nm, respectively, and the absorbance (A_3, A_4, and A_5) of the diluted test solution at wavelengths of 434 nm, 455 nm, and 483 nm, respectively. A_2/A_1 is not less than 1.00, $(A_4 \times 10)/A_1$ is not less than 15.0, A_4/A_3 is 1.30–1.60, and A_4/A_5 is 1.05–1.25.

Loss on Drying Not more than 1.0% (reduced pressure, 4 hours).

Residue on Ignition Not more than 0.1%.

Assay Measure the absorbance (A) of the diluted test solution used in Purity (4) at the absorption maximum at a wavelength of 454–456 nm, and calculate the content by the formula:

$$\text{Conetnet (\%)of β-caroten } (C_{40}H_{56}) = \frac{200}{\text{Weight (g) of the sample}} \times \frac{A}{2500} \times 100$$

Storage Standards Store in a hermetic, light-resistant container under inert gas.

Carrot Carotene

ニンジンカロテン

Definition Carrot Carotene is obtained from the roots of the carrot plant *Daucus carota* L. and consists mainly of carotene. It may contain edible fats or oils.

Content (Color Value) Carrot Carotene contains the equivalent of not less than 0.80% of β-carotene ($C_{40}H_{56}$ = 536.87) or its Color Value ($E^{10\%}_{1cm}$) is not less than 200. The actual value is in the range of 95–115% of the labeled value.

Description Carrot Carotene is a red-brown to brown, turbid, oily substance having a slightly characteristic odor.

Identification

(1) Weigh an amount of Carrot Carotene equivalent to 1 g of carrot carotene with a Color Value 200, and dissolve it in 10 mL of a 1:1 mixture of acetone/cyclohexane. An orange color develops.

(2) Dilute the solution of Carrot Carotene prepared in Identification (1) with acetone to make a 1 in 25 solution. To 5 mL of the solution obtained, add 1 mL of sodium nitrite solution (1 in 20) and 1 mL of sulfuric acid TS (0.5 mol/L). The solution is discolored immediately.

(3) A solution of Carrot Carotene in cyclohexane exhibits an absorption maximum at a wavelength of 445–460 nm or 465–485 nm, or absorption maxima at both of 445–460nm and 465–485nm.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay (Color Value Determination) Proceed as directed under Color Value Determination, using the conditions given blow. Determine the color value or calculate the content of β-carotene by dividing the color value by 250.

Operating Conditions

Solvent: Cyclohexane.

Wavelength: Maximum absorption wavelength of 445–460 nm.

Carthamus Red

ベニバナ赤色素

Definition Carthamus Red is obtained from the flowers of the safflower plant *Carthamus tinctorius* L. and consists mainly of carthamin. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Carthamus Red is not less than 500 and is in the range of 90–110% of the labeled value.

Description Carthamus Red occurs as a dark red to dark purple powder, as lumps, or as a paste. It has a slightly characteristic odor.

Identification

(1) Weigh an amount of Carthamus Red equivalent to 0.1 g of carthamus red with a Color Value 500, and dissolve it in 200 mL of *N, N*-dimethylformamide. The solution is red and exhibits an absorption maximum at a wavelength of 525–535nm.

(2) Weigh an amount of Carthamus Red equivalent to 10 mg of carthamus red with a Color Value 500, and dissolve it in 50 mL of water. The solution is red. This solution turns dark yellow when made alkaline with sodium hydroxide solution (1 in 25), and it turns red when made acidic with 10% hydrochloric acid TS.

(3) Weigh an amount of Carthamus Red equivalent to 1 g of carthamus red with a Color Value 500, dissolve it in 10 mL of *N, N*-dimethylformamide solution, and use the resulting solution as the test solution. Analyze a 2-μL portion of the test solution by thin-layer chromatography, using a 4:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. An orange-red spot is observed at an R_f value of about 0.4. The spot emits a red-purple fluorescence when irradiated with ultraviolet light (around 255 nm).

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead

Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination, using the following conditions.

Operating Conditions

Solvent: *N,N*-Dimethylformamide.

Wavelength: Maximum absorption wavelength of 525–535 nm.

Carthamus Yellow

ベニバナ黄色素

Definition Carthamus Yellow is obtained from the flowers of the safflower plant *Carthamus tinctorius* L. and consists mainly of safflower yellows. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Carthamus Yellow is not less than 100 and is in the range of 90–110% of the labeled value.

Description Carthamus Yellow occurs as a yellow to dark brown powder, as lumps, or as a paste or liquid. It has a slightly characteristic odor.

Identification

(1) Weigh an amount of Carthamus Yellow equivalent to 0.1 g of carthamus yellow with a Color Value 100, and dissolve it in 100 mL of citrate buffer (pH 5.0). The solution is yellow. It exhibits an absorption maximum at a wavelength of 400–408 nm.

(2) To the solution prepared in Identification (1), add sodium hydroxide solution (1 in 25) to make it alkaline. The color becomes orangish.

(3) Weigh an amount of Carthamus Yellow equivalent to 1 g of carthamus yellow with a Color Value of 100, dissolve it in 1 mL of water, and add 10 mL of methanol. After mixing, centrifuge at 3000 rpm for 10 minutes, and use the supernatant as the test solution. Analyze a 2-μL portion of the test solution by thin-layer chromatography, using a 4:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with microcrystalline cellulose for thin-layer chromatography as the solid support and then dried at 60–80°C for 20 minutes. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. Two or more yellow spots are observed at R_f values of about 0.20–0.50.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination, using the following conditions:

Operating Conditions

Solvent: Citrate buffer (pH 5.0).

Wavelength: Maximum absorption wavelength of 400–408 nm.

Casein

カゼイン

Content Casein, when dried, contains 13.8–16.0% of nitrogen (N = 14.01).

Description Casein occurs as a white to light yellow powder, or as granules or flakes. It is odorless and tasteless, or has a slight, characteristic odor and taste.

Identification

(1) Dissolve 0.1 g of Casein in 10 mL of sodium hydroxide solution (1 in 10), and add 8 mL of diluted acetic acid (1 in 2). A white, cotton-like precipitate is formed.

(2) Dissolve 0.1 g of Casein in 10 mL of sodium hydroxide solution (1 in 10), add 1 drop of a solution of copper(II) sulfate pentahydrate (1 in 8), and shake. A blue precipitate is formed, and the solution is purple.

(3) Ignite 0.1 g of Casein at 450–550°C. Fumes are produced, and a characteristic odor develops. When the fumes are no longer evolved, stop heating, and cool. To the black residue, add 5 mL of diluted nitric acid (1 in 10), dissolve it while warming, and filter. To the filtrate, add 1 mL of ammonium molybdate TS, and warm. A yellow precipitate is formed.

pH 3.7–6.5.

Test Solution Weigh 1.0 g of Casein, add 50 mL of water, shake for 10 minutes, and filter.

Purity

(1) Color and clarity of solution Colorless and slightly turbid.

Test Solution Dry Casein in a vacuum desiccator for 4 hours, and make into a fine powder. Weigh 0.1 g of the powder, add 30 mL of water, shake, and allow to stand for about 10 minutes. Add 2 mL of sodium hydroxide solution (1 in 250), dissolve while warming at 60°C for 1 hour and shaking occasionally, cool, and add water to make 100 mL.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method)

(3) Water-soluble substances　Not more than 1.0%.

Weigh 1.5 g of Casein, add 30 mL of water, shake for 10 minutes, and filter. Measure 20 mL of the filtrate, evaporate to dryness on a water bath, dry at 100ºC to constant weight, and weigh.

(4) Fat　Not more than 2.0%.

Dry a flask at 102 ± 2ºC for 1 hour, allow to cool in a desiccator, and weigh accurately. Weigh accurately about 2.5 g of Casein, and wash it out into a Mojonnier tube using about 10 mL of diluted hydrochloric acid (3 in 4). Stopper the tube, dissolve the sample by stirring gently in a water bath, and heat for an additional 20 minutes. After cooling, add 10 mL of ethanol (95), mix gently, add 25 mL of diethyl ether, and agitate for 1 minute. Add 25 mL of petroleum ether, agitate for 30 seconds, and then either allow to stand for at least 30 minutes or centrifuge for 5 minutes at 70 × g on the periphery of the Mojonnier tube. Transfer the supernatant into the flask. Repeat extraction using a 15-mL portion each of diethyl ether and petroleum ether. Filter the supernatant into the flask through a filter paper (5A) covered by a small amount of sodium sulfate. Wash the filter paper and sodium sulfate on the funnel with a small amount of a 1:1 mixture of diethyl ether/petroleum ether, and add the washings to the flask. Whenever glass stopper of the Mojonnier tube is removed and whenever the extract is transferred from the Mojonnier tube to the flask, wash the glass stopper, the mouth of the tube, the mouth of the flask, and the funnel that come into the extract with a small amount of a 1:1 mixture of diethyl ether/petroleum ether. Add the washings to the flask. Remove the solvent in the flask under reduced pressure, and dry the residue at 102 ± 2°C for 1 hour. Allow to cool in a desiccator, and weigh the mass accurately. Repeat drying, allowing to cool, and weighing until weight difference is less than ± 1 mg. Use the smallest value for calculation of fat.

Loss on Drying　Not more than 12.0% (100ºC, 3 hours).

Residue on Ignition　Not more than 2.5% (dried sample).

Assay　Weigh accurately about 0.15 g of Casein, previously dried, and proceed as directed in the Kjeldahl Method under Nitrogen Determination.

Each mL of 0.05 mol/L sulfuric acid = 1.401 mg of N

Catalase

カタラーゼ

Definition　Catalase includes oxidoreductases that decompose hydrogen peroxide. It is derived from the livers of pigs or the culture of filamentous fungi (limited to *Aspergillus aculeatus, Aspergillus awamori*, *Aspergillus foetidus, Aspergillus niger, Aspergillus phoenicis*, and *Penicillium amagasakiense*), yeasts (limited to species of the genus *Saccharomyces*), or bacteria (limited to *Micrococcus luteus,* and *Micrococcus lysodeikticus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives

used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Catalase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown or colorless to dark green liquid. It is odorless or has a characteristic odor.

Identification Catalase complies with the Catalase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Catalase Activity Test

Perform the test using either of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the sample dilution factor, the buffer solution, and the temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Catalase, add water or phosphate buffer (0.05 mol/L) at pH 7.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution To 0.135 mL of hydrogen peroxide, add phosphate buffer (0.05 mol/L) at pH 7.0 to make 100 mL.

Test Solution Condition the spectrophotometer by setting the thermostatic cell holder and wavelength to 25°C and to 240 nm, respectively. Transfer 2.9 mL of the substrate solution into a 10-mm quartz cell, allow to equilibrate at 25°C for 5 minutes, add 0.1 mL of the sample solution to react the substrate solution, and mix. Measure the absorbance of the solution both immediately and at 1 minute after the addition of the sample solution at a wavelength of 240 nm. The absorbance measured immediately is

higher than that 1 minute after that.

Method 2

Sample Solution Weigh 1.0 g of Catalase, add water, cold water, or sodium phosphate buffer (0.01 mol/L, pH 7.0, containing ethylene glycol) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water, cold water, or the same buffer to the resulting solution.

Substrate Solution To 1.25 mL of hydrogen peroxide, add sodium phosphate buffer (0.2 mol/L) at pH 7.0, mix, and make 100 mL. To 10 mL of this solution, add sodium phosphate buffer (0.2 mol/L) at pH 7.0 to make 100 mL.

Test Solution To 1 mL of the sample solution, equilibrated at 30°C for 5 minutes, add 5 mL of the substrate solution, equilibrated at 30°C, and mix. Allow the mixture to incubate for 5 minutes, and add 2 mL of sulfuric acid TS (0.5 mol/L) while agitating it vigorously.

Control Solution Add 2 mL of sulfuric acid TS (0.5 mol/L) to 1 mL of the sample solution, mix, and add 5 mL of the substrate solution.

Procedure To each of the test solution and the control solution, add 1 mL of potassium iodide TS (1 in 10) and 1 drop of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 100), and titrate with 0.005 mol/L sodium thiosulfate (indicator: 5 drops of soluble starch TS). The endpoint is when the blue color of the solution disappears. The amount of 0.005 mol/L sodium thiosulfate consumed by the test solution is less than that of 0.005 mol/L sodium thiosulfate consumed by the control solution.

Cellulase

セルラーゼ

Definition Cellulase includes enzymes that hydrolyze cellulose. It is produced by refining the culture of basidiomycetes (limited to *Pycnoporus coccineus* and species of the genera *Corticium* and *Irpex*), filamentous fungi (limited to *Acremonium cellulolyticus*, *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus niger*, *Humicola insolens*, *Penicillium funiculosum*, *Trichoderma harzianum, Trichoderma insolens*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, and *Trichoderma viride*), actinomycetes (limited to species of the genera *Actinomyces* and *Streptomyces*), or bacteria (limited to *Bacillus circulans* and *Bacillus subtillis*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Cellulase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Cellulase complies with the Cellulase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Cellulase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of Cellulase, add water, acetate buffer (0.05 mol/L) at pH 4.5, acetate buffer (0.l mol/L) at pH 4.5, or acetate buffer (0.1 mol/L) at pH 5.0 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 0.67 g of sodium carboxymethylcellulose, and dissolve it in 50 mL of water by warming. After cooling, add 10 mL of acetate buffer (l mol/L) at pH 4.2, acetate buffer (1 mol/L) at pH 4.5, or acetate buffer (l mol/L) at pH 5.0, and then add water to make 100 mL.

Test Solution Equilibrate 4 mL of the substrate solution at 37°C for 10 minutes, add 1 mL of the sample solution, and shake immediately. Incubate the mixture at 37°C for 30 minutes, add 2 mL of Somogyi TS (I), mix, and heat in a water bath for 30 minutes. After cooling, to this solution, add 2 mL of Nelson TS, and shake well. Add 3 mL of sodium hydroxide TS (0.5 mol/L), dissolve the precipitate by agitating, and allow to stand for 20 minutes. To this solution, add acetate buffer (1 mol/L) at pH 4.5 to make 25 mL. To 1 mL of the resultiung solution, add 9 mL of acetate buffer (1 mol/L) at pH 4.5, and mix.

Control Solution To 1 mL of the sample solution, add 2 mL of Somogyi TS (I), shake, add 4 mL of the substrate solution, and mix. Heat this solution in a water bath for 30 minutes, and cool. Proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 750 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 0.50 g of Cellulase, add water or citrate buffer (0.05 mol/L) at pH 4.8 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Filter Paper Substrate 50 mg of filter paper of about 1×6 cm.

Test Solution Transfer 1 mL of citrate buffer (0.05 mol/L) at pH 4.8 into a test tube, add 0.5 mL of the sample solution, and mix. Put a filter paper substrate into the test tube, shake to immerse it completely into the solution, and incubate at 50°C for 60 minutes. To this solution, add 3 mL of 3,5-dinitrosalicylic acid–phenol TS, shake immediately, and heat in a water bath for 5 minutes. After cooling, add 16 mL of water, and mix.

Control Solution Measure 0.5 mL of the sample solution into a test tube, add 3 mL of 3,5-dinitrosalicylic acid–phenol TS and 1 mL of citrate buffer (0.05 mol/L) at pH 4.8, immediately shake, and heat in a water bath for 5 minutes. After cooling, add 16 mL of water, and mix.

Procedure Measure the absorbance of the test solution and the control solution them at a wavelength of 550 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 3

Sample Solution Weigh 0.50 g of Cellulase, add water or citrate buffer (0.05 mol/L) at pH 4.8 or acetate buffer (1 mol/L) at pH 5.0 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution When sodium carboxymethylcellulose is used as the substrate: Add 10.0 g of sodium carboxymethylcellulose gradually to 800 mL of water while shaking, and add 100 mL of acetic acid TS (1 mol/L). Adjust the pH of the solution to 4.0 or 4.5 with sodium hydroxide TS (0.1 mol/L), add water to make 1000 mL.

When carboxymethylcellulose is used as the substrate: Add 0.75 g of carboxymethylcellulose gradually to 45 mL of water while shaking, and add 5 mL of acetate buffer (1 mol/L) at pH 5.0 to make 50 mL.

Test Solution Transfer 1 mL of the sample solution into a test tube, equilibrate at 40°C for 5 minutes, add 1 mL of the substrate solution, previously equilibrated at the same temperature for 5 minutes, and shake well. Incubate the mixture at 40°C for 10 or 30 minutes, add 4 mL of 3,5-dinitrosalicylic acid–lactose TS or 3,5-dinitrosalicylic acid TS, and mix. Heat in a water bath for 15 minutes with the mouth of the test tube covered

with a glass bead, and cool.

Control Solution Transfer 1 mL of the sample solution into a test tube, add 4 mL of 3,5-dinitrosalicylic acid–lactose TS or 3,5-dinitrosalicylic acid TS, and shake. Add 1 mL of the substrate solution, and shake well. Heat in a water bath for 15 minutes with the mouth of the test tube covered with a glass bead, and cool.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 540 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 4

Sample Solution Weigh 1.0 g of Cellulase, add water or sodium phosphate buffer (0.1 mol/L) at pH 6.0 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold dilution by adding water or the buffer to the resulting solution.

Substrate Solution Add gradually 35 g of carboxymethylcellulose to about 700 mL of sodium phosphate buffer (0.1 mol/L) at pH 6.0, previously warmed to 85°C, while shaking. Warm this solution at 85°C for 30 minutes, and cool it while stirring. To this solution, add sodium phosphate buffer (0.1 mol/L) at pH 6.0 to make 950 mL. Adjust the pH of the resulting solution to 6.0 with hydrochloric acid TS (2 mol/L) or sodium hydroxide TS (2 mol/L), and add sodium phosphate buffer (0.1 mol/L) at pH 6.0 to make 1000 mL. The carboxymethylcellulose must be dissolved completely. Prepare fresh before use. Before use, confirm that the substrate solution has no bubbles.

Test Solution Transfer 0.5 mL of the sample solution into a test tube, add 4 mL of the substrate solution, equilibrated at 25°C, and shake for 25 to 30 seconds. Incubate the mixture at 40°C for 30 minutes.

Control Solution Proceed as directed for the test solution using sodium phosphate buffer (0.1 mol/L) at pH 6.0, instead of the sample solution.

Procedure Install two test tubes separately containing the test solution and control solution in a vibration-type viscometer, place the vibrating sensing terminal in the center of the test tube, and record the readings in 20 seconds. The reading of the test solution is smaller than that of the control solution.

Method 5

Sample Solution Weigh 1.0 g of Cellulase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold dilution by adding water or the buffer to the resulting solution.

Substrate Suspension Weigh 2.0 g of crystalline cellulose and 40 mg of D(+)-glucose, add water, and agitate to make 100 mL. Prepare fresh before use.

Test Solution Transfer 2.5 mL of the substrate suspension into a L-shaped test tube, add 2 mL of acetate buffer (0.05 mol/L) at pH 4.5, and equilibrate at 50°C for 10 minutes while shaking. To this solution, add 0.5 mL of the sample solution, and incubate at 50°C for 30 minutes while shaking. Add 0.5 mL of sodium hydroxide TS (0.5 mol/L), and mix.

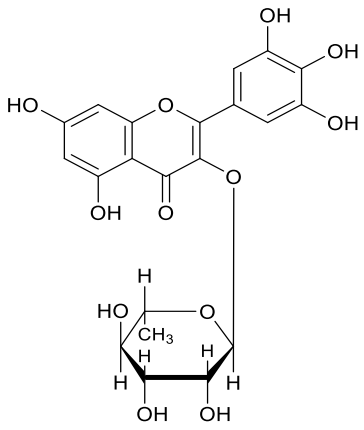
Centrifuge the resulting solution at 3000 rpm for 10 minutes. To 0.5 mL of the supernatant, add 1.5 mL of 3,5-dinitrosalicylic acid–phenol TS (for cellulase activity test), and heat the mixture in a water bath for 5 minutes. Cool the mixture, add 4 mL of water, and mix.

Control Solution Transfer 0.5 mL of the sample solution into a L-shaped test tube, add 0.5 mL of sodium hydroxide TS (0.5 mol/L), and then add 2 mL of acetate buffer (0.05 mol/L) at pH 4.5 and 2.5 mL of the substrate suspension, and mix. Centrifuge the resulting solution at 3000 rpm for 10 minutes. Proceed as directed for the test solution using 0.5 mL of the supernatant.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 540 nm. The absorbance value of the test solution is higher than that of the control solution.

Chinese Bayberry Extract

ヤマモモ抽出物



$C_{21}H_{20}O_{12}$ Mol. Wt. 464.38

5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)-4-oxo-4*H*-chromen-3-yl α-L-rhamnopyranoside [myricitrin anhydrous, 17912-87-7]

Definition Chinese Bayberry Extract is obtained from the fruits, bark, or leaves of Chinese bayberry, *Myrica rubra* (Lour.) Siebold & Zuccarini, by extraction. It consists mainly of myricitrin.

Content Chinese Bayberry Extract, when calculated on the anhydrous basis, contains 95.0–105.0% of myricitrin ($C_{21}H_{20}O_{12}$).

Description Chinese Bayberry Extract occurs as a pale yellow powder or as lumps. It has a slight, characteristic odor.

Identification

(1) Dissolve 5 mg of Chinese Bayberry Extract in 10 mL of ethanol (95). A light yellow to brown color develops. On the addition of 1–2 drops of iron(III) chloride–hydrochloric acid TS, the color changes to greenish black.

(2) Dissolve 5 mg of Chinese Bayberry Extract in 5 mL of ethanol (95). A light yellow to brown color develops. On the addition of 2 mL of hydrochloric acid and 50 mg of magnesium powder, the color gradually changes to red.

(3) A solution of 10 mg of Chinese Bayberry Extract in 1000 mL of methanol exhibits absorption maxima at wavelengths of about 257 nm and 354 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Methanol Not more than 50 μg/g.

(i) Apparatus Use the apparatus specified in Purify (3) for Enju Extract.

(ii) Method

Test Solution Weigh accurately about 5 g of Chinese Bayberry Extract into eggplant-shaped flask A. Add 100 mL of boric acid-sodium hydroxide buffer, mix well, and add a few boiling chips. Put exactly 2 mL of the internal standard in volumetric flask E, and set up the apparatus. Moisten the joint parts with water, distill the mixture at a rate of 2 to 3 mL/minute, and collect about 45 mL of distillate. To the distillate, add water to make exactly 50 mL. Use 2-methyl-2-propanol solution (1 in 1000) as the internal standard.

Standard Solution Weigh accurately about 0.5 g of methanol, and add water to make exactly 100 mL. Measure exactly 5 mL of this solution, and add water to make exactly 100 mL. Then place exactly 2 mL of the resulting solution and 4 mL of the internal standard solution in a volumetric flask, and add water to make exactly 100 mL.

Procedure Analyze 2.0 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratios (Q_T and Q_S) of methanol to 2-methyl-2-propanol for the test solution and the standard solution, and calculate the methanol content by the formula:

$$\text{Content (μg/g) of methanol} = \frac{\text{Weight (g) of methanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 500$$

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube (3 mm internal diameter and 2 m length).

Column packing material: 180- to 250-μm styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Injection port temperature: A constant temperature of about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention time of methanol to about 2 minutes.

Injection: Full filling.

Water Content Not more than 8.0% (0.2 g, Volumetric Titration, Direct Titration).

Assay

Test Solution and Standard Solution Weigh accurately about 50 mg each of Chinese Bayberry Extract and myricitrin for assay, and separately dissolve them in methanol to make 2 solutions of exactly 100 mL each. To exactly 5 mL of each solution, add an 800:200:1 mixture of water/acetonitrile/phosphoric acid to make exactly 50 mL. Use these solutions as the test solution and the standard solution, respectively.

Procedure Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of myricitrin for the test solution and the standard solution, and calculate the myricitrin content using the following formula. Separately, determine the water content of myricitrin for assay as directed under Direct Titration in Volumetric Titration in Water Determination (Karl Fischer Method).

$$\text{Content (\%) of myricitrin } (C_{21}H_{20}O_{12}) = \frac{\text{Anhydrous basis weight (g) of myricitrin for assay}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 254 nm).

Column: A stainless steel tube (3–6 mm internal diameter and 15–25 cm length).

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: An 800:200:1 mixture of water/acetonitrile/phosphoric acid.

Flow rate: Adjust the retention time of myricitrin to about 8–12 minutes.

Chitinase

キチナーゼ

Definition Chitinase includes enzymes that hydrolyze chitin. It is derived from the culture of filamentous fungi (limited to *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma harzianum*, and *Trichoderma reesei*), actinomycetes (limited to *Amycolatopsis orientalis* and species of the genus *Streptomyces*), or bacteria (limited to *Paenibacillus taichungensis* and species of the genus *Aeromonas*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Chitinase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Chitinase complies with the Chitinase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Chitinase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Chitinase, add water or phosphate buffer (0.05 mol/L) at pH 7.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 0.50 g of ethylene glycol chitin, and dissolve it in

phosphate buffer (0.05 mol/L) at pH 7.0 to make 100 mL.

Test Solution Transfer 0.5 mL of the substrate solution into a test tube, equilibrate it at 37°C for 5 minutes, add 0.05 mL of the sample solution, and immediately shake. Incubate the mixture at 37°C for 2 hours. Add 1.65 mL of 3,5-dinitrosalycilic acid–phenol TS, and shake immediately. Cover the mouth of the test tube with a glass bead, heat in a water bath for 15 minutes, and cool. Add 8.8 mL of water.

Control Solution Transfer 1.65 mL of 3,5-dinitrosalycilic acid–phenol TS into a test tube, add 0.5 mL of the substrate solution and 0.05 mL of the sample solution, immediately shake. Cover the mouth of the test tube with a glass bead, heat in a water bath for 15 minutes, and cool. Add 8.8 mL of water.

Procedure Measure the absorbance of the test solution and control solution at a wavelength of 550 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 1.0 g of Chitinase, add water or potassium phosphate buffer (0.2 mol/L) at pH 7.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the buffer to the resulting solution.

Substrate Solution Weigh 17 mg of *p*-nitrophenyl 2-acetamido-2-deoxy- β-D-glucopyranoside, and dissolve it in water to make 100 mL.

Test Solution Transfer 1.5 mL of the substrate solution and 0.4 mL of potassium dihydrogen phosphate TS (0.02 mol/L) into a test tube, equilibrate at 37°C for 5 minutes, add 0.1 mL of the sample solution, and shake. Incubate the mixture at 37°C for 10 minutes. After cooling, add 0.1 mL of 5% trichloroacetic acid solution, shake, add 2.8 mL of potassium phosphate buffer (0.2 mol/L) at pH 7.0, and shake.

Control Solution Proceed as directed for the test solution, using 0.1 mL of water instead of the sample solution.

Procedure Measure the absorbance of the test solution and control solution at a wavelength of 400 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 3

Sample Solution Weigh 1.0 g of Chitinase, add water or Tris buffer (0.05 mol/L) at pH 7.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 55 mg of *p*-nitrophenyl-di-*N*-acetyl-β-chitobioside, and dissolve it in Tris buffer (0.05 mol/L) at pH 7.0 to make 100 mL.

Test Solution Equilibrate 1.4 mL of the substrate solution at 37°C for 5 minutes,

add 0.1 mL of the sample solution, and shake. Incubate the mixture at 37°C for 30 minutes, add 1.5 mL of sodium carbonate TS (0.2 mol/L), and shake.

Control Solution Proceed as directed for the test solution, using 0.1 mL of water instead of the sample solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 405 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Chitosanase

キトサナーゼ

Definition Chitosanase includes enzymes that hydrolyze chitosan. It is derived from the culture of filamentous fungi (limited to *Aspergillus niger, Trichoderma reesei, Trichoderma viride,* and species of the genus *Verticillium*), actinomycetes (limited to *Streptomyces avermitilis*, *Streptomyces cinnamoneus, Streptomyces griseus*, *Streptomyces thermoviolaceus*, and *Streptomyces violaceoruber*), or bacteria (limited to species of the genera *Aeromonas* and *Bacillus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Chitosanase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Chitosanase complies with the Chitosanase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Chitosanase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 1.0 g of Chitosanase, and dissolve it in water to make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 0.50 g of chitosan, and dissolve it in 90 mL of acetic acid TS (0.75 mol/L) by stirring. Adjust its pH to 5.6 with sodium hydroxide TS (10 mol/L), and add water to make 100 mL. Prepare fresh before use.

Test Solution Transfer 0.5 mL of the substrate solution into a test tube, equilibrate it at 40°C for 5 minutes, add 0.5 mL of the sample solution, equilibrated at 40°C for 10 minutes, and shake immediately. Incubate the mixture at 40°C for 10 minutes, add 1 mL of acetylacetone TS, and shake. Cover the mouth of the test tube with a glass bead, heat in a water bath for 20 minutes, cool. Add 3 mL of ethanol (99.5), shake, then add 1 mL of Ehrlich's TS, and shake. Immediately warm in a water bath of 67°C for 10 minutes, and cool. Centrifuge the resulting solution at 3000 rpm for 10 minutes, and use the supernatant.

Control Solution Transfer 0.5 mL of the substrate solution into a test tube, add 1 mL of acetylacetone TS, shake, then add 0.5 mL of the sample solution, and shake. Cover the mouth of the test tube with a glass bead, heat in a water bath for 20 minutes. Proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and control solution at a wavelength of 530 nm. The absorbance value of the test solution is higher than that of the control solution.

Chlorophyll

クロロフィル

Definition Chlorophyll is obtained from green plants and consists mainly of chlorophylls. It may contain edible fats or oils.

Content (Color Value) The Color Value ($E_{1cm}^{10\%}$) of Chlorophyll is not less than 600 and is in the range of 90–110% of the labeled value.

Description Chlorophyll occurs as a green to dark green powder, as lumps, or as a paste or liquid. It has a characteristic odor.

Identification

(1) Weigh an amount of Chlorophyll equivalent to 1 g of chlorophyll with a Color Value 600, and dissolve it in 100 mL of hexane. A green color develops. Add 0.5 mL of hydrochloric acid, and mix thoroughly. The color of the solution changes to greenish yellow.

(2) Weigh an amount of Chlorophyll equivalent to 1 g of chlorophyll with a Color Value 600, and dissolve it in 100 mL of ethyl acetate. A red fluorescence is emitted.

(3) A solution of Chlorophyll in hexane exhibits absorption maxima at wavelengths of both 410–430nm and 660–670nm.

(4) Weigh an amount of Chlorophyll equivalent to 1 g of chlorophyll with a Color Value 600, dissolve it in 30 mL of hexane, and use this solution as the test solution. Analyze a 2-μL portion of the test solution by thin-layer chromatography, using a 10:1:1 mixture of hexane/acetone/2-methyl-2-propanol as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. Three spots are observed: a yellow-green spot (chlorophyll b) at an R_f value of about 0.3, a green spot (chlorophyll a) at an R_f value of about 0.4, and a gray spot (pheophytin) at an R_f value of about 0.65. These spots emit red fluorescence when irradiated with ultraviolet light (around 366 nm) in a dark place. Also, two additional spots are observed: a yellow (xanthophyll) spot at an R_f of approximate 0.25 and a yellow-orange (β-carotene) spot at an R_f of approximate 0.95. These spots do not emit fluorescence when irradiated with ultraviolet light (around 366 nm) in a dark place.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination, using the conditions below.

Operating Conditions

Solvent: Hexane.

Wavelength: Maximum absorption wavelength of 660–670 nm.

Chlorous Acid Water

亜塩素酸水

Definition Chlorous Acid Water is an aqueous solution obtained by the following procedure. A saturated solution of sodium chloride is electrolyzed with hydrochloric acid under acidic condition in an electrolytic cell without a septum ("electrolytic cell without a septum" refers to a cell consisting of an anode and a cathode not separated by a septum) to obtain an aqueous solution. The resulting solution is strongly acidified with sulfuric acid to generate chloric acid, which changes into chlorous acid water by reaction with hydrogen peroxide water.

Content Chlorous Acid Water contains 4.0–6.0% of chlorous acid ($HClO_2$ = 68.46).

Description Chlorous Acid Water occurs as a pale yellow-green to yellow-red, transparent liquid having a chlorine odor.

Identification

(1) To 5 mL of a solution of Hypochlorous Acid Water (1 in 20), add 0.1 mL of potassium permanganate solution (1 in 300). A red-purple color is produced, which changes to light yellow on the addition of 1 mL of diluted sulfuric acid (1 in 20).

(2) A solution of Chlorous Acid Water (1 in 20) exhibits absorption maxima at wavelengths of 258–262 nm and 346–361 nm.

(3) The color of potassium iodide-starch paper changes to blue in Chlorous Acid Water and then fades.

Purity

(1) Lead Not more than 1 μg/g as Pb (5.0 g, Control Solution: Lead Standard Solution 5.0 mL, Flame Method).

Test Solution To the specified amount of the sample, add 2 mL of nitric acid and 20 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue, add diluted nitric acid (1 in 150) to make 10 mL.

Control Solution Exactly measure the specified volume of Lead Standard Solution, and add diluted nitric acid (1 in 150) to make exactly 10 mL.

(2) Arsenic No more than 0.8 μg/g as As (2.5 g, Method 2, Standard Color: Arsenic Standard Solution 4.0 mL, Apparatus B).

Assay

Sample Solution Weigh accurately about 5 g of Chlorous Acid Water, and add water to make exactly 100 mL. Transfer the resulting solution into a gas washing bottle, and blow nitrogen gas into the bottle until the solution is colorless.

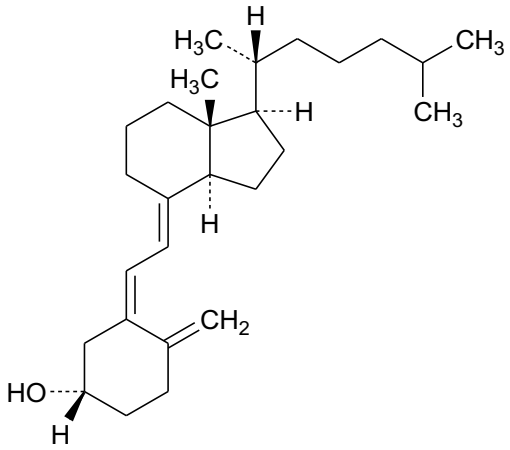
Procedure Place exactly 20 mL of the sample solution in an iodine-flask, add 10 mL of diluted sulfuric acid (1 in 10), and then add 1 g of potassium iodide. Immediately put a stopper tightly on the flask, and shake well. Pour 5 mL of potassium iodide TS in the upper part of the flask without removing the stopper, and allow to stand for 15 minutes in a dark place. Loosen the stopper to pour the potassium iodide TS into the flask, immediately stopper tightly, and shake well. Titrate free iodine with 0.1 mol/L sodium thiosulfate (indicator: 5 mL of starch TS). Add starch TS near the endpoint when the color of the solution changes to light yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test to make a necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 1.711 mg of $HClO_2$

Cholecalciferol

Vitamin D_3

コレカルシフェロール



$C_{27}H_{44}O$ Mol. Wt. 384.64

(3*S*,5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-3-ol [67-97-0]

Description Cholecalciferol occurs as white crystals. It is odorless.

Identification

(1) Proceed as directed in Identification (1) for Ergocalciferol.

(2) Proceed as directed in Identification (2) for Ergocalciferol. The melting point is 133–135°C.

Specific Absorbance $E_{1cm}^{1\%}$ (265 nm): 450–490.

Weigh accurately about 0.1 g of Cholecalciferol, and dissolve it in ethanol (95) to make exactly 200 mL. To exactly 2 mL of this solution, add ethanol (95) to make exactly 100 mL.

Specific Rotation $[\alpha]_D^{20}$: +103.0 to +112.0° (0.1 g, ethanol (95), 20 mL).

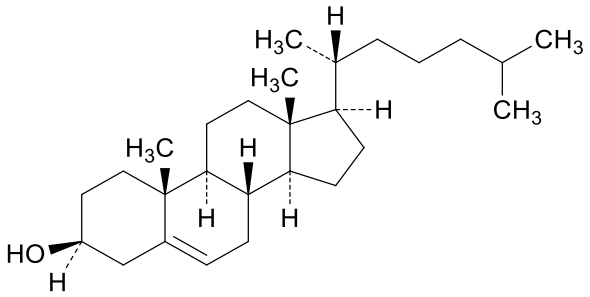
Melting Point 84–88°C.

Purity 7-Dehydrocholesterol Weigh 10 mg of Cholecalciferol, dissolve it in 2 mL of 90% (vol) ethanol, add a solution prepared by weighing 20 mg of digitonin and dissolving in 2 mL of 90% (vol) ethanol, and allow to stand for 18 hours. No precipitate is formed.

Storage Standards Store in a cold place in a hermetic, light-resistant container under inert gas.

Cholesterol

動物性ステロール



$C_{27}H_{46}O$ Mol. Wt. 386.65

Cholest-5-en-3β-ol [57-88-5]

Definition Cholesterol is obtained from fish oil or lanolin* and consists mainly of cholesterol.

Content Cholesterol contains 90.0–102.0% of cholesterol ($C_{27}H_{46}O$).

Description Cholesterol occurs as a white to light yellowish-white power or as granules. It is odorless or a slight, characteristic odor.

Identification Dissolve 5 mg of Cholesterol in 2 mL of hexane. Add 1 mL of acetic anhydride and 1 drop of sulfuric acid, and shake. A red color develops, which changes through blue to green.

Melting Point 145–150°C.

Purity

(1) Clarity of solution Place 0.5 g of Cholesterol into a stoppered flask, dissolve it in 50 mL of warmed ethanol (99.5), and allow to stand in room temperature for 2 hours. No turbidity is produced.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 3.0 % (105°C, 2 hours).

Residue on Ignition Not more than 0.5%.

Assay

Test Solution Add hexane to about 0.1 g of Cholesterol, accurately weighed, to make

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* Lanolin is obtained from the waxy substance of wool of sheep (*Ovis aries* Linnaeus) and consists mainly of esters of higher alcohols and α-hydroxy acids.

exactly 100 mL. To exactly 5 mL of this solution, add exactly 5 mL of the internal standard solution.

Internal Standard Solution A solution (1 in 1000) of 5α-cholestane in hexane.

Standard Solution Add hexane to about 0.1 g of cholesterol for assay, accurately weighed, to make exactly 100 mL. To exactly 5 mL of this solution, add exactly 5 mL of the internal standard solution.

Procedure Analyze 1 µL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratio (Q_T and Q_S) of cholesterol to 5α-cholestane, and calculate the content by the formula:

$$\text{Content (\%) of cholesterol } (C_{27}H_{46}O) = \frac{Q_T}{Q_S} \times \frac{M_S}{M_T} \times 100$$

M_S = Weight (g) of cholesterol for assay,

M_T = Weight (g) of the sample.

Operating Conditions

Detector: Flame-ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 15.0 m length) coated with a 0.10-µm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: 250°C.

Injection port temperature: 280°C.

Detector temperature: 280°C.

Carrier gas: Helium.

Flow rate: Adjust the retention time of 5α-cholestane to 3 minutes.

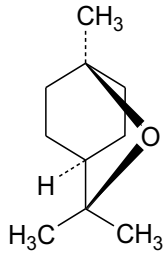
Injection method: Split.

Split ratio: 1:200.

1,8-Cineole

Eucalyptol

1,8-シネオール



$C_{10}H_{18}O$ Mol. Wt. 154.25

1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane [470-82-6]

Content 1,8-Cineole contains not less than 98.0% of 1,8-cineole ($C_{10}H_{18}O$).

Description 1,8-Cineole is a colorless or light yellow, clear liquid having a eucalyptus leaf-like odor.

Identification Determine the absorption spectrum of 1,8-Cineole as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.454–1.460.

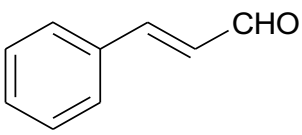
Specific Gravity d_{25}^{25}: 0.921–0.924.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Cinnamaldehyde

Cinnamic Aldehyde

シンナムアルデヒド



C_9H_8O Mol. Wt. 132.16

(2*E*)-3-Phenylprop-2-enal [14371-10-9]

Content Cinnamaldehyde contains not less than 98.0% of cinnamaldehyde (C_9H_8O).

Description Cinnamaldehyde is a colorless to light yellow, clear liquid having a cinnamon-like odor.

Identification Determine the absorption spectrum of Cinnamaldehyde as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum of Cinnamaldehyde. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.619–1.625.

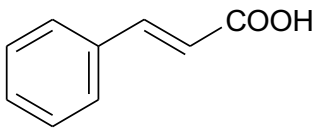
Specific Gravity d_{25}^{25}: 1.046–1.053.

Purity Acid value Not more than 10.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Cinnamic Acid

ケイ皮酸



$C_9H_8O_2$ Mol. Wt. 148.16

(2*E*)-3-Phenylprop-2-enoic acid [140-10-3]

Content Cinnamic Acid contains not less than 98.0% of cinnamic acid ($C_9H_8O_2$).

Description Cinnamic Acid occurs as a white crystalline powder having a characteristic odor.

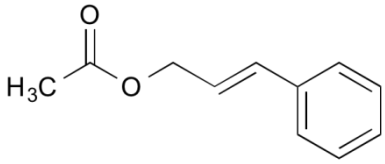
Identification Determine the absorption spectrum of Cinnamic Acid as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point Not less than 132°C.

Assay Using a solution (1 in 100) of Cinnamic Acid in acetone as the test solution, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Cinnamyl Acetate

酢酸シンナミル



$C_{11}H_{12}O_2$ Mol. Wt. 176.21

(2*E*)-3-Phenylprop-2-en-1-yl acetate [21040-45-9]

Content Cinnamyl Acetate contains not less than 98.0% of cinnamyl acetate ($C_{11}H_{12}O_2$).

Description Cinnamyl Acetate is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Cinnamyl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.539–1.544.

Specific Gravity d_{25}^{25}: 1.047–1.054.

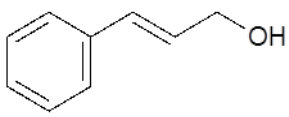
Purity Acid value Not more than 3.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Cinnamyl Alcohol

Cinnamic Alcohol

シンナミルアルコール



$C_9H_{10}O$ Mol. Wt. 134.18

3-Phenylprop-2-en-1-ol [4407-36-7]

Content Cinnamyl Alcohol contains not less than 98.0% of cinnamyl alcohol ($C_9H_{10}O$).

Description Cinnamyl Alcohol occurs as a colorless to light yellow, clear liquid or as

white to light yellow crystalline lumps. It has a characteristic odor.

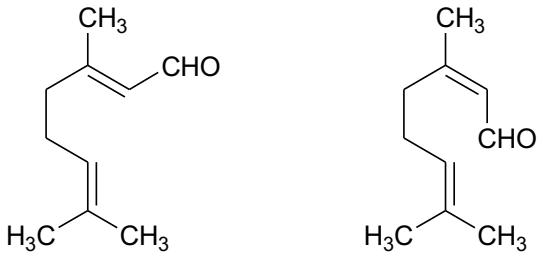
Identification Determine the absorption spectrum of Cinnamyl Alcohol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers. If the sample is a solid, melt it by warming to prepare the test sample.

Melting Point Not less than 30ºC.

Assay Using a solution (1 in 10) of Cinnamyl Alcohol in ethanol (95) as the test solution, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Citral

シトラール



(*trans*-isomer) (*cis*-isomer)

$C_{10}H_{16}O$ Mol. Wt. 152.23

Mixture of (2*E*)-3,7-dimethylocta-2,6-dienal (*trans*-isomer) and (2*Z*)-3,7-dimethylocta-2,6-dienal (*cis*-isomer) [5392-40-5]

Content Citral contains not less than 96.0% of citral ($C_{10}H_{16}O$).

Description Citral is a colorless to light yellow, clear liquid having a lemon-like odor.

Identification Determine the absorption spectrum of Citral as directed in the Liquid Film Method in Infrared Spectrophotometry, and compare with the Reference Spectrum of Citral. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.486–1.490.

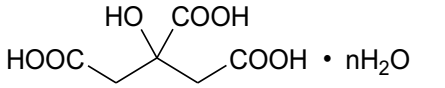
Specific Gravity 0.885–0.891.

Purity Acid Value Not more than 5.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Citric Acid

クエン酸



(n = 1 or 0)

$C_6H_8O_7 \cdot nH_2O$ (n = 1 or 0) Mol. Wt. monohydrate 210.14

anhydrous 192.12

2-Hydroxypropane-1,2,3-tricarboxylic acid monohydrate [5949-29-1]

2-Hydroxypropane-1,2,3-tricarboxylic acid [77-92-9]

Definition Citric Acid occurs in two forms: the crystalline form (monohydrate) called Citric Acid (crystal), and the anhydrous form called Citric Acid (anhydrous).

Content Citric Acid, when calculated on the anhydrous basis, contains not less than 99.5% of citric acid ($C_6H_8O_7$).

Description Citric Acid occurs as colorless, transparent crystals, granules, or lumps, or as a white powder. It is odorless and has a strongly acid taste.

Identification

(1) A solution of Citric Acid (1 in 10) is acidic.

(2) Citric Acid responds to all the tests for Citrate in the Qualitative Tests.

Purity

(1) Sulfate Not more than 0.048% as SO_4 (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(2) Lead Not more than 0.5 μg/g as Pb (8.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Calcium Weigh 1.0 g of Citric Acid, dissolve it in 10 mL of water, neutralize with ammonia TS, and add 1 mL of a solution of ammonium oxalate monohydrate (1 in 30). No turbidity appears.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Oxalate Weigh 1.0 g of Citric Acid, dissolve it in 10 mL of water, and add 2 mL of a solution of calcium chloride dihydrate (2 in 25). No turbidity appears.

(6) Isocitric acid

Test Solution Weigh 0.5 g of Citric Acid, heat at 105°C for 3 hours, cool, and dissolve it in 10 mL of acetone.

Analyze 5 μl of the test solution by paper chromatography without using any control solution. For the filter paper, use a No. 2 filter paper for chromatography. Stop the

development when the developing solvent ascends to a point about 25 cm above the base line. Then air-dry the filter paper, and spray it with bromophenol blue TS for citric acid. No more than one spot is observed. As the developing solvent, use the upper part of an 8:3:2 mixture of 1-butanol/formic acid/water that has been allowed to stand over night.

(7) Readily carbonizable substances To 0.5 g of Citric Acid, add 5 mL of sulfuric acid for the readily carbonizable substances test, and dissolve it while heating at about 90 ± 1°C for 1 hour. The color of the solution is not darker than that of Matching Fluid K.

Residue on Ignition Not more than 0.1%.

Water Content

Crystal: Not more than 8.8% (0.2 g, Volumetric Titration, Direct Titration).

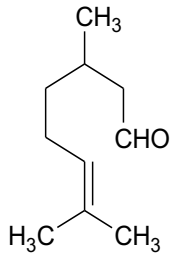
Anhydrous: Not more than 0.5% (2 g, Direct Titration).

Assay Weigh accurately about 1.5 g of Citric Acid, and dissolve it in water to make exactly 250 mL. Measure exactly 25 mL of this solution, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2–3 drops of phenolphthalein TS). Calculate on the anhydrous basis.

Each mL of 0.1 mol/L sodium hydroxide = 6.404 mg of $C_6H_8O_7$

Citronellal

シトロネラール



$C_{10}H_{18}O$ Mol. Wt. 154.25

3,7-Dimethyloct-6-enal [106-23-0]

Content Citronellal contains not less than 85.0% of citronellal ($C_{10}H_{18}O$).

Description Citronellal is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Citronellal as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.446–1.452.

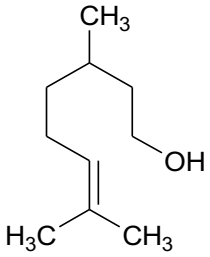
Specific Gravity d_{25}^{25}: 0.850–0.860.

Purity Acid value Not more than 3.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Citronellol

シトロネロール



$C_{10}H_{20}O$ Mol. Wt. 156.27

3,7-Dimethyloct-6-en-1-ol [106-22-9]

Content Citronellol contains not less than 90.0% of citronellol ($C_{10}H_{20}O$).

Description Citronellol is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Citronellol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.453–1.462.

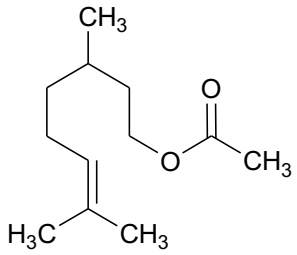
Specific Gravity d_{25}^{25}: 0.850–0.860.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Citronellyl Acetate

酢酸シトロネリル



$C_{12}H_{22}O_2$ Mol. Wt. 198.30

3,7-Dimethyloct-6-en-1-yl acetate [150-84-5]

Content Citronellyl Acetate contains not less than 92.0% of citronellyl acetate ($C_{12}H_{22}O_2$).

Description Citronellyl Acetate is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Citronellyl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.440–1.450.

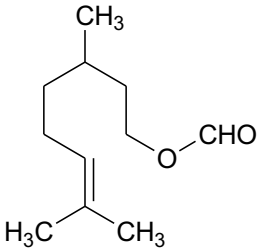
Specific Gravity d_{25}^{25}: 0.883–0.893.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Citronellyl Formate

ギ酸シトロネリル



$C_{11}H_{20}O_2$ Mol. Wt. 184.28

3,7-Dimethyloct-6-en-1-yl formate [105-85-1]

Content Citronellyl Formate contains not less than 90.0% of citronellyl formate ($C_{11}H_{20}O_2$).

Description Citronellyl Formate is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Citronellyl Formate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.443–1.452.

Specific Gravity d_{25}^{25}: 0.890–0.903.

Purity Acid value Not more than 3.0 (Flavoring Substances Tests).

Titrate while cooling in ice water until a light pink color persists for 10 seconds.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Cochineal Extract

Carminic Acid

コチニール色素

Definition Cochineal Extract is obtained from the cochineal insect *Dactylopius coccus* Costa (*Coccus cacti* Linnaeus) and consists mainly of carminic acid.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Cochineal Extract is not less than 80 and is in the range of 95–115% of the labeled value.

Description Cochineal Extract occurs as a red to dark red powder, as lumps, or as a liquid or paste. It has a slight, characteristic odor.

Identification

(1) Weigh an amount of Cochineal Extract equivalent to 0.5 g of cochineal extract with a Color Value 80, dissolve it in 1000 mL of hydrochloric acid TS (0.1 mol/L), and centrifuge. The supernatant is orange and exhibits an absorption maximum at a wavelength of 490–497 nm.

(2) Weigh an amount of Cochineal Extract equivalent to 1 g of cochineal extract with a Color Value 80, and mix with 100 mL of water. An orange-red to dark red-brown color develops. When made alkaline with sodium hydroxide solution (1 in 25), the solution turns purple to purple-red.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Protein Not more than 2.2%.

Weigh accurately about 1 g of Cochineal Extract and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

Each mL of 0.005 mol/L sulfuric acid = 0.8754 mg of protein

Color Value Determination Proceed as directed under Color Value Determination using the conditions below.

Operating Conditions

Solvent: Hydrochloric acid TS (0.1 mol/L).

Wavelength: Maximum absorption wavelength of 490–497 nm.

Copper Chlorophyll

Copper Complexes of Chlorophylls

銅クロロフィル

Description Copper Chlorophyll occurs as a bluish-black to greenish-black powder, as flakes or lumps, or as a viscous substance. It has a characteristic odor.

Identification

(1) Proceed as directed in Identification (1)(ii) for Sodium Copper Chlorophyllin.

(2) Dissolve 10 mg of Copper Chlorophyll in 50 mL of diethyl ether, add 2 mL of a solution of sodium hydroxide in methanol (1 in 100), and shake. Heat under a reflux condenser on a water bath for 30 minutes. Cool, perform extraction 3 to 5 times with 10 mL of water each time, combine the extracts, add phosphate buffer (pH 7.5) to make 200 mL, and measure the absorbance of this solution. The solution exhibits absorption maxima at wavelengths of 403–407 nm and 630–640 nm. When the absorbance values at the absorption maxima are expressed as A_1 and A_2, respectively, A_1/A_2 is not more than 4.0.

Specific Absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 405 nm): Not less than 62.0 (on the dried basis).

This test should be protected from direct light, and the apparatus used in the test should be light-resistant.

Weigh accurately about 0.1 g of Copper Chlorophyll, dissolve it in 50 mL of diethyl ether, add 10 mL of a solution of sodium hydroxide in methanol (1 in 50), and shake. Heat under a reflux condenser on a water bath for 30 minutes. Cool, perform extraction four times with 20 mL of water each time, combine the extracts, and add water to make exactly 100 mL. Filter this solution, measure exactly 5.0 mL of the filtrate, add phosphate buffer (pH 7.5) to make exactly 100 mL, and quickly measure the absorbance of the resulting solution.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Inorganic copper salt Not more than 0.03% as Cu.

Test Solution Weigh 1.0 g of Copper Chlorophyll, and dissolve it in 60 mL of acetone.

Procedure Proceed as directed in Purity (2) for Sodium Copper Chlorophyllin.

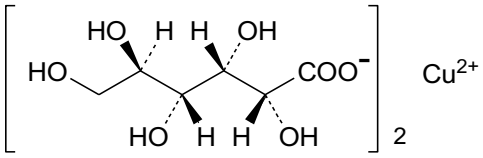
(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Chlorophyllin salt Weigh 1.0 g of Copper Chlorophyll, dissolve it in 30 mL of diethyl ether, add 20 mL of water, and shake. Allow to stand, filter the aqueous layer through a filter paper moistened with water. The filtrate is colorless.

Loss on Drying Not more than 3.0% (105°C, 2 hours).

Copper Gluconate

グルコン酸銅



$C_{12}H_{22}CuO_{14}$ Mol. Wt. 453.84

Monocopper(II) bis(D-gluconate)

Content Copper Gluconate contains 98.0–102.0% of copper gluconate ($C_{12}H_{22}CuO_{14}$).

Description Copper Gluconate occurs as a light blue powder.

Identification

(1) Copper Gluconate responds to tests (1) and (3) for Copper(II) Salt in the Qualitative Tests.

(2) Measure 5 mL of a solution of Copper Gluconate in warm water (1 in 10), and proceed as directed in Identification (2) for Glucono-δ-Lactone.

Purity

(1) Clarity of solution Almost Clear (1.0 g, water 10 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Copper Gluconate in 5 mL of water, add 2 mL of acetic acid and 1.5 g of potassium iodide, and allow to stand for 5 minutes. Dissolve 0.2 g of L(+)-ascorbic acid in the resulting solution.

(4) Reducing sugars Not more than 1.0% as D-glucose.

Weigh 1.0 g of Copper Gluconate, transfer it into a 250-mL Erlenmeyer flask, dissolve it in 10 mL of water, and add 25 mL of copper(II) citrate TS (alkaline). Cover with a small beaker, boil gently for exactly 5 minutes, and cool quickly to room temperature. To this solution, add 25 mL of diluted acetic acid (1 in 10) and exactly 10 mL of 0.05 mol/L iodine, then add 10 mL of diluted hydrochloric acid (1 in 4) and 3 mL of starch TS, and titrate the excess iodine with 0.1 mol/L sodium thiosulfate. The volume of the sodium thiosulfate solution consumed is not less than 6.3 mL.

Assay Weigh accurately about 1.5 g of Copper Gluconate, transfer it into a ground-glass stoppered flask, dissolve it in about 100 mL of water, add 2 mL of acetic acid and 5 g of potassium iodide, immediately stopper tightly, and allow to stand in a dark place for 5 minutes. Titrate this solution with 0.1 mol/L sodium thiosulfate until the color of the solution changes to a light yellow color, dissolve 2 g of ammonium thiocyanate, add 3 mL of starch TS, and titrate again with 0.1 mol/L sodium thiosulfate until the color of the solution changes to an opaque color. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 45.38 mg of $C_{12}H_{22}CuO_{14}$

Crude Magnesium Chloride (Sea Water)*

粗製海水塩化マグネシウム

Definition Crude Magnesium Chloride is obtained by precipitating and separating both potassium chloride and sodium chloride from sea water. It consists mainly of magnesium

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* Note: The standards for Crude Magnesium Chloride (Sea Water) shown in this monograph were newly established and published in Officil Gazzette in March 2007. These standards were to take effect on April 1, 2008. However, the enforcement of them has been postponed until the date the Minister of Health, Labour and Welfare decides.

chloride.

Content Crude Magnesium Chloride contains the equivalent of 12.0–30.0% of magnesium chloride ($MgCl_2$ = 95.21).

Description Crude Magnesium Chloride is a colorless to light yellow liquid having a bitter taste.

Identification

(1) To Crude Magnesium Chloride, add sodium hydroxide TS (1 mol/L). A white gelatinous precipitate is produced. On the addition of iodine TS, the precipitate is stained dark brown and does not dissolve in an excess amount of sodium hydroxide (1 mol/L).

(2) Crude Magnesium Chloride responds to test (1) for Chloride as described in the Quantitative Tests.

Purity

(1) Sulfate Not more than 4.8% as SO_4.

Sample Solution Weigh 0.25 g of Crude Magnesium Chloride, and dissolve it in water to make 100 mL. Use 2.0 mL of this solution for the test.

Control Solution Use 0.50 mL of 0.005 mol/L sulfuric acid.

(2) Bromide Not more than 2.5% as Br.

Test Solution Weigh 1.0 g of Crude Magnesium Chloride, and dissolve it in water to make 500 mL. Measure 10 mL of this solution, and add water to make 100 mL. Next, measure 2 mL of the second solution, add 3 mL of water, 2 mL of phenol red TS (pH4.7), and 1 mL of a solution of sodium *p*-toluenesulfonchloramide trihydrate (1 in 10,000), immediately mix, and allow to stand for 2 minutes. Add 0.15 mL of 0.1 mol/L sodium thiosulfate, mix, and add water to make 10 mL.

Control Solution Weigh 2.979 g of potassium bromide, previously dried at 110°C for 4 hours, and dissolve it in water to make exactly 1000 mL. Measure exactly 1 mL of this solution, and add water to make exactly 1000 mL. Next, exactly measure 5 mL of the second solution, add 2 mL of phenol red TS (pH4.7) and 1 mL of a solution of sodium *p*-toluenesulfonchloramide trihydrate (1 in 10,000), mix immediately, and then proceed as directed for the test solution.

Procedure Measure the absorbance of both solutions at 590 nm using water as the reference. The absorbance of the test solution is not more than that of the control solution.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Crude Magnesium Chloride, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the

sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(4) Zinc Not more than 70 μg/g as Zn.

Sample Solution Weigh 4.0 g of Crude Magnesium Chloride, and dissolve it in water to make 40 mL.

Procedure Measure 30 mL of the sample solution, add 5 drops of acetic acid and 2 mL of a solution of potassium hexacyanoferrate(II) trihydrate (1 in 20), shake, and allow to stand for 10 minutes. The turbidity of this solution is not greater than that of the solution prepared as follows: To 14 mL of Zinc Standard Solution, add 10 mL of the sample solution and water to make 30 mL, add 5 drops of acetic acid and 2 mL of a solution of potassium hexacyanoferrate(II) trihydrate (1 in 20), shake, and allow to stand for 10 minutes.

(5) Calcium Not more than 4.0% as Ca.

Measure exactly 20 mL of Solution A prepared in the Assay, and add water to make 100 mL. Next, add 0.2 mL of L(+)-tartaric acid solution (1 in 5), 10 mL of 2,2′,2″-nitrilotriethanol solution (3 in 10), and 10 mL of potassium hydroxide solution (1 in 10), and allow to stand for 5 minutes. Immediately titrate with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: NN indicator about 0.1 g), and express amount of the solution consumed as b mL. The endpoint is when the red-purple color of the solution disappears completely and changes to blue. Calculate the content by the formula:

$$\text{Content (\%) of calcium (Ca)} = \frac{b \times 0.4008}{\text{Weight (g) of the sample}}$$

(6) Sodium Not more than 4.0% as Na.

Test Solution Weigh 1.0 g of Crude Magnesium Chloride, and dissolve it in water to make 1000 mL. Measure 10 mL of this solution, and add water to make 200 mL.

Control Solution Weigh 2.542 g of sodium chloride, previously dried at 130°C for 2 hours, and dissolve it in water to make exactly 1000 mL. Measure exactly 2 mL of this solution, and add water to make exactly 1000 mL.

Procedure Measure the absorbance of the test solution and the control solution as directed under Atomic Absorption Spectrophotometry according to the conditions given below. The absorbance of the test solution is not greater than that of the control solution.

Operating Conditions

Light source: Sodium hollow cathode lamp.

Analytical line (wavelength): 589.0 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(7) Potassium Not more than 6.0% as K.

Test Solution Use the test solution prepared in Purity (6).

Control Solution Weigh 1.907 g of potassium chloride, previously dried at 105°C for 2 hours, and dissolve it in water to make exactly 1000 mL. Measure exactly 3 mL of this solution, and add water to make exactly 1000 mL.

Procedure Measure the absorbance of the test solution and the control solution as directed under Atomic Absorption Spectrophotometry according to the conditions given below. The absorbance of the test solution is not greater than that of the control solution.

Operating Conditions

Light source: Potassium hollow cathode lamp.

Analytical line (wavelength): 766.5 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(8) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay Weigh accurately about 2 g of Crude Magnesium Chloride, add water to make exactly 200 mL, and refer to the solution obtained as Solution A. Measure exactly 5 mL of Solution A, add 50 mL of water and 5 mL of ammonium buffer (pH 10.7), titrate with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 2 drops of eriochrome black T TS), and determine the amount of the solution consumed (a mL). The endpoint is when the color of the solution changes from red to blue. Calculate the content, using the consumed amount (b mL) obtained in Purity (5) and the following formula:

$$\text{Content (\%) of magnesium chloride } (MgCl_2) = \frac{(a - 0.25b) \times 3.803}{\text{Weight (g) of the sample}}$$

Cupric Sulfate

Copper Sulfate

硫酸銅

$CuSO_4 \cdot 5H_2O$ Mol. Wt. 249.69

Copper(II) sulfate pentahydrate [7758-99-8]

Content Cupric Sulfate contains 98.5–104.5% of cupric sulfate ($CuSO_4 \cdot 5H_2O$).

Description Cupric Sulfate occurs as blue crystals or granules or as a deep blue crystalline powder.

Identification Cupric Sulfate responds to all the tests for Copper(II) Salt and for Sulfate in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear (1.0 g, water 10 mL).

(2) Free acid Weigh 1.0 g of Cupric Sulfate, dissolve it in 20 mL of water, and add 2 drops of methyl orange TS. A green color develops.

(3) Alkali metals and alkali earth metals Not more than 0.30%.

Weigh 6.0 g of Cupric Sulfate, dissolve it in 150 mL of water, add 3 mL of sulfuric acid, and pass hydrogen sulfide through the solution while warming to about 70°C until the solution is saturated. After cooling, add water to make 280 mL, and filter. To the filtrate, add water to make 300 mL. Measure 100 mL of this solution, evaporate to dryness on a sand bath, ignite at 450–550°C to constant weight, and weigh the mass.

(4) Lead Not more than 10 μg/g as Pb (0.40 g, Control Solution: Lead Standard Solution 4.0 mL, Flame Method) .

Test Solution Add diluted nitric acid (1 in 100) to the specified amount of Cupric Sulfate to make 10 mL.

Control Solution Add diluted nitric acid (1 in 100) to the specified amount of Lead Standard Solution, measured exactly, to make exactly 10 mL.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

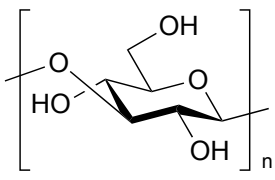
Test Solution Dissolve the specified amount of Cupric Sulfate in 5 mL of water. Add 2 mL of acetic acid and 1.5 g of potassium iodide, allow to stand for 5 minutes, and add 0.2 g of L(+)-ascorbic acid to dissolve.

Assay Weigh accurately about 0.7 g of Cupric Sulfate, and proceed as directed in the Assay for Copper Gluconate.

Each mL of 0.1 mol/L sodium thiosulfate = 24.97 mg of $CuSO_4 \cdot 5H_2O$

Curdlan

カードラン



$(C_6H_{10}O_5)_n$

(3→1)-β-D-Glucopyranan [54724-00-4]

Definition Curdlan is obtained from the culture fluid of *Agrobacterium* biovar 1 or *Rhizobium radiobacter* and consists mainly of β-1,3-glucan.

Content Curdlan contains not less than 80.0% of curdlan.

Description Curdlan occurs as a white to light yellow-brown powder. It is odorless.

Identification

(1) To 0.2 g of Curdlan, add 5 mL of water, stir well, add 1 mL of sodium hydroxide solution (3 in 25), and shake well. It dissolves.

(2) Heat 10 mL of a 2% suspension of Curdlan in a water bath for 10 minutes. A gel is produced.

(3) To 10 mL of a 2% suspension of Curdlan, add 5 mL of sulfuric acid, heat in a water bath for 30 minutes, and cool. To 1 mL of the mixture, add 100 mL of water, and then neutralize with barium carbonate. Centrifuge at 900 × g for 10 minutes. To 5 mL of the supernatant, add 5 mL of Fehling's TS, and heat in a water bath for 5 minutes. A red precipitate is produced.

pH 6.0–7.5 (1% suspension).

Purity

(1) Lead Not more than 0.5 μg/g as Pb (8.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Total nitrogen Not more than 0.3%.

Weigh accurately about 0.5 g of Curdlan, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

Loss on Drying Not more than 10.0% (reduced pressure, 60°C, 5 hours).

Residue on Ignition Not more than 6.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 1000 per gram.

Yeasts and molds: Not more than 100 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid For total plate count and the enumeration of yeasts and molds, prepare as follows: Mix 10 g of Curdlan with 190 mL of phosphate buffer, 0.1% peptone solution, or sodium chloride-peptone buffer to disperse uniformly.

Pre-enrichment Culture For the *Escherichia coli* test, prepare as follows: Mix 10 g of Curdlan with 190 mL of phosphate buffer, 0.1% peptone solution, or sodium chloride-peptone buffer to disperse uniformly, mix 20 mL of the resulting solution with 200 mL of lauryl sulfate broth, and incubate at 35 ± 1°C for 48 ± 2 hours. For the *Salmonella* test, prepare by mixing 25 g of Curdlan with 475 mL of lactose broth to disperse uniformly and incubating at 35 ± 1°C for 24 ± 2 hours.

Assay

Test Solution Weigh accurately about 0.1 g of Curdlan, and dissolve it in sodium hydroxide TS (0.1 mol/L) with shaking to make exactly 100 mL. Measure exactly 5 mL of this solution, and add water to make exactly 100 mL. Measure exactly 1 mL of the second solution, add 1 mL of phenol solution (1 in 20) and 5 mL of sulfuric acid, shake well, and cool in ice water.

Standard Solution Proceed in the same manner as for the test solution, using about 0.1 g of D(+)-glucose, accurately weighed.

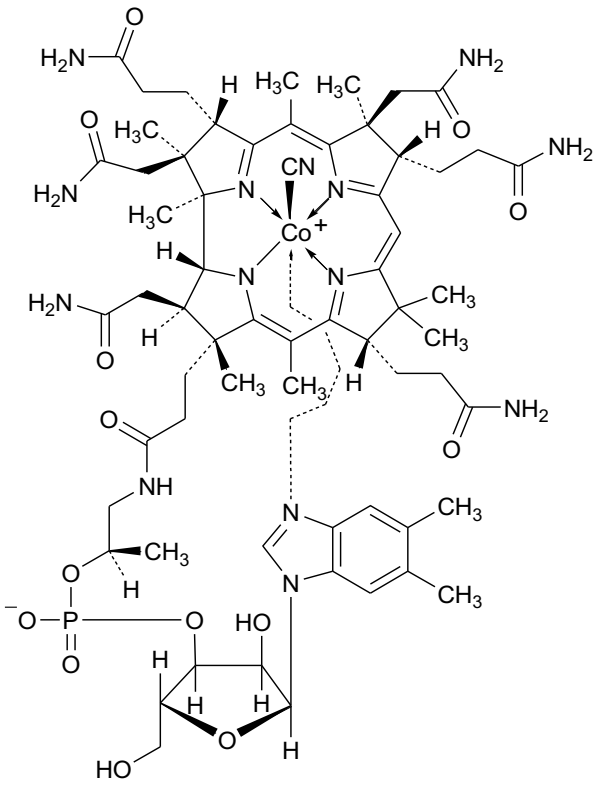
Procedure Measure the absorbance (A_T and A_S) of the test solution and the standard solution at 490 nm against the reference solution prepared as follows: Proceed as directed for the test solution for Assay, using 0.1 mL of water instead of the sample. Calculate the content by the formula:

$$\text{Content (\%) of curdlan} = \frac{\text{Weight (g) of D(+)-glucose}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 0.900 \times 100$$

Cyanocobalamin

Vitamin B_{12}

シアノコバラミン



$C_{63}H_{88}CoN_{14}O_{14}P$ Mol. Wt. 1355.37

Coα-[α-(5,6-Dimethyl-1*H*-benzoimidazol-1-yl)]-Coβ-cyanocobamide [68-19-9]

Definition Cyanocobalamin is obtained by isolation from the culture fluid of actinomycetes (limited to species of the genus *Streptomyces*) or bacteria (limited to species of the genera *Agrobacterium*, *Bacillus*, *Flavobacterium*, *Propionibacterium*, and *Rhizobium*). It consists of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$).

Content Cyanocobalamin, when calculated on the dried basis, contains 96.0–102.0% of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$).

Description Cyanocobalamin occurs as dark red crystals or powder.

Identification

(1) Determine the absorption spectra of the test solution and standard solution prepared in the Assay, given below, as directed under Ultraviolet-visible Spectrophotometry. The spectrum of the test solution exhibits similar intensities of absorption at the same wavelengths as the Reference Standard spectrum.

(2) Mix 1 mg of Cyanocobalamin with 50 mg of potassium hydrogen sulfate, and ignite

to melt. After cooling, crush the melt with a glass rod, add 3 mL of water, and boil to dissolve. Add 1 drop of phenolphthalein TS, and then add sodium hydroxide solution (1 in 20) dropwise until the solution is light red. To this solution, add 0.5 g of sodium acetate trihydrate, 0.5 mL of diluted acetic acid (3 in 50), and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500). A red to orange-red color develops immediately, and the color does not disappear on boiling for one minute with 0.5 mL of hydrochloric acid.

(3) Place 5 mg of Cyanocobalamin in a 50-mL distillation flask, dissolve it in 5 mL of water, and add 2.5 mL of phosphinic acid. Fit a short condenser to the flask, and immerse the lower end of the condenser into 1 mL of sodium hydroxide solution (1 in 50) in a test tube. Boil gently for 10 minutes, and keep distilling until 1 mL of distillate is obtained. To the solution in the test tube, add 4 drops of a saturated solution of iron(II) ammonium sulfate, and shake gently. Add 30 mg of sodium fluoride, heat to boil, and immediately add diluted sulfuric acid (1 in 7) dropwise until the solution becomes clear. On the addition of 3–5 drops of diluted sulfuric acid (1 in 7), a blue to blue-green color is produced.

Purity

(1) Clarity of solution Red and clear (20 mg, water 10 mL).

(2) Related substance The test given below should be conducted using light-resistant containers.

Test Solution Dissolve 10 mg of Cyanocobalamin in 10 mL of the mobile phase.

Standard Solution Diluting 3 mL of the test solution, measured exactly, with the mobile phase solution to make exactly 100 mL.

Procedure Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Continue the chromatography for 4 times the retention time of the main peak, and measure peak areas by the automatic integration method. Exclude the solvent peak from measurement. The sum of the areas of all peaks, other than the cyanocobalamin peak, from the test solution is not greater than the area of the cyanocobalamin peak from the control solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 361 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μm octylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 10 g of disodium hydrogenphosphate in 1000 mL of water, and adjust the pH to 3.5 with phosphoric acid. To 147 mL of this solution, add 53 mL of methanol.

Flow rate: Adjust the retention time of cyanocobalamin to about 7 minutes.

System Suitability

Confirmation of detectability Prepare a solution for the system suitability test by adding the mobile phase to exactly 1 mL of the test solution to make exactly 100 mL. To exactly 1 mL of this solution, add the mobile phase to make exactly 10 mL. The area of the cyanocobalamin peak from 20 μL of the resulting solution is 7–13% of that of the cyanocobalamin peak from the solution for the system suitability test.

System performance The procedure should be conducted promptly after the preparation of a solution for the system performance test. The solution: Add 10 mL of water to 25 mg of Cyanocobalamin, warm if necessary to dissolve, allow to cool, add 0.5 mL of sodium *p*-toluenesulfonchloramide TS and 0.5 mL of hydrochloric acid TS (0.05 mol/L), add water to 25 mL, shake, and allow to stand for 5 minutes. To 1 mL of the resulting solution, add the mobile phase to make 10 mL. Chromatograph 20μL of the resulting solution using the operating conditions given above. The chromatogram shows two main peaks for which the degree of separation is more than 2.5.

System reproducibility When the chromatography is conducted 6 times under the operating conditions given above using 20 μL of the solution for the system suitability test each time, relative standard deviation of the chromatogram peaks obtained is not more than 3.0%.

Loss on Drying Not more than 12.0% (50 mg, not more than 0.67 kPa, desiccant phosphorus oxide(V), 100°C, 4 hours).

Assay

Test Solution Weigh accurately about 20 mg of Cyanocobalamin, and dissolve it in water to make exactly 1000 mL.

Standard Solution Weigh accurately about 20 mg of Cyanocobalamin Reference Standard for which the loss on drying has been already measured, and dissolve it in water to make exactly 1000 mL.

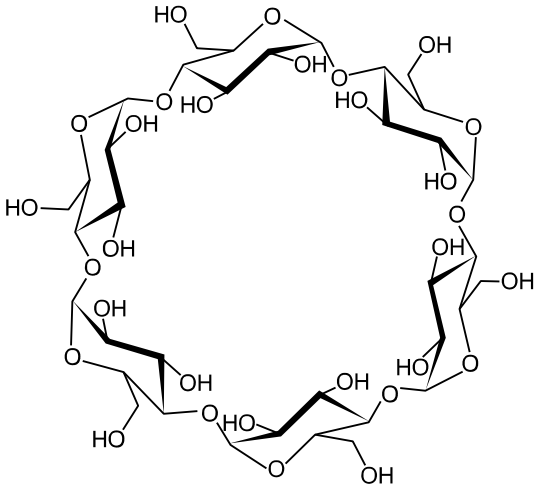
Procedure Measure the absorbance (A_T and A_S) of the test solution and the standard solution at a wavelength of 361 nm, using water as the reference. Calculate the content by the formula:

Content (%) of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$)

$$= \frac{\text{Dry basis weight (g) of Cyanocobalamin Reference Standard}}{\text{Dry basis weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

α-Cyclodextrin

α-シクロデキストリン



$C_{36}H_{60}O_{30}$ Mol. Wt. 972.84

Cyclomaltohexaose [10016-20-3]

Definition α-Cyclodextrin* is a non-reducing cyclic dextrin obtained by enzymatic treatment of starch. It is a cyclic oligosaccharide consisting of six D-glucose units.

Content α-Cyclodextrin, when dried, contains not less than 98.0% of α-cyclodextrin ($C_{36}H_{60}O_{30}$).

Description α-Cyclodextrin occurs as white crystals or crystalline powder. It is odorless and has a slight, sweet taste.

Identification To 0.2 g of α-Cyclodextrin, add 2 mL of iodine TS, dissolve it by heating in a water bath, and cool in cold water. A dark red-purple precipitate is formed.

Specific Rotation $[\alpha]_D^{20}$: +147 to +152° (previously dried, 1 g, water, 100 mL).

Measure the angular rotation of the this solution within 30 minutes

Purity

(1) Clarity of solution Colorless and clear (0.50 g, water 50 mL).

(2) Chloride Not more than 0.018% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.25 mL).

(3) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 1 μg/g as As (1.5 g, Method 2, Standard Color: Arsenic

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* α-Cyclodextrin belongs to the "Cyclodextrin" category contained in the List of Existing Food Additives.

Standard Solution 3.0 mL, Apparatus B).

(5) Reducing substances Weigh 1.0 g of α-Cyclodextrin, previously dried, dissolve it in 25 mL of water, add 40 mL of Fehling's TS, and boil gently for 3 minutes. After cooling, carefully filter the supernatant through a glass filter (1G4) leaving as much precipitate as possible in the flask. Wash the precipitate in the flask with warm water, filter the washings through the glass filter, and discard the filtrate. Repeat this washing and filtering process until the washings are free of alkali. Add 20 mL of ferric iron(III) sulfate TS to the precipitate in the flask to dissolve, filter through the same glass filter, wash the inside of the flask and the glass filter with water, and combine the washings with the filtrate. Heat to 80°C, and titrate with 0.02 mol/L potassium permanganate. The volume of the potassium permanganate solution consumed is not more than 3.2 mL.

Loss on Drying Not more than 14.0% (120°C, 2 hours).

Residue on Ignition Not more than 0.1% (550°C).

Assay

Test Solution Weigh accurately about 0.5 g of α-Cyclodextrin, previously dried, dissolve it in about 35 mL of hot water, and cool. Add water to make exactly 50 mL.

Standard Solutions Prepare three standard solutions with different concentrations by the following procedure: Weigh accurately about 0.7 g of α-cyclodextrin for assay, previously dried, and dissolve it in 45 mL of hot water. After cooling, add water to make exactly 50 mL (Standard Solution 1). Transfer exactly 5 mL of this solution into each of 10-mL and 20-mL volumetric flasks, and add water exactly to volume (standards 2 and 3).

Procedure Analyze 10 μL each of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Prepare a calibration curve by measuring the peak areas of α-cyclodextrin for the standard solutions. Determine the amount (g) of α-cyclodextrin in the test solution from the calibration curve and the peak area of α-cyclodextrin for the test solution. Calculate the content of α-cyclodextrin by the formula:

$$\text{Content (\%) of α-cyclodextrin } (C_{36}H_{60}O_{30}) = \frac{\text{Amount (g) of α-cyclodextrin in the test solution}}{\text{Weight (g) of the sample}} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (5–10 mm internal diameter and 20–50 cm length).

Column packing material: 9- to 30-μm strongly acidic cation exchange resin for liquid chromatography.

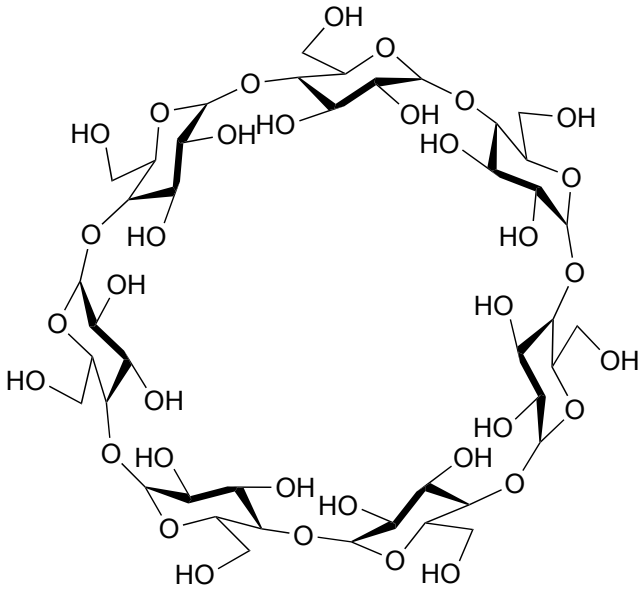
Column temperature: A constant temperature of 50–80°C.

Mobile phase: Water.

Flow rate: A constant rate of 0.3–1.0 mL/min.

β-Cyclodextrin

β-シクロデキストリン



$C_{42}H_{70}O_{35}$ Mol. Wt. 1134.98

Cyclomaltoheptaose [7585-39-9]

Definition β-Cyclodextrin* is a non-reducing cyclic dextrin obtained by enzymatic treatment of starch. It is a cyclic oligosaccharide consisting of seven D-glucose units.

Content β-Cyclodextrin, when dried, contains not less than 98.0% of β-cyclodextrin ($C_{42}H_{70}O_{35}$).

Description β-Cyclodextrin occurs as odorless white crystals or crystalline powder having a slight, sweet taste.

Identification To 0.2 g of β-Cyclodextrin, add 2 mL of iodine TS, dissolve it while heating in a water bath, and cool in cold water. A red-brown precipitate is formed.

Specific Rotation $[\alpha]_D^{20}$: +160 to +164° (previously dried, 1 g, water, 100 mL).

Measure the angular rotation of this solution within 30 minutes.

Purity

(1) Clarity of solution Colorless and clear (0.50 g, water 50 mL).

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* β-Cyclodextrin belongs to the "Cyclodextrin" category contained in the List of Existing Food Additives.

(2) Chloride Not more than 0.018% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.25 mL).

(3) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 1 μg/g as As (1.5 g, Method 2, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Reducing substances Weigh exactly 1.0 g of β-Cyclodextrin, previously dried, dissolve it in 25 mL of water, add 40 mL of Fehling's TS, and boil gently for 3 minutes. After cooling, carefully filter the supernatant through a glass filter (1G4), leaving as much precipitate as possible in the flask. Next, wash the precipitate in the flask with warm water, carefully filter the washings through the glass filter, and discard the filtrate. Repeat this washing and filtering process until the washings are free of alkali. To the precipitate in the flask, add 20 mL of iron(III) sulfate TS to dissolve, and filter through the glass filter. Wash the inside of the flask and the glass filter with water, and combine the washings with the filtrate. Heat the resulting solution to 80°C, and titrate with 0.02 mol/L potassium permanganate. The volume of potassium permanganate solution consumed is not more than 3.2 mL.

Loss on drying Not more than 14.0% (120°C, 2 hours).

Residue on ignition Not more than 0.1% (550°C).

Assay

Test Solution Weigh accurately about 0.5 g of β-Cyclodextrin, previously dried, add about 35 mL of hot water to dissolve completely, and cool. Add water to make exactly 50 mL.

Standard Solutions Prepare 3 standard solutions with different concentrations by the following procedure: Weigh accurately about 0.7 g of β-cyclodextrin for assay, previously dried, add 45 mL of hot water to dissolve completely. After cooling, add water to make exactly 50 mL (Standard Solution 1). Transfer exactly 5 mL of this solution into 10-mL and 20-mL volumetric flasks, and add water exactly to volume (standard solutions 2 and 3).

Procedure Analyze 10 μL each of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Prepare a calibration curve by measuring the peak areas of β-cyclodextrin for the standard solutions. Determine the amount of β-cyclodextrin (g) in the test solution, using the calibration curve and the peak area of β-cyclodextrin for the test solution. Calculate the content of β-cyclodextrin by the formula:

$$\text{Content (\%) of β-cyclodextrin } (C_{42}H_{70}O_{35}) = \frac{\text{Amount (g) of β-cyclodextrin in the test soliution}}{\text{Weight (g) of the sample}} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (5-10 mm internal diameter and 20–50 cm length).

Column packing material: 9- to 30-μm strongly acidic cation exchange resin for liquid chromatography.

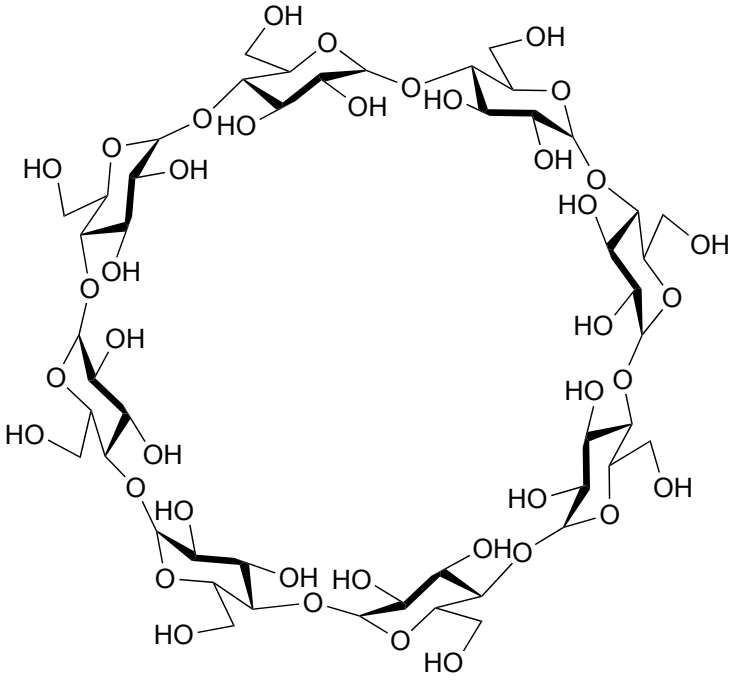
Column temperature: A constant temperature of 50–80°C.

Mobile phase: Water.

Flow rate: A constant rate of 0.3–1.0 mL/min.

γ-Cyclodextrin

γ-シクロデキストリン



$C_{48}H_{80}O_{40}$ Mol. Wt. 1297.12

Cyclomaltooctaose [17465-86-0]

Definition γ-Cyclodextrin* is a non-reducing cyclic dextrin obtained by enzymatic treatment of starch. It is a cyclic oligosaccharide consisting of eight D-glucose units.

Content γ-Cyclodextrin, when dried, contains not less than 98.0% of γ-cyclodextrin ($C_{48}H_{80}O_{40}$).

Description γ-Cyclodextrin occurs as white crystals or crystalline powder. It is odorless

* γ-Cyclodextrin belongs to the "Cyclodextrin" category contained in the List of Existing Food Additives.

and has a slight, sweet taste.

Identification To 0.2 g of γ-Cyclodextrin, add 2 mL of iodine TS, dissolve it by heating in a water bath, and cool in cold water. A brown precipitate is formed.

Specific Rotation $[\alpha]_D^{20}$: +172 to +178° (previously dried, 1 g, water, 100 mL).

Measure the angular rotation of this solution within 30 minutes.

Purity

(1) Clarity of solution Colorless and clear (0.50 g, water 50 mL).

(2) Chloride Not more than 0.018% as Cl (0.50 g, Control Solution: 0.01 mol/L Hydrochloric acid 0.25 mL).

(3) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 1 μg/g as As (1.5 g, Method 2, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Reducing substances Weigh exactly 1.0 g of γ-Cyclodextrin, previously dried, dissolve it in 25 mL of water, add 40 mL of Fehling's TS, and boil gently for 3 minutes. After cooling, carefully filter the supernatant through a glass filter (1G4), leaving as much precipitate as possible in the flask. Wash the precipitate in the flask with warm water, filter the washings through the glass filter, and discard the filtrate. Repeat this washing and filtering process until the washings are free of alkali. To the precipitate in the flask, add 20 mL of iron(III) sulfate TS to dissolve, and filter through the glass filter. Wash the inside of the flask and glass filter with water, and combine the washings with the filtrate. Heat to 80°C, and titrate with 0.02 mol/L potassium permanganate solution. The volume of the potassium permanganate consumed is not more than 3.2 mL.

Loss on Drying Not more than 14.0% (120°C, 2 hours).

Residue on Ignition Not more than 0.1% (550°C).

Assay

Test Solution Weigh accurately about 0.5 g of γ-Cyclodextrin, previously dried, dissolve it in about 35 mL of hot water, and cool. Add water to make exactly 50 mL.

Standard Solutions Prepare three standard solutions with different concentrations by the following procedure: Weigh accurately about 0.7 g of γ-cyclodextrin for assay, previously dried, and dissolve it in 45 mL of hot water. After cooling, add water to make exactly 50 mL (Standard Solution 1). Transfer exactly 5 mL of this solution into 10-mL and 20-mL volumetric flasks, and add water exactly to volume (standard solutions 2 and 3).

Procedure Analyze 10 μL each of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Prepare a calibration curve by measuring the peak areas of γ-cyclodextrin for the standard solutions. Determine the amount (g) of γ-cyclodextrin in the test solution from the calibration curve and the peak area of γ-cyclodextrin for the test solution. Calculate the content of γ-

cyclodextrin by the formula:

$$\text{Content (g) of } \gamma\text{-cyclodextrin } (C_{48}H_{80}O_{40}) = \frac{\text{Amount (g) of } \gamma\text{-cyclodextrin in the test soliution}}{\text{Weight (g) of the sample}} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (5–10 mm internal diameter and 20–50 cm length).

Column packing material: 9- to 30-μm strongly acidic cation exchange resin for liquid chromatography.

Column temperature: A constant temperature of 50–80°C.

Mobile phase: Water.

Flow rate: A constant rate of 0.3–1.0 mL/min.

Cyclodextrin Glucanotransferase

シクロデキストリングルカノトランスフェラーゼ

Definition Cyclodextrin Glucanotransferase includes enzymes that produce cyclodextrin from amylaceous polysaccharides, such as starch. It is derived from the culture of actinomycetes (limited to *Streptomyces thermoviolaceus*) or bacteria (limited to *Anoxybacillus caldiproteolyticus*, *Geobacillus stearothermophilus*, *Paenibacillus campinasensis*, *Paenibacillus macerans*, and species of the genera *Bacillus*, *Brevibacterium*, and *Corynebacterium*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Cyclodextrin Glucanotransferase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Cyclodextrin Glucanotransferase complies with the Cyclodextrin Glucanotransferase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

The requirement of total plate count can be disregarded if unsterilized Cyclodextrin Glucanotransferase is used for captive consumption in the manufacture of food that will be sterilized or pasteurized before completion of the final product.

Cyclodextrin Glucanotransferase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Cyclodextrin Glucanotransferase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold or 100-fold dilution by adding water to the resulting solution.

Substrate Solution Suspend 3.0 g of soluble starch in a small amount of water, add this suspension gradually to about 70 mL of boiling water, and boil for 5 minutes. After cooling, add 10 mL of acetate buffer (1 mol/L) at pH 5.5 and water to make 100 mL.

Procedure Equilibrate 6 mL of the substrate solution at 40°C for 10 minutes, add 3 mL of the sample solution, and shake immediately. While incubating this solution at 40°C, transfer 0.3-mL portions of it into separate test tubes with each containing 0.1 mL of iodide TS cooled in icy water at 1-minute intervals from 3 to 12 minutes after the addition of the sample solution. Place 10-μL portions of these solutions onto separate slide glasses, dry them at 23°C, and microscopically examine at 40- or 100- times magnification. Needle crystals are observed on any of the slides.

Method 2

Sample Solution Weigh 1.0 g of Cyclodextrin Glucanotransferase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 1.0 g of soluble starch, and dissolve it completely in 50 mL of water by heating. Add 12.5 mL of potassium phosphate buffer (0.4 mol/L) at pH 6.0 and water to make 100 mL.

Test Solution Equilibrate 0.9 mL of the substrate solution at 40°C for 5 minutes, add 0.1 mL of the sample solution, and shake immediately. Incubate the mixture at 40°C

for 10 minutes, add 2.5 mL of sodium hydroxide TS (0.04 mol/L), and shake immediately.

Control Solution To 0.9 mL of the substrate solution, add 2.5 mL of sodium hydroxide TS (0.04 mol/L) and then 0.1 mL of the sample solution.

Procedure Add 0.3 mL of phenolphthalein–sodium carbonate TS to the test solution and the control solution, measure the absorbance of each solution immediately at a wavelength of 550 nm. The absorbance value of the test solution is lower than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 3

Sample Solution Weigh 1.0 g of Cyclodextrin Glucanotransferase, add glycine–sodium hydroxide buffer (0.025 mol/L, pH 10.0, containing sodium chloride) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Weigh 1.5 g of soluble starch, and dissolve it completely in 50 mL of water by heating. To this solution, add 10 mL of glycine–sodium hydroxide buffer (0.25 mol/L, pH 10.0, contining sodium chloride) and water to make 100 mL.

Test Solution Equilibrate 0.45 mL of the substrate solution at 40°C for 5 minutes, add 0.05 mL of the sample solution, and shake immediately. Incubate the mixture at 40°C for 10 minutes, add 0.5 mL of hydrochloric acid (0.05 mol/L), and shake immediately. Add 0.1 mL of bromocresol green TS (TS for the cyclodextrin glucanotransferase activity test), and allow to stand at room temperature for 20 minutes. To this solution, add 2 mL of acetic acid–citric acid–sodium hydroxide buffer (pH 4.2), and shake.

Control Solution Mix 0.45 mL of the substrate solution and 0.5 mL of hydrochloric acid (0.05 mol/L), add 0.05 mL of the sample solution, then add 0.1 mL of bromocresol green TS (TS for the cyclodextrin glucanotransferase activity test), and allow to stand for 20 minutes at room temperature. To this solution, add 2 mL of acetic acid–citric acid –sodium hydroxide buffer (pH 4.2), and shake.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 630 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 4

Sample Solution Weigh 1.0 g of Cyclodextrin Glucanotransferase, add water or acetate buffer (0.01 mol/L, pH 5.5, containing calcium chloride) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution To 1.0 g of potato starch, add 20 mL of water, and then add 5 mL of sodium hydroxide TS (1 mol/L) gradually while shaking to make sticky paste. Heat it in a water bath for 3 minutes while shaking, and add 25 mL of water. After cooling, adjust its pH to 5.5 with acetic acid TS (1 mol/L), and add water to make 100 mL. Prepare fresh before use.

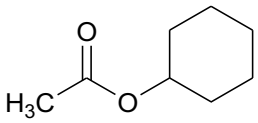
Test Solution Equilibrate 10 mL of the substrate solution at 40°C for 10 minutes, add 1 mL of the sample solution, and shake immediately. Incubate the mixture at 40°C for 10 minutes. To 1 mL of this solution, add 10 mL of hydrochloric acid TS (0.1 mol/L), and shake immediately. To 1 mL of the resulting solution, add 10 mL of iodine–potassium iodide TS (0.4 mmol/L), and shake.

Control Solution Proceed as directed for the test solution using water instead the sample solution.

Procedure Measure the absorbance of the test solution and the control solution at 660 nm. The absorbance value of the test solution is lower than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Cyclohexyl Acetate

酢酸シクロヘキシル



$C_8H_{14}O_2$ Mol. Wt. 142.20

Cyclohexyl acetate [622-45-7]

Content Cyclohexyl Acetate contains not less than 98.0% of cyclohexyl acetate ($C_8H_{14}O_2$).

Description Cyclohexyl Acetate is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Cyclohexyl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.436–1.443.

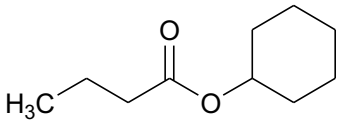
Specific Gravity d_{25}^{25}: 0.965–0.972.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Cyclohexyl Butyrate

酪酸シクロヘキシル



$C_{10}H_{18}O_2$ Mol. Wt. 170.25

Cyclohexyl butanoate [1551-44-6]

Content Cyclohexyl Butyrate contains not less than 98.0% of cyclohexyl butyrate ($C_{10}H_{18}O_2$).

Description Cyclohexyl Butyrate is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Cyclohexyl Butyrate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.439–1.451.

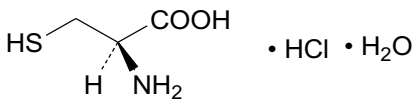
Specific Gravity d_{25}^{25}: 0.936–0.942.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

L-Cysteine Monohydrochloride

L-システイン塩酸塩



$C_3H_7NO_2S \cdot HCl \cdot H_2O$ Mol. Wt. 175.63

(2*R*)-2-Amino-3-sulfanylpropanoic acid monohydrochloride monohydrate [7048-04-6]

Content L-Cysteine Monohydrochloride, when calculated on the dried basis, contains 98.0–102.0% of L-cysteine monohydrochloride ($C_3H_7NO_2S \cdot HCl$ = 157.62).

Description L-Cysteine Monohydrochloride occurs as colorless to white crystals or as a white crystalline powder. It has a characteristic odor and taste.

Identification

(1) To 5 mL of a solution of L-Cysteine Monohydrochloride (1 in 1000), add 0.5 mL of pyridine and 1 mL of ninhydrin solution (1 in 100), and heat for 5 minutes. A purple to purple-brown color develops.

(2) To 10 mL of a solution of L-Cysteine Monohydrochloride (1 in 1000), add 2 mL of sodium hydroxide solution (1 in 25) and 2 drops of a solution of sodium pentacyanonitrosylferrate(III) dihydrate (1 in 20) . A purple-red color develops.

(3) To 10 mL of a solution of L-Cysteine Monohydrochloride (1 in 50), add 1 mL of hydrogen peroxide, and heat in a water bath for 10 minutes. The resulting solution responds to test (2) for Chloride in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +5.0 to +8.0° (4.0 g, hydrochloric acid TS (6 mol/L), 50 mL, on the dried basis).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 20 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of L-Cysteine Monohydrochloride into a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat. Continue heating while occasionally adding 2–3 mL of nitric acid until the solution is colorless to light yellow. After cooling, add 15 mL of a saturated solution of ammonium oxalate, and concentrate to 2–3 mL by heating until white fumes are evolved. After cooling, add water to make 10 mL.

Standard Color Measure 3.0 mL of Arsenic Standard Solution, transfer into a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat until white fumes are evolved. After cooling, add 15 mL of a saturate solution of ammonium oxalate, heat until white fumes are evolved, and evaporate to 2–3mL by heating. After cooling, add water to make 10 mL. Proceed as directed for the test solution in the Method using Apparatus B in the Procedure under the Arsenic Limit Test in the General Tests, starting with "Transfer the test solution into …." in the same manner as the preparation of the test solution.

(4) Cystine

Test Solution Weigh 0.20 g of L-Cysteine Monohydrochloride, dissolve it in *N*-ethylmaleimide solution (1 in 50) to make 100 mL. Measure 2 mL of this solution, add *N*-ethylmaleimide solution (1 in 50) to make 20 mL, and allow to stand for 30 minutes.

Procedure Analyze a 5-μL portion of the test solution by thin-layer chromatography, using a 2:1:1 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the

development when the solvent front has ascended to a point about 15 cm above the starting line, and dry the plate at 80ºC for 30 minutes. Spray with a (1 in 100) solution of ninhydrin in a 97:3 mixture of methanol/acetic acid, and heat at 80ºC for 10 minutes to fix a color. Examine in daylight. Only one spot is observed.

Loss on Drying 8.0–12.0% (not more than 0.7 kPa, 24 hours).

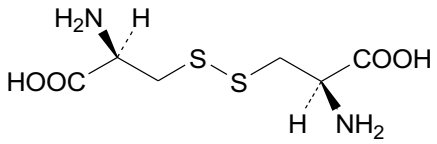
Residue on Ignition Not more than 0.2%.

Assay Weigh accurately about 0.25 g of L-Cysteine Monohydrochloride, dissolve it in 20 mL of water, and add 4 g of potassium iodide to dissolve. To this solution, add 5 mL of diluted hydrochloric acid (1 in 4) and exactly 25 mL of 0.05 mol/L iodine, and allow to stand in ice water for 20 minutes in a dark place. Titrate the excess iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner.

Each mL of 0.05 mol/L iodine = 15.76 mg of $C_3H_7NO_2S \cdot HCl$

L-Cystine

L-シスチン



$C_6H_{12}N_2O_4S_2$ Mol. Wt. 240.30

(2*R*,2*R*)-3,3′-Disulfanylbis[2-amino-3-sulfanylpropanoic acid] [56-89-3]

Content L-Cystine, when calculated on the dried basis, contains 98.0–120.0% of L-cystine ($C_6H_{12}N_2O_4S_2$).

Description L-Cystine occurs as white crystals or crystalline powder. It has a slight, characteristic odor. It is tasteless or has a slight, characteristic taste.

Identification

(1) To 5 mL of a saturated solution of L-Cystine, add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A purple color develops.

(2) To 3 mL of a 1 in 30 solution of L-Cystine in hydrochloric acid TS (2 mol/L), add 40 mg of zinc powder, and heat for 10 minutes in a water bath. After cooling, filter if necessary. Add 10 mL of sodium hydroxide solution (1 in 20), shake, and add 1 drop of sodium pentacyanonitrosylferrate(III) TS. A reddish purple color develops.

Specific Rotation $[\alpha]_D^{20}$: –215 to –230° (2 g, hydrochloric acid TS (1 mol/L), 100 mL, on the dried basis).

pH 5.0–6.5.

Measure the pH of a suspension of 20 mg of L-Cystine in 50 mL of water.

Purity

(1) Clarity of solution　Colorless and clear (1.0 g, 1 mol/L hydrochloric acid 20 mL).

(2) Chloride　Not more than 0.1% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more than 0.3% (105°C, 3 hours).

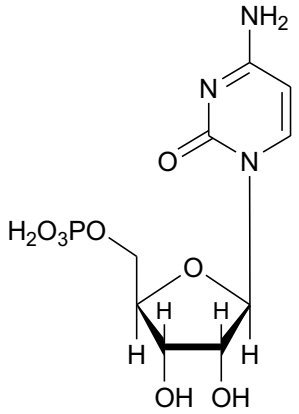
Residue on Ignition　Not more than 0.1%.

Assay　Weigh accurately about 0.3 g of L-Cystine, proceed as directed in the Kjeldahl Method under Nitrogen Determination, and calculate on the dried basis. In decomposing the sample, use 0.2 g of selenium dioxide as a decomposition-promoting agent, and heat for 4 hours.

Each mL of 0.05 mol/L sulfuric acid = 12.02 mg of $C_6H_{12}N_2O_4S_2$

5′-Cytidylic Acid

5′-シチジル酸



$C_9H_{14}N_3O_8P$　Mol. Wt. 323.20

Cytidine 5′-monophosphoric acid　[63-37-6]

Definition　5′-Cytidylic Acid is obtained by enzymatic hydrolysis of the nucleic acids that are water-extracted from cells of the yeasts (limited to *Candida utilis*) in the presence of salt, followed by isolation. It consists of 5′-cytidylic acid ($C_9H_{14}N_3O_8P$).

Content　5′-Cytidylic Acid, when calculated on the dried basis, contains　98.0–102.0%

of 5′-cytidylic acid ($C_9H_{14}N_3O_8P$).

Description 5′-Cytidylic Acid occurs as colorless to white crystals or as a white crystalline powder.

Identification

(1) Dissolve 10 mg of 5′-Cytidylic Acid in 1000 mL of diluted hydrochloric acid (1 in 1000). This solution exhibits an absorption maximum at a wavelength of 277–281 nm.

(2) Dissolve 0.25 g of 5′-Cytidylic Acid in 1 mL of sodium hydroxide TS (1 mol/L), add 5 mL of water, and then add 2 mL of magnesia TS. No precipitate is formed. To this solution, add 7 mL of nitric acid, and boil for 10 minutes. It responds to test (2) for Phosphate as described in the Quantitative Tests.

Purity

(1) Clarity of solution Colorless and almost clear.

Test Solution Weigh 0.50 g of 5′-Cytidylic Acid, dissolve it in 2 mL of sodium hydroxide TS (1 mol/L), and add water to make 20 mL.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of 5′-Cytidylic Acid, and dissolve it in 5 mL of diluted hydrochloric acid (1 in 4).

(4) Absorbance ratio Weigh 10 mg of 5′-Cytidylic Acid, dissolve it in diluted hydrochloric acid (1 in 1000), and make 1000 mL. When the absorbance values of the solution at 250 nm, 260 nm, and 280 nm are expressed as A_1, A_2, and A_3, respectively, A_1/A_2 is 0.40–0.52 and A_3/A_2 is 1.85–2.20.

(5) Other nucleic acid decomposition products

Test Solution Weigh 0.10 g of 5′-Cytidylic Acid, dissolve it in 0.5 mL of sodium hydroxide TS (1 mol/L), and add water to make 20 mL.

Procedure Analyze a 1-μL portion of the test solution by thin-layer chromatography using a 6:5:2 mixture of 1-propanol/ammonia TS/acetone as the developing solvent. No control solution is used. Use a thin-layer plate coated with fluorescent silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, air-dry the plate, and examine under ultraviolet light (around 250 nm) in a dark place. Only one spot is observed.

Loss on Drying Not more than 6.0% (120°C, 4 hours).

Assay Weigh accurately about 0.2 g of 5′-Cytidylic Acid, dissolve it in 1 mL of sodium hydroxide TS (1 mol/L), add water to make exactly 200 mL. Measure exactly 2 mL of this

solution, and add diluted hydrochloric acid (1 in 1000) to make exactly 100 mL. Measure the absorbance (A) of the prepared solution at 280 nm, and calculate the content by the formula:

$$\text{Content (\%) of 5′-cytidylic acid } (C_9H_{14}N_3O_8P) = \frac{0.2 \times 1.224 \times A}{\text{Dry basis weight (g) of the sample}} \times 100$$

5′-Deaminase

5′-デアミナーゼ

Definition 5′-Deaminase includes enzymes that deaminize 5′-adenylic acid to produce 5′-inosinic acid. It is derived from the culture of filamentous fungi (limited to *Aspergillus melleus* and *Aspergillus oryzae*) or actinomycetes (limited to *Streptomyces aureus, Streptomyces avermitilis, Streptomyces cinnamoneus*, *Streptomyces griseus, Streptomyces murinus, Streptomyces thermoviolaceus,* and *Streptomyces violaceoruber*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description 5′-Deaminase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification 5′-Deaminase complies with the 5′-Deaminase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

5′-Deaminase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of

the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 0.5 g of 5′-Deaminase, add water to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 0.33 g of disodium adenosine 5′-monophosphate, previously dried at 105°C for 4 hours, dissolve it in about 25 mL of water, adjust its pH to 5.6 with hydrochloric acid TS (0.1 mol/L) or sodium hydroxide TS (0.1 mol/L), and add water to make 50 mL. To this solution, add phosphate buffer at pH 5.6 (1/15 mol/L) so that the ratio of the solution and the buffer is 1:2.

Test Solution Equilibrate 3 mL of the substrate solution at 37°C for 5 minutes, add 1 mL of the sample solution, and immediately shake. Incubate the mixture at 37°C for 15 minutes, add 4 mL of diluted perchloric acid (1 in 30), and shake. To 2 mL of the resulting solution, add water to make 100 mL. To prepare the diluted perchloric acid, use 60% perchloric acid.

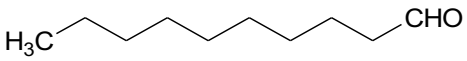
Control Solution To 3 mL of the substrate solution, add 4 mL of perchloric acid (1 in 30), then 1 mL of the sample solution, and shake. To 2 mL of this solution, add water to make 100 mL.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 265 nm. The absorbance of the test solution is lower than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Decanal

Decyl Aldehyde

デカナール



$C_{10}H_{20}O$ Mol. Wt. 156.27

Decanal [112-31-2]

Content Decanal contains not less than 92.0% of decanal ($C_{10}H_{20}O$).

Description Decanal is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Decanal as directed in the Liquid

Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.426–1.430.

Specific Gravity d_{25}^{25}: 0.823–0.832.

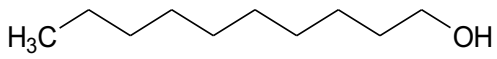
Purity Acid value Not more than 10.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Decanol

Decan-1-ol
Decyl Alcohol

デカノール



$C_{10}H_{22}O$ Mol. Wt. 158.28

Decan-1-ol [112-30-1]

Content Decanol contains not less than 98.0% of decanol ($C_{10}H_{22}O$).

Description Decanol is a colorless or light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Decanol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.435–1.439.

Specific Gravity d_{25}^{25}: 0.826–0.831.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Dextran

デキストラン

Definition Dextran is obtained by isolation from the culture fluid of the bacterium *Leuconostoc mesenteroides* or *Streptococcus equinus*. It consists of dextran.

Description Dextran occurs as a white to light yellow powder or as granules. It has no odor.

Identification To 1 mL of a solution of dextran (1 in 3000), add 2 mL of anthrone TS. A blue-green color develops, and it gradually changes to dark blue-green. The color does not change any more on the addition of 1 mL of diluted sulfuric acid (1 in 2) or acetic acid.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Total nitrogen Not more than 1.0%.

Weigh accurately about 0.5 g of Dextran, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

Loss on Drying Not more than 10.0 % (105°C, 6 hours).

Residue on Ignition Not more than 2.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 1 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture Prepare as directed in Method 1, respectively, for the *Escherichia coli test* and the *Salmonella* test.

Dextranase

デキストラナーゼ

Definition Dextranase includes enzymes that degrade dextran. It is derived from the culture of filamentous fungi (limited to *Chaetomium erraticum*, *Chaetomium gracile*, and *Penicillium lilacinum*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Dextranase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Dextranase complies with the Dextranase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Dextranase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Dextranase, and add phosphate buffer (0.01 mol/L, pH 7.0, containing albumin) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Weigh 2.5 g of dextran (molecular weight: 2,000,000), and dissolve it in acetate buffer (0.1 mol/L) at pH5.1 to make 100 mL. Prepare fresh before use.

Test Solution　Transfer 2 mL of the substrate solution into a test tube, equilibrate it at 40°C for about 10 minutes, add 1 mL of the sample solution, and shake. Incubate the mixture at 40°C for 10 minutes, add 0.5 mL of sulfuric acid TS (1 mol/L), shake it, and allow to stand for about 10 minutes. To this solution, add 1 drop of phenolphthalein–sodium carbonate TS, neutralize with sodium hydroxide TS (5 mol/L), add 5 mL of cupper TS (for xylanase/dextranase activity test), and mix. Heat in a water bath for 20 minutes with the test tube stopped loosely. Cool the solution in running water, and allow to stand while warming at 40°C for 10 minutes or more until a precipitate is formed in the bottom of the test tube. After cooling, add 2 mL of potassium iodide solution (1 in 40), and then add 1.5 mL of sulfuric acid TS (1 mol/L), stir the solution until it is clear brown.

Control Solution　Transfer 2 mL of the substrate solution into a test tube, equilibrate it at 40°C for about 10 minutes, add 0.5 mL of sulfuric acid TS (1 mol/L) and 1 mL of the sample solution, shake the mixture, and allow to stand for about 10 minutes. Proceed as directed for the test solution.

Procedure　Titrate the test solution and the control solution with 0.005 mol/L sodium thiosulfate (indicator: 0.5 mL of soluble starch TS). The amount of 0.005 mol/L sodium thiosulfate consumed by the test solution is less than that of 0.005 mol/L sodium thiosulfate consumed by the control solution.

Method 2

Sample Solution　Weigh 1.0 g of Dextranase, and add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water to the resulting solution.

Substrate Solution　Weigh 1.0 g of dextran (molecular weight: 70,000), and dissolve it in water to make 100 mL. Prepare fresh before use.

Test Solution　To 10 mL of the substrate solution, add 4 mL of acetate buffer (0.1 mol/L) at pH5.8, shake the mixture, and equilibrate it at 37°C for 10–15 minutes. Add 1 mL of the sample solution, mix, and incubate it at 37°C for 30 minutes. To 2 mL of this solution, add 3 mL of water and 5 mL of potassium iron(III) hexacyanate TS (0.025 mol/L), shake well the mixture, and heat it in a water bath for 15 minutes. After cooling, add 5 mL of zinc sulfate–sodium chloride–potassium iodide TS and 3 mL of diluted acetic acid (1 in 20).

Control Solution　Proceed as directed for the test solution using 1 mL of water instead of the sample solution.

Procedure　Titrate the test solution and the control solution with 0.01 mol/L sodium thiosulfate (indicator: 5 drops of starch TS). Continue the titration until the blue color disappears. The amount of 0.01 mol/L sodium thiosulfate consumed by the test solution is less than that of 0.01 mol/L sodium thiosulfate consumed by the control solution.

Diammonium Hydrogen Phosphate

Ammonium Phosphate, Dibasic

リン酸水素二アンモニウム

$(NH_4)_2HPO_4$ Mol. Wt. 132.06

Diammonium hydrogenphosphate [7783-28-0]

Content Diammonium Hydrogen Phosphate contains 96.0–102.0% of diammonium hydrogen phosphate ($(NH_4)_2HPO_4$).

Description Diammonium Hydrogen Phosphate occurs as colorless to white crystals or as a white crystalline powder. It is odorless or has an odor of ammonia.

Identification Diammonium Hydrogen Phosphate responds to all the tests for Ammonium Salt and for Phosphate in the Qualitative Tests.

pH 7.6–8.4 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.035% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.50 mL).

(3) Sulfate Not more than 0.038% as SO_4 (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(4) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Diammonium Hydrogen Phosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay Weigh accurately about 2 g of Diammonium Hydrogen Phosphate, dissolve it in 50 mL of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 3–4 drops of methyl orange–xylene cyanol FF TS).

Each mL of 1 mol/L hydrochloric acid = 132.1 mg of $(NH_4)_2HPO_4$

Diatomaceous Earth

ケイソウ土

Definition Diatomaceous Earth is silicon dioxide derived from diatom and consists mainly of silicon dioxide. There are three types of products: dried, burned, and flux burned. They are called Diatomaceous Earth (dried), Diatomaceous Earth (burned), and Diatomaceous Earth (flux burned), respectively.

Burned products are obtained by burning diatomaceous earth at 800–1200°C, and flux burned products are obtained by burning diatomaceous earth at 800–1200°C with a small amount of alkali salt of carbonic acid. Acid-treated flux burned products should be followed by the directions for burned products.

Description Diatomaceous Earth (dried) occurs as a whitish or light gray powder. Diatomaceous Earth (burned) occurs as a light yellow to light orange or pink to light brown powder. Diatomaceous Earth (flux burned) occurs as a white or light red-brown powder.

Identification

(1) Place 0.2 g of Diatomaceous Earth in a platinum crucible, dissolve it in 5 mL of hydrofluoric acid, and heat. Almost all of it evaporates.

(2) Observe Diatomaceous Earth, magnified 100–200 times under a microscope. The characteristic porous diatom skeleton is observed.

pH

Dried products and burned products: 5.0–10.0.

Flux burned products: 8.0–11.0.

Test Solution Weigh 10.0 g of Diatomaceous Earth, previously dried, into an appropriate container, add 100 mL of water, and boil gently for 2 hours with magnetic stirring while replenishing the evaporated water. After cooling, and filter by suction, using a filter holder equipped with a 47 mm-diameter membrane filter (0.45 µm pore size). If the filtrate is turbid, repeat the suction filtration through the same filter. Wash the container and the residue on the filter with water, combine the washings with the filtrate, and add water to make 100 mL.

Purity

(1) Water-soluble substances Not more than 0.50%.

Measure 50 mL of the test solution prepared for the pH test, evaporate to dryness, dry the residue at 105°C for 2 hours, and weigh.

(2) Hydrochloric acid-soluble substances Not more than 2.5%.

Weigh 2.0 g of Diatomaceous Earth, previous dried, into a flask, add 50 mL of diluted hydrochloric acid (1 in 4), and warm at 50°C for 15 minutes with occasional shaking. Cool and filter the mixture, and wash the flask and the residue on the filter paper with 3 mL of diluted hydrochloric acid (1 in 4). Combine the washings with filtrate. Add 5

mL of diluted sulfuric acid (1 in 20) to this solution, evaporate to dryness, ignite at 450 –550°C to constant weight, and weigh.

(3) Lead Not more than 10 μg/g as Pb (0.40 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Diatomaceous Earth, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, and combine the washings with the filtrate.

(4) Arsenic Not more than 7.5 μg/g as As (2.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Diatomaceous Earth, add 50 mL of diluted hydrocholoric acid (1 in 4), and warm at 70°C for 15 minutes with a watch glass covering it while stirring. After cooling, filter the supernatant through a filter paper (5C), and collect the filtrate. Wash the residue in the container three times with 10 mL of warm water each time, and combine the washings with the filtrate through the filter paper. Wash the filter paper and the residue on the filter paper with 15 mL of water, and add the washings to the filtrate, and add water to make 100 mL. Use 10 mL of this solution as the test solution.

Loss on Drying

Dried products: Not more than 10.0% (105°C, 2 hours).

Burned products and flux burned products: Not more than 3.0% (105°C, 2 hours).

Loss on Ignition Dry Diatomaceous Earth at 105°C for 2 hours, and immediately perform the test, using it as the sample.

Dried products: Not more than 7.0% (1000°C, 30 minutes).

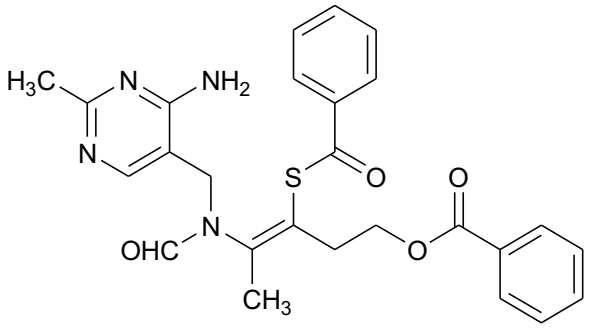
Burned products and flux burned products: Not more than 2.0% (1000°C, 30 minutes).

Hydrofluoric Acid Residue Not more than 25.0%.

Ignite a platinum crucible at 1000°C for 30 minutes, allow to cool in a desiccator, and weigh accurately. Weigh accurately about 0.2 g of Diatomaceous Earth, transfer into the platinum crucible, and weigh accurately. Add 5 mL of hydrofluoric acid and 2 drops of diluted sulfuric acid (1 in 2), evaporate almost completely to dryness on a water bath, and cool. To the residue, add 5 mL of hydrofluoric acid, evaporate to dryness, and heat at 550°C for 1 hour. Raise the temperature gradually, ignite at 1000°C for 30 minutes, allow to cool in a desiccator, and weigh accurately.

Dibenzoyl Thiamine

ジベンゾイルチアミン



$C_{26}H_{26}N_4O_4S$ Mol. Wt. 490.57

4-[*N*-(4-Amino-2-methylpyrimidin-5-ylmethyl)formamido]-3-(benzoylsulfanyl)pent-3-en-1-yl benzoate [299-88-7]

Content Dibenzoyl Thiamine, when dried, contains not less than 97.0% of dibenzoyl thiamine ($C_{26}H_{26}N_4O_4S$).

Description Dibenzoyl Thiamine occurs as a white crystalline powder. It is odorless.

Identification

(1) To 30 mg of Dibenzoyl Thiamine, add 7 mL of diluted hydrochloric acid (1 in 100), and dissolve it while heating in a water bath. To this solution, add 2 mL of a 1:1 mixture of a solution of hydroxylammonium chloride (3 in 20)/sodium hydroxide solution (3 in 20), shake for 1 minute, and add 0.8 mL of hydrochloric acid and 0.5 mL of a solution of iron(III) chloride hexahydrate (1 in 10). A purple color develops.

(2) To 5 mg of Dibenzoyl Thiamine, add 1 mL of methanol, and dissolve it while warming. Add 2 mL of water, 2 mL of a solution of L-cysteine hydrochloride monohydrate (1 in 100), and 2 mL of phosphate buffer (pH 7), shake, and allow to stand for 30 minutes. To this solution, add 1 mL of a freshly prepared solution of potassium hexacyanoferrate(III) (1 in 10), 5 mL of sodium hydroxide solution (1 in 50) and 5 mL of 2-methyl-1-propanol, shake vigorously for 2 minutes, and allow to stand to separate the solution into two layers. Expose the liquid to ultraviolet light from above, and examine the top of the upper-layer solution from perpendicular direction and from the irradiation direction. A blue-purple fluorescence is observed. This fluorescence disappears when the solution is made acidic, and reappears when it is made alkaline.

Melting Point 163–174°C (decomposition).

Purity

(1) Chloride Not more than 0.053% as Cl.

Test Solution Weigh 0.40 g of Dibenzoyl Thiamine, dissolve it in 20 mL of methanol, and add 6 mL of diluted nitric acid (1 in 10) and water to make 50 mL.

Control Solution To 0.60 mL of 0.01 mol/L hydrochloric acid, add 20 mL of methanol, 6 mL of diluted nitric acid (1 in 10) and water to make 50 mL.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Loss on Drying Not more than 3.0% (105°C, 2 hours).

Residue on Ignition Not more than 0.2%.

Assay

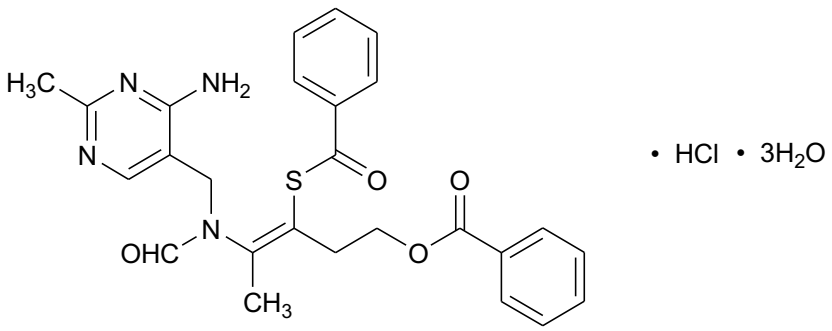
Test Solution Weigh accurately about 0.4 g of Dibenzoyl Thiamine, previously dried, add 40 mL of methanol and 40 mL of diluted hydrochloric acid (1 in 100) to dissolve, and add water to make exactly 1000 mL. Measure exactly 5 mL of this solution, and add diluted hydrochloric acid (1 in 100) to make exactly 250 mL.

Procedure Measure the absorbance (A) of the test solution at a wavelength of 237 nm, using water as the reference. Perform a blank test in the same manner, take the absorbance as A_0, and calculate the content by the formula:

$$\text{Content (\%) of dibenzoyl thiamine } (C_{26}H_{26}N_4O_4S) = \frac{(A - A_0) \times 0.4}{\text{Weight (g) of the sample} \times 0.452} \times 100$$

Dibenzoyl Thiamine Hydrochloride

ジベンゾイルチアミン塩酸塩



$C_{26}H_{26}N_4O_4S \cdot HCl \cdot 3H_2O$ Mol. Wt. 581.08

4-[*N*-(4-Amino-2-methylpyrimidin-5-ylmethyl)formamido]-3-(benzoylsulfanyl)pent-3-en-1-yl benzoate monohydrochloride trihydrate [35660-60-7]

Content Dibenzoyl Thiamine Hydrochloride, when dried, contains not less than 97.0% of dibenzoyl thiamine hydrochloride ($C_{26}H_{26}N_4O_4S \cdot HCl$ = 527.03).

Description Dibenzoyl Thiamine Hydrochloride occurs as a white crystalline powder. It is odorless.

Identification

(1) Proceed as directed in Identification (1) and (2) for Dibenzoyl Thiamine.

(2) Dissolve 0.1 g of Dibenzoyl Thiamine Hydrochloride in 10 mL of methanol, add 1 mL of diluted nitric acid (1 in 10), and add 1 mL of silver nitrate solution (1 in 50).

A white precipitate is formed.

Purity

(1) Clarity of solution Almost clear.

Test Solution Weigh 1.0 g of Dibenzoyl Thiamine Hydrochloride, add 10 mL of water, and dissolve it while heating in a water bath.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Loss on Drying Not more than 11.0% (reduced pressure, 24 hours).

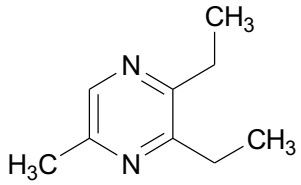
Residue on Ignition Not more than 0.2%.

Assay Weigh accurately about 0.4 g of Dibenzoyl Thiamine Hydrochloride, previously dried, proceed as directed in the Assay for Dibenzoyl Thiamine, and calculate the content by the formula:

$$\text{Content (\%) of dibenzoyl thiamine hydrochloride } (C_{26}H_{26}N_4O_4S\cdot HCl) = \frac{(A - A_0) \times 0.4}{\text{Weight (g) of the sample} \times 0.421} \times 100$$

2,3-Diethyl-5-methylpyrazine

2,3-ジエチル-5-メチルピラジン



$C_9H_{14}N_2$ Mol. Wt. 150.22

2,3-Diethyl-5-methylpyrazine [18138-04-0]

Content 2,3-Diethyl-5-methylpyrazine contains not less than 98.0% of 2,3-diethyl-5-methylpyrazine ($C_9H_{14}N_2$).

Description 2,3-Diethyl-5-methylpyrazine occurs as a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 2,3-Diethyl-5-

methylpyrazine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

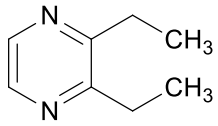
Refractive Index n_D^{20}: 1.493–1.505.

Specific Gravity d_{25}^{25}: 0.938–0.957.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (1).

2,3-Diethylpyrazine

2,3-ジエチルピラジン



$C_8H_{12}N_2$ Mol. Wt. 136.19

2,3-Diethylpyrazine [15707-24-1]

Content 2,3-Diethylpyrazine contains not less than 97.0 % of 2,3-diethylpyrazine ($C_8H_{12}N_2$).

Description 2,3-Diethylpyrazine is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 2,3-Diethylpyrazine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

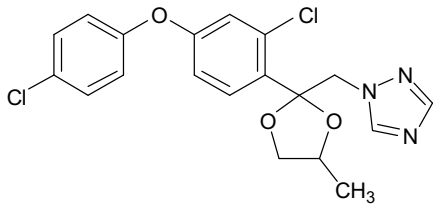
Refractive index n_D^{20}: 1.492–1.509.

Specific gravity d_{25}^{25}: 0.956–0.976.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (1).

Difenoconazole

ジフェノコナゾール



$C_{19}H_{17}Cl_2N_3O_3$ 406.26

3-Chloro-4-[(2*RS*,4*RS*;2*RS*,4*SR*)-4-methyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether [119446-68-3]

Content Difenoconazole contains not less than 94.0% of difenoconazole ($C_{19}H_{17}Cl_2N_3O_3$).

Description Difenoconazole occurs as a white to yellow-brown powder.

Identification

Determine the absorption spectrum of Difenoconazole as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 76–83°C.

Purity

Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Assay

Test Solution and Standard Solution Weigh accurately about 50 mg each of Difenoconazole and difenoconazole for assay. Add exactly 20 mL of the internal standard solution to each, dissolve by adding acetone to make exactly 100 mL.

Internal Standard Solution Dissolve 75 mg of fludioxonil for assay by adding acetone to make exactly 50 mL.

Procedure Analyze 2 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratios (Q_T and Q_S) of difenoconazole to fludioxonil for the test solution and the standard solution, respectively. Determine the content of difenoconazole by the formula:

$$\text{Content (\%) of difenoconazole } (C_{19}H_{17}Cl_2N_3O_3) = \frac{\text{Weight (mg) of difenoconazole for assay}}{\text{Weight (mg) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 30 m length) coated with a 0.25 μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 100°C for 1 minutes, raise at 30°C/minute to 250°C, then raise at 6°C/minute to 300°C, and maintain the temperature at 300°C for 2 minutes.

Injection port temperature: A constant temperature of around 250°C.

Detector temperature: A constant temperature of around 300°C.

Carrier gas: Helium.

Flow rate: Adjust the retention time of difenoconazole to about 10–15 minutes.

Injection method: Split.

Split ratio: 1 : 20.

Diluted Benzoyl Peroxide

希釈過酸化ベンゾイル

Benzoyl peroxide [94-36-0]

Definition Diluted Benzoyl Peroxide is produced by diluting benzoyl peroxide with one or more of the following food additives and food: "Aluminum Potassium Sulfate," "Calcium Salts of Phosphate," "Calcium Sulfate," "Calcium Carbonate," "Magnesium Carbonate," and starch.

Content Diluted Benzoyl Peroxide contains 19.0–22.0% of benzoyl peroxide ($C_{14}H_{10}O_4$ = 242.23).

Description Diluted Benzoyl Peroxide occurs as a white powder.

Identification Place 0.2 g of Diluted Benzoyl Peroxide into a test tube, add 7 mL of chloroform, shake well, and allow to stand. A white insoluble substance remains on the bottom of the test tube. Add 2.0 mL of 4,4′-diaminodiphenylamine TS. The color of the solution and the insoluble substance changes to a blue-green color.

pH 6.0–9.0.

Test Solution Weigh 3.0 g of Diluted Benzoyl Peroxide, add 30 mL of water, shake for 3 minutes, and filter.

Purity

(1) Fineness Weigh 5.0 g of Diluted Benzoyl Peroxide, transfer into a dried 53-μm standard sieve, shake vigorously in all directions for 2 minutes, occasionally tapping the bottom of the receiver, and allow to stand for 1 minute. After the fine powder has settled, weigh the residue on top of the sieve. It is not more than 1.0 g.

(2) Spread of fire Weigh 1.0 g of Diluted Benzoyl Peroxide, transfer on a glass plate so that it is 3 mm high and 10 mm wide, and light one end. The flame does not spread to the other end.

(3) Hydrochloric acid-insoluble substances Weigh 0.20 g of Diluted Benzoyl Peroxide, add 10 mL of diluted hydrochloric acid (1 in 4), shake well, heat gradually, and boil for about 1 minute. Cool, add about 8 mL of diethyl ether, shake well, and allow to stand. Both liquid layers are clear, and no flocculent substances exist at the interface.

(4) Ammonium salt Weigh 0.20 g of Diluted Benzoyl Peroxide, add 3 mL of sodium hydroxide solution (2 in 5), and boil. The evolved gas does not change the color of litmus paper (red) moistened with water to blue.

(5) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(6) Barium Weigh 2.0 g of Diluted Benzoyl Peroxide, add 15 mL of diluted nitric acid (1 in 10), shake, and filter. Wash with water, combine the washings with the filtrate, and add water to make 40 mL. Adjust the pH to 2.4–2.8 with ammonia TS, add water to make 50 mL, add 1 mL of diluted sulfuric acid (1 in 20), and allow to stand for 10 minutes. The solution is not turbid.

(7) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specific amount of Diluted Benzoyl Peroxide, add 5 mL of diluted hydrochloric acid (1 in 4), heat gently, cool quickly in ice water, and filter. Wash the residue with 15 mL of water, combine the washings with the filtrate, and add water to make 40 mL. Use 20 mL of this solution as the test solution.

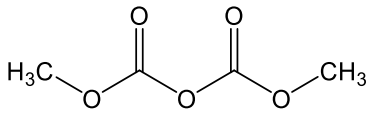
Procedure Proceed as directed in the Arsenic Limit Test, but skip the procedure in which the test solution is neutralized with ammonia solution or ammonia TS.

Assay Weigh accurately about 1 g of Diluted Benzoyl Peroxide, transfer into a ground-glass stoppered flask, add 50 mL of a 1:1 mixture of methanol/chloroform, and shake. Add 0.5 mL of a solution (1 in 10) of citric acid monohydrate in methanol and 2 mL of potassium iodide solution (1 in 2), immediately stopper tightly, allow to stand in a dark place for 15 minutes with occasional shaking. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 12.11 mg of $C_{14}H_{10}O_4$

Dimethyl Dicarbonate

二炭酸ジメチル



$C_4H_6O_5$ Mol. Wt. 134.09

Dimethyl dicarbonate [4525-33-1]

Content Dimethyl Dicarbonate contains not less than 99.8% of dimethyl dicarbonate ($C_4H_6O_5$).

Description Dimethyl Dicarbonate is a colorless liquid.

Identification

Determine the absorption spectrum of Dimethyl Dicarbonate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Lead Not more than 1 μg/g as Pb (Electrothermal Method).

Test Solution Weigh accurately about 1.5 g of Dimethyl Dicarbonate into a container made of polyethylene, quartz, or hard-glass, and add 0.75 mL of nitric acid (for trace metals determination). Stopper loosely, heat up gradually with stirring or occasional shaking, heat at 90°C to 30 minutes, and cool. Add dropwise 0.85 mL of hydrogen peroxide, heat at 95°C to 5–10 minutes with stirring or occasional shaking, and cool. Add dropwise hydrogen peroxide, heat in the same manner, and cool. Transfer this solution into a 25-mL volumetric flask, wash the container with a small amount of water, combine the washings with the solution, and add water to make 25 mL.

Standard Solutions Prepare four standard solutions with different concentrations. Transfer exactly 1, 2.5, 5, 10 mL of Lead Standard Solution into four separate 100-mL volumetric flasks, and to them, add a diluted solution (3 in 100) of nitric acid (for trace metals determination) to make exactly 100 mL.

Procedure Measure exactly a constant portion of the test solution and the standard solutions, and to each, add a quarter volume of a solution of magnesium nitrate hexahydrate (1 in 50), prepared before use. Analyze 25 μL each of these solutions as directed in Lead Limit Test (Atomic Absorption Spectrophotometry) using the operating conditions given below. Determine the concentration (ng/mL) of lead in the test solution from the calibration curve prepared using the standard solutions. Calculate the amount of lead in the sample by the formula given below. Perform a blank test using the blank

test solution prepared with water instead of Dimethyl Dicarbonate in the same manner as for the test solution, and make any necessary correction.

$$\text{Amount (μg/g) of lead} = \frac{\text{Concentration (μg/mL)} \times 25}{\text{Weight (g) of the sample}}$$

Operating Conditions

Light source: Lead hollow cathode lamp.

Wavelength: 283.3 nm.

Temperature for drying: A constant temperature of 200–250°C.

Temperature for incineration: A constant temperature of 700–750°C.

Temperature for atomization: A constant temperature of 1800–2000°C.

(2) Dimethyl carbonate Not more than 0.2%.

The procedure given here should be operated as quickly as possible, protected from moisture.

Test Solution Weigh accurately about 5 g of Dimethyl Dicarbonate, add 0.5 mL of the internal standard solution, and add *tert*-butyl methyl ether to make exactly 5 mL.

Standard Solution Weigh accurately about 10 mg of dimethyl carbonate, add exactly 0.5 mL of the internal standard solution, and add *tert*-butyl methyl ether to make exactly 5 mL.

Internal Standard Solution Dissolve 50 mg of 3-pentanone in *tert*-butyl methyl ether to make exactly 5 mL.

Procedure Analyze 0.5 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratios (Q_T and Q_S) of dimethyl carbonate to 3-pentanone for the test solution and the standard solution, respectively. Determine the amount of dimethyl carbonate by the formula:

$$\text{Amount (\%) of dimethyl carbonate } (C_3H_6O_3) = \frac{\text{Weight (mg) of dimethyl carbonate}}{\text{Weight (g) of the sample} \times 1000} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.53 mm internal diameter and 60 m length) coated with a 1.5 μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 45°C for 7.5 minutes, raise at 10°C/minute to 75°C, then raise at 25°C/minute to 125°C, and maintain the temperature at 125°C for 2 minutes. Raise at 30°C/minute to 260°C, and maintain the temperature at 260°C for 4.5 minutes.

Detector temperature: 300°C.

Carrier gas: Helium.

Flow rate: Adjust so that the peak of 3-pentanone appears in 4–8 minutes after injection.

Injection method: Cold on-column injection method.

Assay The procedure given here should be operated as quickly as possible, protected from moisture.

Weigh accurately 2 g of Dimethyl Dicarbonate, add 100 mL of acetone (dehydrated), and mix. To this solution, add exactly 20 mL of dibutylamine–toluene TS (1 mol/L), stir, and immediately titrate the excess dibutylamine with 1 mol/L hydrochloric acid using an automatic potentiometric titrator. Perform a blank test in the same manner, and determine the content of dimethyl dicarbonate by the formula:

$$\text{Content (\%) of dimethyl dicarbonate } (C_4H_6O_5) = \frac{(a - b) \times 0.1341}{\text{Weight (g) of the sample}} \times 100$$

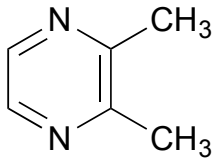
a = volume (mL) of 1 mol/L hydrochloric acid consumed in the blank test.

b = volume (mL) of 1 mol/L hydrochloric acid consumed in the test.

Storage Standards Store in a hermetic container at 20–30°C.

2,3-Dimethylpyrazine

2,3-ジメチルピラジン



$C_6H_8N_2$ Mol. Wt. 108.14

2,3-Dimethylpyrazine [5910-89-4]

Content 2,3-Dimethylpyrazine mainly consists of 2,3-dimethylpyrazine and contains not less than 95.0% of $C_6H_8N_2$ as a mixture of 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, and 2,6-dimethylpyrazine.

Description 2,3-Dimethylpyrazine occurs as a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of 2,3-Dimethylpyrazine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

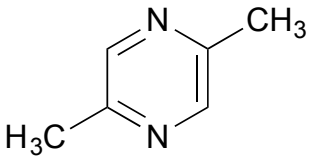
Refractive Index n_D^{20}: 1.501–1.510.

Specific Gravity d_{25}^{25}: 0.997–1.030.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (1).

2,5-Dimethylpyrazine

2,5-ジメチルピラジン



$C_6H_8N_2$ Mol. Wt. 108.14

2,5-Dimethylpyrazine [123-32-0]

Content 2,5-Dimethylpyrazine mainly consists of 2,5-dimethylpyrazine and contains not less than 98.0% of $C_6H_8N_2$ as a mixture of 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, and 2,6-dimethylpyrazine.

Description 2,5-Dimethylpyrazine occurs as a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of 2,5-Dimethylpyrazine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

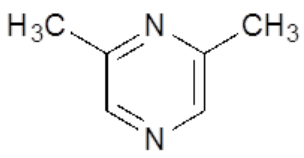
Refractive Index n_D^{20}: 1.497–1.503.

Specific Gravity d_{25}^{25}: 0.982–1.000.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (1).

2,6-Dimethylpyrazine

2,6-ジメチルピラジン



$C_6H_8N_2$ Mol. Wt. 108.14

2,6-Dimethylpyrazine [108-50-9]

Content 2,6-Dimethylpyrazine mainly consists of 2,6-dimethylpyrazine and contains not less than 98.0% of $C_6H_8N_2$ as a mixture of 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, and 2,5-dimethylpyrazine.

Description 2,6-Dimethylpyrazine occurs as white to yellow crystals having a characteristic odor.

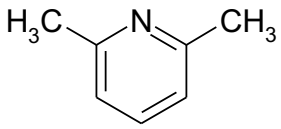
Identification Melt 2,6-Dimethylpyrazine by warming, and hold it between two pre-warmed optical plates. Immediately determine the absorption spectrum, being careful not to become hard, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 35–40 ºC.

Assay Use a solution of 2,6-Dimethylpyrazine in ethanol (95) (1 in 10) as the test solution. Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (1).

2,6-Dimethylpyridine

2,6-ジメチルピリジン



C_7H_9N Mol. Wt. 107.16

2,6-Dimethylpyridine [108-48-5]

Content 2,6-Dimethylpyridine contains not less than 98.5% of 2,6-dimethylpyridine (C_7H_9N).

Description 2,6-Dimethylpyridine occurs as a colorless, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 2,6-Dimethylpyridine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.495–1.501.

Specific Gravity d_{25}^{25}: 0.917–0.923.

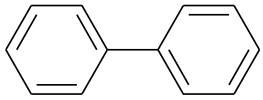
Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions

(2).

Diphenyl

Biphenyl

ジフェニル



$C_{12}H_{10}$ Mol. Wt. 154.21

Biphenyl [92-52-4]

Content Diphenyl contains 98.0–102.0% of diphenyl ($C_{12}H_{10}$).

Description Diphenyl occurs as colorless to white crystals, crystalline powder, or crystalline lumps having a characteristic odor.

Identification

(1) To 2 drops of a solution (1 in 100) of Diphenyl in ethyl acetate, add 0.5 mL of acetic acid and 1 mL of nitric acid, and heat at 70°C for 30 minutes. Cool, add 5 mL of water and 10 mL of ethyl acetate, and shake. Measure 5 mL of the ethyl acetate layer, and evaporate the ethyl acetate. Dissolve the residue in 1 mL of ethanol (95), add 2 mL of diluted hydrochloric acid (1 in 2) and 0.2 g of zinc powder, and heat in a water bath for 10 minutes. Cool and filter the mixture. To the filtrate, add 50 mL of water, then add 1 mL of sodium nitrite solution (1 in 100), and shake. Allow to stand for 10 minutes, add 1 mL of diluted ammonium amidosulfate (1 in 40), and allow to stand for 5 minutes. Add 2 mL of a solution prepared by dissolving 1 g of *N*-1-naphthylethylenediamine dihydrochloride in 100 mL of diluted hydrochloric acid (1 in 4), shake well, and allow to stand for 20 minutes. A purple color develops.

(2) On the surface of 1 mL of a solution (1 in 100) of Diphenyl in ethyl acetate, place 1 mL of formaldhyde solution–sulfuric acid TS. The lower layer shows blue to green-blue color.

Melting Point 69–71°C.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Naphthalene and its derivatives

Test Solution Weigh 2.5 g of Diphenyl, dissolve it in 50 mL of chloroform, add 2.0 mL of a solution (1 in 50) of methyl salicylate in chloroform, and add chloroform to make 100 mL.

Control Solution Measure 5 mL of a solution (1 in 1000) of naphthalene in chloroform, add 2.0 mL of a solution (1 in 50) of methyl salicylate in chloroform, and add chloroform to make 100 mL.

Procedure Analyze equal portions of the test solution and the control solution by gas chromatography, using the conditions given below. The ratio (A/A_S) of the sum (A) of the peak area of the naphthalene and the peak areas of all peaks appearing between the peaks of methyl salicylate and diphenyl for the test solution to the peak area (A_S) of the methyl salicylate does not exceed the ratio (A'/A_S') of the peak area (A') of the naphthalene to the peak area (A_S') of the methyl salicylate for the control solution.

Operating Conditions

Detector: Flame ionization detector.

Column: A stainless steel or glass tube (2–3 m length and 3–4 mm internal diameter).

Column packing material

Liquid phase: 10% Polyethylene glycol 6000 of the amount of support.

Support: 177- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of 160–180°C.

Carrier gas: Use nitrogen.

Flow rate: Adjust so that the peak of the methyl salicylate appears about 5 minutes after the injection.

Assay Weigh accurately about 0.1 g of Diphenyl, and dissolve it in methanol to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add methanol to make exactly 200 mL. Measure the absorbance (A) of the second solution at a wavelength of 248 nm, using methanol for reference, and calculate the content by the formula:

$$\text{Content (\%) of diphenyl } (C_{12}O_{10}) = \frac{A}{1118} \times \frac{20 \times 10}{\text{Weight (g) of the sample}} \times 100$$

Dipotassium Hydrogen Phosphate

Potassium Phosphate, Dibasic

リン酸水素二カリウム

K_2HPO_4 Mol. Wt. 174.18

Dipotassium hydrogenphosphate [7758-11-4]

Content Dipotassium Hydrogen Phosphate, when dried, contains not less than 98.0% of dipotassium hydrogen phosphate (K_2HPO_4).

Description Dipotassium Hydrogen Phosphate occurs as white crystals, powder, or

lumps.

Identification A solution of Dipotassium Hydrogen Phosphate (1 in 20) responds to all the tests for Potassium Salt and for Phosphate in the Qualitative Tests.

pH 8.7–9.3 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution Colorless, very slightly turbid (1.0 g, water 20 mL).

(2) Chloride Not more than 0.011% as Cl (1.0 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(4) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Dipotassium Hydrogen Phosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 5.0% (105°C, 4 hours).

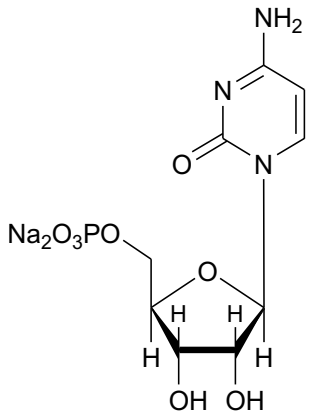
Assay Weigh accurately about 3 g of Dipotassium Hydrogen Phosphate, previously dried, dissolve it in 50 mL of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 2–3 drops of methyl orange–indigo carmine TS).

Each mL of 1 mol/L hydrochloric acid = 174.2 mg of K_2HPO_4

Disodium 5′-Cytidylate

Sodium 5′-Cytidylate

5′-シチジル酸二ナトリウム



$C_9H_{12}N_3Na_2O_8P$ Mol. Wt. 367.16

Disodium cytidine 5′-monophosphate [6757-06-8]

Content Disodium 5′-Cytidylate, when calculated on the anhydrous basis, contains 97.0–102.0% of disodium 5′-cytidylate ($C_9H_{12}N_3Na_2O_8P$).

Description Disodium 5′-Cytidylate occurs as colorless to white crystals or as a white crystalline powder. It has a slight, characteristic taste.

Identification

(1) To 3 mL of a solution of Disodium 5′-Cytidylate (3 in 10,000), add 1 mL of hydrochloric acid and 1 mL of bromine TS, heat in a water bath for 30 minutes, and remove the bromine by blowing with air. Add 0.2 mL of orcinol–ethanol TS, then add 3 mL of ammonium iron(III) sulfate–hydrochloric acid TS, and heat in a water bath for 20 minutes. A green color develops.

(2) To 5 mL of a solution of Disodium 5′-Cytidylate solution (1 in 20), add 2 mL of magnesia TS. No precipitate is formed. Then add 7 mL of nitric acid, boil for 10 minutes, and neutralize with sodium hydroxide solution (1 in 25). The solution responds to test (2) for Phosphate in the Qualitative Tests.

(3) Dissolve 20 mg of Disodium 5′-Cytidylate in 1000 mL of diluted hydrochloric acid (1 in 1000). The solution exhibits an absorption maximum at a wavelength of 277–281 nm.

(4) Disodium 5′-Cytidylate responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 8.0–9.5 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution　Colorless and almost clear (0.50 g, water 10 mL).

(2) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Absorbance ratio　Weigh 20 mg of Disodium 5′-Cytidylate, and dissolve it in diluted hydrochloric acid (1 in 1000) to make 1000 mL. Measure the absorbance (A_1, A_2, and A_3) of this solution at wavelengths of 250 nm, 260 nm, and 280 nm, respectively. A_1/A_2 is 0.40–0.52, and A_3/A_2 is 1.85–2.20.

(5) Other decomposed substances of ribonucleic acids　Proceed as directed in Purity (5) for Disodium 5′-Inosinate.

Water Content　Not more than 26.0% (0.15 g, Volumetric Titration, Back Titration).

Before performing the titration, add water determination TS in excess, and stir for 20 minutes.

Assay

Test Solution　Weigh accurately about 0.5 g of Disodium 5′-Cytidylate, and dissolve it in diluted hydrochloric acid (1 in 1000) to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add diluted hydrochloric acid (1 in 1000) to make exactly 250 mL.

Procedure　Measure the absorbance (A) of the test solution at a wavelength of 280 nm, and calculate the content by the formula:

$$\text{Content (\%) of disodium 5′-cytidylate } (C_9H_{12}N_3Na_2O_8P) = \frac{0.5 \times 1.446 \times A}{\text{Anhydrous basis weight (g) of the sample}} \times 100$$

Disodium Dihydrogen Pyrophosphate

Acid Sodium Pyrophosphate
Sodium Acid Pyrophosphate
Disodium Diphosphate

ピロリン酸二水素二ナトリウム

$Na_2H_2P_2O_7$　　Mol. Wt. 221.94

Sodium dihydrogendiphosphate　[7758-16-9]

Content　Disodium Dihydrogen Pyrophosphate, when dried, contains not less than 95.0% of disodium dihydrogen pyrophosphate ($Na_2H_2P_2O_7$).

Description Disodium Dihydrogen Pyrophosphate occurs as a white crystalline powder.

Identification

(1) To 10 mL of a solution of Disodium Dihydrogen Pyrophosphate (1 in 100), add 1 mL of silver nitrate solution (1 in 50). A white precipitate is formed.

(2) Disodium Dihydrogen Pyrophosphate responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 3.8–4.5 (1.0 g, water 100 mL).

Purity

(1) Water-insoluble substances Not more than 0.80%.

Weigh accurately a glass filter (1G4), previously dried at 110°C for 30 minutes and allowed to cool in a desiccator. Weigh 5.0 g of Disodium Dihydrogen Pyrophosphate, dissolve it in 100 mL of water, and allow to stand for 1 hour with occasional shaking. Collect the insoluble substances by filtration through the glass filter, wash with 30 mL of water, dry at 110°C for 2 hours together with the glass filter, allow to cool in the desiccator, and weigh accurately the glass filter with residue.

(2) Chloride Not more than 0.057% as Cl (0.25 g, Control Solution: 0.01 mol/L hydrochloric acid 0.40 mL).

(3) Orthophosphate Weigh 1.0 g of Disodium Dihydrogen Pyrophosphate, and add dropwise 2–3 drops of silver nitrate solution (1 in 50). No obvious yellow color develops.

(4) Sulfate Not more than 0.038% as SO_4 (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(5) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Disodium Dihydrogen Pyrophosphate, add 5 mL of nitric acid and 25 mL of water, and boil gently for 15 minutes with a watch glass covering it. Allow to cool.

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 5.0% (110°C, 4 hours).

Assay

Test Solution Weigh accurately about 0.2 g of Disodium Dihydrogen Pyrophosphate, previously dried, add 5 mL of nitric acid and 25 mL of water, boil for 30 minutes while replenishing the lost water, and cool. Add water to make exactly 500 mL, and if necessary, filter through a dry filter paper.

Procedure Measure exactly 5 mL of the test solution, add 20 mL of vanadic acid–molybdic acid TS and water to make exactly 100 mL. Shake well, allow to stand for 30 minutes, and measure the absorbance at a wavelength of 400 nm against a reference solution prepared using 5 mL of water instead of the test solution. Measure accurately 10 mL of Phosphate Standard Solution, and add 20 mL of diluted nitric acid (1 in 25)

and water to make exactly 250 mL. Measure exactly 10 mL, 15 mL, and 20 mL of this solution, and proceed in the same manner as for the test solution. Measure the absorbance for each solution, and prepare a calibration curve.

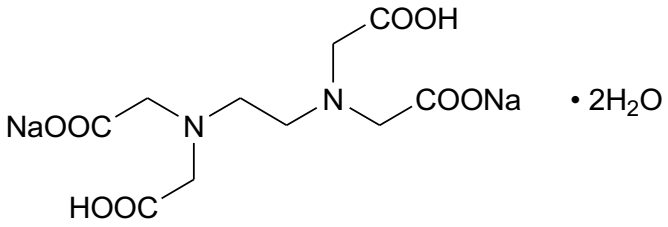
Determine the weight (g) of phosphorus (P) in 5 mL of the test solution from the calibration curve and the absorbance of the test solution, and calculate the content by the formula:

$$\text{Content (\%) of disodium dihydrogen pyrophosphate } (Na_2H_2P_2O_7) = \frac{\text{[Weight (g) of phosphorous (P) in 5 mL of the test solution]} \times 3.583 \times 100}{\text{Weight (g) of the sample}} \times 100$$

Disodium Ethylenediaminetetraacetate

Disodium EDTA

エチレンジアミン四酢酸二ナトリウム



$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ Mol. Wt. 372.24

Disodium dihydrogen ethylenediaminetetraacetate dihydrate [6381-92-6]

Content Disodium Ethylenediaminetetraacetate contains not less than 99.0% of disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$).

Description Disodium Ethylenediaminetetraacetate occurs as a white to whitish crystalline powder. It is odorless.

Identification

(1) A solution of Disodium Ethylenediaminetetraacetate (1 in 20) responds to all the tests for Sodium Salt in the Qualitative Tests.

(2) Proceed as directed in Identification (2) for Calcium Disodium Ethylenediaminetetraacetate.

pH 4.3–4.7.

Test Solution Weigh 1.0 g of Disodium Ethylenediaminetetraacetate, and dissolve it in water to make 100 mL.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Cyanide Not more than 1.0 μg/g as CN.

Test Solution Weigh 1.0 g of Disodium Ethylenediaminetetraacetate into a round-bottom flask, dissolve it in 100 mL of water, add 10 mL of phosphoric acid, and distill. Use a 100-mL measuring cylinder containing 15 mL of sodium hydroxide solution (1 in 50) as the receiver, immerse the end of the condenser in it, and distill until the total amount becomes 100 mL. Transfer 20 mL of this solution to a ground-glass stoppered test tube. Add 1 drop of phenolphthalein TS, neutralize with diluted acetic acid (1 in 20), add 5 mL of phosphate buffer (pH 6.8) and 1 mL of a solution of sodium *p*-toluenesulfonchloramide trihydrate (1 in 500), and immediately stopper. Mix gently, allow to stand for 2–3 minutes, add 5 mL of pyridine–pyrazolone TS, mix thoroughly, and allow to stand at 20–30°C for 50 minutes

Control Solution Measure 1.0 mL of Cyanide Standard Solution, and add 15 mL of sodium hydroxide solution (1 in 50) and water to make 1000 mL. Transfer 20 mL of this solution to a ground-glass stoppered test tube, and proceed as directed for the test solution.

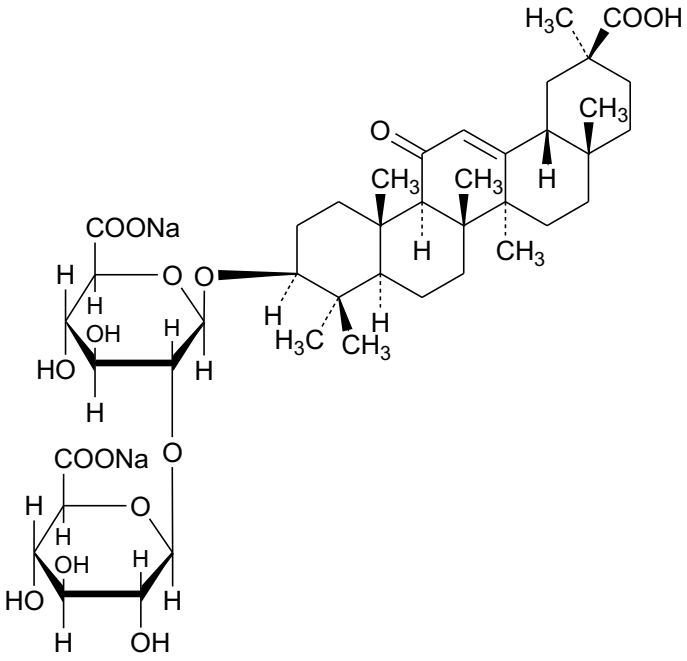
Procedure The color of the solution is not darker than that of the control solution.

Assay Weigh accurately about 0.4 g of Disodium Ethylenediaminetetraacetate, dissolve it in 20 mL of water, add 10 mL of ammonium buffer (pH 10.7), and titrate with 0.05 mol/L zinc (indicator: 2 drops of eriochrome black T TS) until the blue color of the solution changes to red.

Each mL of 0.05 mol/L zinc = 18.61 mg of $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$

Disodium Glycyrrhizinate

グリチルリチン酸二ナトリウム



$C_{42}H_{60}Na_2O_{16}$ Mol. Wt. 866.90

20β-Carboxy-11-oxo-30-norolean-12-en-3β-yl (sodium β-D-glucopyranosyluronate)-(1→2)-(sodium β-D-glucopyranosiduronate)

Content Disodium Glycyrrhizinate, when calculated on the anhydrous basis, contains 95.0–102.0% of disodium glycyrrhizinate ($C_{42}H_{60}Na_2O_{16}$).

Description Disodium Glycyrrhizinate occurs as a white to light yellow powder having an extremely sweet taste.

Identification

(1) To 0.5 g of Disodium Glycyrrhizinate, add 10 mL of diluted hydrochloric acid (1 in 10), boil gently for 10 minutes, cool, and filter. Wash the residue on the filter paper with water thoroughly, and dry at 105°C for 1 hour. To 1 mL of a solution (1 in 1000) of the dried substance in ethanol (95), add 0.5 mL of a solution (1 in 100) of butylated hydroxytoluene in ethanol (95) and 1 mL of sodium hydroxide solution (1 in 5), and heat in a water bath for 30 minutes, allowing the ethanol to vaporize. Red-purple to purple suspended solids are produced in the residual solution.

(2) To 1 mL of the filtrate obtained in Identification (1), add 10 mg of 1,3-dihydroxynaphthalene and 5 drops of hydrochloric acid, boil gently for 1 minute, allow to stand for 5 minutes, and immediately cool. To this solution, add 3 mL of toluene, and shake. The toluene layer turns red-purple.

(3) The residue on ignition of Disodium Glycyrrhizinate responds to all the tests for

Sodium Salt in the Qualitative Tests.

pH 5.5–6.5 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Weigh 0.50 g of Disodium Glycyrrhizinate, and dissolve it in 5 mL of water. The solution is clear, and its color is not darker than that of Matching Fluid I.

(2) Chloride Not more than 0.014% as Cl.

Test Solution Weigh 0.50g of Disodium Glycyrrhizinate, add 6 mL of diluted nitric acid (1 in 10) and 10 mL of water, boil gently for 10 minutes, and filter. Wash the residue on the filter paper twice with a small amount of water, and combine the filtrate and the washings. If the solution is colored, add 1 mL of hydrogen peroxide, and heat on a water bath for 10 minutes. Cool, filter the deposit, and wash the residue on the filter paper twice with a small amount of water. Combine the filtrate and the washings, and add water to make 50 mL.

Control Solution Measure 0.20 mL of 0.01 mol/L hydrochloric acid, add 6 mL of diluted nitric acid (1 in 10), and then add water to make 50 mL.

(3) Sulfate Not more than 0.029% as SO_4.

Test Solution Weigh 0.50 g of Disodium Glycyrrhizinate, add 5 mL of diluted hydrochloric acid (1 in 4) and 10 mL of water, boil gently for 10 minutes, and filter. Wash the residue on the filter paper twice with a small amount of water, combine the filtrate and the washings, and neutralize with ammonia TS. If the solution is colored, add 1 mL of hydrogen peroxide, and heat on a water bath for 10 minutes. After cooling, filter if necessary, wash the residue on the filter paper twice with a small amount of water, combine the filtrate and the washings, and add water to make 50 mL.

Control Solution Measure 0.30 mL of 0.005 mol/L sulfuric acid, and add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (1.5 g, Standard Color: Arsenic Standard Solution 9.0 mL, Apparatus B).

Test Solution Weigh the specific amount of Disodium Glycyrrhizinate, transfer into a Kjeldahl flask, add 10 mL of sulfuric acid and 10 mL of nitric acid, and heat until white fumes are evolved. If the solution still remains brown, cool, add 2 mL of nitric acid, and heat. Repeat until the solution becomes colorless to light yellow. After cooling, add 15 mL of a solution of ammonium oxalate monohydrate (1 in 25), and heat until white fumes are evolved again. Cool, and add water to make 25 mL. Use 10 mL of this solution as the test solution.

Standard Color Measure the specified volume of Arsenic Standard Solution, transfer into a Kjeldahl flask, add 10 mL of sulfuric acid and 10 mL of nitric acid, and heat until white fumes are evolved. Allow it to cool, add 15 mL of a solution of ammonium oxalate

monohydrate (1 in 25), and heat until white fumes are evolved again. After cooling, add 25 mL of water. Measure 10 mL of this solution, and proceed in the same manner as for the sample.

Water Content Not more than 13.0% (0.2 g, Volumetric Titration, Back Titration).

Residue on Ignition 15.0–18.0% (calculated on the anhydrous basis).

Assay

Test Solution Weigh accurately about 0.1 g of Disodium Glycyrrhizinate, and dissolve it in water to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add water to make exactly 25 mL.

Standard Solution Weigh accurately about 50 mg of Nicotinamide Reference Standard, previously dried for 4 hours in a vacuum desiccator, and dissolve it in water to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add water to make exactly 25 mL.

Procedure Measure the absorbance (A_T) of the test solution at a wavelength of 259 nm, using water as the reference solution. Measure the absorbance (A_S) of the standard solution at a wavelength of 261 nm, using water as the reference solution and calculate the content by the formula:

$$\text{Content (g) of disodium glycyrrhizinate } (C_{42}H_{60}Na_2O_{16}) = \frac{\text{Weight (g) of nicotinamide}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{2A_T}{A_S \times F}$$

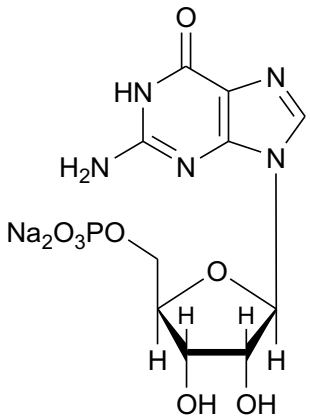
F = 1.093.

Disodium 5′-Guanylate

Disodium Guanylate

Sodium 5′-Guanylate

5′-グアニル酸二ナトリウム



$C_{10}H_{12}N_5Na_2O_8P$ Mol. Wt. 407.18

Disodium guanosine 5′-monophosphate [5550-12-9]

Content Disodium 5′-Guanylate, when dried, contains 97.0–102.0% of disodium 5′-guanylate ($C_{10}H_{12}N_5Na_2O_8P$).

Description Disodium 5′-Guanylate occurs as colorless to white crystals or powder having a characteristic taste.

Identification

(1) To 3 mL of a solution of Disodium 5′-Guanylate (3 in 10,000), add 0.2 mL of a solution of orcinol–ethanol TS), then add 3 mL of ammonium iron(III) sulfate–hydrochloric acid TS, and heat in a water bath for 10 minutes. A green color develops.

(2) To 5 mL of a solution of Disodium 5′-Guanylate (1 in 100), add 2 mL of magnesia TS. No precipitate is formed. Then add 7 mL of nitric acid, boil for 10 minutes, and neutralize with sodium hydroxide solution (1 in 25). The solution responds to test (2) for Phosphate in the Qualitative Tests.

(3) Dissolve 20 mg of Disodium 5′-Guanylate in 1000 mL of diluted hydrochloric acid (1 in 1000). The solution exhibits an absorption maximum at a wavelength of 254–258 nm.

(4) Disodium 5′-Guanylate responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 7.0–8.5 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and almost clear (0.10 g, water 10 mL).

(2) Lead Not more than 1 μg/g as Pb (4.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Absorbance ratio　Weigh 20 mg of Disodium 5′-Guanylate, and dissolve it in diluted hydrochloric acid (1 in 1000) to make 1000 mL. Measure the absorbance (A_1, A_2, and A_3) of this solution at wavelengths of 250 nm, 260 nm, and 280 nm, respectively. A_1/A_2 is 0.95–1.03, and A_3/A_2 is 0.63–0.71.

(5) Other decomposed substances of nucleic acid　Proceed as directed in Purity (5) for Disodium 5′-Inosinate.

Loss on Drying　Not more than 25.0% (120°C, 4 hours).

Assay　Weigh accurately about 0.5 g of Disodium 5′-Guanylate, and dissolve it in diluted hydrochloric acid (1 in 1000) to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add diluted hydrochloric acid (1 in 1000) to make exactly 250 mL. Use the second solution as the test solution. Measure the absorbance (A) of the test solution at a wavelength of 260 nm, and calculate the content by the formula:

$$\text{Content (g) of Disodium 5′-Guanylate } (C_{10}H_{12}N_5Na_2O_8P) = \frac{250}{\text{dry basis weight (g) of the sample}} \times \frac{A}{289.8} \times 100$$

Disodium Hydrogen Phosphate

Disodium Phosphate
Sodium Phosphate, Dibasic

リン酸水素二ナトリウム

$Na_2HPO_4 \cdot nH_2O$ (n = 12, 10, 8, 7, 5, 2, or 0)　　Mol. Wt. dodecahydrate　358.14
anhydrous　141.96

Disodium hydrogenphosphate dodecahydrate　[10039-32-4]

Disodium hydrogenphosphate decahydrate

Disodium hydrogenphosphate octahydrate

Disodium hydrogenphosphate heptahydrate　[7782-85-6]

Disodium hydrogenphosphate pentahydrate

Disodium hydrogenphosphate dihydrate　[10028-24-7]

Disodium hydrogenphosphate　[7558-79-4]

Definition　Disodium Hydrogen Phosphate occurs in two forms: the crystalline form (dodeca-, deca-, octa-, hepta-, penta-, or dihydrate) called Disodium Hydrogen Phosphate (crystal) and the anhydrous form called Disodium Hydrogen Phosphate (anhydrous).

Content Disodium Hydrogen Phosphate, when dried, contains not less than 98.0% of disodium hydrogen phosphate (Na_2HPO_4).

Description Disodium Hydrogen Phosphate (crystal) occurs as colorless to white crystals or crystalline lumps. Disodium Hydrogen Phosphate (anhydrous) occurs as a white powder.

Identification A solution of Disodium Hydrogen Phosphate (1 in 20) responds to all the tests for Sodium Salt and for Phosphate in the Qualitative Tests.

pH 9.0–9.6 (1.0 g, water 100 mL).

Purity For Disodium Hydrogen Phosphate (crystal), dry the sample before performing the tests.

(1) Clarity of solution Colorless and almost clear (0.50 g, water 20 mL).

(2) Chloride Not more than 0.21% as Cl (0.10 g, Control Solution: 0.01 mol/L hydrochloric acid 0.60 mL).

(3) Sulfate Not more than 0.038% as SO_4 (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(4) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Disodium Hydrogen Phosphate , add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying

Crystal: Not more than 61.0% (40°C, 3 hours, then 120°C, 4 hours).

Anhydrous: Not more than 2.0% (120°C, 4 hours).

Assay Weigh accurately about 3 g of Disodium Hydrogen Phosphate, previously dried, dissolve it in 50 mL of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 2–3 drops of methyl orange–indigo carmine TS).

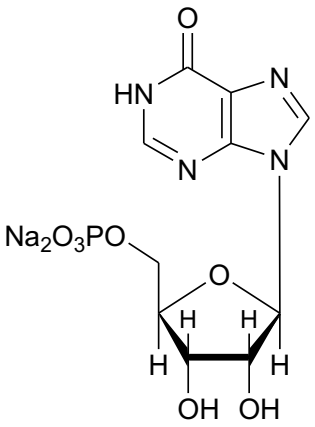
Each mL of 1 mol/L hydrochloric acid = 142.0 mg of Na_2HPO_4

Disodium 5′-Inosinate

Disodium Inosinate

Sodium 5′-Inosinate

5′-イノシン酸二ナトリウム



$C_{10}H_{11}N_4Na_2O_8P$ Mol. Wt. 392.17

Disodium inosine 5′-monophosphate [4691-65-0]

Content Disodium 5′-Inosinate, when calculated on the anhydrous basis, contains 97.0–102.0% of disodium 5′-inosinate ($C_{10}H_{11}N_4Na_2O_8P$).

Description Disodium 5′-Inosinate occurs as colorless to white crystals or as a white crystalline powder. It has a characteristic taste.

Identification

(1) To 3 mL of a solution of Disodium 5′-Inosinate (3 in 10,000), add 0.2 mL of orcinol–ethanol TS, then add 3 mL of ammonium iron(III) sulfate–hydrochloric acid TS, and heat in a water bath for 10 minutes. A green color develops.

(2) To 5 mL of a solution of Disodium 5′-Inosinate (1 in 20), add 2 mL of magnesia TS. No precipitate is formed. Then add 7 mL of nitric acid, boil for 10 minutes, and neutralize with sodium hydroxide solution (1 in 25). The solution responds to test (2) for Phosphate in the Qualitative Tests.

(3) Dissolve 20 mg of Disodium 5′-Inosinate in 1000 mL of diluted hydrochloric acid (1 in 1000). The solution exhibits an absorption maximum at a wavelength of 248–252 nm.

(4) Disodium 5′-Inosinate responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 7.0–8.5 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and almost clear (0.50 g, water 10 mL).

(2) Lead Not more than 1 μg/g as Pb (4.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Absorbance ratio　Weigh 20 mg of Disodium 5′-Inosinate, and dissolve it in diluted hydrochloric acid (1 in 1000) to make 1000 mL. Measure the absorbance (A_1, A_2, and A_3) of this solution at wavelengths of 250 nm, 260 nm, and 280 nm, respectively. A_1/A_2 is 1.55–1.65, and A_3/A_2 is 0.20–0.30.

(5) Other decomposed substances of nucleic acid

Test Solution　Weigh 0.10 g of Disodium 5′-Inosinate and dissolve it in water to make 20 mL.

Procedure　Analyze a 1-μL portion of the test solution by thin-layer chromatography, using a 6:5:2 mixture of 1-propanol/ammonia TS/acetone as the developing solvent. No control solution is used. Use a thin-layer plate that is coated with fluorescent silica gel for thin-layer chromatography as the support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. Examine under ultraviolet light (around 250 nm) in a dark place. Only one spot is observed.

Water Content　Not more than 29.0% (0.15 g, Volmetric Titration, Back Titration).

Before titrating, add water determination TS in excess and stir for 20 minutes.

Assay　Weigh accurately about 0.5 g of Disodium 5′-Inosinate, and dissolve it in diluted hydrochloric acid (1 in 1000) to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add diluted hydrochloric acid (1 in 1000) to make exactly 250 mL. Use the second solution as the test solution. Measure the absorbance (A) of the test solution at a wavelength of 250 nm, and calculate the content by the formula:

$$\text{Content (\%) of disodium 5′-Inosinate } (C_{10}H_{11}N_4Na_2O_8P) = \frac{250 \times A}{\text{Anhydrous basis weight (g) of the sample} \times 310.0} \times 100$$

Disodium 5′-Ribonucleotide

Sodium 5′-Ribonucleotide

5′-リボヌクレオチド二ナトリウム

Definition　Disodium 5′-Ribonucleotide is a mixture of disodium 5′-inosinate, disodium 5′-guanylate, disodium 5′-cytidylate, and disodium 5′-uridylate, or a mixture of disodium 5′-inosinate and disodium 5′-guanylate.

Content　Disodium 5′-Ribonucleotide, when calculated on the anhydrous basis, contains 97.0–102.0% of disodium 5′-ribonucleotide, of which not less than 95.0% consists of disodium 5′-inosinate and disodium 5′-guanylate.

Description Disodium 5′-Ribonucleotide occurs as white to whitish crystals or powder. It is odorless and has a characteristic taste.

Identification

(1) To 1 mL of a solution of Disodium 5′-Ribonucleotide (1 in 2000), add 0.2 mL of orcinol–ethanol TS, then add 3 mL of ammonium iron(II) sulfate–hydrochloric acid TS, and heat in a water bath for 10 minutes. A green color develops.

(2) To 1 mL of a solution of Disodium 5′-Ribonucleotide (1 in 1000), add 2 mL of diluted hydrochloric acid (1 in 4) and 0.1 g of zinc powder, heat in a water bath for 10 minutes, and filter. Cool the filtrate in ice water, add 1 mL of sodium nitrite solution (3 in 1000), shake, and allow to stand for 10 minutes. Add 1 mL of ammonium amidosulfate solution (1 in 200), shake well, and allow to stand for 5 minutes. To the mixture, add 1 mL of *N*-1-naphthylethylenediamine dihydrochloride solution (1 in 500). A purple-red color develops.

(3) To 1 mL of a solution of Disodium 5′-Ribonucleotide (1 in 5000), add 1 mL of diluted hydrochloric acid (1 in 4), heat in a water bath for 10 minutes, and cool. Add 0.5 mL of Folin's TS and 2 mL of sodium carbonate saturated solution. A blue color develops.

(4) To 5 mL of a solution of Disodium 5′-Ribonucleotide (1 in 20), add 2 mL of magnesia TS. No precipitate is formed. Add 7 mL of nitric acid, boil for 10 minutes, and neutralize with sodium hydroxide solution (1 in 25). The solution responds to test (2) for Phosphate in the Qualitative Tests.

(5) A solution of Disodium 5′-Ribonucleotide (1 in 10) responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 7.0–8.5 (1.0 g, water 20 mL).

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Water Content Not more than 27.0% (0.15 g, Volumetric Titration, Back Titration).

Before titrating, add water determination TS in excess, and stir for 20 minutes.

Assay Calculate the content of disodium 5′-ribonucleotide and the total content of disodium 5′-inosinate ($C_{10}H_{11}N_4Na_2O_8P$) and disodium 5′-guanylate ($C_{10}H_{12}N_5Na_2O_8P$), using the following formulae from the values (I, G, and P) determined as directed in (1), (2), and (3) below.

$$\text{Content (\%) of disodium 5′-ribonucleotide} = \frac{(I + G + P)}{100 - \text{Water content (\%)}} \times 100$$

Content (%) of disodium 5′-inosinate ($C_{10}H_{11}N_4Na_2O_8P$)
and disodium 5′-guanylate ($C_{10}H_{12}N_5Na_2O_8P$)

$$= \frac{I + G}{100 - \text{Water content (\%)}} \times 100$$

(1) Disodium 5′-inosinate

Test Solution Weigh accurately about 0.65 g of Disodium 5′-Ribonucleotide, dissolve it in water to make exactly 500 mL, and use this solution as the sample solution. Measure exactly 1 mL of the sample solution, and add 4 mL of diluted hydrochloric acid (1 in 2) and water to make exactly 10 mL. Heat the mixture in a water bath for 40 minutes, and then cool. Add 0.4 g of zinc powder, allow to stand for 50 minutes with occasional vigorous shaking, add water to make exactly 20 mL, and filter. Measure exactly 10 mL of the filtrate, add 1 mL of diluted hydrochloric acid (1 in 2), add 1 mL of sodium nitrite solution (3 in 1000) while cooling in ice, shake well, and allow to stand for 10 minutes. Add 1 mL of ammonium amidosulfate solution (1 in 200), shake well, and allow to stand for 5 minutes. To this solution, add 1 mL of *N*-1-naphthylethylenediamine dihydrochloride solution (1 in 500), shake well, allow to stand for 15 minutes, and add water to make exactly 20 mL.

Standard Solutions and *Calibration* Weigh accurately about 30 mg each of disodium 5′-inosinate *n*-hydrate and disodium 5′-guanylate *n*-hydrate, dissolve separately in diluted hydrochloric acid (1 in 1000) to prepare two standard solutions of exactly 1000 mL each, and measure the absorbance of each solution. Use a wavelength of 250 nm for disodium 5′-inosinate and a wavelength of 260 nm for disodium 5′-guanylate. Calculate the molecular extinction coefficients (E_I and E_G) from each absorbance measured, and determine the contents of disodium 5′-inosinate and disodium 5′-guanylate using the following formulae:

$$\text{Content (\%) disodium 5′-inosinate } (C_{10}H_{11}N_4Na_2O_8P) = \frac{E_I}{12{,}160} \times 100$$

$$\text{Content (\%) disodium 5′-guanylate } (C_{10}H_{12}N_5Na_2O_8P) = \frac{E_G}{11{,}800} \times 100$$

Based on each content above, weigh accurately an amount equivalent to about 50 mg each of disodium 5′-inosinate *n*-hydrate and disodium 5′-guanylate *n*-hydrate on the anhydrous basis, combine them, and dissolve in water to make a standard stock solution of exactly 200 mL. Measure exactly 1 mL, 2 mL, and 3 mL of the standard stock solution, and add 4 mL of diluted hydrochloric acid (1 in 2) and water to each to make exactly 10 mL. Proceed as directed for the test solution to prepare standard solutions. Measure the absorbance of each standard solution at a wavelength of 515 nm, using the same reference solution as used for the test solution under Procedure to prepare a calibration

curve.

Procedure Measure the absorbance of the test solution at a wavelength of 515 nm against a reference solution prepared in the same manner as the preparation of the test solution, using 1 mL of water instead of the sample solution. From the calibration curve and the absorbance of the test solution, calculate the content (I (%)) of disodium 5′-inosinate ($C_{10}H_{11}N_4Na_2O_8P$) in the sample.

(2) Disodium 5′-guanylate

Test Solution Measure exactly 1 mL of the sample solution obtained in (1), and add 4 mL of diluted hydrochloric acid (1 in 6) and water to make exactly 10 mL. Heat the mixture in a water bath for 30 minutes, and then cool. Add 2 mL of Folin's TS and 5 mL of a saturated solution of sodium carbonate, allow to stand for 15 minutes, and add water to make exactly 50 mL. Centrifuge the solution if necessary. *Standard Solutions and Calibration* Transfer exactly 1 mL, 2 mL, and 3 mL of the standard stock solution obtained in (1) into separate 10-mL volumetric flasks, add 4 mL of diluted hydrochloric acid (1 in 6) to each, and add water to volume. Using these solutions, proceed as directed in the preparation of the test solution. Measure each absorbance at a wavelength of 750 nm using the same reference solution as used for the test solution to prepare a calibration curve.

Procedure Measure the absorbance of the test solution at a wavelength of 750 nm against a reference solution prepared as directed for the test solution using 1 mL of water instead of the sample solution. From the calibration curve and the absorbance of the test solution, calculate the content (G (%)) of disodium 5′-guanylate ($C_{10}H_{12}N_5Na_2O_8P$) in the sample.

(3) Disodium 5′-cytidylate and disodium 5′-uridylate

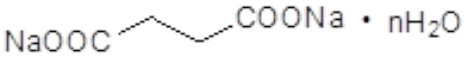
Test Solution Weigh accurately about 1.5 g of Disodium 5′-Ribonucleotide, add water to make exactly 50 mL, and use this solution as the sample solution. Measure exactly 1 mL of the sample solution, add 2 mL of hydrazine monohydrate, heat in a water bath for 1 hour, and then cool. Next, add diluted hydrochloric acid (1 in 10) to make the solution slightly acidic, and add diluted hydrochloric acid (1 in 1000) to make exactly 100 mL. Measure exactly 10 mL of this solution, and add diluted hydrochloric acid (1 in 1000) to make exactly 100 mL.

Procedure Measure the absorbance (A_{260} and A_{280}) of the test solution at wavelengths of 260 nm and 280 nm, respectively, using a reference solution prepared as directed for the test solution with 1 mL of water instead of the sample solution. Separately, measure exactly 1 mL of the sample solution, and add diluted hydrochloric acid (1 in 1000) to make exactly 100 mL. Measure exactly 10 mL of the second solution, and add diluted hydrochloric acid (1 in 1000) to make exactly 100 mL. Measure the absorbance (A'_{260} and A'_{280}) of the last solution at wavelengths of 260 nm and 280 nm, respectively, and calculate the total content (P (%)) of disodium 5′-cytidylate ($C_9H_{12}N_3Na_2O_8P$) and disodium 5′-uridylate ($C_9H_{11}N_2Na_2O_9P$) in the sample by the formula:

$$P\ (\%) = \frac{170.5 \times (A'_{260} - A_{260}) + 68.6 \times (A'_{280} - A_{280})}{\text{Weight (g) of the sample}}$$

Disodium Succinate

コハク酸二ナトリウム



n = 6 or 0

$C_4H_4Na_2O_4 \cdot nH_2O$ (n = 6 or 0) Mol. Wt. hexahydrate 270.14

anhydrous 162.05

Disodium butanedioate hexahydrate

Disodium butanedioate [150-90-3]

Definition Disodium Succinate occurs in two forms: the crystalline form (hexahydrate) called Disodium Succinate (crystal) and the anhydrous form called Disodium Succinate (anhydrous).

Content Disodium Succinate, when dried, contains not less than 98.0% of disodium succinate ($C_4H_4Na_2O_4$).

Description Disodium Succinate occurs as colorless to white crystals or as a white powder. It is odorless and has a characteristic taste.

Identification Disodium Succinate responds to all the tests for Sodium Salt and for Succinate in the Qualitative Tests.

pH 7.0–9.0 (1.0 g, water 20 mL).

Purity

(1) Sulfate Not more than 0.019% as SO_4.

Test Solution Weigh 1.0 g of Disodium Succinate, dissolve it in 30 mL of water, and neutralize with diluted hydrochloric acid (1 in 40).

Control Solution Use 0.40 mL of 0.005 mol/L sulfuric acid.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Readily oxidizable substances Weigh 2.0 g of Disodium Succinate, dissolve it in 20 mL of water and 30 mL of diluted sulfuric acid (1 in 20), and add 4.0 mL of 0.02 mol/L

potassium permanganate. The pink color of the solution does not disappear within 3 minutes.

Loss on Drying

Crystal: 37.0–41.0% (120°C, 2 hours).

Anhydrous: Not more than 2.0% (120°C, 2 hours).

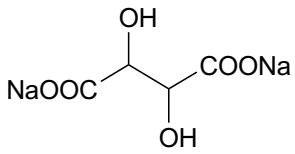
Assay Weigh accurately about 0.15 g of Disodium Succinate, previously dried, dissolve it in 30 mL of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid (indicator: 1 mL of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 8.103 mg of $C_4H_4Na_2O_4$

Disodium DL-Tartrate

Disodium *dl*-Tartrate

DL-酒石酸ナトリウム



$C_4H_4Na_2O_6$ Mol. Wt. 194.05

Disodium 2,3-dihydroxybutanedioate

Content Disodium DL-Tartrate, when dried, contains not less than 98.5% of disodium DL-tartrate ($C_4H_4Na_2O_6$).

Description Disodium DL-Tartrate occurs as colorless crystals or as a white crystalline powder.

Identification

(1) A solution of Disodium DL-Tartrate solution (1 in 10) has no optical rotation.

(2) Disodium DL-Tartrate responds to all the tests for Sodium Salt and for Tartrate in the Qualitative Tests.

pH 7.0–9.0 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Almost clear (1.0 g, water 20 mL).

(2) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(3) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Readily oxidizable substances　Weigh 2.0 g of Disodium DL-Tartrate, add 20 mL of water and 30 mL of diluted sulfuric acid (1 in 20) to dissolve, and then add 4.0 mL of 0.02 mol/L potassium permanganate while keeping at 20°C. The pink color of the solution does not disappear within 3 minutes.

Loss on Drying　Not more than 0.5% (105°C, 4 hours).

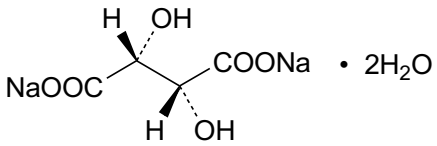
Assay　Weigh accurately about 0.2 g of Disodium DL-Tartrate, previously dried, add 3 mL of formic acid, dissolve it by warming, add 50 mL of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid. The endpoint is usually confirmed using a potentiometer. When crystal violet–acetic acid TS (1 mL) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 9.703 mg of $C_4H_4Na_2O_6$

Disodium L-Tartrate

Disodium *d*-Tartrate
Disodium Tartrate

L-酒石酸ナトリウム



$C_4H_4Na_2O_6 \cdot 2H_2O$　　Mol. Wt. 230.08

Disodium (2*R*,3*R*)-2,3-dihydroxybutanedioate dihydrate　[6106-24-7]

Content　Disodium L-Tartrate, when dried, contains not less than 98.5% of disodium L-tartrate ($C_4H_4Na_2O_6$ = 194.05).

Description　Disodium L-Tartrate occurs as colorless crystals or as a white crystalline powder.

Identification

(1) A solution of Disodium L-Tartrate (1 in 10) is dextrorotary.

(2) Disodium L-Tartrate responds to all the tests for Sodium Salt and for Tartrate in the Qualitative Tests.

Specific Rotation　$[\alpha]_D^{20}$: +25.0 to +27.5° (5 g, water, 50 mL).

pH 7.0–9.0. Proceed as directed in the pH test for Disodium DL-Tartrate.

Purity

(1) Clarity of solution Almost clear (1.0 g, water 20 mL).

(2) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Oxalate Weigh 1.0 g of Disodium L-Tartrate, dissolve it in 10 mL of water, and add 2 mL of a solution of calcium chloride dihydrate (2 in 25). The solution is not turbid.

Loss on Drying 14.0–17.0% (150°C, 3 hours).

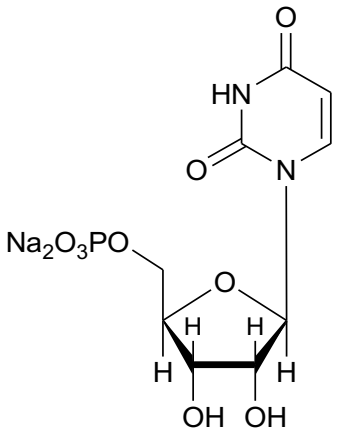
Assay Proceed as directed in the Assay for Disodium DL-Tartrate.

Each mL of 0.1 mol/L perchloric acid = 9.703 mg of $C_4H_4Na_2O_6$

Disodium 5′-Uridylate

Sodium 5′-Uridylate

5′-ウリジル酸二ナトリウム



$C_9H_{11}N_2Na_2O_9P$ Mol. Wt. 368.14

Disodium uridine 5′-monophosphate [3387-36-8]

Content Disodium 5′-Uridylate, when calculated on the anhydrous basis, contains 97.0–102.0% of disodium 5′-uridylate ($C_9H_{11}N_2Na_2O_9P$).

Description Disodium 5′-Uridylate occurs as colorless to white crystals or as a white crystalline powder. It has a slight, characteristic taste.

Identification

(1) To 3 mL of a solution of Disodium 5′-Uridylate (3 in 10,000), add 1 mL of hydrochloric acid and 1 mL of bromine TS, heat on a water bath for 30 minutes, remove the bromine by blowing with air, and add 0.2 mL of orcinol–ethanol TS. To this solution, add 3 mL of ammonium iron(III) sulfate–hydrochloric acid TS, and heat in a water bath for 20 minutes. A green color develops.

(2) To 5 mL of a solution of Disodium 5′-Uridylate (1 in 20), add 2 mL of magnesia TS. No precipitate is formed. Then add 7 mL of nitric acid, boil for 10 minutes, and neutralize with sodium hydroxide solution (1 in 25). The solution responds to test (2) for Phosphate in the Qualitative Tests.

(3) Dissolve 20 mg of Disodium 5′-Uridylate in 1000 mL of diluted hydrochloric acid (1 in 1000). The resulting solution exhibits an absorption maximum at a wavelength of 260–264 nm.

(4) Disodium 5′-Uridylate responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 7.0–8.5 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and almost clear (0.50 g, water 10 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Absorbance ratio Weigh 20 mg of Disodium 5′-Uridylate, and dissolve it in diluted hydrochloric acid (1 in 1000) to make 1000 mL. Measure the absorbance (A_1, A_2, and A_3) of this solution at wavelengths of 250 nm, 260 nm, and 280 nm, respectively. A_1/A_2 is 0.70–0.78, and A_3/A_2 is 0.34–0.42.

(5) Other decomposed substances of ribonucleic acids

Weigh 0.10 g of Disodium 5′-Uridylate, dissolve it in water to make 10 mL, and use this solution as the test solution. Analyze a 1-μL portion of the test solution by thin-layer chromatography, using a 2:2:1 mixture of ethanol (95)/2-methoxyethanol/diluted hydrochloric acid (1 in 10) as the developing solvent. No control solution is used. Use a thin-layer plate coated with microcrystalline cellulose for thin-layer chromatography as the solid support and then dried at 60–80°C for 20 minutes. Stop the development when the solvent front has ascended to a point 10 cm above the starting line, and air-dry the plate. Examine under ultraviolet light (around 250 nm) in a dark place. Only one spot is observed.

Water Content Not more than 26.0% (0.15 g, Volmetric Titration, Back Titration).

Before titrating, add water determination TS in excess, and stir for 20 minutes.

Assay Weigh accurately about 0.5 g of Disodium 5′-Uridylate, and dissolve it in diluted hydrochloric acid (1 in 1000) to make exactly 1000 mL. Measure exactly 10 mL of this

solution, and add diluted hydrochloric acid (1 in 1000) to make exactly 250 mL. Use the second solution as the test solution. Measure the absorbance (A) of the test solution at a wavelength of 260 nm, and calculate the content by the formula:

$$\text{Content (\%) of Disodium 5′-Uridylate } (C_9H_{11}N_2Na_2O_9P) = \frac{0.5 \times 1.859 \times A}{\text{Anhydrous basis weight (g) of the sample}} \times 100$$

Distarch Phosphate

リン酸架橋デンプン

[55963-33-2]

Definition Distarch Phosphate is obtained by esterifying starch with sodium trimetaphosphate or phosphorus oxychloride.

Description Distarch Phosphate occurs as a white to off-white powder, or as flakes or granules. It is odorless.

Identification

(1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

Purity

(1) Phosphorous Not more than 0.5% as P.

Proceed as directed in Purity (3) for Acetylated Distrach Phosphate.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (not more than 13.3 kPa, 120°C, 4 hours).

Dry Formed Vitamin A

粉末ビタミンA

Definition Dry Formed Vitamin A is produced by powdering vitamin A esters of fatty acids or vitamin A in oil.

Content Dry Formed Vitamin A contains the equivalent of 90–120% of the labeled value of vitamin A.

Description Dry Formed Vitamin A occurs as a light yellow to light red-brown powder.

Identification Weigh an amount of Dry Formed Vitamin A equivalent to 1500 units of vitamin A, and grind in a mortar. Add 10 mL of warm water, stir thoroughly to make a milky emulsion, and add 10 mL of ethanol (95) to dissolve the emulsion. Transfer it to a flask, add 20 mL of hexane, shake well, and let it separate into two layers either by allowing to stand or by centrifuging. Collect the hexane layer, wash with 20 mL of water by shaking well, separate the aqueous layer, and evaporate the hexane layer to dryness under reduced pressure. Dissolve the residue in 5 mL of petroleum ether, and use as the test solution. Proceed as directed in Identification (1) for Vitamin A Esters of Fatty Acids.

Purity

(1) Decay Dry Formed Vitamin A has no unpleasant odor.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (1.5 g, Standard Color: Arsenic Standard Solution 9.0 mL, Apparatus B).

Test Solution Weigh the specified amount of Dry Formed Vitamin A into a Kjeldahl flask, add 20 mL of nitric acid, and heat weakly until the contents is flowable. After cooling, add 5 mL of sulfuric acid, and heat until white fumes are evolved. If the solution is still brown, add another 5 mL of nitric acid after cooling, and heat. Repeat this procedure until the solution is colorless or light yellow. After cooling, add 15 mL of a solution of ammonium oxalate monohydrate (1 in 25), heat until white fumes are evolved again, and cool. Add water to make 25 mL, and use 10 mL of the resulting solution as the test solution.

Standard Color Transfer the specified amount of Arsenic Standard Solution into a Kjeldahl flask, and add 20 mL of nitric acid and 5 mL of sulfuric acid, and heat until while fumes are evolved. Allow to cool, add 15 mL of a solution of ammonium oxalate monohydrate (1 in 25), and heat until white fumes are evolved again. Allow to cool, and add water to make 25 mL. For 10 mL of this solution, proceed as directed for the test solution under the Arsenic Limit Test in the General Tests.

Loss on Drying Not more than 5.0% (reduced pressure, 4 hours).

Residue on Ignition Not more than 5.0%.

Assay Weigh accurately about 5 g of Dry Formed Vitamin A, add a small amount of

warm water, shake thoroughly to obtain milky emulsion, transfer into a flask, and proceed as directed in the Assay for Vitamin A in Oil.

Storage Standards Store in a hermetic, light-resistant container.

Dunaliella Carotene

デュナリエラカロテン

Definition Dunaliella Carotene is obtained from the entire part of the alga *Dunaliella bardawil* or *Dunaliella salina* and consists mainly of β-carotene. It may contain edible fats or oils.

Content (Color Value) Dunaliella Carotene contains the equivalent of not less than 10% of β-carotene ($C_{40}H_{56}$ = 536.88) or its Color Value ($E_{1cm}^{10\%}$) is not less than 2500. The actual value is in the range of 95–115% of the labeled value.

Description Dunaliella Carotene is a dark orange to red-brown, suspended oily substance having a slight, characteristic odor.

Identification

(1) Weigh an amount of Dunaliella Carotene equivalent to 50 mg of dunaliella carotene with a Color Value 2500, dissolve it in 5 mL of a 1:1 mixture of acetone/cyclohexane. An orange color develops.

(2) Prepare a solution of Dunaliella Carotene in a 1:1 mixture of acetone/cyclohexane at the concentration equivalent to either about 1 mg β-carotene per mL or about a Color Value 1 per mL, calculated from the labeled value. To 1 mL of the solution, add acetone to make 5 mL. To the resulting solution, add 1 mL of sodium nitrite solution (1 in 20) and 1 mL of sulfuric acid TS (0.5 mol/L). The solution immediately discolors.

(3) A solution of Dunaliella Carotene in cyclohexane exhibits an absorption maximum at a wavelength of either 446–457 nm or 472–486 nm or absorption maxima at both 446–457 nm and 472–486 nm.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50g, Method 4, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay (Color Value Determination) Proceed as directed under Color Value Determination, using the conditions below. Obtain the color value or determine the content of β-carotene by dividing color value by 250.

Operating Conditions

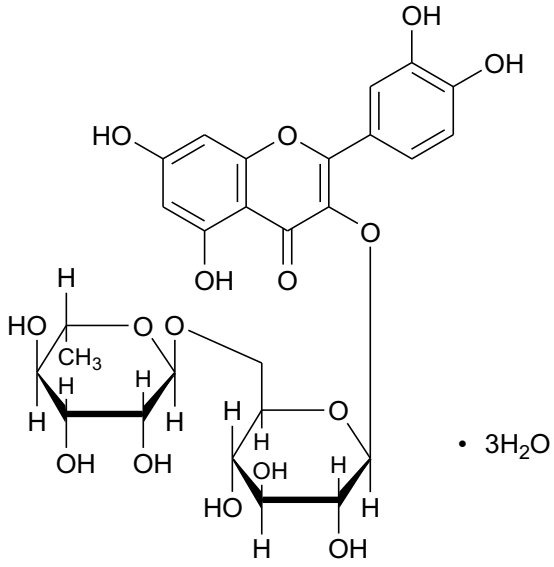
Solvent: Cyclohexane.

Wavelength: Maximum absorption wavelength of 446–457nm.

Enju Extract

Japanese Pagoda Tree Extract

エンジュ抽出物



$C_{27}H_{30}O_{16}\cdot 3H_2O$ Mol. Wt. 664.56

5,7-Dihydroxy-2-(3,4-dihydroxyphenyl)-4-oxo-4*H*-chromen-3-yl α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside trihydrate [rutin trihydrate, 250249-75-3]

Definition Enju Extract* is obtained from the buds or flowers of the plant *Styphnolobium japonicum* (L.) Schott (*Sophora japonica* L.) by extraction with water, ethanol, or methanol and then removal of the solvent used. It consists mainly of rutin.

Content Enju Extract, when dried, contains 95.0–105.0% of rutin ($C_{27}H_{30}O_{16}$).

Description Enju Extract occurs as a light yellow to light yellow-green crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Dissolve 20 mg of Enju Extract in 10 mL of ethanol (95). A yellow color develops, and on the addition of 1 to 2 drops of a solution of iron (III) chloride hexahydrate (1 in 50), the color changes to greenish brown.

(2) Dissolve 20 mg of Enju Extract in 5 mL of ethanol (95) by warming. A yellow color develops, and on the addition of 2 mL of hydrochloric acid and 50 mg of magnesium

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* Enju Extract is one of the substances belonging to the "Rutin (extract)" category. For the definition of the Rutin (extract), see Enzymatically Decomposed Rutin.

powder, the color gradually changes to red.

(3) Dissolve 20 mg of Enju Extract in 100 mL of ethanol (95) and to 2 mL of this solution, add ethanol (95) to make 20 mL. The solution exhibits absorption maxima at wavelengths of approximately 257 nm and 361 nm.

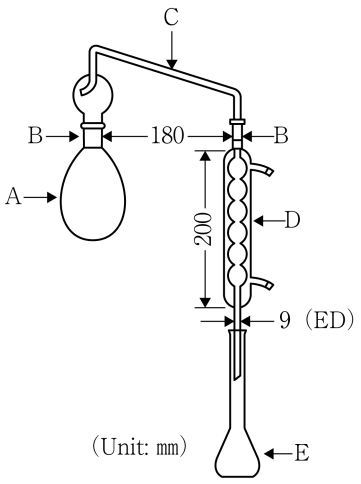
Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Methanol Not more than 0.015%.

(i) Apparatus Use the apparatus as illustrated in the figure.



A: Eggplant-shaped flask (200 mL)

B: Ground-glass joint

C: Delivery tube with a spray trap

D: Condenser

E: Volumetric flask (50 mL)

(ii) Method

Test Solution Weigh accurately about 5 g of Enju Extract in eggplant-shaped flask A, add 100 mL of boric acid-sodium hydroxide buffer, mix well, and add 2–3 boiling chips. Place exactly 2 mL of the internal standard solution in volumetric flask E. Assemble the apparatus, and moisten the ground-glass joints with water. Distill at a rate of 2–3 mL/minute until about 45 mL of distillate is obtained. To the distillate, add water to make exactly 50 mL. Use this solution as the test solution. Use 2-methyl-2-propanol solution (1 in 1000) as the internal standard.

Standard Solution Weigh accurately about 0.5 g of methanol, and add water to make exactly 100 mL. Measure exactly 5 mL of this solution, and add water to make exactly 100 mL. Next, measure exactly 3 mL of the second solution and exactly 2 mL of the internal standard solution, and add water to make exactly 50 mL.

Procedure Analyze 2.0 μL each of the test solution and the standard solution by gas chromatography using the operating conditions below. Determine the peak area ratios

(Q_T and Q_S)of methanol to 2-methyl-2-propanol for the test solution and the standard solution, and calculate the methanol content by the formula:

$$\text{Content (\%) of methanol} = \frac{\text{Weight (g) of methanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 0.15$$

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube (3 mm internal diameter and 2 m length).

Column packing material: 180- to 250-μm styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Injection port temperature: A constant temperature of about 200°C.

Injection: Full filling.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention time of methanol to about 2 minutes.

Loss on Drying Not more than 9.0 % (135°C, 2 hours).

Residue on Ignition Not more than 0.3 % (550°C, 4 hours).

Assay

Test Solution and Standard Solution Weigh accurately about 50 mg each of Enju Extract and rutin for assay, previously dried at 135°C for 2 hours, and separately dissolve them in methanol to make 2 solutions of exactly 50 mL each. Measure exactly 5 mL of each solution, and add an 800:200:1 mixture of water/acetonitrile/phosphoric acid to each to make exactly 50 mL. Use these solutions as the test solution and the standard solution, respectively.

Procedure Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions below. Measure the peak areas (A_T and A_S) of the test solution and the standard solution, and calculate the rutin content by the formula:

$$\text{Content (\%) of rutin } (C_{27}H_{30}O_{16}) = \frac{\text{Weight (g) of rutin for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Ultraviolet absorption spectrophotometer (wavelength: 254 nm).

Column: A stainless steel tube (3–6 mm internal diameter and 15–25 cm length).

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid

chromatography.

Column temperature: 40°C.

Mobile phase: An 800:200:1 mixture of water/acetonitrile/phosphoric acid.

Flow rate: Adjust the retention time of rutin to about 8–12 minutes.

Enzymatically Decomposed Lecithin

酵素分解レシチン

Definition Enzymatically Decomposed Lecithin is obtained from vegetable lecithin, which is derived from the seeds of the rape plant *Brassica rapa* var. *oleifera* DC. or *Brassica napus* L. or the soybean plant *Glycine max* (L.) Merr., or obtained from yolk lecithin, which is derived from egg yolk. It consists mainly of phosphatidic acid and lysolecithin. There are two types of commercial products: enzymatically decomposed vegetable lecithin and enzymatically decomposed yolk lecithin.

Description Enzymatically Decomposed Lecithin occurs as white to brown granules powder, or lumps, or as a light yellow to dark brown viscous liquid. It has a characteristic odor.

Identification

(1) Place 1 g of Enzymatically Decomposed Lecithin in a Kjeldahl flask, add 5 g of powdered potassium sulfate, 0.5 g of copper(II) sulfate pentahydrated, and 20 mL of sulfuric acid. Heat gently with a tilt of about 45° until effervescence almost stops. Raise the temperature, and boil until the content becomes a clear blue liquid. Heat for an additional 1 to 2 hours. After cooling, add an equal volume of water. To 5 mL of this solution, add 10 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 5), and heat. A yellow precipitate is formed.

(2) Fatty acids To 1 g of Enzymatically Decomposed Lecithin, add 25 mL of 3.5% (w/v) potassium hydroxide–ethanol TS, reflux for 1 hour, and cool with ice. A precipitate or turbidity of potassium soap is formed.

Purity

(1) Acid value Not more than 65.

Test Solution For vegetable lecithin, dissolve about 2 g of the sample, accurately weighed, in 50 mL of toluene. For yolk lecithin, to about 2 g of the sample, accurately weighed, add 50 mL of methanol, and dissolve it by warming in a water bath at 60°C or less.

Procedure Proceed as directed in the Acid Value Test in the Fats and Related

Substances Tests.

(2) Acetone-soluble substances Not more than 60%.

Weigh accurately about 2 g of Enzymatically Decomposed Lecithin into a 50-mL stoppered graduated centrifuge tube, dissolve it in 3 mL of toluene for vegetable lecithin or in 3 mL of methanol for yolk lecithin, and warm in a water bath at 60°C or less if necessary. To this solution, add 15 mL of acetone, stir well, and allow to stand in icy water for 15 minutes. Add acetone, previously cooled to 0–5°C, to make 50 mL, stir well, and allow to stand in icy water for 15 minutes. Centrifuge the solution obtained at about 3000 rpm for 10 minutes, and transfer the upper layer solution into a flask. To the residue in the centrifuge tube, add acetone cooled to a temperature of 0–5°C to make 50 mL, and stir well while cooling in icy water. Centrifuge in the same manner, and transfer the upper layer solution into the flask to combine them. Distill the combined solution on a water bath, dry the residue at 105°C for 1 hour, and weigh accurately.

(3) Peroxide value Not more than 10.

Weigh accurately about 5 g of Enzymatically Decomposed Lecithin into a 250-mL stoppered Erlenmeyer flask, add 35 mL of a 2:1 mixture of chloroform/acetic acid, and shake gently to uniformly disperse or dissolve. Replace the air in the flask with clean nitrogen, and add exactly 1 mL of potassium iodine TS under nitrogen. Stop the nitrogen flow, immediately stopper the flask, shake for 1 minute, and allow to stand for 5 minutes in a dark place. To this solution, add 15 mL of water, stopper, and shake vigorously. Titrate with 0.01 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Calculate the peroxide value, using the following formula. Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Conduct a blank test to make any necessary correction.

$$\text{Peroxide Value} = \frac{\text{Volume (mL) of 0.01 mol/L of sodium thiosulfate consumed}}{\text{Weight (g) of the sample}} \times 10$$

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 4.0% (105°C, 1 hour).

If the sample is a powder, proceed as directed under Loss on Drying in the General Tests. If the sample consists of granules, lumps, or a viscous liquid, proceed as directed in the following procedure. Place about 3 g of the sample in a weighing bottle, along with about 15 g of sea sand, weighed accurately, and a small glass rod, weighed accurately. Accurately weigh the bottle containing the sand and rod. Promptly grind the sample into particles of 2 mm or less using the rod if it is solid, or mix well if it is liquid. Finally, heat

the bottle with the glass rod, and determine the loss.

Enzymatically Decomposed Rutin

ルチン酵素分解物

Definition Enzymatically Decomposed Rutin is obtained by enzymatically treating and purifying rutin (extract).* Its principal constituent is isoquercitrin.

Content Enzymatically Decomposed Rutin, when dried, contains 91.0–103.0% of isoquercitrin ($C_{21}H_{20}O_{12}$ = 464.38).

Description Enzymatically Decomposed Rutin occurs as a light yellow to yellow powder, as lumps, or a paste. It has a slight, characteristic odor.

Identification

(1) Dissolve 5 mg of Enzymatically Decomposed Rutin in 10 mL of ethanol (95). A yellow color develops, and when 1 to 2 drops of a solution of iron(III) chloride hexahydrate (1 in 50) are added, the color changes to greenish brown.

(2) Dissolve 5 mg of Enzymatically Decomposed Rutin in 5 mL of ethanol (95). A yellow color develops, and when 2 mL of hydrochloric acid and 50 mg of magnesium powder are added, it gradually changes to red.

(3) Dissolve 10 mg of Enzymatically Decomposed Rutin in 500 mL of ethanol (95). The solution exhibits absorption maxima at wavelengths of approximately 258 nm and 362 nm.

(4) Prepare a test solution by dissolving 1.0 g of Enzymatically Decomposed Rutin in 20 mL of methanol. If necessary, filter before testing. Analyze a 2-μL portion of the test solution by thin-layer chromatography, using a 2-μL portion of a solution (1 in 20) of rutin for assay in methanol as the control solution and a 4:2:1 mixture of 1-butanol/acetic acid/water as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 15 cm above the starting line, and air-dry the plate. Spray with iron(III) chloride–hydrochloric acid TS. A brown main spot with an R_f value greater than that of the main spot of rutin for assay is observed.

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* "Rutin (extract)" is defined in the List of Existing Food Additives as a substance that is obtained from the entire part of the *azuki* plant *Vigna angularis* (Willd.) Ohwi & H. Ohashi, the buds or flowers of the plant *Styphnolobium japonicum* (L.), or the entire part of the buckwheat plant *Fagopyrum esculentum* Moench and that consists mainly of rutin.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 50.0% (135°C, 2 hours).

Assay

Test Solution Weigh accurately about 50 mg of Enzymatically Decomposed Rutin, previously dried, and dissolve it in methanol to make exactly 100 mL. If necessary, filter the solution obtained. Measure exactly 4 mL of the solution, and add diluted phosphoric acid (1 in 1000) to make exactly 100 mL.

Standard Solution Weigh accurately about 50 mg of rutin for assay, previously dried at 135°C for 2 hours, and dissolve it in methanol to make exactly 100 mL. Measure exactly 4 mL of this solution, and add diluted phosphoric acid (1 in 1000) to make exactly 100 mL.

Procedure Measure the absorbance (A_T and A_S) of the test solution and the standard solution as directed under Ultraviolet-Visible Spectrophotometry at 351 nm. Use diluted phosphoric acid (1 in 1000) as the reference. Calculate the content by the formula:

$$\text{Content (\%) of isoquercitrin } (C_{21}H_{20}O_{12}) = \frac{\text{Weight (g) of the rutin for assay} \times 0.761}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Enzymatically Hydrolyzed Licorice Extract

酵素分解カンゾウ

Definition Enzymatically Hydrolyzed Licorice Extract is obtained by enzymatically hydrolyzing licorice extract* and consists mainly of glycyrrhetinic acid 3-*O*-glucuronide.

Content Enzymatically Hydrolyzed Licorice contains the equivalent of not less than 40% of glycyrrhetinic acid glycosides, not less than 25% of which is made up by glycyrrhetinic acid 3-*O*-glucuronide.

Description Enzymatically Hydrolyzed Licorice Extract occurs as a white to yellow-brown powder.

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* Licorice Extract is defined in the List of Existing Food Additives as a substance that is obtained from the roots or rhizomes of *Glycyrrhiza uralensis* Fisch. ex DC., *Glycyrrhiza inflata* Batalin, or *Glycyrrhiza glabra* L., or their allied plants and consists mainly of glycyrrhizic acid.

Identification Analyze Enzymatically Hydrolyzed Licorice Extract by liquid chromatography using the operating conditions specified under the Assay. The retention times of the two peaks from the test solution correspond to respective peaks of glycyrrhetinic acid 3-*O*-glucuronide and glycyrrhizic acid in the standard solution.

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 8.0% (105°C, 1 hour).

Residue on Ignition Not more than 15.0%.

Assay

Test Solution Weigh accurately about 0.1 g of dried Enzymatically Hydrolyzed Licorice Extract, and dissolve it in 50% (vol) ethanol to make exactly 100 mL.

Standard Solution Weigh accurately about 20 mg each of glycyrrhetinic acid 3-*O*-glucuronide for assay (measure the water content in advance) and Glycyrrhizic Acid Reference Standard (measure the water content in advance) into a 100-mL volumetric flask, and dissolve in 50% (vol) ethanol to make exactly 100 mL.

Procedure Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_{T1}, A_{S1}) of glycyrrhetinic acid 3-*O*-glucuronide and the peak areas (A_{T2}, A_{S2}) of glycyrrhizic acid for both the test solution and standard solution, respectively. Calculate the content of glycyrrhetinic acid glycosides by the following formula, and then determine the ratio of glycyrrhetinic acid 3-*O*-glucuronide to glycyrrhetinic acid glycosides.

$$\text{Content (\%) of glycyrrhetinic acid 3-}O\text{-glucuronide} = \frac{\text{Anhydorous basis weight (g) of glycyrrhetinic acid 3-}O\text{-glucuronide for assay}}{\text{Weight (g) of the sample}} \times \frac{A_{T1}}{A_{S1}} \times 100$$

$$\text{Content (\%) of glycyrrhizic acid} = \frac{\text{Anhydorous basis weight (g) of Glycyrrhizic Acid Reference Standard}}{\text{Weight (g) of the sample}} \times \frac{A_{T2}}{A_{S2}} \times 100$$

Content (%) of glycyrrhetinic acid glycosides

= content (%) of glycyrrhetinic acid 3-*O*-glucuronide

+ content (%) glycyrrhizic acid

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 254 nm).

Column: A stainless steel tube (4–6 mm internal diameter and 15–30 cm).

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 42°C.

Mobile phase: A 1:1 mixture of 2% acetic acid/acetonitrile.

Flow rate: Adjust the retention time of glycyrrhetinic acid 3-*O*-glucuronide to about 15 minutes.

Column selection: Dissolve 5 mg of glycyrrhetinic acid 3-*O*-glucuronide for assay, 5 mg of glycyrrhizic acid for thin-layer chromatography, and 1 mg of propyl *p*-hydroxybenzoate in 50% ethanol (95) to make 20 mL. Use a column capable of producing well-resolved peaks of glycyrrhizic acid, propyl *p*-hydroxybenzoate, and glycyrrhetinic acid 3-*O*-glucuronide, in this order, when 20 μL of the prepared solution is chromatographed using the above operating conditions.

Enzymatically Modified Hesperidin

酵素処理ヘスペリジン

Definition Enzymatically Modified Hesperidin is obtained by glucosylating hesperidin, which is extracted from the peels, juice, or seeds of citrus fruits with an alkaline aqueous solution, using cyclodextrin glucosyltransferase.

Content Enzymatically Modified Hesperidin, when dried, contains the equivalent of not less than 30.0% of total hesperetin glycosides.

Description Enzymatically Modified Hesperidin occurs as a pale yellow to yellow-brown powder having a slight, characteristic odor.

Identification

(1) Dissolve 5 mg of Enzymatically Modified Hesperidin in 10 mL of water, and add 1–2 drops of 0.2% (w/v) iron(III) chloride TS. A brown color develops.

(2) Dissolve 0.5 g of Enzymatically Modified Hesperidin in 100 mL of an 80:20:0.01 mixture of water/acetonitrile/acetic acid, and use this solution as the test solution. Separately, dissolve 50 mg of monoglucosyl hesperidin for assay in 250 mL of an

80:20:0.01 mixture of water/acetonitrile/acetic acid, and use the resulting solution as the standard solution. Analyze 10 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Enzymatically Modified Hesperidin exhibits a peak at the position corresponding to monoglucosyl hesperidin, having an absorption maximum at a wavelength of 280–286 nm.

Operating Conditions

Detector: Photodiode array detector (wavelength: 280 nm, 200–400 nm).

Column: A stainless steel tube (3.9–4.6 mm internal diameter and 15–30 cm length).

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: An 80:20:0.01 mixture of water/acetonitrile/acetic acid.

Flow rate: Adjust the retention time of monoglucosyl hesperidin to about 15 minutes.

Purity

(1) Clarity Clear (0.5 g, water 100 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 6.0% (Not more than 2.7 kPa, 120°C, 2 hours).

Assay The total content of hesperetin glycosides is obtained as the sum of the contents of hesperidin, monoglucosyl hesperidin, and α-glucosyl residues released by glucoamylase treatment.

(i) Hesperidin and monoglucosyl hesperidin

Test Solution Weigh accurately about 1 g of Enzymatically Modified Hesperidin, previously dried, and dissolve it in 100 mL of water. Pour this solution into a glass tube (25 mm internal diameter) packed with 50 mL of acrylic acid ester resin, allow it to flow through at a rate of 2.5 mL/minute or less, and wash the resin with 250 mL of water. Next, allow 200 mL of 50% (vol) ethanol to flow through at a rate of 2.5 mL/minute or less to elute the absorbed fraction. Evaporate the eluate obtained to about 40 mL, add 10,000 units of glucoamylase, and allow the mixture to stand at 55°C for exactly 30 minutes. Heat it at 95°C for 30 minutes, cool to room temperature, and add water to make exactly 50 mL. Refer to this as Solution A. Measure exactly 3 mL of Solution A, and add an 80:20:0.01 mixture of water/acetonitrile/acetic acid to make exactly 50 mL.

Standard Solution Weigh accurately about 50 mg of monoglucosyl hesperidin for assay, previously dried, and dissolve it in an 80:20:0.01 mixture of water/acetonitrile/acetic acid to make exactly 250 mL.

Procedure Analyze 10 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_{TH}, A_{TM}) of hesperidin and monoglucosyl hesperidin for the test solution and the peak area (A_S) of monoglucosyl hesperidin for the standard solution. Calculate the contents of hesperidin and monoglucosyl hesperidin using the following formulae. The relative retention time of hesperidin to monoglucosyl hesperidin is about 1.1.

$$\text{Content (\%) of hesperidin} = \frac{\text{Weight (g) of dried monoglucosyl hesperidin for assay}}{\text{Weight (g) of the dried sample}} \times \frac{A_{TH}}{A_S} \times \frac{10}{3} \times 0.790 \times 100$$

$$\text{Content (\%) of monoglucosyl hesperidin} = \frac{\text{Weight (g) of dried monoglucosyl hesperidin for assay}}{\text{Weight (g) of the dried sample}} \times \frac{A_{TM}}{A_S} \times \frac{10}{3} \times 100$$

Operating Conditions

Detector: Ultraviolet absorption spectrophotometer (wavelength: 280 nm).

Column: A stainless steel tube (3.9–4.6 mm internal diameter and 15–30 cm length).

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: An 80:20:0.01 mixture of water/acetonitrile/acetic acid.

Flow rate: Adjust the retention time of monoglucosyl hesperidin to about 15 minutes.

(ii) α-Glucosyl residues released by glucoamylase treatment

Test Solution Use Solution A prepared in section (i) above.

Blank Test Solution Add 10,000 units of glucoamylase to about 40 mL of water, allow to stand at 55°C for 30 minutes, heat at 95°C for about 30 minutes, and cool to room temperature. Add water to make exactly 50 mL.

Standard Solutions Weigh accurately about 1g of D(+)-glucose, and dissolve it in water to make exactly 100 mL. Transfer exactly 5 mL, 10 mL, 20 mL, and 30 mL of this solution into separate 100-mL volumetric flasks, and dilute each with water to volume.

Procedure Measure 20 μL of the test solution, add exactly 3 mL of color fixing TS for

D-glucose determination, and shake. Allow the solution to stand at 37°C exactly for 5 minutes, and then cool to room temperature. Measure the absorbance at a wavelength of 505 nm against a reference solution prepared as directed for the test solution using 20 μL of water instead of the test solution. Perform a blank test by measuring the absorbance of the blank test solution in the same manner as for the test solution, and make any necessary correction. Prepare a calibration curve by measuring the absorbance of the standard solutions in the same manner as for the test solution. Determine the concentration of D(+)-glucose in the test solution from the calibration curve and the corrected absorbance of the test solution, and calculate the content of α-glucosyl residue released by glucoamylase treatment content by the formula:

$$\text{Content (\%) of α-glucosyl residue} = \frac{\text{Concentration (mg/mL) of D(+)-glucose in the test solution} \times 50}{\text{Weight (g) of the dried sample} \times 1000} \times 0.900 \times 100$$

(iii) Total content of hesperetin glycosides (dry matter)

Calculate the total content of hesperetin glycosides by the formula:

Total content (%) of hesperetin glycosides (dry matter)

= Content (%) of hesperidin + Content (%) of monoglucosyl hesperidin

+ Content (%) of α-glucosyl residue

Enzymatically Modified Isoquercitrin

酵素処理イソクエルシトリン

Definition Enzymatically Modified Isoquercitrin is obtained by glucosylating a mixture of "Enzymatically Decomposed Rutin" and starch or dextrin with cyclodextrin glucosyltransferase. It consists mainly of α-glucosylisoquercitrin.

Content Enzymatically Modified Isoquercitrin, when dried, contains α-glucosylisoquercitrin, equivalent to not less than 60.0% of rutin ($C_{27}H_{30}O_{16}$ = 610.52).

Description Enzymatically Modified Isoquercitrin occurs as a yellow to yellow-orange powder, as lumps, or as a paste. It has a slight, characteristic odor.

Identification

(1) Dissolve 5 mg of Enzymatically Modified Isoquercitrin in 10 mL of water. A yellow to yellow-orange color develops, and on the addition of 1 to 2 drops of a solution of iron(III) chloride hexahydrate (1 in 50), the color changes to blackish-brown.

(2) Dissolve 5 mg of Enzymatically Modified Isoquercitrin in 5 mL of water. A yellow

to yellow-orange color is produced, and on the addition of 2 mL of hydrochloric acid and 50 mg of magnesium powder, the color gradually changes to orange to red.

(3) Dissolve 0.1 g of Enzymatically Modified Isoquercitrin in 100 mL of sulfuric acid TS (0.5 mol/L), boil for 2 hours, and cool. A yellow precipitate is formed.

(4) Dissolve 10 mg of Enzymatically Modified Isoquercitrin in 500 mL of diluted phosphoric acid (1 in 1000). The solution obtained has absorption maxima at wavelengths of approximately 255 nm and 350 nm.

(5) Dissolve 0.1 g of Enzymatically Modified Isoquercitrin in 20 mL of water, and use the resulting solution as the test solution. Analyze a 5-μL portion of the test solution by thin-layer chromatography, using a 2-μL portion of a solution (1 in 20) of rutin for assay in methanol as the control solution and a 4:2:1 mixture of 1-butanol/acetic acid/water as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 15 cm above the starting line, air-dry the plate, and spray with iron(III) chloride–hydrochloric acid TS. Several brown spots are observed: one having an R_f value greater than that of the main spot of rutin for assay and others having R_f values the same as or smaller than that of the main spot of rutin for assay.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 50.0% (135°C, 2 hours).

Assay

Test Solution Weigh accurately about 50 mg of Enzymatically Modified Isoquercitrin, previously dried, and dissolve it in water to make exactly 100 mL. Filter if necessary. Measure exactly 4 mL of this solution, add diluted phosphoric acid (1 in 1000), and make exactly 100 mL.

Standard Solution Weigh accurately about 50 mg of rutin for assay, dried at 135°C for 2 hours, and dissolve it in methanol to make exactly 100 mL. Measure 4 mL of this solution, and add diluted phosphoric acid (1 in 1000) to make exactly 100 mL.

Procedure Measure the absorbance (A_T and A_S) of the test solution and the standard solution at 351 nm as directed under Spectrophotometry, using diluted phosphoric acid (1 in 1000) as the reference solution. Calculate the α-glucosylisoquercitrin content as rutin ($C_{27}H_{30}O_{16}$) by the formula:

$$\text{Content (\%) of } \alpha\text{-glucosylisoquercitrin as rutin } (C_{27}H_{30}O_{16}) = \frac{\text{Weight (g) of the rutine for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Enzymatically Modified Rutin (Extract)

酵素処理ルチン(抽出物)

Definition Enzymatically Modified Rutin (Extract) is obtained from rutin (extract)* and consists mainly of α-glucosylrutin.

Content Enzymatically Modified Rutin (Extract), when dried, contains not less than 70 % of quercetin glycosides (α-glucosylrutin, rutin, and isoquercitrin) and not less than 50% of α-glucosylrutin.

Description Enzymatically Modified Rutin (Extract) occurs as a yellow to yellow-brown powder.

Identification

(1) Dissolve 5 mg of Enzymatically Modified Rutin (Extract) in 10 mL of water, and add 1 to 2 drops of a solution of iron(III) chloride hexahydrate (1 in 50). A brown to blackish brown color develops.

(2) Prepare a test solution by dissolving about 0.2 g of Enzymatically Modified Rutin (Extract) in the mobile phase specified in the operating condition for Assay to make 100 mL. Prepare a standard solution by dissolving 10 mg of monoglucosylrutin in the mobile phase to make 10 mL. Analyze 10 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions specified under the Assay. Chromatograph at a wavelength of 254 nm using a photodiode array detector. The retention time of a peak from the test solution corresponds to that of monoglucosylrutin from the standard solution. Compare the absorption spectrum of this peak at 200–400 nm with that of the peak of monoglucosylrutin from the standard solution, both spectra exhibit the absorption maximum at the same wavelength.

Purity

(1) Clarity of solution Clear (0.5 g, water 100 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

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* "Rutin (extract)" is defined in the List of Existing Food Additives as a substance that is obtained from the entire part of the *azuki* plant *Vigna angularis* (Willd.) Ohwi & H. Ohashi, the buds or flowers of the plant *Styphnolobium japonicum* (L.) Schott (*Sophora japonica* L.) or the entire part of the buckwheat plant *Fagopyrum esculentum* Moench and that consists mainly of rutin.

Loss on Drying Not more than 6.0% (not more than 2.7 kPa, 120°C, 2 hours).

Assay The content of quercetin glycosides is obtained as the sum of the contents of quercetin glycosides obtained after glucoamylase treatment and α-glucosyl residues released by glucoamylase treatment. The content of α-glucosylrutin is calculated by subtracting the amounts of rutin and isoquercitrin from the content of quercetin glycosides.

(i) Quercetin glycosides obtained after glucoamylase treatment

Test Solution Weigh accurately about 0.5 g of dried Enzymatically Modified Rutin (Extract), dissolve it in 50 mL of water. Pour this solution into a glass tube (25 mm internal diameter) packed with 50 mL of acrylic acid ester resin, allow it to flow through at a rate of 2.5 mL/minute or less, and wash the resin with 250 mL of water. Next, allow 200 mL of 80% (vol) ethanol to flow through at a rate of 2.5 mL/minute or less to elute the absorbed fraction. Evaporate the eluate to about 40 mL, add 50,000 units of glucoamylase, and allow the mixture to stand at 55°C for about 60 minutes. Heat it at 95°C for 30 minutes, cool to room temperature, and add water to make exactly 100 mL. Refer to this as Solution A. Measure exactly 5 mL of Solution A, and add the mobile phase specified in the operating conditions to make exactly 50 mL.

Standard Solutions Prepare three standard solutions. Standard Solution 1: Dissolve about 20 mg of dried rutin for assay, accurately weighed, in 20 mL of methanol, and add the mobile phase to make exactly 100 mL. Standard Solution 2: Dissolve about 10 mg of monoglucosylrutin in the mobile phase to make 10 mL. Standard Solution 3: Dissolve about 10 mg of isoquercitrin in a small amount of ethanol, and add the mobile phase to make 10 mL .

Procedure Analyze 10 μL each of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Identify the peaks of rutin, monoglucosylrutin, and isoquercitrin from the test solution by comparing with their respective retention times of three substances in the standard solutions. Measure the peak areas (A_{TR}, A_{TM}, A_{TI}) of respective substances in the test solution and the peak area (A_S) of rutin in Standard Solution 1. Calculate the amounts of rutin, monoglucosylrutin, and isoquercitrin obtained after glucoamylase treatment by the respective formulae. Then, calculate the content of quercetin glycosides obtained after glucoamylase treatment.

$$\text{Content (\%) of rutin obtained after glucoamylase treatment} = \frac{\text{Weight (g) of dried rutin for assay}}{\text{Weight (g) of the dried sample}} \times \frac{A_{TR}}{A_S} \times \frac{50}{5} \times 100$$

$$\text{Content (\%) of monoglucosylrutin obtained after glucoamylase treatment} = \frac{\text{Weight (g) of dried rutin for assay}}{\text{Weight (g) of the dried sample}} \times \frac{A_{TM}}{A_S} \times \frac{50}{5} \times 1.266 \times 100$$

Content (%) of isoquercitrin obtained after glucoamylase treatment

$$= \frac{\text{Weight (g) of dried rutin for assay}}{\text{Weight (g) of the dried sample}} \times \frac{A_{TI}}{A_S} \times \frac{50}{5} \times 0.7606 \times 100$$

Content (%) of quercetin glycosides obtained after glucoamylase treatment

= Content (%) of rutin obtained after glucoamylase treatment

+ Content (%) of monoglucosylrutin obtained after glucoamylase treatment

+ Content (%) of isoquercitrin obtained after glucoamylase treatment

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 254 nm).

Column: A stainless steel tube (3.9–4.6 mm internal diameter and 15–30 cm length).

Column packing material: Octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: An 80 : 20 : 0.1 mixture of water/acetonitrile/phosphoric acid.

Flow rate: 0.5 mL/minutes.

(ii) α-Glucosyl residue released by glucoamylase treatment

Test Solution Use Solution A prepared in section (i) above.

Blank Test Solution Add 50,000 units of glucoamylase to about 40 mL of water, allow to stand at 55°C for 60 minutes, heat at 95°C for 30 minutes, and cool to room temperature. Add water to make exactly 100 mL.

Standard Solutions Dissolve about 1g of D(+)-glucose, accurately weighed, in water to make exactly 100 mL. Transfer exactly 5 mL, 10 mL, 20 mL, and 30 mL of this solution into separate 100-mL volumetric flasks, and add water to make exactly 100 mL each.

Procedure To 20 μL of the test solution, add exactly 3 mL of color fixing TS for D-glucose determination, and shake. Allow this solution to stand at 37°C exactly for 5 minutes, and then cool to room temperature. Measure the absorbance at a wavelength of 505 nm against a reference solution prepared as directed for the test solution using 20 μL of water instead of the test solution. Perform a blank test by measuring the absorbance of the blank test solution in the same manner as for the test solution, and make any necessary correction. Prepare a calibration curve by measuring the absorbance of the standard solutions in the same manner as for the test solution. Determine the concentration of D(+)-glucose in the test solution from the calibration curve, and calculate the content of α-glucosyl residue released by glucoamylase treatment by the formula:

Content (%) of α-Glucosyl residue released by glucoamylase treatment

$$= \frac{\text{Concentration (mg/mL) of D(+)-glucose in the test solution}}{\text{Weight (g) of the dried sample}} \times 0.900 \times 100$$

(iii) Quercetin glycosides

Determine the content of quercetin glycosides by the formula:

Content (%) of quercetine glycosides (dried)

= Content (%) of quercetin glycosides obtained after glucoamylase treatment

+ Content (%) of α-glucosyl residue released by glucoamylase treatment

(iv) α-Glucosylrutin

Test Solution Weigh accurately about 0.2 g of Enzymatically Modified Rutin (Extract), and dissolve it in the mobile phase specified in the operating conditions of (i) to make exactly 100 mL.

Procedure Analyze 10 μL each of the test solution and Standard Solutions 1 and 3 prepared in (i) by liquid chromatography using the operating conditions, and measure the peak areas of rutin and isoquercitrin. Calculate the respective contents of rutin and isoquercitrin by the following formula, and determine the content of α-glucosylrutin.

$$\text{Content (\%) of rutin} = \frac{\text{Weight (g) of dried rutin for assay}}{\text{Weight (g) of the dried sampe}} \times \frac{A_{TR}}{A_S} \times 100$$

$$\text{Content (\%) of isoquercitrin} = \frac{\text{Weight (g) of dried rutin for assay}}{\text{Weight (g) of the dried sampe}} \times \frac{A_{TI}}{A_S} \times 0.7606 \times 100$$

Content (%) of α-glucosylrutin = Content (%) of quercetin glycosides (%)

− Content (%) of rutin

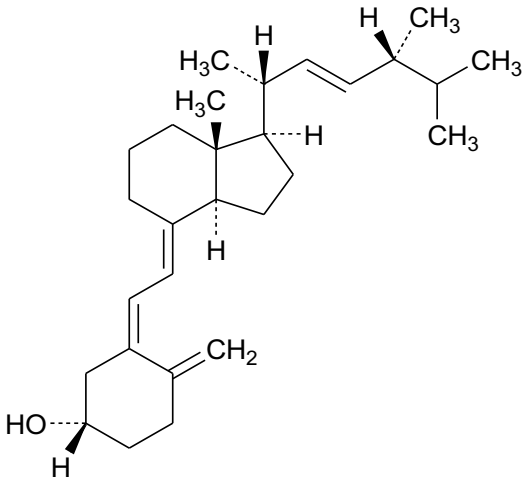
− Content (%) of isoquercitrin

Ergocalciferol

Calciferol

Vitamin D_2

エルゴカルシフェロール



$C_{28}H_{44}O$ Mol. Wt. 396.65

(3*S*,5*Z*,7*E*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol [50-14-6]

Description Ergocalciferol occurs as white crystals. It is odorless.

Identification

(1) Dissolve 0.5 mg of Ergocalciferol in 5 mL of toluene, and add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and shake. A red color develops, which immediately changes through purple and blue eventually to green.

(2) Dissolve 50 mg of Ergocalciferol in 1 mL of pyridine (dehydrated), add a solution of 50 mg of 3,5-dinitrobenzoyl chloride in 1 mL of dehydrated pyridine, heat under a reflux condenser on a water bath for 10 minutes, and cool to room temperature. Transfer the solution to a separating funnel, and extract with 15 mL of diluted hydrochloric acid (1 in 10) and 30 mL of diethyl ether by shaking. Wash the diethyl ether extract three times with 15 mL of diluted hydrochloric acid (1 in 10) each time, and wash with 30 mL of water. Add 5 g of sodium sulfate, allow to stand for 20 minutes, filter through absorbent cotton, and wash with a small amount of diethyl ether. Combine the filtrate and the washings, and evaporate the diethyl ether under reduced pressure. Recrystallize the residue twice from acetone, and dry in a desiccator for 2 hours (under reduced pressure). The melting point is 147–149°C.

Specific Absorbance $E_{1cm}^{1\%}$ (265 nm): 445–485.

Weigh accurately about 0.1 g of Ergocalciferol, and dissolve it in ethanol (95) to make exactly 200 mL. Measure exactly 2 mL of this solution, and add ethanol (95) to make exactly 100 mL. Measure the absorbance of the second solution.

Specific Rotation $[\alpha]_D^{20}$: +102.0 to +107.0° (0.3 g, ethanol (95), 20 mL).

Melting Point 115–118°C

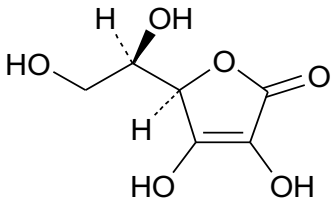
Purity Ergosterol Weigh 10 mg of Ergocalciferol, and add 2 mL of 90% (vol) ethanol to dissolve. To the resulting solution, add a solution prepared by dissolving 20 mg of digitonin in 2 mL of 90% (vol) ethanol, and allow to stand for 18 hours. No precipitate is formed.

Storage Standards Store in a cold place in a hermetic, light-resistant container under inert gas.

Erythorbic Acid

Isoascorbic Acid

エリソルビン酸



$C_6H_8O_6$ Mol. Wt. 176.12

(5*R*)-5-[(1*R*)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one [89-65-6]

Content Erythorbic Acid, when dried, contains not less than 99.0% of erythorbic acid ($C_6H_8O_6$).

Description Erythorbic Acid occurs as white to yellowish-white crystals or crystalline powder. It is odorless and has an acid taste.

Identification

(1) Dissolve 0.1 g of Erythorbic Acid in 100 mL of metaphosphoric acid solution (1 in 50). To 5 mL of this solution, add iodine TS dropwise until a slightly yellow color develops. To the solution, add 1 drop of a solution of copper(II) sulfate pentahydrate (1 in 1000) and 1 drop of pyrrole, and warm in a water bath at 50–60°C for 5 minutes. A blue to blue-green color develops.

(2) To 10 mL of a solution of Erythorbic Acid (1 in 100), add 1 mL of potassium permanganate solution (1 in 300). A pink color develops, which disappears immediately.

Specific Rotation $[\alpha]_D^{20}$: −16.2 to −18.2° (previously dried, 1 g, freshly boiled and cooled water, 10 mL).

Melting Point 166–172°C (decomposition).

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.4% (reduced pressure, 3 hours).

Residue on Ignition Not more than 0.3%.

Assay Weigh accurately about 0.4 g of Erythorbic Acid, dried previously, dissolve it in metaphosphoric acid solution (1 in 50) to make exactly 100 mL. Measure exactly 50 mL of this solution, and titrate with 0.05 mol/L iodine (indicator: starch TS).

Each mL of 0.05 mol/L iodine = 8.806 mg of $C_6H_8O_6$

Esterase

エステラーゼ

Definition Esterase includes enzymes that hydrolyze esters. It is obtained from the livers of animals, fishes, or the culture of filamentous fungi (limited to species of the genus *Aspergillus*), yeasts (limited to species of the genera *Candida* and *Torulopsis*), or bacteria (limited to species of the genus *Pseudomonas*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Esterase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Esterase complies with the Esterase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Esterase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 0.50 g of Esterase, add water or phosphate buffer (0.05 mol/L) at pH 6.5 to dissolve it or disperse it uniformly, and make 30 or 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the buffer to the resulting solution.

Substrate Solution Weigh 50 mg of chlorogenic acid hemihydrate, dissolve it in 1.0 mL of methanol, and add phosphate buffer (0.05 mol/L) at pH 6.5 to make 100 mL.

Test Solution Allow 0.5 mL of the substrate solution to equilibrate at 30°C for 2 minutes, add 0.03 mL of the sample solution, equilibrated to 30°C, shake immediately, and allow to incubate at 30°C for 30 minutes. To this solution, add 10 mL of 80% (vol) methanol, and immediately shake.

Control Solution Allow 0.5 mL of the substrate solution to equilibrate at 30°C for 30 minutes, add 10 mL of 80% (vol) methanol, and immediately shake.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 350 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Ester Gum

エステルガム

Definition Ester Gum is an ester compound composed of derivatives of rosin or its polymers. There are several types of ester gum products, according to the alcohols used, including glycerol ester gum, pentaerythritol ester gum, and methanol ester gum.

Description Ester Gum occurs as a white to yellow-whitish powder, as light yellow to light brown glassy lumps, or as a clear, viscous liquid. It is odorless or has a slight, characteristic odor.

Identification

(1) Dissolve 0.1 g of Ester Gum in 10 mL of acetic anhydride while heating in a water bath, cool, and add 1 drop of sulfuric acid. A purple-red color develops.

(2) To 1 g of Ester Gum, add 5 mL of sodium hydroxide solution (1 in 25) and 5 mL of water, and shake vigorously. Light yellow turbidity appears, and persistent foams are produced.

(3) In the case of glycerol ester gum compounds or pentaerythritol ester gum compounds

Test Solution Weigh about 5 g of Ester Gum, place into a 100-mL flask, add 40 mL

of a solution (1 in 10) of potassium hydroxide in 1-hexanol, and reflux under a condenser for 2 hours. Add 40 mL of diethyl ether and 40 mL of water, mix, and transfer to a separating funnel. Adjust the pH to 1.0 to 1.5 with hydrochloric acid (1 in 4), and allow to stand. After the solution is separated into two layers, collect the aqueous layer (lower layer), remove water by heating under reduced pressure, and evaporate to dryness. To about 0.1 g of the residue, add 1 mL of silylation TS, and silylate by heating at 70°C for 20 minutes.

Standard Solution Weigh about 50 mg of glycerol for glycerol ester gum or of pentaerythritol for pentaerythritol ester gum, add 1 mL of silylation TS, and silylate in the same manner as for the test solution.

Procedure Analyze equal portions of the test solution and the standard solution by gas chromatography using the operating conditions given below. The retention time of the main peak from the test solution corresponds to that of the peak of silylated glycerol or silylated pentaerythritol from the standard solution. The peak of the solvent should be excluded.

Operating Conditions

Detector: Flame ionization detector.

Column: A glass or stainless steel tube (2 mm internal diameter and 2 m length).

Column packing material

Liquid phase: 5 % Methyl silicone polymer of the amount of support.

Support: 149- to 177- μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of around 150°C.

Carrier gas: Nitrogen.

Flow rate: About 50 mL/minute.

(4) In the case of methanol ester gum compounds

Test Solution Weigh about 5 g of Ester Gum, place into a 100-mL flask, add 40 mL of a solution (1 in 10) of potassium hydroxide in 1-hexanol, and reflux under a condenser for 2 hours. Distil under reduced pressure (15 kPa), and take the distillate at 50°C. Add 5 g of 1-hexanol to the distillate.

Standard Solution Prepare a solution (1 in 10) of methanol in 1-hexanol, and use as the standard solution.

Procedure Analyze equal portions of the test solution and the standard solution by gas chromatography using the operating conditions. The retention time of the main peak from the test solution corresponds to that of the peak of methanol from the standard solution. The peak of the solvent should be excluded.

Operating Conditions

Detector: Flame ionization detector.

Column: A glass or stainless steel tube (2 mm internal diameter and 2 m length).

Column packing material

Liquid phase: 5% Methyl silicon polymer of the amount of support.

Support: 149- to 177- μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of around 50°C.

Carrier gas: Nitrogen.

Flow rate: About 50 mL/minute.

Purity

(1) Clarity of solution　Clear.

Test Solution　Weigh 10 g of Ester Gum, add 10 mL of toluene, dissolve it by warming to 70–75°C, filter while warm, and allow to stand for 24 hours.

(2) Acid value

Glycerol ester gum compounds: Not more than 8.0.

Pentaerythritol ester gum compounds: Not more than 18.0.

Methanol ester gum compounds: Not more than 8.0.

Test Solution　Weigh accurately about 3 g of Ester Gum, dissolve it in 50 mL of a 2:1 mixture of toluene/ethanol (95).

Procedure　Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests.

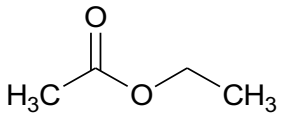
(3) Lead　Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10.0 mL, Flame Method).

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color:　Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition　Not more that 0.1%.

Ethyl Acetate

酢酸エチル



$C_4H_8O_2$　　Mol. Wt. 88.11

Ethyl acetate　[141-78-6]

Content　Ethyl Acetate contains not less than 98.0% of ethyl acetate ($C_4H_8O_2$).

Description Ethyl Acetate is a colorless, clear liquid having a fruity odor.

Identification

(1) To 1 mL of Ethyl Acetate, add 25 mL of sodium hydroxide solution (1 in 25), heat in a water bath for 5 minutes. After cooling, neutralize with diluted hydrochloric acid (1 in 4) and add 5 drops of a solution of iron(III) chloride hexahydrate (1 in 10). A deep red color develops.

(2) To 1 mL of Ethyl Acetate, add 5 mL of sodium hydroxide solution (1 in 5), and heat in a water bath while shaking. The fruity odor disappears. Acidify this solution with diluted sulfuric acid (1 in 20), and heat again in a water bath while shaking. An odor of acetic acid is evolved.

Refractive Index n_D^{20}: 1.370–1.375.

Specific Gravity d_{20}^{20}: 0.900–0.904.

Purity Acid value Not more than 0.1

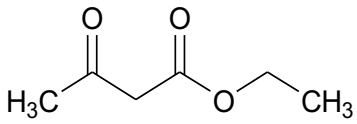
Weigh 20 g of Ethyl Acetate, and proceed as directed in the Acid Value Test in the Flavoring Substances Tests.

Assay Place 10 mL of ethanol (95) into a 100-mL flask, and weigh the flask with the ethanol accurately. To the flask, add about 1 g of Ethyl Acetate, and weigh it accurately. Add exactly 40 mL of 0.5 mol/L ethanolic potassium hydroxide, and heat under a reflux condenser in a water bath at 78–82°C for 20 minutes. After cooling, titrate the excess alkali with 0.5 mol/L hydrochloric acid (indicator: 2–3 drops of phenolphthalein TS). Perform a blank test in the same manner.

Each mL of 0.5 mol/L ethanolic potassium hydroxide = 44.05 mg of $C_4H_8O_2$

Ethyl Acetoacetate

アセト酢酸エチル



$C_6H_{10}O_3$ Mol. Wt. 130.14

Ethyl 3-oxobutanoate [141-97-9]

Content Ethyl Acetoacetate contains not less than 97.5% of ethyl acetoacetate ($C_6H_{10}O_3$).

Description Ethyl Acetoacetate is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Ethyl Acetoacetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same

wavenumbers.

Refractive Index n_D^{20}: 1.418–1.421.

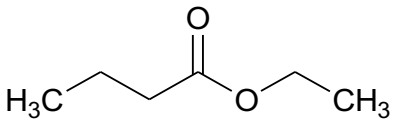
Specific Gravity d_{25}^{25}: 1.024–1.029.

Purity Acid value Not more than 5.0 (Flavoring Substances Tests). To confirm the endpoint with an indicator, use bromocresol purple TS. The endpoint is when the color of the solution changes from yellow to bluish purple.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests using operation conditions (1).

Ethyl Butyrate

酪酸エチル



$C_6H_{12}O_2$ Mol. Wt. 116.16

Ethyl butanoate [105-54-4]

Content Ethyl Butyrate contains not less than 98.0% of ethyl butyrate ($C_6H_{12}O_2$).

Description Ethyl Butyrate is a colorless to light yellow, clear liquid having a fruity odor.

Identification Determine the absorption spectrum of Ethyl Butyrate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.391–1.394.

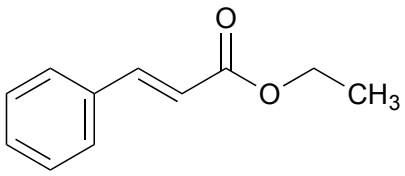
Specific Gravity d_{25}^{25}: 0.873–0.880.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (2).

Ethyl Cinnamate

ケイ皮酸エチル



$C_{11}H_{12}O_2$ Mol. Wt. 176.21

Ethyl(*2E*)-3-phenylprop-2-enoate [4192-77-2]

Content Ethyl Cinnamate contains not less than 99.0% of ethyl cinnamate ($C_{11}H_{12}O_2$).

Description Ethyl Cinnamate is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Ethyl Cinnamate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.558–1.562.

Specific Gravity d_{25}^{25}: 1.044–1.051.

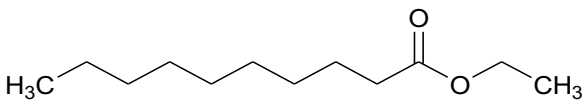
Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Ethyl Decanoate

Ethyl Caprate

デカン酸エチル



$C_{12}H_{24}O_2$ Mol. Wt. 200.32

Ethyl decanoate [110-38-3]

Content Ethyl Decanoate contains not less than 98.0% of ethyl decanoate ($C_{12}H_{24}O_2$).

Description Ethyl Decanoate is a colorless, clear liquid having a brandy-like odor.

Identification Determine the absorption spectrum of Ethyl Decanoate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.424–1.427.

Specific Gravity d_{25}^{25}: 0.864–0.865.

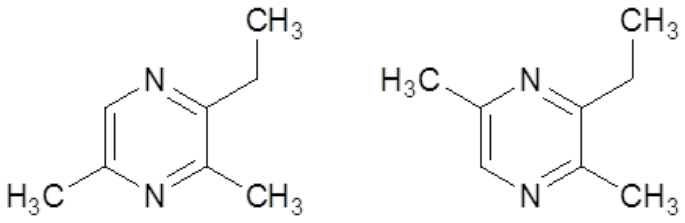
Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

2-Ethyl-3,(5 or 6)-dimethylpyrazine

2-Ethyl-3,5(6)-dimethylpyrazine

2-エチル-3,5-ジメチルピラジン及び 2-エチル-3,6-ジメチルピラジンの混合物



$C_8H_{12}N_2$ Mol. Wt.136.19

Mixture of 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine [27043-05-6]

Content 2-Ethyl-3,(5 or 6)-dimethylpyrazine contains not less than 95.0% of a mixture of 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine ($C_8H_{12}N_2$).

Description 2-Ethyl-3,(5 or 6)-dimethylpyrazine is a colorless to light yellow, clear liquid. It has a characteristic odor.

Identification Determine the absorption spectrum of 2-Ethyl-3,(5 or 6)-dimethylpyrazine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.496–1.506.

Specific Gravity d_{20}^{20}: 0.950–0.980.

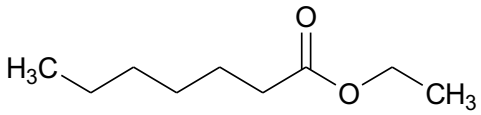
Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests,

using operating conditions (1).

Ethyl Heptanoate

Ethyl Enanthate

ヘプタン酸エチル



$C_9H_{18}O_2$ Mol. Wt. 158.24

Ethyl heptanoate [106-30-9]

Content Ethyl Heptanoate contains not less than 98.0% of ethyl heptanoate ($C_9H_{18}O_2$).

Description Ethyl Heptanoate is a colorless to light yellow, clear liquid having a wine-like odor.

Identification Determine the absorption spectrum of Ethyl Heptanoate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum of Ethyl heptanoate. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.411–1.415.

Specific Gravity d_{25}^{25}: 0.864–0.869.

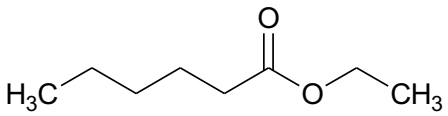
Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Ethyl Hexanoate

Ethyl Caproate

ヘキサン酸エチル



$C_8H_{16}O_2$ Mol. Wt. 144.21

Ethyl hexanoate [123-66-0]

Content Ethyl Hexanoate contains not less than 98.0% of ethyl hexanoate ($C_8H_{16}O_2$).

Description Ethyl Hexanoate is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Ethyl Hexanoate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.406–1.409.

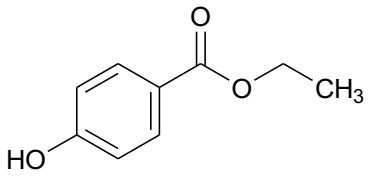
Specific Gravity d_{25}^{25}: 0.867–0.871.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Ethyl *p*-Hydroxybenzoate

パラオキシ安息香酸エチル



$C_9H_{10}O_3$ Mol. Wt. 166.17

Ethyl 4-hydroxybenzoate [120-47-8]

Content Ethyl *p*-Hydroxybenzoate, when dried, contains not less than 99.0% of ethyl *p*-hydroxybenzoate ($C_9H_{10}O_3$).

Description Ethyl *p*-Hydroxybenzoate occurs as colorless crystals or as a white crystalline powder. It is odorless.

Identification

(1) Proceed as directed in Identification (1) for Isobutyl *p*-Hydroxybenzoate.

(2) To 50 mg of Ethyl *p*-Hydroxybenzoate, add 2 drops of acetic acid and 5 drops of sulfuric acid, and warm for 5 minutes. An odor of ethyl acetate is evolved.

Melting Point 115–118°C.

Purity

(1) Free acid Not more than 0.55% as *p*-hydroxybenzoic acid.

Proceed as directed in Purity (1) for Isobutyl *p*-Hydroxybenzoate.

(2) Sulfate Not more than 0.024% as SO_4.

Proceed as directed in Purity (2) for Isobutyl *p*-Hydroxybenzoate.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.5% (80°C, 2 hours).

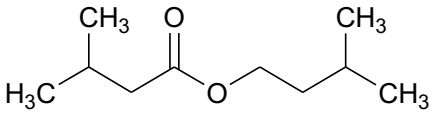
Residue on Ignition Not more than 0.05% (5 g).

Assay Proceed as directed in the Assay for Isobutyl *p*-Hydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide = 166.2 mg of $C_9H_{10}O_3$

Ethyl Isovalerate

イソ吉草酸エチル



$C_7H_{14}O_2$ Mol. Wt. 130.18

Ethyl 3-methylbutanoate [108-64-5]

Content Ethyl Isovalerate contains not less than 98.0% of ethyl isovalerate ($C_7H_{14}O_2$).

Description Ethyl Isovalerate is a colorless to light yellow, clear liquid having a fruity odor.

Identification Determine the absorption spectrum of Ethyl Isovalerate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.395–1.399.

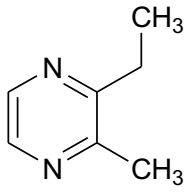
Specific Gravity d_{25}^{25}: 0.861–0.865.

Purity Acid value Not more than 2.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (2).

2-Ethyl-3-methylpyrazine

2-エチル-3-メチルピラジン



$C_7H_{10}N_2$ Mol. Wt. 122.17

2-Ethyl-3-methylpyrazine [15707-23-0]

Content 2-Ethyl-3-methylpyrazine contains not less than 98.0% of 2-Ethyl-3-methylpyrazine ($C_7H_{10}N_2$).

Description 2-Ethyl-3-methylpyrazine is a colorless or yellow liquid having a characteristic odor.

Identification Determine the absorption spectrum of 2-Ethyl-3-methylpyrazine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

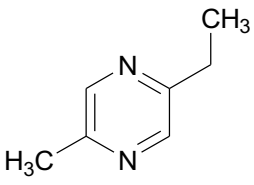
Refractive Index n_D^{20}: 1.502–1.505.

Specific Gravity d_{25}^{25}: 0.978–0.988.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operating conditions (1).

2-Ethyl-5-methylpyrazine

2-エチル-5-メチルピラジン



$C_7H_{10}N_2$ Mol. Wt. 122.17

2-Ethyl-5-methylpyrazine [13360-64-0]

Content 2-Ethyl-5-methylpyrazine contains not less than 95.0% of 2-ethyl-5-methylpyrazine ($C_7H_{10}N_2$).

Description 2-Ethyl-5-methylpyrazine occurs as a colorless to light yellow, clear

liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 2-Ethyl-5-methylpyrazine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

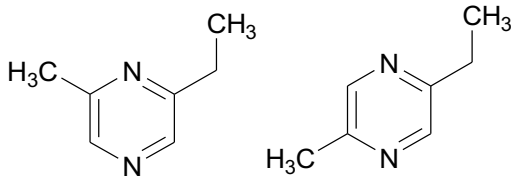
Refractive Index n_D^{20}: 1.491–1.501.

Specific Gravity d_{25}^{25}: 0.960–0.970.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operating conditions (1), except for the column. Use a fused silica tube (0.25–0.53 mm in internal diameter and 30–60 m in length) coated with a 0.25–1 μm thick layer of polyethylene glycol for gas chromatography.

2-Ethyl-6-methylpyrazine

2-エチル-6-メチルピラジン



$C_7H_{10}N_2$ Mol. Wt. 122.17

Mixture of 2-ethyl-6-methylpyrazine and 2-ethyl-5-methylpyrazine [36731-41-6]

Definition 2-Ethyl-6-methylpyrazine occurs as the mixture of 2-ethyl-6-methylpyrazine and 2-ethyl-5-methylpyrazine.

Content 2-Ethyl-6-methylpyrazine contains not less than 95.0% of the sum of 2-ethyl-6-methylpyrazine and 2-ethyl-5-methylpyrazine ($C_7H_{10}N_2$).

Description 2-Ethyl-6-methylpyrazine occurs as a colorless to pale yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 2-Ethyl-6-methylpyrazine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.492–1.502.

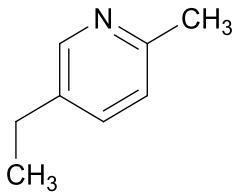
Specific Gravity d_{25}^{25}: 0.960–0.973.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use

operating conditions (1).

5-Ethyl-2-methylpyridine

5-エチル-2-メチルピリジン



$C_8H_{11}N$ Mol. Wt. 121.18

5-Ethyl-2-methylpyridine [104-90-5]

Content 5-Ethyl-2-methylpyridine contains not less than 96.5% of 5-ethyl-2-methylpyridine ($C_8H_{11}N$).

Description 5-Ethyl-2-methylpyridine occurs as a colorless, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 5-Ethyl-2-methylpyridine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.495–1.502.

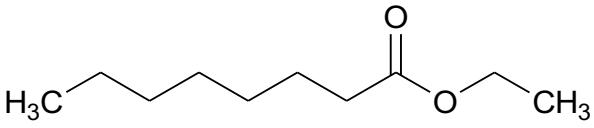
Specific Gravity d_{20}^{20}: 0.917–0.923.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operating conditions (1).

Ethyl Octanoate

Ethyl Caprylate

オクタン酸エチル



$C_{10}H_{20}O_2$ Mol. Wt. 172.26

Ethyl octanoate [106-32-1]

Content Ethyl Octanoate contains not less than 98.0% of ethyl octanoate ($C_{10}H_{20}O_2$).

Description Ethyl Octanoate is a colorless or slightly yellow, clear liquid having a brandy-like odor.

Identification Determine the absorption spectrum of Ethyl Octanoate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.417–1.419.

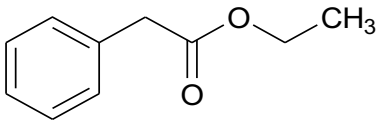
Specific Gravity d_{25}^{25}: 0.863–0.866.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Ethyl Phenylacetate

フェニル酢酸エチル



$C_{10}H_{12}O_2$ Mol. Wt. 164.20

Ethyl 2-phenylacetate [101-97-3]

Content Ethyl Phenylacetate contains not less than 97.0% of ethyl phenylacetate ($C_{10}H_{12}O_2$).

Description Ethyl Phenylacetate is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Ethyl Phenylacetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.494–1.500.

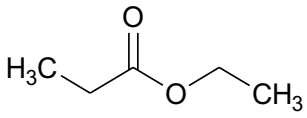
Specific Gravity d_{25}^{25}: 1.027–1.032.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Ethyl Propionate

プロピオン酸エチル



$C_5H_{10}O_2$ Mol. Wt. 102.13

Ethyl propanoate [105-37-3]

Content Ethyl Propionate contains not less than 97.0% of ethyl propionate ($C_5H_{10}O_2$).

Description Ethyl Propionate is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Ethyl Propionate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.383–1.385.

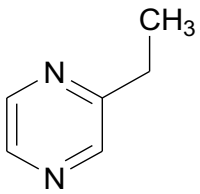
Specific Gravity d_{25}^{25}: 0.886–0.889.

Purity Acid value Not more than 2.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (2).

2-Ethylpyrazine

2-エチルピラジン



$C_6H_8N_2$ Mol. Wt. 108.14

2-Ethylpyrazine [13925-00-3]

Content 2-Ethylpyrazine contains not less than 98.0% of 2-ethylpyrazine ($C_6H_8N_2$).

Description 2-Ethylpyrazine occurs as a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of 2-Ethylpyrazine, as directed in

the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

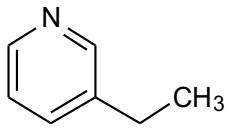
Refractive Index n_D^{20}: 1.493–1.508.

Specific Gravity d_{25}^{25}: 0.981–1.000.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operating conditions (1).

3-Ethylpyridine

3-エチルピリジン



C_7H_9N Mol. Wt. 107.15

3-Ethylpyridine [536-78-7]

Content 3-Ethylpyridine contains not less than 98.0% of 3-ethylpyridine (C_7H_9N).

Description 3-Ethylpyridine occurs as a colorless to brown liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 3-Ethylpyridine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.499–1.505.

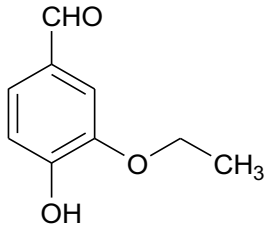
Specific Gravity d_{25}^{25}: 0.937–0.943.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operating conditions (1).

Ethylvanillin

Ethyl Vanillin

エチルバニリン



$C_9H_{10}O_3$ Mol. Wt. 166.17

3-Ethoxy-4-hydroxybenzaldehyde [121-32-4]

Content Ethylvanillin contains not less than 98.0% of ethylvanillin ($C_9H_{10}O_3$).

Description Ethylvanillin occurs as white to light yellow, flaky crystals or crystalline powder having a vanilla-like odor and taste.

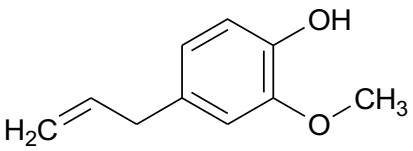
Identification Determine the absorption spectrum of Ethylvanillin as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 76–78°C.

Assay Using a solution (1 in 10) of Ethylvanillin in acetone as the test solution, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Eugenol

オイゲノール



$C_{10}H_{12}O_2$ Mol. Wt. 164.20

4-Allyl-2-methoxyphenol [97-53-0]

Content Eugenol contains not less than 98.0% of eugenol ($C_{10}H_{12}O_2$).

Description Eugenol is a colorless to light yellow-brown, clear liquid having a clove-like odor.

Identification Determine the absorption spectrum of Eugenol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.540–1.542.

Specific Gravity d_{25}^{25}: 1.062–1.068.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Exomaltotetraohydrolase

エキソマルトテトラオヒドロラーゼ

Definition Exomaltotetraohydrolase includes enzymes that hydrolyze starch to remove maltotetraose unites from the non-reducing terminus. It is derived from the culture of actinomycetes (limited to *Streptomyces thermoviolaceus* and *Streptomyces violaceoruber*) or bacteria (limited to *Pseudomonas stutzeri*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Exomaltotetraohydrolase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Exomaltotetraohydrolase complies with the Exomaltotetraohydrolase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli*

test and Method 2 for the *Salmonella* test.

The requirement of total plate count can be disregarded if unsterilized Exomaltotetraohydrolase is used for captive consumption in the manufacture of food that will be sterilized or pasteurized before the completion of the final product.

Exomaltotetraohydrolase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by either method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Exomaltotetraohydrolase, add sodium phosphate buffer (0.004 mol/L) at pH 7.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Suspend 1.0 g of soluble starch in 50 mL of water, heat with occasional shaking not to let starch precipitate, boil for 5 minutes, and cool. To this solution, add 10 mL of sodium phosphate buffer (0.2 mol/L) at pH 7.0 and water to make 100 mL.

Test Solution Add 0.5 mL of the sample solution to 10 mL of the substrate solution, equilibrated at 40°C, and incubate the mixture at 40°C for 20 minutes while shaking. Heat this solution in a water bath for 10 minutes, cool it to room temperature, and filter with a membrane filter (0.45 μm pore size). Use the filtrate as the test solution.

Control Solution Add 0.5 mL of the sample solution to 10 mL of the substrate solution, immediately heat the mixture in a water bath for 10 minutes, cool it to room temperature, and filter with a membrane filter (0.45 μm pore size). Use the filtrate as the control solution .

Standard Solution Weigh 50 mg of maltotetraose, and dissolve it in water to make 10 mL.

Procedure Analyze 20 μL each of the test solution, the control solution, and standard solution by liquid chromatography using the operating conditions given below. A peak is observed at the retention time of maltotetraose for the test solution. The peak area is larger than that of the peak corresponding to the retention time of maltotetraose in the control solution.

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (5–20 mm internal diameter and 20–40 cm length).

Column packing material: About 25-μm Ag-form cation exchange resin for liquid chromatography.

Column temperature: A constant temperature of 50–80°C.

Mobile phase: Water

Flow rate: 0.3–1.0 mL/min.

Method 2

Sample Solution Weigh 0.50 g of Exomaltotetraohydrolase, add water or sodium phosphate buffer (0.004 mol/L) at pH 7.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh an amount of soluble starch equivalent to 5.0 g of its dried substance, suspend it in 300 mL of water, boil the suspension for 5 minutes with occasional shaking not to let starch precipitate, and cool. Add 50 mL of sodium phosphate buffer (0.004 mol/L) at pH7.0 and water to make 500 mL.

Test Solution To 5 mL of the substrate solution, equilibrated at 40°C, add 0.2 mL of the sample solution, and mix. Incubate the mixture at 40°C for 20 minutes. Transfer 1 mL of this solution immediately into a test tube containing 2 mL of Somogyi copper TS, and mix. Cover the mouth of the test tube with a glass bead, heat it in a water bath for 10 minutes, and cool. Add 2 mL of Nelson TS, mix, allow the solution to stand at room temperature for 30 minutes, and add 5 mL of water.

Control Solution To 5 mL of the substrate solution, equilibrated at 40°C, add 0.2 mL of the sample solution, and mix. Transfer 1 mL of this solution immediately into a test tube containing 2 mL of Somogyi copper TS, and mix. Proceed as directed for the test solution, beginning with "Cover the mouth of the test tube with a glass bead,…"

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 520 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Ferric Ammonium Citrate

Ferric Ammonium Citrate, Brown
Ferric Ammonium Citrate, Green

クエン酸鉄アンモニウム

Ammonium iron(III) salt of 2-hydroxypropane-1,2,3-tricarboxylic acid [1185-57-5]

Content Ferric Ammonium Citrate contains 14.5–21.0% of iron (Fe = 55.85).

Description Ferric Ammonium Citrate occurs as green, red-brown, deep red, brown, or brownish-yellow, transparent flaky crystals, powder, granules, or lumps. It is odorless or has a slight odor of ammonia and a weak iron taste.

Identification

(1) To 5 mL of a solution of Ferric Ammonium Citrate (1 in 10), add 5 mL of sodium hydroxide solution (1 in 25), and heat. An odor of ammonia is evolved, and a red-brown

precipitate is formed.

(2) To a solution of Ferric Ammonium Citrate (1 in 100), add ammonia TS. A black color develops, and no precipitate is formed.

(3) To 10 mL of a solution of Ferric Ammonium Citrate (1 in 10), add 4 mL of potassium hydroxide solution (1 in 15), heat, and filter. Measure 4 mL of the filtrate, add diluted acetic acid (1 in 4) to make slightly acidic, and cool. Add 2 mL of a solution of calcium chloride dihydrate (3 in 40), and boil. A white crystalline precipitate is formed.

Purity

(1) Sulfate Not more than 0.48% as SO_4.

Proceed as directed in Purity (1) for Sodium Ferrous Citrate.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Ferric Ammonium Citrate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(3) Arsenic Not more than 3 μg/g as As (1.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Ferric Ammonium Citrate, add 5 mL of water, 1 mL of sulfuric acid, and 10 mL of sulfurous acid solution, evaporate to about 2 mL, and add water to make 10 mL. Use 5 mL of this solution as the test solution.

(4) Iron(III) citrate Weigh 0.10 g of Ferric Ammonium Citrate, dissolve it in 10 mL of water, and add 1 drop of a freshly prepared solution of potassium hexacyanoferrate(II) trihydrate (1 in 10). No blue precipitate is formed.

Assay Weigh accurately about 1 g of Ferric Ammonium Citrate into a ground-glass stoppered flask, dissolve it in 25 mL of water, add 5 mL of hydrochloric acid and 4 g of potassium iodide, immediately stopper tightly, and allow to stand for 15 minutes in a dark place. Add 100 mL of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 5.585 mg of Fe

Ferric Chloride

塩化第二鉄

$FeCl_3 \cdot 6H_2O$ Mol. Wt. 270.30

Iron(III) chloride hexahydrate [10025-77-1]

Content Ferric Chloride contains 98.5–102.0% of ferric chloride ($FeCl_3 \cdot 6H_2O$).

Description Ferric Chloride occurs as deliquescent, yellow-brown crystals or lumps.

Identification Ferric Chloride responds to all the tests for Iron(III) Salt and for Chloride in the Qualitative Tests.

Purity

(1) Clarity of solution Very slightly turbid.

Weigh 1.0 g of Ferric Chloride, add 10 mL of diluted hydrochloric acid (1 in 100), and dissolve it by heating.

(2) Free acid Weigh 2.0 g of Ferric Chloride, dissolve it in 5 mL of water, and bring a glass rod wetted with ammonia solution (28) close to it. No white fumes are evolved.

(3) Nitrate Weigh 5.0 g of Ferric Chloride, dissolve it in 25 mL of water, boil, and add 25 mL of ammonia solution (28). After cooling, add water to make 100 mL, and filter. Use this solution as the sample solution. Measure 5.0 mL of the sample solution, and add 5 mL of water, 0.1 mL of indigo carmine TS, and 10 mL of sulfuric acid. A blue color persists for not less than 5 minutes.

(4) Sulfate Not more than 0.019% as SO_4.

Test Solution Measure 20 mL of the sample solution prepared in Purity (3) above, add 3 mL of sodium carbonate solution (1 in 8), evaporate to dryness in a water bath, and heat over a small flame until the white fumes are no longer evolved. After cooling, add 10 mL of water and 3 mL of diluted hydrochloric acid (1 in 4), and evaporate to dryness in a water bath. Dissolve the residue by adding 0.3 mL of diluted hydrochloric acid (1 in 4) and a small amount of water, and then add water to make 50 mL.

Control Solution To 0.40 mL of 0.005 mol/L sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(5) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Ferric Chloride, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Gently boil for 5 minutes, and allow to cool.

(6) Zinc Not more than 30 μg/g as Zn.

Test Solution Measure 20 mL of the sample solution prepared in Purity (3) above, transfer into a Nessler tube, neutralize with hydrochloric acid, add water to make 30 mL, and add 3 mL of diluted hydrochloric acid (1 in 4) and 0.2 mL of a freshly prepared solution of potassium hexacyanoferrate(II) trihydrate (1 in 10).

Control Solution Measure 3.0 mL of Zinc Standard Solution, transfer into a Nessler tube, add water to make 30 mL, and then proceed as directed for the test solution.

Procedure Allow the test solution and the control solution to stand for 15 minutes. The test solution is not more turbid than the control solution.

(7) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Ferric Chloride in 20 mL of water, and add 0.2 g of L(+)-ascorbic acid to dissolve in the solution.

Standard Color To the specified amount of Arsenic Standard Solution, add 20 mL of water., and then add and dissolve 0.2 g of L(+)-ascorbic acid. Proceed as directed for the test solution.

Procedure Perform the test without neutralizing with ammonia solution.

(8) Free chlorine Weigh 2.0 g of Ferric Chloride, dissolve it in 5 mL of water, heat, and bring a filter paper wetted with zinc iodide–starch TS close to it. No blue color develops.

Assay Weigh accurately about 0.6 g of Ferric Chloride into a ground-glass stoppered flask, dissolve it in about 50 mL of water, add 3 mL of hydrochloric acid and 3 g of potassium iodide, and immediately stopper tightly. Allow to stand for 15 minutes in a dark place, and titrate with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 27.03 mg of $FeCl_3 \cdot 6H_2O$

Ferric Citrate

クエン酸鉄

Iron(III) salt of 2-hydroxypropane-1,2,3-tricarboxylic acid

Content Ferric Citrate contains 16.5–18.5% of iron (Fe = 55.85).

Description Ferric Citrate occurs as a brown powder or as transparent, red-brown laminae.

Identification Ferric Citrate responds to all the tests for Iron(III) Salt and to test (2) for Citrate in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear.

Test Solution Weigh 1.0 g of Ferric Citrate, add 20 mL of water, and dissolve by heating in a water bath.

(2) Sulfate Not more than 0.48% as SO_4.

Proceed as directed in Purity (1) for Sodium Ferrous Citrate.

(3) Ammonium salt Weigh 1.0 g of Ferric Citrate, add 10 mL of water and 5 mL of potassium hydroxide solution (1 in 15), and boil. No odor of ammonia is evolved.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Ferric Citrate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, and use as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(5) Arsenic Not more than 3 μg/g as As (1.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Ferric Citrate, add 5 mL of water, 1 mL of sulfuric acid, and 10 mL of sulfurous acid solution, evaporate to about 2 mL, and add water to make 10 mL. Use 5 mL of this solution as the test solution.

Assay Weigh accurately about 1 g of Ferric Citrate, transfer it into a ground-glass stoppered flask, add 5 mL of hydrochloric acid and 30 mL of water, dissolve by heating, and cool. Add 4 g of potassium iodide, immediately stopper tightly, and allow to stand for 15 minutes in a dark place. Add 100 mL of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 5.585 mg of Fe

Ferric Pyrophosphate

ピロリン酸第二鉄

$Fe_4(P_2O_7)_3$ Mol. Wt. 745.21

Iron(III) diphosphate

Content Ferric Pyrophosphate, when ignited, contains not less than 95.0% of ferric pyrophosphate ($Fe_4(P_2O_7)_3$).

Description Ferric Pyrophosphate occurs as a yellow to yellow-brown powder. It is odorless and has a slight, iron taste.

Identification

(1) To 0.2 g of Ferric Pyrophosphate, add 10 mL of sodium hydroxide solution (1 in 25), and filter the red-brown precipitate formed. Dissolve the residue on the filter paper by adding diluted hydrochloric acid (1 in 4). The resulting solution responds to all the tests for Iron(III) Salt.

(2) Add diluted nitric acid (1 in 10) to the filtrate obtained in Identification (1) to make

slightly acidic, and add silver nitrate solution (1 in 50). A white precipitate is formed.

Purity

(1) Clarity of solution Very slightly turbid.

Weigh 0.10 g of Ferric Pyrophosphate, dissolve it in 5.0 mL of diluted hydrochloric acid (1 in 2), and add water to make 20 mL.

(2) Chloride Not more than 3.55% as Cl.

Sample Solution Weigh 1.00 g of Ferric Pyrophosphate, add 5 mL of diluted nitric acid (1 in 2), and dissolve it by heating in a water bath. Add several drops of phenolphthalein TS and 50 mL of sodium hydroxide solution (1 in 25), shake well, add water to make 100 mL, allow to stand for about 10 minutes, and filter through a dry filter paper. Measure 10 mL of the filtrate, and add water to make 100 mL. Measure 2.0 mL of this solution, and neutralize with diluted nitric acid (1 in 10).

Control Solution Use 0.20 mL of 0.01 mol/L hydrochloric acid.

(3) Sulfate Not more than 0.12% as SO_4.

Sample Solution Measure 40 mL of the filtrate obtained in Purity (2), and neutralize with diluted hydrochloric acid (1 in 4).

Control Solution Use 1.0 mL of 0.005 mol/L sulfuric acid.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Ferric Pyrophosphate, add 5 mL of nitric acid and 25 mL of water, and boil gently for 15 minutes with a watch glass covering it. Allow to cool.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Ferric Pyrophosphate in 5 mL of diluted hydrochloric acid (1 in 2), and then add 0.2 g of L(+)-ascorbic acid to dissolve.

Standard Color Add 5 mL of diluted hydrochloric acid (1 in 2) to the specified amount of Arsenic Standard Solution, and dissolve 0.2 g of L(+)-ascorbic acid in the resulting solution.

Procedure Proceed as directed in the Arsenic Limit Test for the test solution and the standard color. In the procedure, omit the neutralization of the test solution with ammonia solution.

Loss on Ignition Not more than 20.0% (1 hour).

Assay Immediately weigh accurately about 0.3 g Ferric Pyrophosphate, previously ignited, dissolve it in 20 mL of diluted hydrochloric acid (1 in 2), and transfer the solution to a ground-glass stoppered flask with 20 mL of water. Add 3 g of potassium iodide, immediately stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 mL of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution

is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner.

Each mL of 0.1 mol/L sodium thiosulfate = 18.63 mg of $Fe_4(P_2O_7)_3$

Ferric Pyrophosphate Solution

ピロリン酸第二鉄液

Content Ferric Pyrophosphate Solution contains 2.5–3.5% of ferric pyrophosphate ($Fe_4(P_2O_7)_3$ = 745.21).

Description Ferric Pyrophosphate Solution is a white to light yellow, milky liquid. It is odorless and has a slight, iron taste.

Identification

(1) To Ferric Pyrophosphate Solution, add sodium hydroxide solution (1 in 25) in excess, and filter the red-brown precipitate formed. Dissolve the residue on the filter paper with diluted hydrochloric acid (1 in 4). The solution responds to all the tests for Iron(III) Salt in the Qualitative Tests.

(2) Add diluted nitric acid (1 in 10) to the filtrate obtained in Identification (1) to make slightly acidic, and add silver nitrate solution (1 in 50). A white precipitate is formed.

Purity

(1) Clarity of solution Very slightly turbid.

Weigh 2.0 g of Ferric Pyrophosphate Solution, dissolve it in 5.0 mL of diluted hydrochloric acid (1 in 2), and add water to make 20 mL.

(2) Chloride Not more than 0.35% as Cl.

Sample Solution Weigh 10 g of Ferric Pyrophosphate Solution, add several drops of phenolphthalein TS and 7 mL of sodium hydroxide solution (1 in 25), shake well, add water to make 100 mL, allow to stand for about 10 minutes, and filter through a dry filter paper. Measure 10 mL of the filtrate, add water to make 100 mL, then measure 2.0 mL of the solution obtained, and neutralize with diluted nitric acid (1 in 10).

Control Solution Use 0.20 mL of 0.01 mol/L hydrochloric acid.

(3) Sulfate Not more than 0.002% as SO_4.

Test Solution Measure 40 mL of the filtrate obtained in Purity (2), and neutralize with diluted hydrochloric acid (1 in 4).

Control Solution 0.20 mL of 0.005 mol/L sulfuric acid.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Ferric Pyrophosphate Solution, add 5 mL of nitric acid and 25 mL of water, and boil gently for 15 minutes with a watch glass

covering it. Allow to cool.

(5) Arsenic Not more than 0.2 μg/g as As (7.5 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve 0.2 g of L(+)-ascorbic acid in the specified amount of Ferric Pyrophosphate Solution.

Standard Color Add 4 mL of water to the specified amount of Arsenic Standard Solution, dissolve 0.1 g of L(+)-ascorbic acid in the resulting solution.

Procedure Proceed as directed as the Arsenic Limit Test for the test solution and standard color. In the procedure, omit the neutralization of the test solution with ammonia solution.

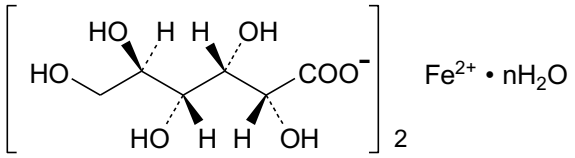
Assay Weigh accurately about 10 g of Ferric Pyrophosphate Solution, transfer it into a ground-glass stoppered flask with about 30 mL of water, and dissolve by adding 10 mL of hydrochloric acid. Add 3 g of potassium iodide, immediately stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 mL of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner.

Each mL of 0.1 mol/L sodium thiosulfate = 18.63 mg of $Fe_4(P_2O_7)_3$

Ferrous Gluconate

Iron Gluconate

グルコン酸第一鉄



$C_{12}H_{22}FeO_{14}\cdot nH_2O$ (n = 2 or 0) Mol. Wt. dihydrate 482.17

anhydrous 446.14

Monoiron(II) bis(D-gluconate) dihydrate

Monoiron(II) bis(D-gluconate) [299-29-6]

Content Ferrous Gluconate, when dried, contains not less than 95.0% of ferrous gluconate ($C_{12}H_{22}FeO_{14}$).

Description Ferrous Gluconate occurs as a yellow-gray to green-yellow powder or as granules. It has a slight, characteristic odor.

Identification

(1) Measure 5 mL of a solution (1 in 10) of Ferrous Gluconate in warm water, and proceed as directed in Identification (2) for Glucono-δ-Lactone.

(2) A solution of Ferrous Gluconate (1 in 20) responds to all the tests for Iron(II) Salt in the Qualitative Tests.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Ferrous Gluconate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(2) Iron(III) salt Not more than 2.0% as Fe^{3+}.

Weigh 5.0 g of Ferrous Gluconate, add 100 mL of water and 10 mL of hydrochloric acid to dissolve, then add 3 g of potassium iodide, and shake. Allow to stand in a dark place for 5 minutes. Titrate with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. The volume of the sodium thiosulfate consumed is not more than 18 mL.

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Oxalate Weigh 1.0 g of Ferrous Gluconate, add 10 mL of water and 2 mL of hydrochloric acid to dissolve, and transfer into a separating funnel. Perform extraction twice with 50 mL and 20 mL of diethyl ether. Combine the extracts, add 10 mL of water, evaporate the diethyl ether on a water bath, and add 1 drop of acetic acid and 1 mL of a solution of calcium acetate monohydrate (1 in 20). No turbidity appears within 5 minutes.

(5) Sucrose or reducing sugars Weigh 0.5 g of Ferrous Gluconate, add 10 mL of water, dissolve it by warming, and add 1 mL of ammonia TS. Pass hydrogen sulfide through the solution, allow to stand for 30 minutes, and filter. Wash the residue on the filter paper twice with 5 mL of water each time, combine the filtrate and the washings, neutralize with hydrochloric acid, and add 2 mL of diluted hydrochloric acid (1 in 4). Concentrate the solution to about 10 mL, cool, add 5 mL of sodium carbonate solution (1 in 8) and 20 mL of water, and filter. Add water to the filtrate to make 100 mL. To 5 mL of this solution, add 2 mL of Fehling's TS, and boil for 1 minute. A orange-yellow to red precipitate is not formed immediately.

Loss on Drying Not more than 10.0% (105°C, 4 hours).

Assay Weigh accurately about 1.5 g of Ferrous Gluconate, previously dried, add 75 mL of water and 15 mL of diluted sulfuric acid (1 in 20) to dissolve, and then add 0.25 g of zinc powder. Allow to stand for 20 minutes, filter with suction, through a crucible-shaped glass filter (1G4) on which zinc powder is placed in a layer, and wash the residue with

10 mL of diluted sulfuric acid (1 in 20) and 10 mL of water. Combine the filtrate and the washings, add 2 drops of 1,10-phenanthroline TS, filter with suction if necessary, and titrate immediately with 0.1 mol/L diammonium cerium(IV) nitrate. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L diammonium cerium(IV) nitrate = 44.61 mg of $C_{12}H_{22}FeO_{14}$

Ferrous Sulfate

硫酸第一鉄

$FeSO_4$

Iron(II) sulfate hydrate [13463-43-9]

Definition Ferrous Sulfate occurs in crystalline form (heptahydrate), called Ferrous Sulfate (crystal), and in dried form (monohydrate to sesquihydrate), called Ferrous Sulfate (dried).

Content Ferrous Sulfate (crystal) contains 98.0–104.0% of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$ = 278.01), and Ferrous Sulfate (dried) contains not less than 85.0% of ferrous sulfate ($FeSO_4$ = 151.91).

Description Ferrous Sulfate (crystal) occurs as whitish-green crystals or crystalline powder. Ferrous Sulfate (dried) occurs as a gray-white powder.

Identification A solution of Ferrous Sulfate (1 in 100) responds to all the tests for Iron(II) Salt and for Sulfate in the Qualitative Tests.

pH Acidic, not less than 3.4 (crystal 1.0 g, water 10 mL).

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Ferrous Sulfate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate the solution to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay Weigh accurately about 0.5 g of Ferrous Sulfate, dissolve it in a mixture of 25 mL of diluted sulfuric acid (1 in 25) and 25 mL of freshly boiled and cooled water, and titrate with 0.02 mol/L potassium permanganate.

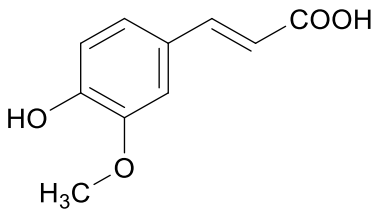
Ferrous Sulfate (crystal): Each mL of 0.02 mol/L potassium permanganate = 27.80 mg of $FeSO_4 \cdot 7H_2O$

Ferrous Sulfate (dried): Each mL of 0.02 mol/L potassium permanganate = 15.19 mg

of $FeSO_4$

Ferulic Acid

フェルラ酸



$C_{10}H_{10}O_4$ Mol. Wt. 194.18

(2*E*)-3-(4-Hydroxy-3-methoxyphenyl)prop-2-enoic acid [537-98-4]

Content Ferulic Acid, when dried, contains 98.0–102.0% of ferulic acid ($C_{10}H_{10}O_4$).

Description Ferulic Acid occurs as a white to yellowish-white powder. It is odorless or has a slight, characteristic odor.

Identification

(1) To 10 mg of Ferulic Acid, add 10 mL of 3.5% (w/v) potassium hydroxide–ethanol TS, and dissolve it by warming. A light yellow color produced.

(2) Dissolve 10 mg of Ferulic Acid in 2 mL of acetone, add 0.1 mL of a solution (1 in 50) of iron(III) chloride hexahydrate in ethanol (95). A red-brown color is produced.

(3) A solution (1 in 100,000) of Ferulic Acid in methanol exhibits absorption maxima at wavelengths of 231–235 nm and 318–322 nm.

(4) Prepare a test solution by dissolving 60 mg of Ferulic Acid in 10 mL of ethyl acetate. Prepare a control solution by dissolving 15 mg of ferulic acid for assay in ethyl acetate to make 50 mL. Analyze a 5-μL portion each of the test solution and the control solution by thin-layer chromatography as directed in Identification (4) for γ-Oryzanol. The main spot from the test solution corresponds to the spot of ferulic acid in the control solution.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Related substances Perform thin-layer chromatography as directed in Identification (4) above. The main spot from the test solution is at the same position as the spot of ferulic acid in the control solution and any other single spot from the test solution is not more intense than that from the control solution.

Loss on Drying Not more than 0.5% (105°C, 3 hours).

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.5 g of Ferulic Acid, previously dried, add 50 mL of 50% (vol) ethanol solution, and dissolve it by heating on a water bath. After cooling, titrate with 0.1 mol/L sodium hydroxide (indicator: 3 drops of bromothymol blue TS). Separately, perform a blank to make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide = 19.42 mg of $C_{10}H_{10}O_4$.

Ficin

フィシン

Definition Ficin includes enzymes that degrade proteins. It is derived from the sap of the fig tree *Ficus carica* L. or *Ficus insipida* Willd. (*Ficus glabrata* Kunth). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Ficin occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Ficin complies with the Ficin Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

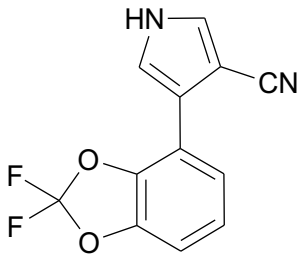
Ficin Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 0.50 g of Ficin, and add the diluent prepared in Enzyme Activity Determination of the monograph for Papain to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, or 100-fold, or 1000-fold dilution by adding the same diluent to the resulting solution.

Procedure Proceed as directed in the Procedure in Enzyme Activity Determination in the monograph for Papain to measure the absorbance (A_T andA_b). A_T is larger than A_b. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Fludioxonil

フルジオキソニル



$C_{12}H_6F_2N_2O_2$ Mol. Wt. 248.19

4-(2,2-difluorobenzo[*d*][1,3]dioxol-4-yl)-1*H*-pyrrole-3-carbonitrile [131341-86-1]

Content Fludioxonil contains not less than 97.0% of fludioxonil ($C_{12}H_6F_2N_2O_2$).

Description Fludioxonil occurs as colorless to white crystals or as a white to soft yellow powder. It is odorless.

Identification Determine the infrared absorption spectrum of Fludioxonil, as directed in the Paste Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 199–201°C.

Purity Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Water Content Not more than 0.50% (2 g, Volumetric Titration, Direct Titration).

Assay

Test Solution and Standard Solution Weigh accurately about 60 mg each of Fludioxonil and fludioxonil for assay, dissolve them separately in methanol to make exactly 100 mL of each, and use them as the test solution and as standard solution, respectively.

Procedure Analyze 10 μL each of the test solution and the standard solution by

liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of fludioxonil for the test solution and the standard solution, and determine the content by the formula:

$$\text{Content (\%) of fuldioxonil } (C_{12}H_6F_2N_2O_2) = \frac{\text{Weight (g) of fuldioxonil for assay}}{\text{Weight (g) the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Ultraviolet absorption spectrophotometer (determination wavelength: 270 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 15 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

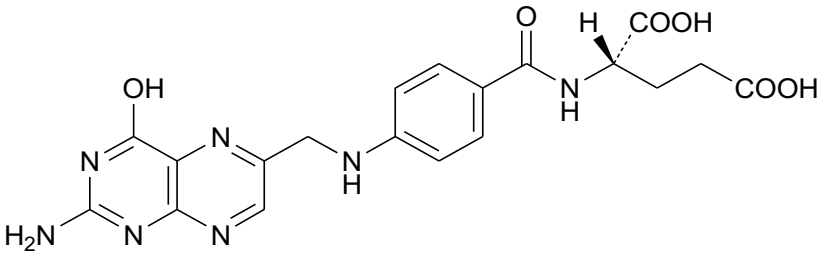
Column temperature: A constant temperature of 25–40°C.

Mobile phase: Use a solution prepared as follows: Dissolve 3.8 g of potassium dihydrogen phosphate and 5.8 g of disodium hydrogenphosphate in water to make 1L. To 100 mL of this solution, add 500 mL of water, 300 mL of acetonitrile, and 350 mL of methanol.

Flow rate: 1 mL/minute.

Folic Acid

葉酸



$C_{19}H_{19}N_7O_6$ Mol. Wt. 441.40

N-{4-[(2-Amino-4-hydroxypteridin-6-ylmethyl)amino]benzoyl}-L-glutamic acid [59-30-3]

Content Folic Acid contains 98.0–102.0% of folic acid ($C_{19}H_{19}N_7O_6$).

Description Folic Acid occurs as a yellow to orange-yellow crystalline powder. It is odorless.

Identification Dissolve 1.5 mg of Folic Acid in sodium hydroxide solution (1 in 250) to make 100 mL. The solution exhibits absorption maxima at wavelengths of 255–257 nm, 281–285 nm, and 361–369 nm.

Purity Free amine Not more than 1.0%.

Weigh accurately about 50 mg of *p*-Aminobenzoylglutamic Acid Reference Standard, previously dried for 4 hours under reduced pressure in a desiccator, and dissolve it in 40% (vol) ethanol to make exactly 100 mL. Measure exactly 3 mL of this solution, and add water to make exactly 1000 mL. Using exactly 4 mL of the resulting solution, prepare a solution as directed for the preparation of Solution S_3 in the Assay (preparation of Solution S_3 from Solution S_2), and measure the absorbance (A_S'). Calculate the amount of free amine by the formula below from A_S' and A_C obtained in the Assay.

$$\text{Amount of free amine} = \frac{\text{Weight (g) of } p\text{-Aminobenzoylglutamic Acid Reference Standard}}{\text{Anhydrous basis weight (g) of the sample used in Assay}} \times \frac{A_C}{A_S'}$$

Water Content Not more than 8.5% (0.2 g, Volumetric Titration, Back Titration).

Place 5 mL of pyridine for water determination and 20 mL of methanol for water determination in a titration vessel. Add a constant amount of excess water determination TS, and stir for 30 minutes before back titration.

Residue on Ignition Not more than 0.5%.

Assay Weigh accurately about 50 mg each of Folic Acid and Folic Acid Reference Standard (the water content should be measured previously in the same manner as for Folic Acid), add 50 mL of sodium hydroxide solution (1 in 250) to each, and dissolve it by shaking well. Add sodium hydroxide solution (1 in 250) to make exactly 100 mL of each. Refer to these solutions as Solution T_1 and Solution S_1, respectively.

Measure exactly 30 mL each of Solution T_1 and Solution S_1, add 20 mL of diluted hydrochloric acid (1 in 4), then add water to make exactly 100 mL of each. Measure exactly 60 mL of each, add 0.5 g of zinc powder to each, and allow to stand for 20 minutes with occasional shaking. Filter each solution through a dry filter paper, discard the initial 10 mL of filtrate, measure exactly the subsequent 10 mL of filtrate, and add water to make exactly 100 mL of each. Refer to these solutions as Solution T_2 and Solution S_2, respectively.

Measure exactly 4 mL each of Solution T_2 and Solution S_2, add 1 mL of water, 1 mL of diluted hydrochloric acid (1 in 4), and 1 mL of sodium nitrite solution (1 in 1000) to each, mix, and allow to stand for 2 minutes. Add 1 mL of ammonium amidosulfate solution (1 in 200) to each, shake well, and allow to stand for another 2 minutes. Then add 1 mL of *N*,*N*-diethyl-*N*′-1-naphthylethylenediamine oxalate solution (1 in 1000), shake, allow to stand for 10 minutes, and add water to make exactly 20 mL of each. Refer to these solutions as Solution T_3 and Solution S_3, respectively.

Measure exactly 30 mL of Solution T_1, and add 20 mL of diluted hydrochloric acid (1 in 4) and water to make exactly 100 mL. With exactly 4 mL of this solution, prepare

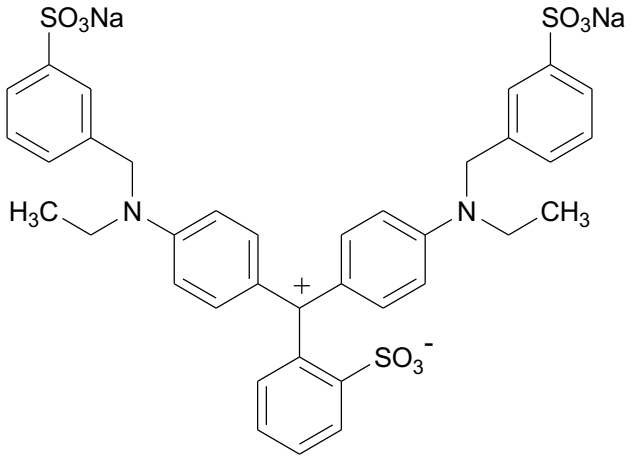
solution C, according to the direction in which Solution T_3 was prepared using solution T_2. Measure the absorbance (A_T, A_S, and A_C) of Solutions T_3, S_3, and C at a wavelength of 550 nm against a reference prepared with 4 mL of water, according to the direction in which Solution T_3 was prepared using Solution T_2. Calculate the content by the formula:

$$\text{Content (\%) of folic acid } (C_{19}H_{19}N_7O_6) = \frac{\text{Anhydrous basis weight (g) Folic Acid Reference Standard}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T - 0.1 \times A_C}{A_S} \times 100$$

Food Blue No. 1

Brilliant Blue FCF
FD&C Blue No. 1

食用青色 1 号



$C_{37}H_{34}N_2Na_2O_9S_3$ Mol. Wt. 792.85

Disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}methyliumyl)-benzenesulfonate [3844-45-9]

Definition Food Blue No. 1 consists mainly of disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}methyliumyl)benzenesulfonate.

Content Food Blue No. 1 contains the equivalent of not less than 85.0% of disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}methyliumyl)-benzenesulfonate ($C_{37}H_{34}N_2Na_2O_9S_3$).

Description Food Blue No. 1 occurs as a dark purple to dark purplish-red powder or as granules. It is odorless and has a metallic luster.

Identification A solution of 0.1 g of Food Blue No. 1 in 200 mL of ammonium acetate TS (0.02 mol/L) is brilliant blue to deep blue. To 1 mL of this solution, add ammonium

acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 628–632 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 4.0% as the sum (Coloring Matter Tests).

(3) Lead Not more than 2 μg/g as Pb (Coloring Matter Tests, Method 1).

(4) Manganese Not more than 50 μg/g as Mn (Coloring Matter Tests, Manganese and Chromium).

(5) Chromium Not more than 50 μg/g as Cr (Coloring Matter Tests, Manganese and Chromium)

(6) Arsenic Not more than 3 μg/g as As (Coloring Matter Tests).

(7) Subsidiary colors Not more than 6%.

Proceed as directed in the Coloring Matter Tests (Subsidiary Colors (2)) using the operating conditions given below.

Operating Conditions

Wavelength: 630 nm.

Concentration gradient (A/B): Run a linear gradient from 90/10 to 40/60 in 25 minutes, and maintain for 5 minutes.

Measurement time: 0–30 minutes after sample injection.

(8) Unreacted raw materials and products of side reactions

Not more than 1.5% as the sum of 2-, 3-, and 4-formyl-benzenesulfonic acid.

Not more than 0.3% of 3-[*N*-ethyl-*N*-(4-sulfophenyl)amino]methylbenzenesulfonate.

Test Solution Weigh accurately about 0.1 g of Food Blue No. 1, dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL.

Standard Solutions Weigh accurately about 10 mg of sodium 2-formyl-benzenesulfonate and an amount of calcium 3-[*N*-ethyl-*N*- (4-sulfophenyl)amino]methylbenzenesulfonate equivalent to about 10 mg of $C_{15}H_{15}CaNO_6S_2$,both previously dried in a vacuum desiccator for 24 hours. Dissolve them separately in ammonium acetate TS (0.02 mol/L) to make 100 mL of each. Use them as the standard stock solutions. Prepare the standard solutions of sodium 2-formyl-benzenesulfonate by transferring exactly 0.5 mL, 5, mL, 10 mL, and 20 mL of the standard stock solution, and adding ammonium acetate TS (0.02 mol/L) to make exactly 100 mL of each. For the standard solution of calcium 3-[*N*-ethyl-*N*- (4-sulfophenyl)amino]methylbenzenesulfonate, proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions). *Procedure* Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions) to determine the amounts of 2-, 3-, and 4-formyl-benzenesulfonic acid sodium salts and calcium 3-[*N*-ethyl-*N*- (4-sulfophenyl)amino]methylbenzenesulfonate in the

test solution. The peaks of sodium 3-formyl-benzenesulfonate and sodium 4-formyl-benzenesulfonate should be identified from the relative retention times to sodium 2-formyl-benzenesulfonate (the relative retention time of sodium 3-formyl-benzenesulfonate and sodium 4-formyl-benzenesulfonate are about 0.68 and about 0.72, respectively). Determine the amount of each salt from the calibration curve of sodium 2-formyl-benzenesulfonate. Determine the amounts of the three substances (2-formyl-benzenesulfonic acid, 3-formyl-benzenesulfonic acid, 4-formyl-benzenesulfonic acid) by multiplying the amounts of sodium salts of them by 0.894. Similarly, determine the amount of 3-[*N*-ethyl-*N*-(4-sulfophenyl)amino]methylbenzenesulfonate by multiplying the amount of its calcium salt by 0.9073.

Operating Conditions

Wavelength: 254 nm.

Concentration gradient (A/B): Run a linear gradient from 90/10 to 40/60 in 25 minutes, and maintain for 5 minutes.

(9) Color precursor (leuco base) Not more than 5%.

Test Solution Use the test solution for Purity (8).

Procedure Proceed as directed in the Coloring Matter Tests (Color Precursor) using Food Blue No. 1 Color Precursor Standard Stock Solution. Perform liquid chromatography using the operating conditions given in Purity (8).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 4.8 g of Food Blue No. 1, and dissolve it in water to make exactly 250 mL. Use exactly 50 mL of this solution as the test solution. Proceed as directed in Titanium(III) Chloride Method (ii) in the Assay in the Coloring Matter Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 39.64 mg of $C_{37}H_{34}N_2Na_2O_9S_3$

Food Blue No. 1 Aluminium Lake

(Food Blue No. 1 Aluminum Lake)

Brilliant Blue FCF Aluminum Lake

食用青色１号アルミニウムレーキ

Definition Food Blue No. 1 Aluminum Lake is prepared by adsorbing "Food Blue No. 1" to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Blue No. 1 Aluminum Lake contains the equivalent of not less than 10.0% of disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}-methyliumyl)benzenesulfonate ($C_{37}H_{34}N_2Na_2O_9S_3$ = 792.85).

Description Food Blue No. 1 Aluminum Lake occurs as a brilliant blue fine powder. It

is odorless.

Identification

(1) To 0.1 g of Food Blue No. 1 Aluminum Lake, add 5 mL of diluted sulfuric acid (1 in 20), stir well, and add ammonium acetate TS (0.02 mol/L) to make 200 mL. If the solution is not clear, centrifuge. Measure 1 to 10 mL of this solution so that the absorbance is between 0.2 and 0.7, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 628–632 nm.

(2) To 0.2 g of Food Blue No. 1 Aluminum Lake, add 20 mL of diluted hydrochloric acid (1 in 4), heat for 5 minutes in a water bath, shake well to dissolve the sample, add 1.0 g of active carbon, shake well, and filter. Adjust the pH of the colorless filtrate to 3–4 with sodium hydroxide solution (1 in 10). To confirm the pH value, use a pH paper. The solution responds to all the tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Lead Not more than 5 μg/g as Pb (Coloring Matter Aluminum Tests).

(3) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(4) Arsenic Not more than 3 μg/g as As (Coloring Matter Aluminum Lake Test).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

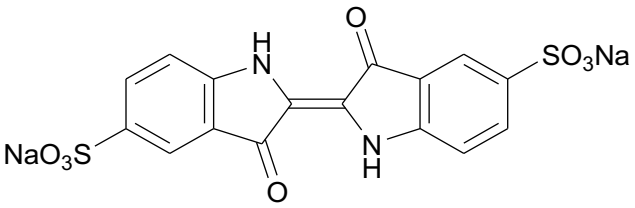
Assay Weigh accurately an amount of Food Blue No. 1 Aluminum Lake so that the volume of 0.1 mol/L titanium(III) chloride consumed is about 20 mL, and proceed as directed in Assay (2) in the Coloring Matter Aluminum Lake Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 39.64 mg of $C_{37}H_{34}N_2Na_2O_9S_3$

Food Blue No. 2

Indigo Carmine
Indigotine
FD&C Blue No.2

食用青色 2 号



$C_{16}H_8N_2Na_2O_8S_2$ Mol. Wt. 466.35

Disodium 2,2′-bi(3-oxo-1*H*-indolin-2-ylidene)-5,5′-disulfonate [860-22-0]

Definition Food Blue No. 2 consists principally of disodium 2,2′-bi(3-oxo- 1*H*-indolin-2-ylidene)-5,5′-disulfonate.

Content Food Blue No. 2 contains the equivalent of not less than 85.0% of disodium 2,2′-bi(3-oxo-1*H*-indolin-2-ylidene)-5,5′-disulfonate ($C_{16}H_8N_2Na_2O_8S_2$).

Description Food Blue No. 2 occurs as a very dark purplish-blue to very dark purple powder or as granules. It is odorless.

Identification A solution of 0.1 g of Food Blue No. 2 in 100 mL of ammonium acetate TS (0.02 mol/L) is deep greenish blue to deep blue or very dark greenish blue to very dark blue. To 1 mL of this solution, add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 610–614 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 7.0% as the sum (Coloring Matter Tests).

(3) Lead Not more than 2 μg/g as Pb (Coloring Matter Tests, Method 1).

(4) Iron Not more than 500 μg/g as Fe (Coloring Matter Tests, Zinc and Iron (2)).

(5) Arsenic Not more than 3 μg/g as As (Coloring Matter Tests).

(6) Isomer (disodium 2,2′-bi(3-oxo-1*H*-indolin-2-ylidene)-5,7′-disulfonate) Not more than 18%.

Test Solution Weigh accurately about 0.1 g of Food Blue No. 2, dissolve in diluted acetic acid (1 in 1000) to make exactly 100 mL. To exactly 2 mL of this solution, add diluted acetic acid (1 in 1000) to make exactly 20 mL. Prepare fresh before use.

Procedure Analyze a certain amount of the test solution by liquid chromatography using the operating conditions given below. Refer to the area one thousandth of the peak of the main pigment in the test solution as A. Record the areas of the peaks appearing within the measurement time after the injection of the test solution. Refer to the sum of areas that are larger than A as A_T. Refer to the peak area of disodium 2,2′-bi(3-oxo-1*H*-indolin-2-ylidene)-5,7′-disulfonate as A_B. The relative retention time of this substance to food blue No.2 is about 1.22. Determine the amount by the formula:

Amount (%) of disodium 2,2′-bi(3-oxo-1*H*-indolin-2-ylidene)-5,7′-disulfonate

$$= \frac{A_B}{A_T} \times \text{content (\%)}$$

Operating Conditions

Detector: Visible spectrophotometer or photodiode array detector (wavelength 610 nm).

Column: A stainless steel (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid

chromatography.

Column temperature: A constant temperature of around 40°C.

Mobile phase

A: Ammonium acetate TS (0.02 mol/L).

B: 7:3 mixture of acetonitrile/water.

Concentration gradient (A/B): Maintain at 95/5 for 5 minutes, run a linear gradient from 95/5 to 30/70 in 25 minutes, and maintain for 5 minutes.

Flow rate: 1 mL/ minute.

Measurement time: 0–35 minutes after the injection of the test solution.

(7) Subsidiary colors Not more than 1% (excluding disodium 2,2′-bi(3-oxo-1*H*-indolin-2-ylidene)-5,7′-disulfonate).

Proceed as directed in the Coloring Matter Tests (Subsidiary Colors (2)) using the operating conditions given below.

Test Solution Use the test solution for Purity (6).

Procedure Refer to the sum of the areas excluding the main pigment peak and the peak of disodium 2,2′-bi(3-oxo-1*H*-indolin-2-ylidene)-5,7′-disulfonate as A_S.

Operating Conditions

Wavelength: 610 nm.

Concentration gradient (A/B): Maintain at 95/5 for 5 minutes, run a linear gradient from 95/5 to 30/70 in 25 minutes, and maintain for 5 minutes.

Measurement time: 0–35 minutes after the injection of the test solution.

(8) Unreacted raw materials and products of side reactions

Not more than 0.5% as the sum of

2,3-dihydro-2,3-dioxo-1*H*-indole-5-sulfonic acid;

2-amino-5-sulfobenzoic acid; and

2-aminobenzoic acid.

Test Solution Weigh accurately about 0.1 g of Food Blue No. 2, dissolve it in diluted acetic acid (1 in 1000) to make exactly 100 mL.

Standard Solutions Weigh accurately about 10 mg each of 2,3-dihydro-2,3-dioxo-1*H*-indole-5-sulfonic acid sodium salt dihydrate, 2-amino-5-sulfobenzoic acid, and 2-aminobenzoic acid, dried previously in a vacuum desiccator for 24 hours. Dissolve 2,3-dihydro-2,3-dioxo-1*H*-indole-5-sulfonic acid sodium salt dihydrate and 2-amino-5-sulfobenzoic acid separately in diluted acetic acid (1 in 1000) to make exactly 100 mL. Dissolve 2-aminobenzoic acid in 5 mL of acetonitrile, and add diluted acetic acid (1 in 1000) to make exactly 100 mL. Use them as the standard stock solutions. Transfer exactly 0.5 mL, 1 mL, 2 mL, and 5 mL of each standard stock solution, and add diluted acetic acid (1 in 1000) to make exactly 100 mL of standard solutions. Prepare fresh the

test solution and the standard solutions before use.

Procedure Analyze the constant amount of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Measure peak areas for the standard solutions to prepare calibration curves for these substances. Measure peak areas of unreacted raw materials and products of side reactions (2,3-dihydro-2,3-dioxo-1*H*-indole-5-sulfonic acid sodium salt dihydrate, 2-amino-5-sulfobenzoic acid, and 2-aminobenzoic acid) in the test solution to determine the amounts of them from the calibration curves. The amount of 2,3-dihydro-2,3-dioxo-1*H*-indole-5-sulfonic should be calculated by multiplying the amount of 2,3-dihydro-2,3-dioxo-1*H*-indole-5-sulfonic acid sodium salt dihydrate by the factor 0.923.

Operating Conditions

Wavelength: 254 nm.

Concentration gradient (A/B): Maintain at 95/5 for 5 minutes, run a linear gradient from 95/5 to 30/70 in 25 minutes, and maintain for 5 minutes.

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 2.7 g of Food Blue No. 2, and dissolve it in water to make exactly 500 mL. Use exactly 100 mL of this solution as the test solution. Proceed as directed in Titanium(III) Chloride Method (ii) in the Assay in the Coloring Matter Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 23.32 mg of $C_{16}H_8N_2Na_2O_8S_2$

Food Blue No. 2 Aluminium Lake

(Food Blue No. 2 Aluminum Lake)

Indigo Carmine Aluminum Lake

食用青色 2 号アルミニウムレーキ

Definition Food Blue No. 2 Aluminum Lake is prepared by adsorbing "Food Blue No. 2" to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Blue No. 2 Aluminum Lake contains the equivalent of not less than 10.0% of disodium 2,2′-bi(3-oxo-1*H*-indolin-2-ylidene)-5,5′-disulfonate ($C_{16}H_8N_2Na_2O_8S_2$ = 466.35).

Description Food Blue No. 2 Aluminum Lake occurs as a deep blue fine powder. It is odorless.

Identification

(1) To 0.1 g of Food Blue No. 2 Aluminum Lake, add 5 mL of diluted sulfuric acid (1 in 20), stir well, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. If the solution is not clear, centrifuge. Measure 1 to 10 mL of this solution so that the

absorbance is between 0.2 to 0.7, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 610–614 nm.

(2) To 0.2 g of Food Blue No. 2 Aluminum Lake, add 20 mL of diluted hydrochloric acid (1 in 4), heat in a water bath for 5 minutes, shake well to dissolve most of it, add 1.0 g of active carbon, shake well, and filter. Adjust the pH of the colorless filtrate to 3–4 with sodium hydroxide solution (1 in 10). To confirm the pH value, use a pH paper. The solution responds to all the tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Lead Not more than 5 μg/g as Pb (Coloring Matter Aluminum Lake Tests).

(3) Iron Not more than 250 μg/g as Fe (Coloring Matter Aluminum Lake Tests, Zinc and Iron (2)).

(4) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(5) Arsenic Not more than 3 μg/g as As (Coloring Matter Aluminum Lake Tests).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

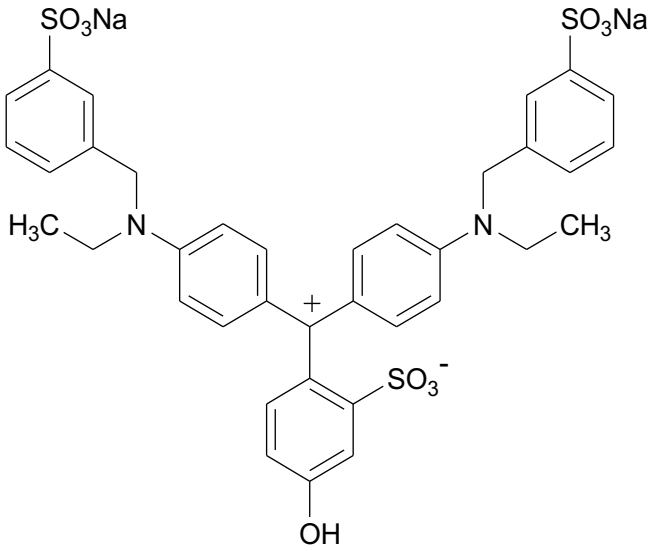
Assay Weigh accurately an amount of Food Blue No. 2 Aluminum Lake so that the volume of 0.1 mol/L titanium(III) chloride consumed is about 20 mL, and proceed as directed in Assay (2) in the Coloring Matter Aluminum Lake Tests.

Each mL of 0.1 mol/L titanium(III) chloride solution = 23.32 mg of $C_{16}H_8N_2Na_2O_8S_2$

Food Green No. 3

Fast Green FCF
FD&C Green No.3

食用緑色 3 号



$C_{37}H_{34}N_2Na_2O_{10}S_3$ Mol. Wt. 808.85

Disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}methyliumyl)- 5-hydroxybenzenesulfonate [2353-45-9]

Definition Food Green No. 3 consists principally of disodium 2-(bis{4-[*N*-ethyl- *N*-(3-sulfonatophenylmethyl)amino]phenyl}methyliumyl)-5-hydroxybenzenesulfonate.

Content Food Green No. 3 contains the equivalent of not less than 85.0% of disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}methyliumyl)-5-hydroxybenzenesulfonate ($C_{37}H_{34}N_2Na_2O_{10}S_3$).

Description Food Green No. 3 occurs as a very dark reddish yellow to very dark yellow-red or dark green to dark blue-green powder or as granules. It is odorless.

Identification A solution of 0.1 g of Food Green No. 3 in 200 mL of ammonium acetate TS (0.02 mol/L) is dark blue-green to deep blue-green. To 1 mL of this solution, add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 622–626 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 5.0% as the sum (Coloring Matter Tests).

(3) Lead Not more than 2 μg/g as Pb (Coloring Matter Tests, Method 1).

(4) Manganese Not more than 50 μg/g as Mn (Coloring Matter Tests, Manganese

and Chromium).

(5) Chromium Not more than 50 μg/g as Cr (Coloring Matter Tests, Manganese and Chromium).

(6) Arsenic Not more than 3 μg/g as As (Coloring Matter Tests).

(7) Subsidiary colors Not more than 6%.

Proceed as directed in the Coloring Matter Tests (Subsidiary Colors (2)) using the operating conditions given below.

Operating Conditions

Wavelength: 625 nm.

Concentration gradient (A/B): Maintain at 85/15 for 5 minutes, run a linear gradient from 85/15 to 65/35 in 10 minutes, and maintain at 65/35 for 20 minutes.

Measurement time: 0–35 minutes after sample injection.

(8) Unreacted raw materials and products of side reactions

Not more than 0.5% as the sum of 2-, 3-, and 4-formyl-benzenesulfonic acid.

Not more than 0.3% of 3-[*N*-ethyl-*N*-(4-sulfophenyl)amino]methylbenzenesulfonic acid.

Not more than 0.5% of 2-formyl-5-hydroxy-benzenesulfonic acid.

Test Solution Weigh accurately about 0.1 g of Food Green No. 3, dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL.

Standard Solutions Weigh accurately about 10 mg each of sodium 2-formyl-benzenesulfonate and sodium 2-formyl-5-hydroxy-benzenesulfonate, both previously dried in in a vacuum desiccator for 24 hours. Also, weigh an amount of calcium 3-[*N*-ethyl-*N*-(4-sulfophenyl)amino]methylbenzenesulfonate equivalent to about 10 mg of $C_{15}H_{15}CaNO_6S_2$. Dissolve them separately in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL of each. Use them as the standard stock solutions.

Procedure Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions) to determine the amounts of 2-, 3-, and 4-formyl-benzenesulfonic acid sodium salts, calcium 3-[*N*-ethyl-*N*-(4-sulfophenyl)amino]methylbenzenesulfonate, and sodium 2-formyl-5-hydroxy-benzenesulfonate in the test solution. The peaks of sodium 3-formyl-benzenesulfonate and sodium 4-formyl-benzenesulfonate should be identified from the relative retention times to sodium 2-formyl-benzenesulfonate (the relative retention time of sodium 3-formyl-benzenesulfonate and sodium 4-formyl-benzenesulfonate are about 0.66 and about 0.69, respectively). Determine the amount of each salt from the calibration curve of sodium 2-formyl-benzenesulfonate. Determine the amounts of the three substances (2-formyl-benzenesulfonic acid, 3-formyl-benzenesulfonic acid, 4-formyl- benzenesulfonic acid) by multiplying the amounts of sodium salts of them by 0.894. Similarly, determine the amount of 3-[*N*-ethyl-*N*-(4- sulfophenyl)amino]methylbenzenesulfonic acid) by multiplying the amount of its calcium salt by 0.907 and determine the amount of 2-

formyl-5-hydroxy-benzenesulfonic acid by multiplying the amount of its sodium salt by 0.9023.

Operating Conditions

Wavelengths:

2-, 3-, and 4-formyl-benzenesulfonic acid; and 3-[*N*-ethyl-*N*-(4-sulfophenyl)amino]methylbenzenesulfonic acid: 254 nm.

2-formyl-5-hydroxy-benzenesulfonic acid: 300 nm.

Concentration gradient (A/B): Maintain at 85/15 for 5 minutes, run a linear gradient from 85/15 to 65/35 in 10 minutes, and maintain at 65/35 for 20 minutes.

(9) Color precursor (leuco base) Not more than 5%.

Test Solution Use the test solution for Purity (8).

Control Solution Prepare a control solution as directed in Coloring Matter Tests (Color Precursor), using Food Green No. 3 Color Precursor Standard Stock Solution.

Procedure Analyze the test solution and the control solution by liquid chromatography using the operating conditions specified in Purity (8).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 4.7 g of Food Green No. 3, and dissolve it in water to make exactly 250 mL. Use exactly 50 mL of this solution as the test solution, and proceed as directed in Titanium(III) Chloride Method (ii) in the Assay in the Coloring Matter Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 40.44 mg of $C_{37}H_{34}N_2Na_2O_{10}S_3$

Food Green No. 3 Aluminium Lake

(Food Green No. 3 Aluminum Lake)

Fast Green FCF Aluminum Lake

食用緑色 3 号アルミニウムレーキ

Definition Food Green No. 3 Aluminum Lake is prepared by adsorbing "Food Green No. 3" to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Green No. 3 Aluminum Lake contains the equivalent of not less than 10.0% of disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}-methyliumyl)-5-hydroxybenzenesulfonate ($C_{37}H_{34}N_2Na_2O_{10}S_3$ = 808.85).

Description Food Green No. 3 Aluminum Lake occurs as a dark green-blue fine powder. It is odorless.

Identification

(1) To 0.1 g of Food Green No. 3 Aluminum Lake, add 5 mL of diluted sulfuric acid (1 in 20), stir well, and add ammonium acetate TS (0.02 mol/L) to make 200 mL. If the solution is not clear, centrifuge. Measure 1 to 10 mL of the solution so that the absorbance is between 0.2 and 0.7, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 622–626 nm.

(2) To 0.2 g of Food Green No. 3 Aluminum Lake, add 20 mL of diluted hydrochloric acid (1 in 4), heat in a water bath for 5 minutes, and shake well to dissolve the sample. Add 1.0 g of active carbon, shake well, and filter. Adjust the pH of the colorless filtrate to 3–4 with sodium hydroxide solution (1 in 10). To confirm the pH value, use a pH paper. The solution responds to all the tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Lead Not more than 5 μg/g as Pb (Coloring Matter Aluminum Lake Tests).

(3) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(4) Arsenic Not more than 3 μg/g as As (Coloring Matter Aluminum Lake Tests).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

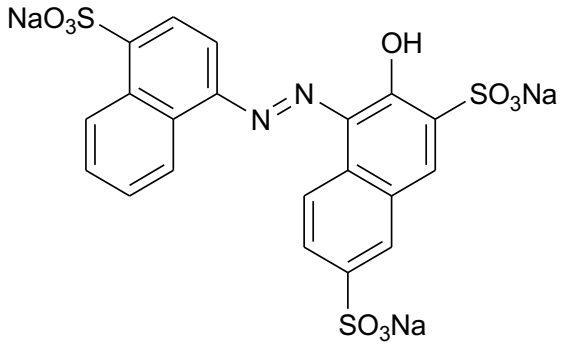
Assay Weigh accurately Food Green No. 3 Aluminum Lake so that the volume of 0.1 mol/L titanium(III) chloride consumed is about 20 mL, and proceed as directed in Assay (2) in the Coloring Matter Aluminum Lake Tests.

Each mL of 0.1 mol/L titanium(III) chloride solution = 40.44 mg of $C_{37}H_{34}N_2Na_2O_{10}S_3$

Food Red No. 2

Amaranth

食用赤色 2 号



$C_{20}H_{11}N_2Na_3O_{10}S_3$ Mol. Wt. 604.47

Trisodium 3-hydroxy-4-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-2,7-disulfonate [915-67-3]

Definition Food Red No. 2 is obtained by diazotizing 4-amino-1-naphthalenesulfonic acid, coupling the obtained diazo compound with 3-hydroxy-2,7-naphthalenedisulfonic acid, and then salting out and refining the resulting dye. It consists principally of trisodium 3-hydroxy-4-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-2,7-disulfonate.

Content Food Red No. 2 contains the equivalent of not less than 85.0% of trisodium 3-hydroxy-4-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-2,7-disulfonate ($C_{20}H_{11}N_2Na_3O_{10}S_3$).

Description Food Red No. 2 occurs as a very dark yellow-red to very dark red powder or as granules. It is odorless.

Identification A solution of 0.1 g of Food Red No. 2 in 100 mL of ammonium acetate TS (0.02 mol/L) is deep red to deep purplish red. To 1 mL of this solution, add ammonium acetate TS (0.02 mol/L) to make 100 mL. The resulting solution exhibits an absorption maximum at a wavelength of 518–522 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 5.0% as the sum (Coloring Matter Tests).

(3) Lead Not more than 2 μg/g as Pb (Coloring Matter Tests, Method 1).

(4) Arsenic Not more than 3 μg/g as As (Coloring Matter Tests).

(5) Subsidiary colors Not more than 3%.

Proceed as directed in the Coloring Matter Tests (Subsidiary Colors (2)) using the operating conditions given below.

Operating Conditions

Wavelength: 510 nm.

Concentration gradient (A/B): Maintain at 100/0 for 10 minutes, run a linear gradient from 100/0 to 50/50 in 20 minutes, and maintain at 50/50 for 5minutes.

Measurement time: 0–35 minutes after sample injection.

(6) Unreacted raw materials and products of side reactions

Not more than 0.5% as the sum of

monosodium 4-amino-1-naphthalenesulfonate tetrahydrate;

disodium 7-hydroxy-1,3-naphthalenedisulfonate;

disodium 3-hydroxy-2,7-naphthalenedisulfonate;

monosodium 6-hydroxy-2-naphthalenesulfonate; and

trisodium 7-hydroxy-1,3,6-naphthalenetrisulfonate.

Test Solution Weigh accurately about 0.1 g of Food Red No. 2, dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL.

Standard Solutions Weigh accurately about 10 mg each of monosodium 4-amino-1-naphthalenesulfonate tetrahydrate, disodium 7-hydroxy-1,3- naphthalenedisulfonate, disodium 3-hydroxy-2,7-naphthalenedisulfonate, monosodium 6-hydroxy-2-naphthalenesulfonate, and trisodium 7-hydroxy-1,3,6- naphthalenetrisulfonate, dried previously in a vacuum desiccator for 24 hours. Dissolve them separately in ammonium acetate TS (0.02 mol/L) to make standard stock solutions of exactly 100 mL each. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Determine the amount of each salt in the test solution as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions), and calculate the total amount.

Operating Conditions

Wavelength: 238 nm.

Concentration gradient (A/B): Maintain at 100/0 for 10 minutes, run a linear gradient from 100/0 to 50/50 in 20 minutes, and maintain at 50/50 for 5 minutes.

(7) Unsulfonated primary aromatic amines

Not more than 0.01% calculated as aniline,

Not more than 1.0 μg/g as 1-naphthylamine (Coloring Matter Tests).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 1.7 g of Food Red No. 2, and dissolve it in water to make exactly 250 mL. Use exactly 50 mL of this solution as the test solution, and proceed as directed under Titanium(III) Chloride Method (i) in the Assay in the Coloring Matter Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 15.11 mg of $C_{20}H_{11}N_2Na_3O_{10}S_3$

Food Red No. 2 Aluminium Lake

(Food Red No. 2 Aluminum Lake)

Amaranth Aluminum Lake

食用赤色 2 号アルミニウムレーキ

Definition Food Red No. 2 Aluminum Lake is prepared by adsorbing “Food Red No. 2” to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Red No. 2 Aluminum Lake contains the equivalent of not less than 10.0% of trisodium 3-hydroxy-4-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene- 2,7-

disulfonate ($C_{20}H_{11}N_2Na_3O_{10}S_3$ = 604.47).

Description Food Red No. 2 Aluminum Lake occurs as a purplish red, fine powder. It is odorless.

Identification

(1) To 0.1 g of Food Red No. 2 Aluminum Lake, add 5 mL of diluted sulfuric acid (1 in 20), stir well, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. If the solution is not clear, centrifuge. Measure 1 to 10 mL of this solution so that the absorbance is between 0.2 and 0.7, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 518–522 nm.

(2) To 0.2 g of Food Red No. 2 Aluminum Lake, add 20 mL of diluted hydrochloric acid (1 in 4), heat in a water bath for 5 minutes, shake well until most of it dissolves, add 1.0 g of active carbon, shake well, and filter. Adjust the pH of the colorless filtrate to 3–4 with sodium hydroxide solution (1 in 10). To confirm the pH value, use a pH paper. The resulting solution responds to all the tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Lead Not more than 5 μg/g as Pb (Coloring Matter Aluminum Lake Tests).

(3) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(4) Arsenic Not more than 3 μg/g as As (Coloring Matter Aluminum Lake Tests).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

Assay Weigh accurately an amount of Food Red No. 2 Aluminum Lake so that the volume of 0.1 mol/L titanium(III) chloride consumed is about 20 mL, and proceed as directed in Assay (1) in the Coloring Matter Aluminum Lake Tests.

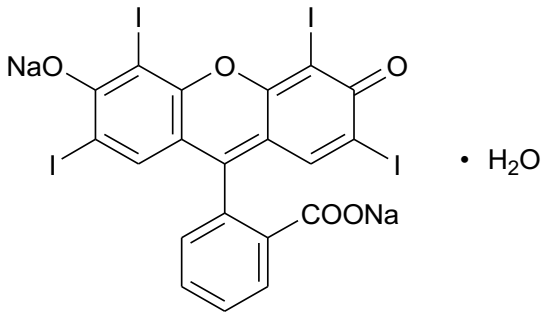
Each mL of 0.1 mol/L titanium(III) chloride = 15.11 mg of $C_{20}H_{11}N_2Na_3O_{10}S_3$

Food Red No. 3

Erythrosine

FD&C Red No. 3

食用赤色 3 号



$C_{20}H_6I_4Na_2O_5 \cdot H_2O$ Mol. Wt. 897.87

Disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate monohydrate [anhydrous 16423-68-0]

Definition Food Red No. 3 consists principally of disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate monohydrate.

Content Food Red No. 3 contains the equivalent of not less than 85.0% of disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate monohydrate ($C_{20}H_6I_4Na_2O_5 \cdot H_2O$).

Description Food Red No. 3 occurs as a deep yellow-red to deep red powder or as granules. It is odorless.

Identification A solution of 0.1 g of Food Red No. 3 in 500 mL of ammonium acetate TS (0.02 mol/L) is brilliant yellowish red. To 3 mL of this solution, add ammonium acetate TS (0.02 mol/L) to make 200 mL. The resulting solution exhibits an absorption maximum at a wavelength of 524–528 nm.

pH 6.5–10.0 (1.0 g, water 100 mL).

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 2.0% as the sum.

Test Solution Weigh accurately about 0.1 g of Food Red No. 3, dissolve it in water to make exactly 100 mL. Dilute exactly 20 mL of this solution, with water to make exactly 50 mL. Proceed as directed in the Coloring Matter Tests.

(3) Iodide Not more than 0.4% (Coloring Matter Tests).

(4) Lead Not more than 2 μg/g as Pb (Coloring Matter Tests, Method 2).

(5) Zinc Not more than 200 μg/g as Zn (Coloring Matter Tests, Zinc and Iron (1)).

(6) Arsenic Not more than 3 μg/g as As (Coloring Matter Tests).

(7) Subsidiary colors Not more than 4%.

Proceed as directed in the Coloring Matter Tests (Subsidiary Colors (2)) using the operating conditions given below.

Operating Condition

Wavelength: 530 nm.

Concentration gradient (A/B): Maintain at 80/20 for 30 minutes, run a linear gradient from 80/20 to 30/70 in 8 minutes, and maintain at 30/70 for 12 minutes.

Measurement time: 0–50 minutes after sample injection.

(8) Unreacted raw materials and products of side reactions

Not more than 0.1% as the sum of phthalic acid, resorcinol, and fluorescein.

Not more than 0.2% of 2-(2,4-dihydroxy-3,5-diiodobenzoyl)benzoic acid.

Test Solution Weigh accurately about 0.1 g of Food Red No. 3, dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL.

Standard Solutions Weigh accurately about 10 mg each of phthalic acid, resorcinol, fluorescein, and 2-(2,4-dihydroxy-3,5-diiodobenzoyl)benzoic acid, all previously dried in a vacuum desiccator for 24 hours. Dissolve the three substances (phthalic acid, resorcinol, and 2-(2,4-dihydroxy-3,5-diiodobenzoyl)benzoic acid) separately in 5 mL of acetonitrile and fluorescein in 5 mL of ammonia solution (1 in 25). To each solution, add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL each. Measure exactly 10 mL each of them, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Use them as the standard stock solutions. Transfer exactly 1 mL, 5 mL, 10 mL, and 50 mL of each stock solution, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL of standard solutions.

Procedure Measure the constant amount of the test solution and the standard solutions, and proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions) to determine the amount of each substance in the test solution.

Operating Conditions

Wavelength: 223 nm.

Concentration gradient (A/B): Maintain at 80/20 for 30 minutes, run a linear gradient from 80/20 to 30/70 in 8 minutes, and maintain at 30/70 for 12 minutes.

Loss on Drying Not more than 12.0% (135°C, 6 hours).

Assay Weigh accurately about 1 g of Food Red No. 3, and dissolve it in water to make exactly 100 mL. Use exactly 50 mL of this solution as the test solution, and proceed as directed in the Mass Method in the Assay in the Coloring Matter Tests.

$$\text{Content (\%) of Food Red No. 3 } (C_{20}H_6I_4Na_2O_5 \cdot H_2O) = \frac{\text{Mass (g) of the precipitate} \times 2.148}{\text{Weight (g) of the sample}} \times 100$$

Food Red No. 3 Aluminium Lake

(Food Red No. 3 Aluminum Lake)

Erythrosine Aluminum Lake

食用赤色 3 号アルミニウムレーキ

Definition Food Red No. 3 Aluminum Lake is prepared by adsorbing "Food Red No. 3" to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Red No. 3 Aluminum Lake contains the equivalent of not less than 10.0% of disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate monohydrate ($C_{20}H_6I_4Na_2O_5 \cdot H_2O$ = 897.87).

Description Food Red No. 3 Aluminum Lake occurs as a brilliant purplish red to bright red, fine powder. It is odorless.

Identification

(1) To 0.1 g of Food Red No. 3 Aluminum Lake, add 5 mL of sodium hydroxide solution (1 in 10), dissolve it by heating in a water bath, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. If the solution is not clear, centrifuge. Measure 0.5 to 5 mL of this solution so that the absorbance is between 0.2–0.7, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits absorption maximum at a wavelength of 524–528 nm.

(2) To 0.2 g of Food Red No. 3 Aluminum Lake, add 20 mL of diluted hydrochloric acid (1 in 4), heat for 5 minutes in a water bath, shake well until most of it dissolves, add 1.0 g of active carbon, shake well, and filter. Adjust the pH of the colorless filtrate to 3–4 with sodium hydroxide solution (1 in 10). To confirm the pH value, use a pH paper. The solution responds to all the tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Iodide Not more than 0.2% (Coloring Matter Aluminum Lake Tests).

(3) Lead Not more than 5 μg/g as Pb (Coloring Matter Aluminum Lake Tests).

(4) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(5) Zinc Not more than 50 μg/g as Zn (Coloring Matter Aluminum Lake Tests).

(6) Arsenic Not more than 3 μg/g as As (Coloring Matter Aluminum Lake Tests).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

Assay

Test Solution Weigh accurately about 0.1 g of Food Red No. 3 Aluminum Lake, transfer into a 100-mL beaker, add 50 mL of sodium hydroxide solution (1 in 250) to

dissolve, and transfer into a 500-mL volumetric flask. Wash the beaker with ammonium acetate TS (0.02 mol/L), add the washings to the volumetric flask, and add ammonium acetate TS (0.02 mol/L) to make exactly 500 mL. Use this solution as the sample solution. Measure exactly a certain volume, between 10 and 20 mL, of the sample solution so that the absorbance is between 0.2 and 0.7, and add ammonium acetate TS (0.02 mol/L) to make exactly 200 mL.

Procedure Measure the absorbance (A) of the test solution at a wavelength of 526 nm, and calculate the content by the formula:

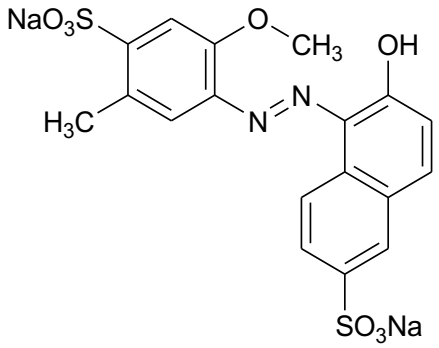
$$\text{Content (\%) of Food Red No. 3 } (C_{20}H_6I_4Na_2O_5 \cdot H_2O) = \frac{A \times 0.1}{0.111 \times S \times \text{Weight (g) of the sample}} \times 100$$

S = Number of mL of the sample solution used for the preparation of the test solution.

Food Red No. 40

Allura Red AC
FD&C Red No. 40

食用赤色 40 号



$C_{18}H_{14}N_2Na_2O_8S_2$ Mol. Wt. 496.42

Disodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfonatophenyl)diazenyl]-naphthalene-2-sulfonate [25956-17-6]

Definition Food Red No. 40 is obtained by diazotizing 4-amino-5-methoxy-2-methylbenzenesulfonic acid, coupling the obtained diazo compound with 6-hydroxy-2-naphthalenesulfonic acid, and then salting out and refining the resulting dye. It consists principally of disodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate.

Content Food Red No. 40 contains the equivalent of not less than 85.0% of disodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate

($C_{18}H_{14}N_2Na_2O_8S_2$).

Description Food Red No. 40 occurs as a dark yellow-red to dark red or deep yellowish red powder or as granules. It is odorless.

Identification A solution of 0.1 g of Food Red No. 40 in 100 mL of ammonium acetate TS (0.02 mol/L) is brilliant yellowish red or brilliant red. To 1 mL of this solution, add ammonium acetate TS (0.02 mol/L) to make 100 mL. This solution exhibits an absorption maximum at a wavelength of 497–501 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 5.0% as the sum (Coloring Matter Tests).

(3) Lead Not more than 2 μg/g as Pb (Coloring Matter Tests, Method 1).

(4) Arsenic Not more than 3 μg/g as As (Coloring Matter Tests).

(5) Lower sulfonated subsidiary colors Not more than 1.0%.

Test Solution Weigh accurately about 0.1 g of Food Red No. 40, dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL.

Standard Solutions Weigh accurately about10 mg each of cresidine sulfonic acid azo β-naphthol and cresidine azo Schaeffer's salt, dried previously in a vacuum desiccator for 24 hours. dissolve separately in ammonium acetate TS (0.02 mol/L) to prepare standard stock solutions of exactly 100 mL each. Proceed as directed in the Coloring Matter Tests (Subsidiary Colors (1)).

Procedure Determine the amounts of cresidine sulfonic acid azo β-naphthol and cresidine azo Schaeffer's salt in the test solution as directed in the Coloring Matter Tests (Subsidiary Colors (1)), and calculate the total amount.

Operating Conditions

Wavelength: 510 nm.

Mobile phase

A: Ammonium acetate TS (0.02 mol/L).

B: A 7:3 mixture of acetonitrile/water.

Concentration gradient (A/B): Maintain at 100/0 for 10 minutes, run a linear gradient from 100/0 to 40/60 in 40 minutes, and maintain for 10 minutes.

(6) Higher sulfonated subsidiary colors Not more than 1.0%.

Test Solution Use the test solution prepared in Purity (5).

Standard Solutions Weigh accurately about 10 mg of each of cresidine sulfonic acid azo G salt and cresidine sulfonic acid azo R salt, dried previously in a vacuum desiccator for 24 hours, dissolve them separately in ammonium acetate TS (0.02 mol/L) to prepare standard stock solutions of exactly 100 mL each. Proceed as directed in the Coloring Matter Tests (Subsidiary Colors (1)).

Procedure Analyze equal portions of the test solution and the standard solutions by liquid chromatography using the operating conditions specified in Purity (5), as directed in the Coloring Matter Tests (Subsidiary Colors (1)). Determine the amounts of cresidine sulfonic acid azo G salt and cresidine sulfonic acid azo R salt in the test solution, and calculate the total amount.

(7) Monosodium 6-hydroxy-2-naphthalenesulfonate Not more than 0.3%.

Test Solution Use of the test solution prepared in Purity (5).

Standard Solutions Weigh accurately about 10 mg of monosodium 6-hydroxy-2-naphthalenesulfonate, dried in a vacuum desiccator for 24 hours, dissolve it in ammonium acetate TS (0.02 mol/L) to prepare a standard stock solution of exactly 100 mL. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Determine the amount of monosodium 6-hydroxy-2-naphthalenesulfonate in the test solution, as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Operating Conditions

Wavelength: 238 nm.

Mobile phase

A: Ammonium acetate TS (0.02 mol/L).

B: A 7:3 mixture of acetonitrile/water.

Concentration gradient (A/B): Maintain at 100/0 for 10 minutes, run a linear gradient from 100/0 to 40/60 in 40 minutes, and maintain for 10 minutes.

(8) 4-Amino-5-methoxy-2-methylbenzenesulfonic acid Not more than 0.2%.

Test Solution Use the test solution prepared in Purity (5).

Standard Solutions Weigh accurately about 10 mg of 4-amino-5-methoxy-2-methylbenzenesulfonic acid, dried in a vacuum desiccator for 24 hours, and dissolve it in ammonium acetate TS (0.02 mol/L) to prepare a standard stock solution of exactly 100 mL. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Analyze equal portions of the test solution and the standard solutions by liquid chromatography using the operating conditions specified in Purity (7), as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions), and determine the amount of 4-amino-5-methoxy-2-methylbenzenesulfonic acid in the test solution.

(9) Disodium 6,6′-oxybis(2-naphthalenesulfonate) Not more than 1.0%.

Test Solution Use the test solution prepared in Purity (5).

Standard Solutions Weigh accurately 10 mg of disodium 6,6′-oxybis(2-naphthalenesulfonate), dried in a vacuum desiccator for 24 hours, and dissolve it in ammonium acetate TS (0.02 mol/L) to prepare a standard stock solution of exactly 100

mL. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Analyze equal portions of the test solution and the standard solutions by liquid chromatography using the operating conditions specified under Purity (7), as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions), and determine the amount of disodium 6,6′-oxybis(2- naphthalenesulfonate) in the test solution.

(10) Unsulfonated primary aromatic amines

Not more than 0.01% as aniline (Coloring Matter Tests).

Not more than 10 μg/g as 2-methoxy-5-athylanirin (Coloring Matter Tests).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 1.5 g of Food Red No. 40, and dissolve it in water to make exactly 250 mL. Use exactly 50 mL of this solution as the test solution, and proceed as directed in Titanium(III) Chloride Method (i) in the Assay in the Coloring Matter Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 12.41 mg of $C_{18}H_{14}N_2Na_2O_8S_2$

Food Red No. 40 Aluminium Lake

(Food Red No. 40 Aluminum Lake)

Allura Red AC Aluminum Lake

食用赤色 40 号アルミニウムレーキ

Definition Food Red No. 40 Aluminum Lake is prepared by adsorbing “Food Red No. 40” to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Red No. 40 Aluminum Lake contains the equivalent of not less than 10.0% of disodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfonatophenyl)diazenyl]-naphthalene-2-sulfonate ($C_{18}H_{14}N_2Na_2O_8S_2$ = 496.42).

Description Food Red No. 40 Aluminum Lake occurs as a brilliant yellow-red to brilliant yellowish red, fine powder. It is odorless.

Identification

(1) To 0.1 g of Food Red No. 40 Aluminum Lake, add 60 mL of diluted ammonia solution (1 in 25), heat to boil, and concentrate to about 40 mL. Cool and centrifuge the liquid, and take the supernatant. To the residue, add 10 mL of water, mix, centrifuge again, and take the supernatant. Combine both supernatants, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. Measure 1 to 10 mL of this solution so that the absorbance is between 0.2 and 0.7, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 497–501 nm.

(2) To 0.2 g of Food Red No. 40 Aluminum Lake, add 20 mL of diluted hydrochloric acid (1 in 4), heat for 5 minutes in a water bath, shake well dissolve the sample, add 1.0 g of active carbon, shake well, and filter. Adjust the pH of the colorless filtrate to 3–4 with sodium hydroxide solution (1 in 10). To confirm the pH value, use a pH paper. The solution responds to all the tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Lead Not more than 5 μg/g as Pb (Coloring Matter Aluminum Lake Tests).

(3) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(4) Arsenic Not more than 3 μg/g as As (Coloring Matter Aluminum Lake Tests).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

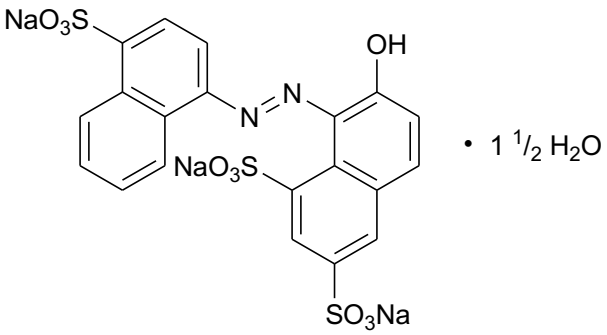
Assay Weigh accurately an amount of Food Red No. 40 Aluminum Lake so that the volume of 0.1 mol/L titanium(III) chloride consumed is about 20 mL, and proceed as directed in Assay (1) in the Coloring Matter Aluminum Lake Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 12.41 mg of $C_{18}H_{14}N_2Na_2O_8S_2$

Food Red No. 102

New Coccine
Ponceau 4R

食用赤色 102 号



$C_{20}H_{11}N_2Na_3O_{10}S_3 \cdot 1\,{}^1/_2H_2O$ Mol. Wt. 631.50

Trisodium 7-hydroxy-8-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-1,3- disulfonate sesquihydrate [anhydrous 2611-82-7]

Definition Food Red No. 102 is obtained by diazotizing 4-amino-1- naphthalenesulfonic acid, coupling the obtained diazo compound with 7-hydroxy-1,3-naphthalenedisulfonic acid, and then salting out and refining the resulting dye. It consists principally of

trisodium 7-hydroxy-8-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-1,3-disulfonate sesquihydrate.

Content Food Red No. 102 contains the equivalent of not less than 85.0% of trisodium 7-hydroxy-8-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-1,3-disulfonate sesquihydrate ($C_{20}H_{11}N_2Na_3O_{10}S_3 \cdot 1\,{}^1/_2H_2O$).

Description Food Red No. 102 occurs as a deep yellow-red to deep red powder or as granules. It is odorless.

Identification A solution of 0.1 g of Food Red No. 102 in 100 mL of ammonium acetate TS (0.02 mol/L) is brilliant yellow-red to brilliant red. To 1 mL of this solution, add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 506–510 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 8.0% as the sum (Coloring Matter Tests).

(3) Lead Not more than 2 μg/g as Pb (Coloring Matter Tests, Method 1).

(4) Arsenic Not more than 3 μg/g as As (Coloring Matter Tests).

(5) Subsidiary colors Not more than 1%.

Proceed as directed in the Coloring Matter Tests (Subsidiary Colors (2)) using the operating conditions given below.

Operating Conditions

Wavelength: 510 nm.

Concentration gradient (A/B): Run a linear gradient from 100/0 to 40/60 in 30 minutes, and maintain at 40/60 for 5 minutes.

Measurement time: 0–35 minutes after sample injection.

(6) Unreacted raw materials and products of side reactions

Not more than 0.5% as the sum of

monosodium 4-amino-1-naphthalenesulfonate tetrahydrate;

disodium 7-hydroxy-1,3-naphthalenedisulfonate;

disodium 3-hydroxy-2,7-naphthalenedisulfonate;

monosodium 6-hydroxy-2-naphthalenesulfonate; and

trisodium 7-hydroxy-1,3,6-naphthalenetrisulfonate.

Test Solution Weigh accurately about 0.1 g of Food Red No. 102, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL.

Standard Solutions Weigh accurately about 10 mg each of monosodium 4-amino-1-naphthalenesulfonate tetrahydrate, disodium 7-hydroxy-1,3-naphthalenedisulfonate, disodium 3-hydroxy-2,7-naphthalenedisulfonate, monosodium 6-hydroxy-2-

naphthalenesulfonate, and trisodium 7-hydroxy-1,3,6- naphthalenetrisulfonate, dried previously in a vacuum desiccator for 24 hours. Dissolve separately in ammonium acetate TS (0.02 mol/L) to prepare standard stock solutions of exactly 100 mL each. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Determine the amounts of these salts as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions), and calculate the total amount.

Operating Conditions

Wavelength: 238 nm.

Concentration gradient (A/B): Run a linear gradient from 100/0 to 40/60 in 30 minutes, and maintain at 40/60 for 5 minutes.

(7) Unsulfonated primary aromatic amines

Not more than 0.01% calculated as aniline.

Not more than 1.0 μg/g as 1-naphtylamine. (Coloring Matter Tests)

Loss on Drying Not more than 10.0% (135°C, 6 hours).

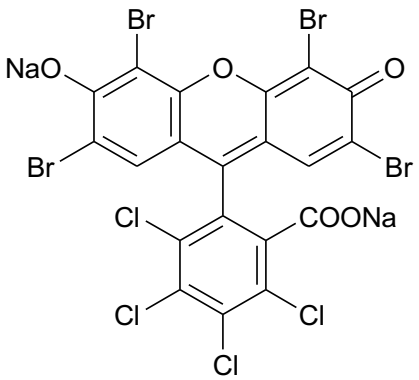
Assay Weigh accurately about 1.7 g of Food Red No. 102, and dissolve it in water to make 250 mL. Use exactly 50 mL of this solution as the test solution, and proceed as directed in Titanium(III) Chloride Method (i) in the Assay in the Coloring Matter Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 15.79 mg of $C_{20}H_{11}N_2Na_3O_{10}S_3 \cdot 1\frac{1}{2}H_2O$

Food Red No. 104

Phloxine

食用赤色 104 号



$C_{20}H_2Br_4Cl_4Na_2O_5$ Mol. Wt. 829.63

Disodium 3,4,5,6-tetrachloro-2-(2,4,5,7-tetrabromo-6-oxido-3-oxo-3*H*-xanthen-9-yl)-

benzoate [18472-87-2]

Definition Food Red No. 104 consists principally of disodium 3,4,5,6-tetrachloro-2-(2,4,5,7-tetrabromo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate.

Content Food Red No. 104 contains the equivalent of not less than 85.0% of disodium 3,4,5,6-tetrachloro-2-(2,4,5,7-tetrabromo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate ($C_{20}H_2Br_4Cl_4Na_2O_5$).

Description Food Red No. 104 occurs as a deep yellow-red to deep red powder or as granules. It is odorless.

Identification A solution of 0.1 g of Food Red No. 104 in 200 mL of ammonium acetate TS (0.02 mol/L) is brilliant yellowish red having a brilliant yellow-red fluorescence. To 1 mL of this solution, add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 536–540 nm.

pH 6.5–10.0 (1.0 g, water 100 mL).

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 5.0% as the sum (Coloring Matter Tests).

(3) Bromide Not more than 1.0% (Coloring Matter Tests).

(4) Lead Not more than 2 μg/g as Pb (Coloring Matter Tests, Method 2).

(5) Zinc Not more than 200 μg/g as Zn (Coloring Matter Tests, Zinc and Iron (1)).

(6) Arsenic Not more than 3 μg/g as As (Coloring Matter Tests).

(7) Subsidiary colors, unreacted raw materials and products of side reactions

Not more than 6%.

Test Solutions Weigh accurately about 0.1 g of Food Red No. 104, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL.

Procedure Analyze a certain amount of the test solution by liquid chromatography using the operating conditions given below. Refer to the area one thousandth of the peak of the main pigment in the test solution as A. Record the areas of the peaks appearing within 30 minutes after the injection of the test solution. Refer to the sum of areas that are larger than A as A_T. Deem the peaks other than the main pigment peak as subsidiary colors, unreacted raw materials, and products of side reactions, and refer to the sum of them as A_O. Determine the amount by the formula:

Amount (%) of subsidiary colors, unreacted raw materials, and products of side reactions

$$= \frac{A_O}{A_T} \times \text{content}$$

Operating Conditions

Detector: Ultraviolet spectrophotometer or photodiode array detector (wavelength: 254 nm).

Column: A stainless steel (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of around 40°C.

Concentration gradient (A/B): Run a linear gradient from 75/25 to 10/90 in 25 minutes, and maintain at 10/90 for 5 minutes.

Flow rate: 1 mL/ minute.

Measurement time: 0–30 minutes after the injection of the test solution.

(8) Hexachlorobenzene Not more than 5.0 μg/g.

Test Solution Weigh accurately about 20 mg of Food Red No. 104 into a 50-mL centrifuge tube, and dissolve it in 30 mL of water. Add exactly 10 m of hexane, and shake for 5 minutes. Transfer the hexane layer into a stoppered test tube, add 0.5 g of sodium sulfate to the hexane layer, and shake. Use the hexane layer as the test solution.

Standard Solutions Weigh accurately about 10 mg of hexachlorobenzene, and dissolve it in hexane to make exactly 100 mL. Measure exactly 5 mL of this solution, and add hexane to make exactly 100 mL. Measure exactly 1 mL of the second solution, and add hexane to make exactly 100 mL. Next, transfer exactly 1 mL, 1 mL, 2 mL, 3 mL, and 6 mL of the last solution into separate volumetric flasks. To each, add hexane to make exactly 50 mL, 10 mL, 10 mL, 10 mL, and 10 mL, respectively .

Procedure Analyze 1 μL each of the test solution and the standard solutions by gas chromatography using the conditions given below. Measure the peak areas of hexachlorobenzene for the standard solutions to prepare a calibration curve. Obtain the content of hexachlorobenzene in the test solution from the calibration curve and the peak area of hexachlorobenzene for the test solution.

Operating Conditions

Detector: Electron-capture detector.

Column: A fused silicate tube (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of 5% diphenyl/95% dimethyl polysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 60°C for 1 minute, thereafter raise to 280°C, and maintain at 280°C for 5 minutes. The temperature should be adjusted so that the peak of hexachlorobenzene is separated from the peaks of other components and appears 10–15 minutes after injection.

Injection port temperature: 260°C.

Detector temperature: 300°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the peak of hexachlorobenzene appears 10–15 minutes after injection.

Injection method: Splitless.

Loss on Drying Not more than 10.0% (135°C, 6 hours).

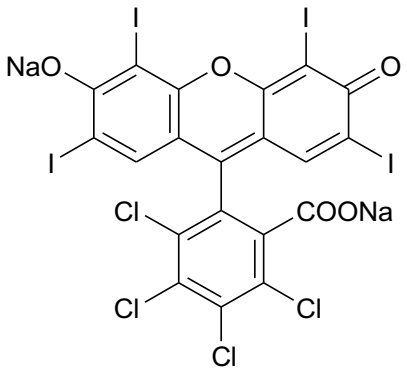
Assay Weigh accurately about 1 g of Food Red No. 104, and dissolve it in water to make exactly 100 mL. Use exactly 50 mL of this solution as the test solution, and proceed as directed in the Mass Method in the Assay in the Coloring Matter Tests.

$$\text{Content (\%) of Food Red No. 104 } (C_{20}H_2Br_4C_{14}Na_2O_5) = \frac{\text{Mass (g) of the precipitate} \times 2.112}{\text{Weight (g) of the sample}} \times 100$$

Food Red No. 105

Rose Bengal

食用赤色 105 号



$C_{20}H_2Cl_4I_4Na_2O_5$ Mol. Wt. 1017.64

Disodium 3,4,5,6-tetrachloro-2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)-benzoate [632-69-9]

Definition Food Red No. 105 consists principally of disodium 3,4,5,6-tetrachloro- 2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate.

Content Food Red No. 105 contains the equivalent of not less than 85.0% of disodium 3,4,5,6-tetrachloro-2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate ($C_{20}H_2Cl_4I_4Na_2O_5$).

Description Food Red No. 105 occurs as a very dark yellow-red to dark purplish red powder or as granules. It is odorless.

Identification A solution of 0.1 g of Food Red No. 105 in 200 mL of ammonium acetate

TS (0.02 mol/L) is brilliant yellowish red to red. To 1 mL of this solution, add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 546–550 nm.

pH 6.5–10.0 (1.0 g, water 100 mL).

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 5.0% as the sum (Coloring Matter Tests).

(3) Iodide Not more than 0.4% (Coloring Matter Tests).

(4) Lead Not more than 2 μg/g as Pb (Coloring Matter Tests, Method 1).

(5) Zinc Not more than 200 μg/g as Zn (Coloring Matter Tests, Zinc and Iron (1)).

(6) Arsenic Not more than 3 μg/g as As (Coloring Matter Tests).

(7) Subsidiary colors, unreacted raw materials and products of side reactions

Not more than 4.5%.

Test Solutions Weigh accurately about 0.1 g of Food Red No. 105, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL.

Procedure Analyze a certain amount of the test solution by liquid chromatography using the operating conditions given below. Refer to the area one thousandth of the peak of the main pigment in the test solution as A. Record the areas of the peaks appearing within 30 minutes after the injection of the test solution. Refer to the sum of areas that are larger than A as A_T. Deem the peaks other than the main pigment peak as subsidiary colors, unreacted raw materials, and products of side reactions, and refer to the sum of them as A_O. Determine the amount by the formula:

Amount
(%) of subsidiary colors, unreacted raw materials, and products of side reactions

$$= \frac{A_O}{A_T} \times \text{content}$$

Operating Conditions

Detector: Ultraviolet spectrophotometer or photodiode array detector (wavelength : 254 nm).

Column: A stainless steel (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of around 40°C.

Concentration gradient (A/B): Run a linear gradient from 75/25 to 10/90 in 25 minutes, and maintain at 10/90 for 5 minutes.

Flow rate: 1 mL/ minute.

Measurement time: 0–30 minutes after the injection of the test solution.

(8) Hexachlorobenzene Not more than 6.5 μg/g.

Proceed as directed in Purity (8) for Food Red No. 104.

Loss on Drying Not more than 10.0% (135°C, 6 hours).

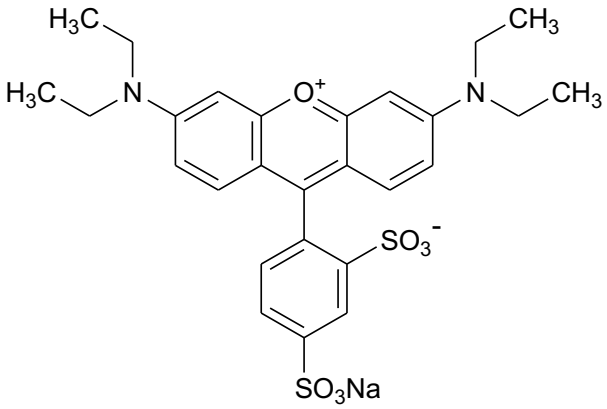
Assay Weigh accurately about 1 g of Food Red No. 105, and dissolve it in water to make exactly 100 mL. Use exactly 50 mL of this solution as the test solution, and proceed as directed in the Mass Method in the Assay in the Coloring Matter Tests.

$$\text{Content (\%) of Food Red No. 105 } (C_{20}H_2Cl_4I_4Na_2O_5) = \frac{\text{Mass (g) of the precipitate} \times 2.090}{\text{Weight (g) of the sample}} \times 100$$

Food Red No. 106

Acid Red

食用赤色 106 号



$C_{27}H_{29}N_2NaO_7S_2$ Mol. Wt. 580.65

Monosodium 6-[3,6-bis(diethylamino)xanthenium-9-yl]benzene-1,3-disulfonate [3520-42-1]

Definition Food Red No. 106 consists principally of monosodium 6-[3,6-bis(diethylamino)xanthenium-9-yl]benzene-1,3-disulfonate.

Content Food Red No. 106 contains the equivalent of not less than 85.0% of monosodium 6-[3,6-bis(diethylamino)xanthenium-9-yl]benzene-1,3-disulfonate ($C_{27}H_{29}N_2NaO_7S_2$).

Description Food Red No. 106 occurs as a dark yellow-red to dark yellowish red or very dark reddish purple to very dark red-purple powder or as granules. It is odorless.

Identification A solution of 0.1 g of Food Red No. 106 in 500 mL of ammonium acetate TS (0.02 mol/L) is deep red-purple. To 3 mL of this solution, add ammonium acetate TS (0.02 mol/L) to make 200 mL. The solution exhibits an absorption maximum at a wavelength of 564–568 nm.

pH 6.5–10.0 (1.0 g, water 100 mL).

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 5.0% as the sum (Coloring Matter Tests).

(3) Lead Not more than 2 μg/g as Pb (Coloring Matter Tests, Method 1).

(4) Manganese Not more than 50 μg/g as Mn (Coloring Matter Tests, Manganese and Chromium).

(5) Chromium Not more than 25 μg/g as Cr (Coloring Matter Tests, Manganese and Chromium).

Test Solution To 20 mL of the sample solution, add 10 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

Blank Test Solution Prepare the solution in the same manner as for the test solution without using the sample solution.

Control Solution To 4 mL of Chromium Standard Solution, add 10 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

Procedure Proceed as directed in the Coloring Matter Tests. The difference in absorbance between the test solution and the blank test solution is less than the absorbance of the control solution.

(6) Arsenic Not more than 3 μg/g as As (Coloring Matter Tests).

(7) Subsidiary colors, unreacted raw materials and products of side reactions

Not more than 10%.

Test Solutions Weigh accurately about 0.1 g of Food Red No. 106, add ammonium acetate TS (0.02 mol/L), and dissolve it by ultrasonic treatment if necessary. Add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL.

Procedure Analyze a certain amount of the test solution by liquid chromatography using the operating conditions given below. Refer to the area one thousandth of the peak of the main pigment in the test solution as A. Record the areas of the peaks appearing within 35 minutes after the injection of the test solution. Refer to the sum of areas that are larger than A as A_T. Deem the peaks other than the main pigment peak as subsidiary colors, unreacted raw materials, and products of side reactions, and refer to the sum of them as A_O. Determine the amount by the formula:

Amount
(%) of subsidiary colors, unreacted raw materials, and products of side reactions

$$= \frac{A_O}{A_T} \times \text{content}$$

Operating Conditions

Detector: Ultraviolet spectrophotometer or photodiode array detector (wavelength 254 nm).

Column: A stainless steel (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of around 40°C.

Concentration gradient (A/B): Run a linear gradient from 70/30 to 20//80 in 30 minutes, and maintain at 20/80 for 5 minutes.

Flow rate: 1 mL/ minute.

Measurement time: 0–35 minutes after the injection of the test solution.

Loss on Drying Not more than 10.0% (135°C, 6 hours).

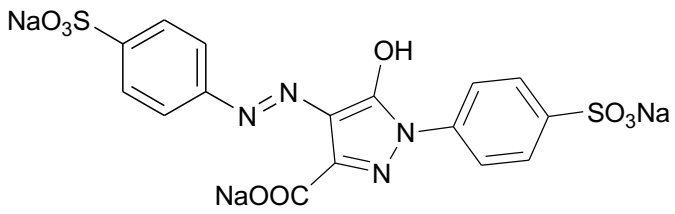
Assay Weigh accurately about 3 g of Food Red No. 106, and dissolve it in water to make exactly 250 mL. Use exactly 50 mL of this solution as the test solution, and proceed as directed in Titanium(III) Chloride Method (iv) in the Assay in the Coloring Matter Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 29.03 mg of $C_{27}H_{29}N_2NaO_7S_2$

Food Yellow No. 4

Tartrazine
FD&C Yellow No. 5

食用黄色 4 号



$C_{16}H_9N_4Na_3O_9S_2$ Mol. Wt. 534.36

Trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)diazenyl]-1*H*-pyrazole-3-carboxylate [1934-21-0]

Definition Food Yellow No. 4 is obtained by diazotizing 4-aminobenzenesulfonic acid, coupling the obtained diazo compound with 5-hydroxy-1-(4-sulfophenyl)-3-pyrazolecarboxylic acid, and then salting out and refining the resulting dye. It consists essentially of trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)diazenyl]- 1*H*-pyrazole-3-carboxylate.

Content Food Yellow No. 4 contains the equivalent of not less than 85.0% of trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)diazenyl]-1*H*-pyrazole-3-carboxylate ($C_{16}H_9N_4Na_3O_9S_2$).

Description Food Yellow No. 4 occurs as a brilliant reddish yellow to brilliant yellow-red powder or as granules. It is odorless.

Identification A solution of 0.1 g of Food Yellow No. 4 in 100 mL of ammonium acetate TS (0.02 mol/L) is brilliant yellow. To 1 mL of this solution, add ammonium acetate (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 426–430 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 6.0% as the sum (Coloring Matter Tests).

(3) Lead Not more than 2 μg/g as Pb (Coloring Matter Tests, Method 1).

(4) Arsenic Not more than 3 μg/g as As (Coloring Matter Tests).

(5) Subsidiary colors Not more than 1%.

Proceed as directed in the Coloring Matter Tests (Subsidiary Colors (2)) using the operating conditions given below.

Operating Conditions

Wavelength: 430 nm.

Concentration gradient (A/B): Run a linear gradient from 100/0 to 65/35 in 30 minutes, and maintain at 65/35 for 5 minutes.

Measurement time: 0–35 minutes after sample injection.

(6) Unreacted raw materials and products of side reactions

Not more than 0.5% as the sum of

4-aminobenzenesulfonic acid;

5-hydroxy-1-(4-sulfophenyl)-3-pyrazolecarboxylic acid;

4-hydrazinobenzenesulfonic acid; and

disodium 4,4′-(diazoamino)dibenzenesulfonate.

Test Solution Weigh accurately about 0.1 g of Food Yellow No. 4, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL.

Standard Solutions Weigh accurately about 10 mg each of 4-aminobenzenesulfonic

acid, 5-hydroxy-1-(4-sulfophenyl)-3-pyrazolecarboxylic acid, 4-hydrazinobenzenesulfonic acid, and disodium 4,4′-(diazoamino)dibenzenesulfonate, dried previously in a vacuum desiccator for 24 hours. Dissolve them separately in ammonium acetate TS (0.02 mol/L) to prepare standard stock solutions of exactly 100 mL each. The standard stock solutions of 4-hydrazinobenzenesulfonic acid and disodium 4,4′-(diazoamino)dibenzenesulfonate should be prepared fresh. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Determine the amounts of these substances in the test solution as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions), and calculate the total amount.

Operating Conditions

Wavelength: 238 nm.

Concentration gradient (A/B): Run a linear gradient from 100/0 to 65/35 in 30 minutes, and maintain at 65/35 for 5 minutes.

(7) Unsulfonated primary aromatic amines Not more than 0.01% calculated as aniline (Coloring Matter Tests).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 1.5 g of Food Yellow No. 4, and dissolve it in water to make exactly 250 mL. Use exactly 50 mL of this solution as the test solution, and proceed as directed in Titanium(III) Chloride Method (iii) in the Assay in the Coloring Matter Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 13.36 mg of $C_{16}H_9N_4Na_3O_9S_2$

Food Yellow No. 4 Aluminium Lake

(Food Yellow No. 4 Aluminum Lake)

Tartrazine Aluminum Lake

食用黄色 4 号アルミニウムレーキ

Definition Food Yellow No. 4 Aluminum Lake is prepared by adsorbing "Food Yellow No. 4" to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Yellow No. 4 Aluminum Lake contains the equivalent of not less than 10.0% of trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)diazenyl]-1*H*-pyrazole-3-carboxylate ($C_{16}H_9N_4Na_3O_9S_2$ = 534.36).

Description Food Yellow No. 4 Aluminum Lake occurs as a brilliant yellow to bright yellow-red, fine powder. It is odorless.

Identification

(1) To 0.1 g of Food Yellow No. 4 Aluminum Lake, add 5 mL of diluted sulfuric acid (1 in 20), stir well, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. If the solution is not clear, centrifuge. Measure 1 to 10 mL of this solution so that the absorbance is between 0.2 and 0.7, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 426–430 nm.

(2) To 0.2 g of Food Yellow No. 4 Aluminum Lake, add 20 mL of diluted hydrochloric acid (1 in 4), heat for 5 minutes in a water bath, shake well to dissolve the sample, add 1.0 g of active carbon, shake well, and filter. Adjust the pH of the colorless filtrate to 3–4 with sodium hydroxide solution (1 in 10). To confirm the pH value, use a pH paper. The solution responds to all the tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Lead Not more than 5 μg/g as Pb (Coloring Matter Aluminum Lake Tests).

(3) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(4) Arsenic Not more than 3 μg/g as As (Coloring Matter Aluminum Lake Tests).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

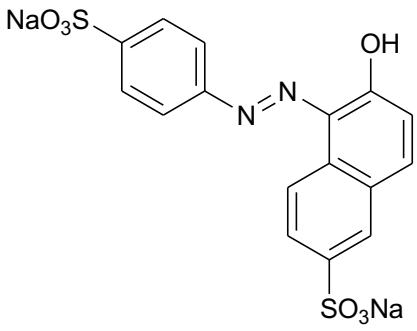
Assay Weigh accurately an amount of Food Yellow No. 4 Aluminum Lake so that the volume of 0.1 mol/L titanium(III) chloride consumed is about 20 mL, and proceed as directed in Assay (3) in the Coloring Matter Aluminum Lake Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 13.36 mg of $C_{16}H_9N_4Na_3O_9S_2$

Food Yellow No. 5

Sunset Yellow FCF
FD&C Yellow No. 6

食用黄色 5 号



$C_{16}H_{10}N_2Na_2O_7S_2$ Mol. Wt. 452.37

Disodium 6-hydroxy-5-[(4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate [2783-94-0]

Definition Food Yellow No. 5 is obtained by diazotizing 4-aminobenzenesulfonic acid, coupling the obtained diazo compound with 6-hydroxy-2-naphthalenesulfonic acid, and then salting out and refining the resulting dye. It consists principally of disodium 6-hydroxy-5-[(4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate .

Content Food Yellow No. 5 contains the equivalent of not less than 85.0% of disodium 6-hydroxy-5-[(4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate ($C_{16}H_{10}N_2Na_2O_7S_2$).

Description Food Yellow No. 5 occurs as a brilliant yellow-red to brilliant yellowish red or deep yellowish red to deep red powder or as granules. It is odorless.

Identification A solution of 0.1 g of Food Yellow No. 5 in 100 mL of ammonium acetate TS (0.02 mol/L) is brilliant yellow-red. To 1 mL of this solution, add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 480–484 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 5.0% as the sum (Coloring Matter Tests).

(3) Lead Not more than 2 μg/g as Pb (Coloring Matter Tests, Method 1).

(4) Arsenic Not more than 3 μg/g as As (Coloring Matter Tests).

(5) Subsidiary colors

Not more than 5% as the sum of sulfanilic acid azo R salt, sulfanilic acid azo G salt, sulfanilic acid azo β-naphthol, and aniline azo Schaeffer's salt.

Not more than 2% of colors other than sulfanilic acid azo R salt.

Test Solution Weigh accurately about 0.1 g of Food Yellow No. 5, dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL.

Standard Solutions Weigh accurately about 10 mg of each of sulfanilic acid azo R salt, sulfanilic acid azo G salt, and sulfanilic acid azo β-naphthol, all previously dried in a vacuumed desiccator for 24 hours. Dissolve them separately in ammonium acetate TS (0.02 mol/L) to prepare standard stock solutions of exactly 100 mL each. Proceed as directed in the Coloring Matter Tests (Subsidiary Colors (1)).

Procedure Determine the amounts of these colors in the test solution as directed in the Coloring Matter Tests (Subsidiary Colors (1)), and calculate the total amount. Aniline azo Schaeffer's salt and sulfanilic acid azo β-naphthol is both calculated as sulfanilic acid azo β-naphthol, because in the operating conditions, aniline azo Schaeffer's salt and sulfanilic acid azo β-naphthol cannot be separated.

Operating Conditions

Wavelength: 482 nm.

Concentration gradient (A/B): Maintain at 100/0 for 10 minutes, run a linear

gradient from 100/0 to 40/60 in 40 minutes, and maintain for 10 minutes.

(6) Unreacted raw materials and products of side reactions

Not more than 0.5% as the sum of

4-aminobenzenesulfonic acid;

disodium 7-hydroxy-1,3-naphthalenedisulfonate;

disodium 3-hydroxy-2,7-naphthalenedisulfonate;

monosodium 6-hydroxy-2-naphthalenesulfonat;

disodium 6,6′-oxybis(2-naphthalenesulfonate); and

disodium 4,4′-(diazoamino)dibenzenesulfonate.

Test Solution Weigh accurately about 0.1 g of Food Yellow No. 5, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL.

Standard Solutions Weigh accurately about 10 mg each of 4-aminobenzenesulfonic acid, disodium 7-hydroxy-1,3-naphthalenedisulfonate, disodium 3-hydroxy-2,7-naphthalenedisulfonate, monosodium 6-hydroxy-2- naphthalenesulfonate, disodium 6,6′-oxybis(2-naphthalenesulfonate), and disodium 4,4′-(diazoamino)dibenzenesulfonate, dried previously in a vacuum desiccator for 24 hours. Dissolve them separately in ammonium acetate TS (0.02 mol/L) to prepare standard stock solutions of exactly 100 mL each. The solution of disodium 4,4′-(diazoamino)dibenzenesulfonate should be prepared fresh. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Determine the amounts of these substances in the test solution as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions), and calculate the total amount.

Operating Conditions

Wavelength: 238 nm.

Concentration gradient (A/B): Maintain at 100/0 for 10 minutes, run a linear gradient from 100/0 to 40/60 in 40 minutes, and maintain for 10 minutes.

(7) 1-Phenylazo-2-naphthalenol (sudan-1) Not more than 1 μg/g.

Test Solution Weigh accurately about 0.5 g of Food Yellow No. 5 into a 50-mL centrifuge tube, add 10 mL of water, and dissolve it by ultrasonic treatment. To this solution, add 5 mL of acetonitrile, and mix well. Add 20 mL of ethyl acetate, shake for 1 minute, centrifuge at 3000 rpm for 1 minute, and collect the upper layer. To the remaining lower layer, add 20 mL of ethyl acetate, shake for 1 minute, centrifuge again, and collect the upper layer to combine both upper layers. Evaporate it to dryness under reduced pressure at 40°C. Dissolve the residue in a 7:3 mixture of acetonitrile/water to make exactly 2 mL. Filter it through a polytetrafluoroethylene membrane filter (0.45 μm pore size).

Standard Solutions Weigh accurately about 10 mg of 1-phenylazo-2-naphthalenol, previously dried under reduced pressure for 24 hours. Add acetonitrile, and dissolve the

sample completely by ultrasonic treatment to make exactly 100 mL. Measure exactly 1 mL of this solution, and add a 7:3 mixture of acetonitrile/water to make exactly 100 mL. Then, measure exactly graded amounts of the resulting solution, add a 7:3 mixture of acetonitrile/water to prepare several solutions containing graded concentrations of 1-phenylazo-2-naphthalenol in the range of 0.05–0.5 μg/mL.

Procedure Analyze 20 μL each of the standard solutions by liquid chromatography using the operation conditions given below. Measure the peak areas of them to prepare a calibration curve. Measure the peak area of 1-phenylazo-2-naphthalenol in the test solution to determine its amount from the calibration curve.

Operation conditions

Detector: Ultraviolet spectrophotometer or photodiode array detector (wavelength 485 nm).

Column: A stainless steel of 4.6 mm internal diameter and 15–25 cm length.

Column packing material: 5-μm octadecylsilanized silica gel.

Column temperature: A constant temperature of around 40°C.

Mobile phase: A 7:3 mixture of acetonitrile/water.

Flow rate: 1 mL/ minute.

(8) Unsulfonated primary aromatic amines Not more than 0.01% as aniline (Coloring Matter Tests).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 1.3 g of Food Yellow No. 5, and dissolve it in water to make exactly 250 mL. Measure exactly 50 mL of this solution, use as the test solution, and proceed as directed in Titanium(III) Chloride Method (i) in the Assay in the Coloring Matter Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 11.31 mg of $C_{16}H_{10}N_2Na_2O_7S_2$

Food Yellow No. 5 Aluminium Lake

(Food Yellow No. 5 Aluminum Lake)

Sunset Yellow FCF Aluminum Lake

食用黄色 5 号アルミニウムレーキ

Definition Food Yellow No. 5 Aluminum Lake is prepared by adsorbing "Food Yellow No. 5" to a solution of aluminum salt that was reacted with alkali. Following lake formulation, the product is filtered, dried, and crushed.

Content Food Yellow No. 5 Aluminum Lake contains the equivalent of not less than 10.0% of disodium 6-hydroxy-5-[(4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate

($C_{16}H_{10}N_2Na_2O_7S_2$ = 452.37).

Description Food Yellow No. 5 Aluminum Lake occurs as a brilliant yellow-red to brilliant yellowish red or deep yellowish red to deep red, fine powder. It is odorless.

Identification

(1) To 0.1 g of Food Yellow No. 5 Aluminum Lake, add 5 mL of diluted sulfuric acid (1 in 20), stir well, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. If the solution is not clear, centrifuge. Measure 1 to 10 mL of this solution so that the absorbance to be measured is in the range of 0.2 to 0.7, and add ammonium acetate TS (0.02 mol/L) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 480–484 nm.

(2) To 0.2 g of Food Yellow No. 5 Aluminum Lake, add 20 mL of diluted hydrochloric acid (1 in 4), heat for 5 minutes in a water bath, and shake well to dissolve the sample, add 1.0 g of active carbon, shake well, and filter. Adjust the pH of the colorless filtrate to 3–4 with sodium hydroxide solution (1 in 10). To confirm the pH value, use a pH paper. The solution responds to all the tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Lead Not more than 5 μg/g as Pb (Coloring Matter Aluminum Lake Tests).

(3) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(4) Arsenic Not more than 3 μg/g as As (Coloring Matter Aluminum Lake Tests).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

Assay Weigh accurately an amount of Food Yellow No. 5 Aluminum Lake so that the volume of 0.1 mol/L titanium(III) chloride consumed is about 20 mL, and proceed as directed in Assay (1) in the Coloring Matter Aluminum Lake Tests.

Each mL of 0.1 mol/L titanium(III) chloride = 11.31 mg of $C_{16}H_{10}N_2Na_2O_7S_2$

Fructosyl Transferase

フルクトシルトランスフェラーゼ

Definition Fructosyl Transferase includes enzymes that transfer the fructosyl group of sugars. It is derived from the culture of filamentous fungi (limited to *Penicillium roqueforti* and species of the genera *Aspergillus* and *Aureobasidium*) or bacteria (limited to *Microbacterium saccharophilum*, *Zymomonas mobilis*, and species of the genera *Arthrobacter* and *Bacillus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Fructosyl Transferase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Fructosyl Transferase complies with the Fructosyl Transferase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

The requirement of total plate count can be disregarded if unsterilized Fructosyl Transferase is used for captive consumption in the manufacture of food that will be sterilized or pasteurized before the completion of the final product.

Fructosyl Transferase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Fructosyl Transferase, add water or sodium phosphate buffer (0.1 mol/L) at pH 6.5 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 40 g of xylose, and dissolve it in 50 mL of sodium phosphate buffer (0.1 mol/L) at pH 6.5 by warmingat 40°C. After cooling, adjust the pH to 6.5 with hydrochloric acid TS (1 mol/L) or sodium hydroxide TS (1 mol/L), add 20 g of sucrose, and warm at 40°C to dissolve it. After cooling, adjust the pH to 6.5 with hydrochloric acid TS (1 mol/L) or sodium hydroxide TS (1 mol/L), and add water to make 100 mL. If solid matter is observed, filter the solution through a filter paper.

Test Solution Equilibrate 0.2 mL of the sample solution at 40°C for 2 minutes, add 0.2 mL of the substrate solution, equilibrated at 40°C, and mix. Incubate the mixture at

40°C for 10 minutes. Add 0.1 mL of this solution to 1.9 mL of water, previously heated in a water bath for about 10 minutes, heat the mixture in a water bath for 20 minutes, and allow to cool to room temperature. To 0.04 mL of this solution, add 1.168 mL of TS for D-glucose and D-fructose determination, mix, and allow to stand at room temperature for 10–15 minutes.

Control Solution To 1.9 mL of water, add 0.05 mL of the sample solution, heat the mixture in a water bath for 10 minutes, add 0.05 mL of the substrate solution, heat in a water bath for 20 minutes, and allow to cool to room temperature. To 0.04 mL of this solution, add 1.168 mL of TS for D-glucose and D-fructose determination, mix, and allow to stand at room temperature for 10–15 minutes.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 340 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 1.0 g of Fructosyl Transferase, add water or citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.5 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold or 100-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 10 g of inulin (a product derived from dahlia or chicory), and dissolve it in water by warming. After cooling, make 100 mL.

Test Solution To 0.5 mL of the substrate solution, add 0.45 mL of citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.5, mix, and equilibrate the mixture at 60°C for 10 minutes. Add 0.05 mL of the sample solution, mix, and incubate the mixture at 60°C for 10 minutes. Heat this solution in a water bath for 5 minutes, and filter through a membrane filter (0.45 μm pore size). Use the filtrate as the test solution.

Control Solution Proceed as directed for the test solution using water or citric acid–sodium hydroxide buffer (0.1 mol/L) at pH 5.5 instead of the sample solution.

Standard Solution Dissolve 0.5 g of α-D-fructfranose β-D-fructofuranose 1,2′: 2,3′ dianhydride in water to make 100 mL. Filter this solution through a membrane filter (0.45 μm pore size), and use the filtrate.

Procedure Analyze 5 μL each of the test solution, the control solution, and the standard solution by liquid chromatography using the operating condition given below. A peak is observed at the retention time of α-D-fructfranose β-D-fructofuranose 1,2′: 2,3′ dianhydride for the test solution. The peak area is larger than that of the peak corresponding to the retention time of α-D-fructfranose β-D-fructofuranose 1,2′: 2,3′ dianhydride in the control solution.

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (4–8 mm internal diameter and 25–35 cm length).

Column packing material: About 6-μm Na-form cation exchange resin for liquid chromatography.

Column temperature: A constant temperature of 60–80°C.

Mobile phase: Water

Flow rate: 0.5–1.2 mL/min. Adjust the retention time of α-D-fructfranose β-D-fructofuranose 1,2′: 2,3′ dianhydride to about 7 minutes.

Method 3

Sample Solution Weigh 1.0 g of Fructosyl Transferase, and add water or McIlvain buffer to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 25.0 g of sucrose, and dissolve it in water to make 100 mL.

Test Solution To 2.0 mL of McIlvain buffer (0.1 mol/L) at pH5.0, add 1.0 mL of the sample solution, mix, and equilibrate the mixture at 40°C for 2 minutes. To this solution, add 2.0 mL of the substrate solution, equilibrated at 40°C. Shake the mixture for 1 hour (30 times reciprocation/minute) while incubating at 40°C, and immediately heat in a water bath for 10 minutes. After cooling, filter this solution through a membrane filter (pore size of 0.45 μm), and use the filtrate as the test solution.

Control Solution Proceed as directed for the test solution using water or McIlvain buffer (0.1 mol/L) at pH5.0 instead of the sample solution.

Standard Solution Dissolve 0.40 g of 1-kestose in water to make 20 mL.

Procedure Analyze 10 μL each of the test solution, the control solution, and the standard solution by liquid chromatography using the operating condition given below. A peak is observed at the retention time of 1-kestose for the test solution. The peak area is larger than that of the peak corresponding to the retention time of 1-kestose in the control solution.

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (4 mm internal diameter and 25 cm length).

Column packing material: About 5-μm aminopropyl-bonded silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 7:3 mixture of acetonitrile/water.

Flow rate: 1.0 mL/min.

Fukuronori Extract

フクロノリ抽出物

Definition Fukuronori Extract is obtained from the whole *fukurofunori* algae, *Gloiopeltis furcata*, and consists mainly of polysaccharides. It may contain sucrose, glucose, lactose, dextrin, or maltose.

Description Fukuronori Extract occurs as a white to brown powder or as granules. It has little or no odor.

Identification

(1) To 200 mL of water, add 4 g of Fukuronori Extract, and keep at about 80°C in a water bath while stirring until a homogenous viscous liquid is formed. Replenish the lost water, and cool to room temperature. It remains viscous.

(2) To 50 mL of the viscous solution obtained in Identification (1), add 0.2 g of potassium chloride, warm again, stir well, and cool to room temperature. The solution remains viscous.

(3) To 20 mL of water, add 0.1 g of Fukuronori Extract, add 3 mL of a solution of barium chloride dihydrate (3 in 25) and 5 mL of diluted hydrochloric acid (2 in 5), and mix well. If necessary, remove any precipitate that has been produced. Boil the mixture for 10 minutes. A white crystalline precipitate is formed.

Viscosity Not less than 5.0 mPa·s (1.5%, 75°C).

To 450 mL of water, add an amount equivalent to 7.5 g of Fukuronori Extract on the dried basis, and disperse it by stirring for 10–20 minutes. Add water to make 500 g of a dispersion, and heat to 80°C while stirring constantly in a water bath. Replenish the water lost by evaporation, and measure the viscosity at 75°C as directed in Method 2 of Viscosity in the General Tests. In measurement, first attach rotor No. 1 and the adaptor, heated to about 75°C, to the viscometer. Immerse the rotor into the dispersed sample up to a specified level. Start the measurement at 60 rounds per minute, and take the reading in 60 seconds. If the viscosity is too low, use an adaptor for low viscosity, and if it is too high, use rotor No. 2.

Purity

(1) Sulfuric group 5–30%.

Proceed as directed in Purity (3) for Semirefined Carrageenan.

(2) Acid-insoluble matter Not more than 2.0%.

Proceed as directed in Purity (4) for Semirefined Carrageenan.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 12.0% (105°C, 5 hours).

Ash 5–30% (on the dried basis).

Acid-insoluble Ash Not more than 1.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 10,000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

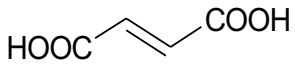
Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 2 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture Prepare as directed in Method 2 for the *Escherichia coli* test. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 5 g of Fukuronori Extract with 500 mL of lactose broth to disperse completely, and incubate it at 35 ± 1°C for 24 ± 2 hours.

Fumaric Acid

フマル酸



$C_4H_4O_4$ Mol. Wt. 116.07

(2*E*)-But-2-enedioic acid [110-17-8]

Content Fumaric Acid contains not less than 99.0% of fumaric acid ($C_4H_4O_4$).

Description Fumaric Acid occurs as a white crystalline powder. It is odorless and has a characteristic acid taste.

Identification

(1) Heat Fumaric Acid. It sublimates.

(2) Dry Fumaric Acid at 105°C for 3 hours. The melting point is 287–302°C (in sealed tube, decomposition).

(3) To 0.5 g of Fumaric Acid, add 10 mL of water, dissolve it by boiling, and add 2–3 drops of bromine TS while hot. The color of the solution disappears.

(4) Place 50 mg of Fumaric Acid into a test tube, add 2–3 mg of resorcinol and 1 mL of sulfuric acid, and shake. Heat at 120–130°C for 5 minutes, cool, and add water to make 5 mL. To this solution, add sodium hydroxide solution (3 in 10) dropwise while cooling to

make alkaline, and add water to make 10 mL. A green-blue fluorescence appears under ultraviolet light.

Purity

(1) Clarity of solution Colorless and clear (0.50 g, sodium hydroxide solution (1 in 25) 10 mL).

(2) Sulfate Not more than 0.010% as SO_4.

Sample Solution Weigh 1.0 g of Fumaric Acid, add 30 mL of water, and shake. Add 1 drop of phenolphthalein TS, then add ammonia TS dropwise until the color of the solution changes to a slightly pink color.

Control Solution 0.20 mL of 0.005 mol/L sulfuric acid.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Fumaric Acid, add 10 mL of water, dissolve it by heating, and cool.

Procedure Use 10 mL of tin(II) chloride TS (acidic) and 3 g of zinc for arsenic analysis.

Residue on Ignition Not more than 0.05% (5 g).

Assay Weigh accurately about 1 g of Fumaric Acid, and dissolve it in water to make exactly 250 mL. Measure exactly 25 mL of this solution, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide = 5.804 mg of $C_4H_4O_4$

α-Galactosidase

α-ガラクトシダーゼ

Definition α-Galactosidase includes enzymes that hydrolyze α-D-galactosidic linkages in the non-reducing terminals of saccharides. It is derived from the culture of filamentous fungi (limited to *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus niger*, *Aspergillus phoenicis*, and species of the genus *Mortierella*) or bacteria (limited to *Bacillus stearothermophilus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description α-Galactosidase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification α-Galactosidase complies with the α-Galactosidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

α-Galactosidase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of α-Galactosidase, add water to dissolve it or disperse it uniformly, and make 250 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 1.0 g of melibiose, dissolve it in acetic acid–sodium hydroxide buffer (0.05 mol/L) at pH5.0, and make 100 mL. Prepare fresh before use.

Test Solution Equilibrate 0.5 mL of the substrate solution at 40°C for 5 minutes, add 0.5 mL of the sample solution, and immediately shake. Incubate the mixture at 40°C for 30 minutes. Heat it in a water bath for 10 minutes, and cool to room temperature with running water. To this solution, add 6 mL of TS for D-glucose determination (containing mutarotase), shake well, and warm at 40°C for 5 minutes.

Control Solution To 0.5 mL of the substrate solution, add 0.5 mL of the sample solution, and immediately shake well. Then immediately heat the mixture in a water bath for 10 minutes, and cool it to room temperature with running water. Proceed as directed for the test solution.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 505 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 0.50 g of α-Galactosidase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, 10,000-fold, or 100,000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 0.21 g of *p*-nitrophenyl-α-D-galactopyranoside, dissolve it in acetic acid–sodium hydroxide buffer (0.05 mol/L) at pH5.5, and make 100 mL. Prepare fresh before use.

Test Solution Equilibrate 2 mL of the substrate solution at 37°C for 5 minutes, add 1 mL of the sample solution, and shake immediately. Incubate the mixture at 37°C for 15 minutes. To this solution, add 5 mL of sodium carbonate solution (11 in 1000), and mix immediately.

Control Solution To 2 mL of the substrate solution, add 5 mL of sodium carbonate solution (11 in 1000), and immediately shake well. Add 1 mL of the sample solution, and mix.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 405 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

β-Galactosidase

β-ガラクトシダーゼ

Definition β-Galactosidase includes enzymes that hydrolyze β-D-galactosidic linkages. It is derived from animal organs or the culture of filamentous fungi (limited to *Aspergillus niger*, *Aspergillus oryzae*, *Penicillium multicolor*, and *Rhizopus oryzae*), yeasts (*Cryptococcus laurentii, Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Sporobolomyces singularis*, and species of the genus *Saccharomyces*), or bacteria (limited to *Bacillus circulans* and species of the genus *Streptococcus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description β-Galactosidase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification β-Galactosidase complies with the β-Galactosidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic

Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 fort the *Escherichia coli* test and Method 2 for the *Salmonella* test.

The requirement of total plate count can be disregarded if unsterilized β-Galactosidase is used for captive consumption in the manufacture of food that will be sterilized or pasteurized before completion of the final product.

β-Galactosidase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of β-Galactosidase, add acetate buffer (0.1 mol/L, pH 6.0, containing polyoxyethylene (10) octylphenyl ether and sodium chloride) to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Weigh 12.63 g of lactose monohydrate, and dissolve it in 80 mL of water while heating in a water bath. Cool the solution with running water, add 10 mL of acetate buffer (1 mol/L) at pH 6.0 and water to make 100 mL. Prepare fresh before use.

Test Solution Equilibrate 5 mL of the substrate solution at 40°C for 10 minutes, add 1 mL of the sample solution, shake immediately. Incubate the mixture at 40°C for 10 minutes. Add 1 mL of sodium hydroxide solution (43 in 500), and immediately mix. Warm this solution at 40°C for 5 minutes. Cool it in icy water, add 1 mL of diluted hydrochloric acid (9 in 50), shake, and cool the mixture in icy water. To 0.1 mL of this solution, add 3 mL of TS for D-glucose determination (containing mutarotase), mix, and warm the mixture at 40°C for 20 minutes.

Control Solution To 5 mL of the substrate solution, add 1 mL of sodium hydroxide solution (43 in 500), and shake. Equilibrate the mixture at 40°C for 10 minutes, and add 1 mL of the sample solution, and shake immediately. Warm this solution at 40°C for 5 minutes. Proceed as directed for the test solution.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 505 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 0.14 g of β-Galactosidase, add potassium phosphate buffer (pH 6.5, containing magnesium sulfate–disodium dihydrogen ethylenediaminetetraacetate) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Weigh 0.25 g of *o*-nitrophenyl-β-D-galactopyranoside, dissolve it in potassium phosphate buffer (pH 6.5, containing magnesium sulfate–disodium dihydrogen ethylenediaminetetraacetate), and make 100 mL. Prepare fresh before use.

Test Solution To 1 mL of the sample solution, equilibrated at 30°C for 5–15 minutes, add 5 mL of the substrate solution, equilibrated at 30°C, and mix. Incubate the mixture at 30°C for 10 minutes. To this solution, add 2 mL of sodium carbonate–disodium dihydrogen ethylenediaminetetraacetate TS.

Control Solution To 1 mL of the sample solution, equilibrated at 30°C for 5–15 minutes, add 2 mL of sodium carbonate–disodium dihydrogen ethylenediaminetetraacetate TS and 5 mL of the substrate solution, and mix.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 420 nm within 30 minutes after preparation of them. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 3

Sample Solution Weigh 1.0 g of β-Galactosidase, add water to dissolve it or disperse it uniformly, and make 250 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000 dilution by adding the same buffer to the resulting solution.

Substrate Solution Weigh 0.37 g of *o*-nitophenyl-β-D-galactopyranoside, dissolve it in acetic acid–sodium hydroxide buffer (0.1 mol/L) at pH 4.5, and make 100 mL. Prepare fresh before use.

Test Solution Equilibrate 2 mL of the substrate solution at 37°C for 10 minutes, add 0.5 mL of the sample solution, and shake immediately. Incubate the mixture at 37°C for 15 minutes. Add 2.5 mL of sodium carbonate solution (1 in 10), shake immediately, and add 20 mL of water.

Control Solution Proceed as directed for the test solution, using water instead of the sample solution.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 420 nm within 15 minutes after preparation of them. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Gardenia Blue

クチナシ青色素

Definition Gardenia Blue is obtained from the fruits of *Gardenia jasminoides* J. Ellis (*Gardenia augusta Merr.*). It is produced by adding β-glucosidase to a mixture of iridoid glycosides from gardenia fruits and protein degradation products. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Gardenia Blue is not less than 50 and is in the range of 90–110% of the labeled value.

Description Gardenia Blue occurs as a dark purple to blue powder, as lumps, or as a paste or liquid. It has a slight characteristic odor.

Identification

(1) Weigh an amount of Gardenia Blue equivalent to 0.2 g of gardenia blue with a Color Value 50, and dissolve it in 100 mL of citrate buffer (pH 7.0). A blue to blue-purple color develops.

(2) A solution of Gardenia Blue in citrate buffer (pH 7.0) exhibits an absorption maximum at a wavelength of 570–610 nm.

(3) Weigh an amount of Gardenia Blue equivalent to 0.2 g of gardenia blue with a Color Value 50, and add water to make 100 mL. To 5 mL of this solution, add 1 to 2 drops of hydrochloric acid, and then add 1 to 3 drops of sodium hypochlorite TS. The solution is immediately decolorized.

(4) Weigh an amount of Gardenia Blue equivalent to 0.2 g of gardenia blue with a Color Value 50, and add water to make 100 mL. To 5 mL of this solution, add 5 mL of sodium hydroxide solution (1 in 25), and heat at 40–43°C for 20 minutes. No definite color change is observed.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Methanol Not more than 0.10% (on the basis of a Color Value 50).

Test Solution Weigh exactly an amount of Gardenia Blue equivalent to 1.00 g of gardenia blue with a Color Value 50, into a 10-mL volumetric flask, dissolve it in water, and add exactly 2 mL of the internal standard solution and water to volume. Use this solution as the sample solution. Prepare a 500-mg graphite carbon cartridge by pouring 4 mL of ethanol (95) and 10 mL of water and discarding the effluent. To the cartridge, pour exactly 1 mL of the sample solution, and collect the effluent in a 5-mL volumetric

flask. Pour water into the cartridge at a flow rate that does not allow the blue color to elute until the total volume of effluent reaches 5 mL.

Control Solution Weigh 0.50 g of methanol into a 100-mL volumetric flask, and add water to volume. Measure exactly 10 mL of this solution into a 100-mL volumetric flask, and add water to make 100 mL. Then measure exactly 2 mL of the second solution into a 50-mL volumetric flask, add exactly 2 mL of the internal standard solution, and add water to volume.

Internal Standard Solution Weigh 0.50 g of 2-propanol into a 100-mL volumetric flask, and add water to volume. Measure exactly 10 mL of this solution into a 100-mL volumetric flask, and add water to volume.

Procedure Analyze 2.0 μL each of the test solution and the control solution by gas chromatography using the operating conditions given below. The peak area ratio of methanol to 2-propanol for the test solution is not larger than that for the control solution.

Operating Conditions

Detector: Ionization detector.

Column: A glass or stainless steel tube (3–4 mm internal diameter and 1–2 m length).

Column packing material: 180- to 250-μm styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Injection port temperature: 160–200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention time of methanol to 2–4 minutes.

Color Value Determination Proceed as directed under Color Value Determination, according to the following operating conditions:

Operating Conditions

Solvent: Citrate buffer (pH 7.0).

Wavelength: Maximum absorption wavelength of 570–610 nm.

Gardenia Red

クチナシ赤色素

Definition Gardenia Red is obtained from the fruits of *Gardenia jasminoides* J. Ellis *(Gardenia augusta* Merr.). It is produced by adding β-glucosidase to a mixture of ester-hydrolysis products of iridoid glycosides from gardenia fruits and protein degradation

products. It may contain dextrin or lactose.

Color Value The color value ($E_{1cm}^{10\%}$) of Gardenia Red is not less than 50 and is in the range of 90–110% of the labeled value.

Description Gardenia Red occurs as a dark red-purple to red powder, as lumps, or as a paste or liquid. It has a slight characteristic odor.

Identification

(1) Weigh an amount of Gardenia Red equivalent to 0.2 g of gardenia red with a Color Value 50, and dissolve it in 100 mL of acetate buffer (pH 4.0). A red to purple-red color develops.

(2) A solution of Gardenia Red in acetate buffer (pH 4.0) exhibits an absorption maximum at a wavelength of 520–545 nm.

(3) Weigh an amount of Gardenia Red equivalent to 0.2 g of gardenia red with a Color Value 50, and add water to make 100 mL. To 5 mL of this solution, add 1 to 2 drops of hydrochloric acid and 1 to 3 drops of sodium hypochlorite TS. The solution is immediately decolorized.

(4) Weigh an amount of Gardenia Red equivalent to 0.2 g of gardenia red with a Color Value 50, add water to make 100 mL, and use the solution obtained as the test solution. To 5 mL of the test solution, add 5 mL of sodium hydroxide solution (1 in 25) to make it alkaline. The solution may become turbid but no definite color change is observed. To 5 mL of the test solution, add 1 to 3 drops of hydrochloric acid. The solution may become turbid, but no definite color change is observed.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination, using the following operating conditions.

Operating Conditions

Solvent: Acetate buffer (pH 4.0).

Wavelength: Maximum absorption wavelength of 520–545 nm.

Gardenia Yellow

クチナシ黄色素

Definition Gardenia Yellow is obtained from the fruits of *Gardenia jasminoides* J. Ellis

(*Gardenia augusta* Merr.) and consists mainly of crocin and crocetin. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Gardenia Yellow is not less than 100 and is in the range of 90–120% of the labeled value.

Description Gardenia Yellow occurs as a yellow to dark red powder, as lumps, or as a paste or liquid. It has a slight characteristic odor.

Identification

(1) Weigh an amount of Gardenia Yellow equivalent to 0.1 g of gardenia yellow with a Color Value 100, and add 100 mL of sodium hydroxide TS (0.02 mol/L). A yellow color develops.

(2) Weigh an amount of Gardenia Yellow equivalent to 0.1 g of gardenia yellow with a Color Value 100, and add 100 mL of sodium hydroxide TS (0.02 mol/L). Dissolve it with constant shaking while warming in a water bath at 50°C for 20 minutes. The resulting solution exhibits an absorption maximum at a wavelength of 410–425 nm.

(3) Weigh an amount of Gardenia Yellow equivalent to 0.1 g of gardenia yellow with a Color Value 100, and if necessary, evaporate to dryness on a water bath. Cool, and add 5 mL of sulfuric acid. A blue color develops, which changes through purple to brown.

(4) Weigh an amount of Gardenia Yellow equivalent to 1 g of gardenia yellow with a Color Value 100. Add 100 mL of sodium hydroxide TS (0.02 mL/L), warm in a water bath at 50°C for 20 minutes, and shake if necessary to dissolve. Use this solution as the test solution. Analyze a 5-μL portion of the test solution by thin-layer chromatography using an 8:7:7 mixture of tetrahydrofuran/acetonitrile/a solution of oxalic acid dihydrate (1 in 80) as the developing solvent. No control solution is used. Use a thin-layer plate coated with octadecylsilanized silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, air-dry the plate, and examine. A yellow spot is observed at an R_f value of about 0.4–0.6.

Purity

(1) Lead Not more than 8 μg/g as Pb (0.50 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method)

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Geniposide Not more than 0.5% (in terms of a Color Value 100).

Test Solution Weigh an amount of Gardenia Yellow equivalent to 1.0 g of gardenia yellow with a Color Value 100, and add a 17:3 mixture of water/acetonitrile to make exactly 25 mL. Centrifuge if necessary, and use the supernatant as the test solution.

Standard Solutions Weigh accurately about 10 mg of geniposide for assay, previously

dried for 24 hours in a desiccator, and dissolve it in a 17:3 mixture of water/acetonitrile to make exactly 100 mL. Transfer exactly 1 mL, 5 mL, and 10 mL of this solution into separate 100-mL volumetric flasks, and dilute each with a 17:3 mixture of water/acetonitrile to volume.

Procedure Analyze 10 μL each of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Measure the peak areas of geniposide for the standard solutions, and prepare a calibration curve. Determine the concentration (μg/mL) of geniposide in the test solution from the calibration curve and the peak area of geniposide for the test solution. Calculate the amount of geniposide by the formula:

Amount (%) of geniposide in terms of Color Value 100

= concentration (μg/mL) of geniposide in the test solution × 0.0025

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 238 nm).

Column: A stainless steel tube (4–5 mm internal diameter and 15–30 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 17:3 mixture of water/acetonitrile.

Flow rate: Adjust the retention time of geniposide to about 15 minutes.

Color Value Determination Weigh accurately an amount of Gardenia Yellow equivalent to about 5 g of gardenia yellow with a Color Value 100, add 50 mL of sodium hydroxide TS (0.02 mol/L), and warm in a water bath at 50°C for 20 minutes to dissolve while shaking if necessary. To this solution, add water to make exactly 100 mL. Measure exactly 1 mL of this solution, add 50% (vol) ethanol to make exactly 100 mL, and use this as the test solution. If necessary, centrifuge the test solution, and use the supernatant for the test. Measure the absorbance (A) in a 1-cm cell at the maximum at 410 nm–425 nm. Use 50% (vol) ethanol as the reference solution. Calculate the Color Value by the formula:

$$\text{Color Value} = \frac{A \times 1000}{\text{Weight (g) of the sample}}$$

Gellan Gum

ジェランガム

[71010-52-1]

Definition Gellan Gum is obtained from the culture fluid of *Sphingomonas elodea* and consists mainly of polysaccharides.

Content Gellan Gum, when dried, contains 85.0–108.0 % of gellan gum .

Description Gellan Gum occurs as a white to brownish powder having a slight, particular odor.

Identification

(1) When dissolved in water, Gellan Gum forms a viscous liquid.

(2) To 1 g of Gellan Gum, add 100 mL of water, and stir for 2 hours. Pipet a small amount of the solution into a solution of calcium chloride dihydrate (1 in 10). Immediately a linear gel is formed.

(3) To 90 mL of the solution obtained in Identification (2), add 0.50 g of sodium chloride. Heat the solution to 80°C while stirring, and allow for 1 minutes. Cool to room temperature without stir. A gel is formed.

Purity

(1) Total Nitrogen Not more than 3%.

Weigh accurately about 1 g of Gellan Gum, and proceed as directed in the Kjeldahl Method under Nitrogen Determination.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) 2-Propanol Not more than 0.075%.

(i) Apparatus Use the apparatus illustrated in Purity (7) for Semirefined Carrageenan.

(ii) Method

Test Solution Weigh accurately about 2 g of Gellan Gum in eggplant-shaped flask A, add 200 mL of water, a few boiling chips, and about 1 mL of silicon resin, and stir well. Place exactly 4 mL of the internal standard solution in volumetric flack E, and moisten the joint parts with water, and set the apparatus. Distill it at a rate of 2 to 3 mL/minute, controlling the heat so that bubbles do not come in delivery tube C, and collect about 90 mL of distillate. To the distillate, add water to make exactly 100 mL. Use 2-methyl-2-propanol solution (1 in 1000) as the internal standard solution.

Standard Solution Weigh accurately about 0.5 g of 2-propanol, and add water to

make exactly 50 mL. Measure exactly 5 mL of this solution, and add water to make exactly 50 mL. Then measure exactly 3 mL of the second solution and 8 mL of the internal standard solution in a volumetric flask, and add water to make exactly 200 mL.

Procedure Analyze 2.0 μL each of the test solution and the standard solution by gas chromatography using the operating conditions below. Obtain the peak area ratios (Q_T and Q_S) of 2-propanol to 2-methyl-2-propanol for the test solution and the standard solution, and calculate the amount by the formula:

$$\text{Amount (\%) of 2-propanol} = \frac{\text{Weight (g) of 2-propanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 0.3$$

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube (3 mm internal diameter and 2 m length).

Column packing material: 180- to 250-μm styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Injection port temperature: A constant temperature of about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention time of 2-propanol to about 10 minutes.

Loss on Drying Not more than 15.0% (105°C, 2.5 hours).

Ash Not more than 16.0% (on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 10,000 per gram.

Yeasts and molds: Not more than 400 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid For total plate count and the enumeration of yeasts and molds prepare as follows: Mix 1 g of Gellan Gum with 200 mL of phosphate buffer, 0.1% peptone solution, or sodium chloride-peptone buffer to disperse uniformly.

Pre-enrichment Culture For the *Escherichia coli* test, prepare as follows: Mix 1 g of Gellan Gum with 200 mL of lauryl sulfate broth to disperse uniformly, and incubate at 35 ± 1°C for 48 ± 2 hours. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 1 g of Gellan Gum with 200 mL of lactose broth to disperse uniformly, and incubate at 35 ± 1°C for 24 ± 2 hours.

If the sample is not dispersed uniformly in the preparation of the sample fluid or pre-

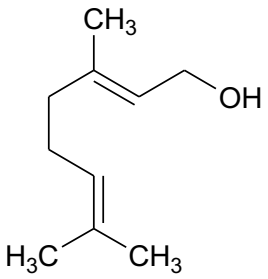
enrichment culture, use 500 mL of the specified diluent or medium. In the enumeration of yeasts and molds, the volume of the sample solution to be dispensed to each plate should be 2 mL. In the *Salmonella* test, prepare five pre-enrichment cultures according to this condition, and perform the test for each culture.

Assay Weigh about 1.0 g of diatomaceous earth for chromatography into a glass filter (1G3), and spread uniformly. Dry the glass with the diatomaceous earth at 105°C for 5 hours, allow to cool in a desiccator, and weigh accurately. Weigh accurately about 0.2 g of dried Gellan Gum, add 50 mL of water, and dissolve it while stirring in a water bath. Add 200 mL of 2-propanol, previously warmed at 60–70°C, mix well, and allow to stand overnight. Wash down the produced precipitate with 78% (vol) 2-propanol into the glass filter, and filter. Wash the residue 3 times with 20 mL of 78% (vol) 2-peopanol each time, and twice with 10 mL of 78% (vol) 2-propanol each time. Dry the glass filter with residue at 105°C over night, and weigh accurately. Calculate the content by the following formula:

$$\text{Content (\%) of gellan gum} = \frac{\text{Weight (g) of the residue}}{\text{Weight (g) of the sample}} \times 100$$

Geraniol

ゲラニオール



$C_{10}H_{18}O$ Mol. Wt. 154.25

(2*E*)-3,7-Dimethylocta-2,6-dien-1-ol [106-24-1]

Content Geraniol contains not less than 85.0% of geraniol ($C_{10}H_{18}O$).

Description Geraniol is a colorless to light yellow, clear liquid having a characteristic odor.

Identification To 1 mL of Geraniol, add 1 mL of acetic anhydride and 1 drop of phosphoric acid, keep lukewarm for 10 minutes, add 1 mL of water, and shake in warm water for 5 minutes. Cool, and make slightly alkaline with sodium carbonate solution (1 in 8). An odor of geranyl acetate is evolved.

Refractive Index n_D^{20}: 1.469–1.478.

Specific Gravity d_{20}^{20}: 0.870–0.885.

Purity

(1) Acid value　Not more than 1.0 (Flavoring Substances Tests).

(2) Clarity of solution　Clear (1.0 mL, 70% (vol) ethanol 3.0 mL).

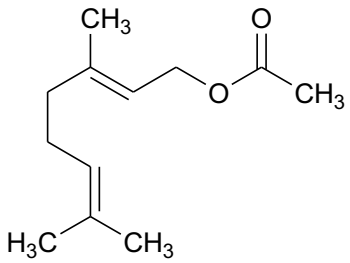
(3) Ester value　Not more than 3.0 (5.0 g, Flavoring Substances Tests).

(4) Aldehyde　Weigh accurately about 5 g of Geraniol, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, allow the mixture to stand for 15 minutes before titrating. The volume of consumed 0.5 mol/L hydrochloric acid is not more than 0.65 mL.

Assay　Proceed as directed in the Alcohol Content Test in the Flavoring Substances Tests, using 1 g of acetylated oil.

Geranyl Acetate

酢酸ゲラニル



$C_{12}H_{20}O_2$　Mol. Wt. 196.29

(2*E*)-3,7-Dimethylocta-2,6-dien-1-yl acetate　[105-87-3]

Content　Geranyl Acetate contains not less than 90.0% of geranyl acetate ($C_{12}H_{20}O_2$).

Description　Geranyl Acetate is a colorless to light yellow, clear liquid having a characteristic odor.

Identification　To 1 mL of Geranyl Acetate, add 5 mL of 10% (w/v) potassium hydroxide–ethanol TS, and heat in a water bath. The characteristic odor disappears, and an odor of geranyl is evolved. After cooling, add 2 mL of diluted hydrochloric acid (1 in 4) and 2 mL of water. The solution responds to test (3) for Acetate in the Qualitative Tests.

Refractive Index　n_D^{20}: 1.457–1.464.

Specific Gravity　d_{20}^{20}: 0.903–0.917.

Purity

(1) Acid value　Not more than 1.0 (Flavoring Substances Tests).

(2) Clarity of solution　Clear (1.0 mL, 80% (vol) ethanol 4.0 mL).

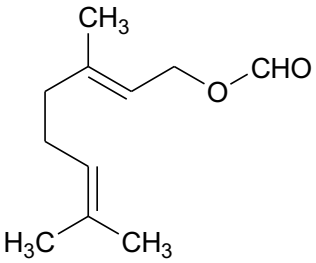
Assay　Weigh accurately about 1 g of Geranyl Acetate, and proceed as directed in the

Ester Content Test in the Flavoring Substances Tests.

Each mL of 0.5 mol/L ethanolic potassium hydroxide = 98.14 mg of $C_{12}H_{20}O_2$.

Geranyl Formate

ギ酸ゲラニル



$C_{11}H_{18}O_2$ Mol. Wt. 182.26

(2*E*)-3,7-Dimethylocta-2,6-dien-l-yl formate [105-86-2]

Content Geranyl Formate contains not less than 85.0% of geranyl formate ($C_{11}H_{18}O_2$).

Description Geranyl Formate is a colorless to pale yellow, clear liquid having a characteristic odor.

Identification

(1) To 1 mL of Geranyl Formate, add 10 mL of 10% (w/v) potassium hydroxide–ethanol TS, and heat in a water bath for 5 minutes while shaking. The characteristic odor disappears, and an odor of geraniol is evolved.

(2) To 1 mL of Geranyl Formate, add 10 mL of sodium hydroxide solution (1 in 25), heat in a water bath for 5 minutes while shaking, and allow to stand. To 1 mL of an aqueous solution of the lower layer, add 1.5 mL of diluted hydrochloric acid (1 in 4), and add 20 mg of magnesium powder in several divided portions. After effervescence ceases, add 3 mL of diluted sulfuric acid (3 in 5) and 10 mg of disodium chromotropic acid dihydrate, shake, and warm in a warm water for 10 minutes. A pink-purple color develops.

Refractive Index n_D^{20}: 1.457–1.466.

Specific Gravity d_{20}^{20}: 0.909–0.917.

Purity

(1) Acid value Not more than 1.0 (Flavoring Substances Tests).

In the test, titrate while cooling in ice water, and continue the titration until a light pink color persists for 10 seconds.

(2) Clarity of solution Clear (1.0 mL, 80% (vol) ethanol 3.0 mL).

Assay Weigh accurately about 1 g of Geranyl Formate, and perform the tests as

directed in the Saponification Value Test and the Acid Value Test, respectively, in the Flavoring Substances Tests. Calculate the content by the formula:

$$\text{Content (\%) of geranyl formate } (C_{11}H_{18}O_2) = \frac{\text{Saponification value} - \text{Acid value}}{561.1} \times 182.3$$

Glucanase

グルカナーゼ

Definition Glucanase includes enzymes that hydrolyze β-D-glucan. It is derived from the culture of basidiomycetes (limited to *Pycnoporus coccineus*), filamentous fungi (limited to *Aspergillus aculeatus*, *Aspergillus niger*, *Geosmithia emersonii*, *Humicola insolens, Penicillium emersonii*, *Penicillium funiculosum*, *Rasamsonia emersonii*, *Rhizopus delemar*, *Trichoderma harzianum*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, and *Trichoderma viride*), yeasts (limited to species of the genus Saccharomyces), actinomycetes (limited to *Streptomyces avermitilis*, *Streptomyces griseus*, *Streptomyces thermoviolaceus*, and *Streptomyces violaceoruber*), or bacteria (limited to *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Cellulosimicrobium cellulans*, *Lysobacter enzymogenes*, *Paenibacillus curdlanolyticus*, *Pseudomonas paucimobilis,* and species of the genus *Arthrobacter*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Glucanase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Glucanase complies with the Glucanase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Glucanase Activity Test Perform the test using one of the methods given below. If the test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of Glucanase, add water to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water to the resulting solution.

Substrate Suspension Weigh 2.0 g of curdlan, and suspend it uniformly in 100 mL of water by agitating. Prepare fresh before use.

Test Solution Transfer 1 mL of the substrate suspension into an L-shaped test tube, add 5 mL of sodium phosphate buffer (0.1 mol/L) at pH 7.0 or acetate buffer (0.1 mol/L) at pH 4.0, equilibrate at 37°C for 5 minutes, and add 1 mL of the sample solution while shaking. Incubate the mixture at 37°C for 30 minutes while shaking, add 1 mL of hydrochloric acid TS (0.5 mol/L), mix, and centrifuge this solution at 3500 rpm for 15 minutes. To 1 mL of the supernatant, add 1 mL of phenol solution (1 in 20), then add 5 mL of sulfuric acid promptly, and agitate.

Control Solution To 1 mL of the substrate suspension, add 5 mL of sodium phosphate buffer (0.1 mol/L) at pH 7.0 or acetate buffer (0.1 mol/L) at pH 4.0, then add 1 mL of hydrochloric acid TS (0.5 mol/L), mix, and add 1 mL of the sample solution. Centrifuge this solution at 3500 rpm for 15 minutes. Proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and control solution at a wavelength of 490 nm. The absorbance value of the test solution is higher than that of the control solution.

Method 2

Sample Solution Weigh 0.50 g of Glucanase, add water or acetate buffer (0.1 mol/L)at pH 5.0 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Suspend 3.75 g of β-glucan (barley-derived) in 150 mL of water, and dissolve it by heating in a water bath for 10 minutes while shaking. After cooling, add 25 mL of acetate buffer (1 mol/L) at pH 5.0 and water to make 250 mL. Store in a refrigerator and use within 2 weeks.

Test Solution Transfer 1.75 mL of the substrate solution into a test tube, equilibrate at 50°C for 5 minutes, and add 0.25 mL of the sample solution, mix immediately. Incubate the mixture at 50°C for 10 minutes. To this solution, add 2 mL of 3,5-dinitrosalicilic acid TS, and shake well. Cover the mouth of the test tube with a glass bead, heat in a water bath for 15 minutes. After cooling in water, add 10 mL of water.

Control Solution Transfer 1.75 mL of the substrate solution into a test tube, add 2 mL of 3,5-dinitrosalicilic acid TS, shake well, and add 0.25 mL of the sample solution. Cover the mouth of the test tube with a glass bead, heat in a water bath for 15 minutes. After cooling in water, add 10 mL of water.

Procedure Measure the absorbance of the test solution and control solution at a wavelength of 540 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 3

Sample Solution Weigh 0.50 g of Glucanase, add water to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water to the resulting solution.

Substrate Suspension Suspend dry yeast (for the glucanase activity test) in phosphate buffer (0.005 mol/L) at pH 7.0. Adjust each amount of the dry yeast and phosphate buffer so that the absorbance of the substrate suspension at a wavelength of 660 nm is the range of 0.45–0.55. Store in icy water, and use within 15 minutes after preparation.

Test Solution Transfer 10 mL of the substrate suspension into a test tube, equilibrate at 40°C for 5 minutes, add 1 mL of the sample solution, and shake. Incubate the mixture at 40°C for 15 minutes.

Control Solution Proceed as directed for the test solution using water instead of the sample solution.

Procedure After 15 minutes warming at 40°C, shake well the test solution and the control solution, and measure the absorbance at a wavelength of 660 nm. The absorbance of the test solution is less than that of the control solution.

Method 4

Sample Solution Weigh 0.50 g of Glucanase, add water or acetate buffer (0.1 mol/L, pH 6.0, containing albumin) to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the buffer to the resulting solution.

Substrate Solution Suspend 1.0 g of β-glucan (barley-derived) in 60 mL of water, and dissolve it by heating in a water bath for 5 minutes while shaking. After cooling, add 10 mL of acetate buffer (1 mol/L) at pH 6.0. Adjust the pH of the solution to 6.0 with sodium hydroxide TS (1 mol/L), and add water to make 100 mL. Prepare fresh before use.

Test Solution Transfer 0.5 mL of the sample solution into a test tube, equilibrate it at 40°C for 10 minutes, add 0.5 mL of the substrate solution, equilibrated at 40°C, agitate immediately. Incubate the mixture at 40°C for 30 minutes. To this solution, add 1 mL of Somogyi TS (III), and shake well. Cover the test tube with a glass bead, and heat in a water bath for 30 minutes. After cooling, add 1 mL of Nelson TS, dissolve the red precipitate by gently shaking, allow to stand for 30 minutes, add 2 mL of water, and mix. Centrifuge this solution at 3000 rpm for 10 minutes, and use the supernatant.

Control Solution Transfer 0.5 mL of the sample solution into a test tube, add 1 mL of Somogyi TS (III), shake, and add 0.5 mL of the substrate solution, and shake. Cover the test tube with a glass bead, and heat in a water bath for 30 minutes. Proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 520 nm. The absorbance value of the test solution is higher than that of the control solution.

Method 5

Sample Solution Weigh 0.50 g of Glucanase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water to the resulting solution.

Substrate Suspension Add 30 mL of water to 1.0 g of β-glucan (barley-derived), shake for 1 hour, and dissolve it by heating in a water bath for 5 minutes. After cooling, add 10 mL of potassium phosphate–phosphoric acid buffer (1 mol/L) at pH 5.0 and water to make 100 mL. Prepare fresh before use.

Test Solution Equilibrate 15 mL of the substrate solution at 45°C for 20 minutes, add 2 mL of the sample solution, and shake. Incubate the mixture at 45°C for 15 minutes.

Control Solution Proceed as directed for the test solution using water instead of the sample solution.

Procedure Warm the test solution and the control solution at 45°C for 15 minutes, and proceed as directed under Method 1 Viscosity Measurement by Capillary Tube Viscometer in Viscosity in General Tests. Perform the test at 45°C. The flow time of the test solution is less than that of the control solution.

Glucoamylase

グルコアミラーゼ

Definition Glucoamylase includes enzymes that hydrolyze glucosidic linkages in amylaceous polysaccharides, such as starch, to produce glucose. It is derived from the culture of basidiomycetes (limited to *Corticium rolfsii*), filamentous fungi (limited to *Humicola grisea*, *Rhizopus delemar*, *Rhizopus niveus*, *Rhizopus oryzae*, and species of the genera *Acremonium* and *Aspergillus*), yeasts (limited to species of *the genus Saccharomyces*), or bacteria (limited to species from the genuses *Bacillus* and *Pseudomonas*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Glucoamylase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Glucoamylase complies with the Glucoamylase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Solution Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Glucoamylase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of Glucoamylase, add water, salt TS, or cooled salt TS to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water, salt TS, or cooled salt TS to the resulting solution.

Substrate Solution Add 20 mL of water to 2.0 g of soluble starch, and add the resulting mixture gradually to about 40 mL of boiling water while stirring well. Keep boiling for about 2 minutes after the start of boiling. After cooling, add water to make 100 mL. Prepare fresh before use.

Test Solution To 1 mL of the substrate solution, add 0.2 mL of acetate buffer (0.2 mol/L) at pH 5.0, equilibrate at 40°C for 5 minutes, add 0.1 mL of the sample solution, and shake immediately. Incubate the mixture at 40°C for 20 minutes, add 0.1 mL of sodium hydroxide TS (1 mol/L), and shake immediately. Allow the solution to stand at room temperature for 30 minutes. Neutralize it with 0.1 mL of hydrochloric acid TS (1 mol/L). To 0.2 mL of the resulting solution, add 6 mL of TS for D-glucose determination (containing mutarotase), mix, warm the solution at 40°C for 40 minutes, and cool to room temperature.

Control Solution To 1 mL of the substrate solution, add 0.2 mL of acetate buffer (0.2 mol/L) at pH 5.0, equilibrate at 40°C for 5 minutes, add 0.1 mL of sodium hydroxide TS (1 mol/L) and 0.1 mL of the sample solution, and immediately shake. Allow this solution

to stand at room temperature for 30 minutes. Then proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 505 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 0.50 g of Glucoamylase, add water or a solution of polyoxyethylene(10) octylphenylether (1 in 1000) to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or a solution of polyoxyethylene(10) octylphenylether (1 in 1000) to the resulting solution.

Substrate Solution Weigh 2.16 g of D(+)-maltose monohydrate, and dissolve it in acetate buffer (0.1 mol/L, pH 4.3, containing polyoxyethylene(10) octylphenylether) to make 100 mL. Prepare fresh before use.

Test Solution Equilibrated 0.1 mL of the substrate solution at 37°C for 8 minutes, add 0.02 mL of the sample solution, and incubate at 37°C for 6 minutes. Add 0.02 mL of sodium hydroxide TS (0.5 mol/L), and 1 minute later add 0.11 mL of TS for D-glucose determination (containing hexokinase), and shake immediately.

Control Solution Proceed as directed for the test solution using water or the solution used to prepare the sample solution, instead of the sample solution.

Procedure Warm the test solution and the control solution at 37°C for 7 minutes, and measure the absorbance of them at a wavelength of 340 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 3

Sample Solution Weigh 0.50 g of Glucoamylase, add water or acetic acid–sodium hydroxide buffer (0.1 mol/L, pH4.3, containing sodium chloride) to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 55 mg of *p*-nitrophenyl α-D-glucopyranoside, and dissolve it in acetic acid–sodium hydroxide buffer (0.1 mol/L, pH4.3, containing sodium chloride) to make 500 mL. Prepare fresh before use.

Test Solution To 0.2 mL of the sample solution, add 0.25 mL acetic acid–sodium hydroxide buffer (0.1 mol/L, pH4.3, containing sodium chloride), and mix. Equilibrate it at 30°C for 5 minutes, add 0.5 mL of the substrate solution, and shake immediately. Incubate the mixture at 30°C for 10 minutes, and add 1 mL of a solution of sodium tetraborate decahydrate (1 in 50).

Control Solution Proceed as directed for the test solution using the diluent used to dilute the sample solution, instead of the sample solution.

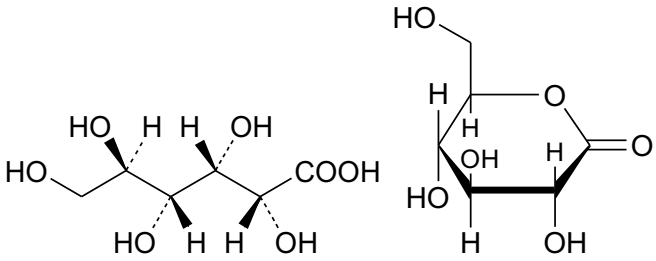
Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 400 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 4 Proceed as directed in Method 1 of β-Amylase Activity Test for β-Amylase.

Method 5 Proceed as directed in Method 2 of β-Amylase Activity Test for β-Amylase.

Gluconic Acid

グルコン酸



Definition Gluconic Acid is an aqueous solution of gluconic acid and glucono-δ-lactone.

Content Gluconic Acid contains the equivalent of 50.0–52.0% gluconic acid ($C_6H_{12}O_7$ = 196.16).

Description Gluconic Acid is a colorless to light yellow, clear syrupy liquid. It is odorless or has a slight odor, and has a sour taste.

Identification

(1) To 1 mL of a solution of Gluconic Acid (1 in 25), add 1 drop of a solution of iron(III) chloride hexahydrate (1 in 10). A deep yellow color develops.

(2) To 1 mL of Gluconic Acid, add 4 mL of water, and proceed as directed in Identification (2) for Glucono-δ-Lactone.

Purity

(1) Chloride Not more than 0.035% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.50 mL).

(2) Sulfate Not more than 0.024% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Sucrose or reducing sugars Weigh 1.0 g of Gluconic Acid, and proceed as directed

in Purity (6) for Glucono-δ-Lactone.

Residue on Ignition Not more than 0.1% (5 g).

Assay Weigh accurately about 1 g of Gluconic Acid, add 30 mL of water and exactly 40 mL of 0.1 mol/L sodium hydroxide shake, and allow to stand for 20 minutes. Titrate the excess alkali with 0.05 mol/L sulfuric acid (indicator: 3 drops of phenolphthalein TS). Perform a blank test in the same manner.

Each mL of 0.1 mol/L sodium hydroxide = 19.62 mg of $C_6H_{12}O_7$

Glucono-δ-Lactone

Gluconolactone

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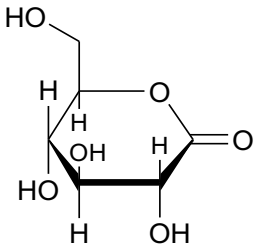


$C_6H_{10}O_6$ Mol. Wt. 178.14

D-Glucono-1,5-lactone [90-80-2]

Content Glucono-δ-Lactone, when dried, contains not less than 99.0% of glucono-δ-lactone ($C_6H_{10}O_6$).

Description Glucono-δ-Lactone occurs as white crystals or crystalline powder. It is odorless or has a slight odor. It has a sweet taste at first and changes to a slight acid taste.

Identification

(1) To 1 mL of a solution of Glucono-δ-Lactone (1 in 50), add 1 drop of a solution of iron(III) chloride hexahydrate(1 in 10). A deep yellow color develops.

(2) To 5 mL of a solution of Glucono-δ-Lactone (1 in 10), add 0.7 mL of acetic acid and 1 mL of freshly distilled phenylhydrazine, and heat on a water bath for 30 minutes. After cooling, rub the inner wall with a glass rod. Crystals are deposited. Collect the crystals by filtration, dissolve them in 10 mL of boiling water, add a small amount of active carbon, and filter. After cooling, rub the inner wall with a glass rod, and dry the deposited crystals. The melting point is 192–202°C (decomposition).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.035% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.50 mL).

(3) Sulfate Not more than 0.024% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(6) Sucrose or reducing sugars Weigh 0.50 g of Glucono-δ-Lactone, add 10 mL of water and 2 mL of diluted hydrochloric acid (1 in 4), boil for 2 minutes. Cool, add 5 mL of sodium carbonate solution (1 in 8), allow to stand for 5 minutes, and add water to make 20 mL. Measure 5 mL of this solution, add 2 mL of Fehling's TS, and boil for 1 minute. An orange–yellow to red precipitate is not formed immediately.

Loss on Drying Not more than 1.0% (105°C, 2 hours).

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.3 g of Glucono-δ-Lactone, previously dried, dissolve it in exactly 30 mL of 0.1 mol/L sodium hydroxide allow to stand for 20 minutes, and titrate the excess alkali with 0.05 mol/L sulfuric acid (indicator: 3 drops of phenolphthalein TS). Perform a blank test in the same manner.

Each mL of 0.1 mol/L sodium hydroxide = 17.81 mg of $C_6H_{10}O_6$

Glucose Isomerase

グルコースイソメラーゼ

Definition Glucose Isomerase includes enzymes that isomerizeglucose. It is derived from the culture of filamentous fungi (limited to species of the genus *Aspergillus*), actinomycetes (limited to *Actinoplanes missouriensis*, *Streptomyces griseofuscus*, *Streptomyces griseus*, *Streptomyces murinus*, *Streptomyces phaeochromogenes*, *Streptomyces rubiginosus*, *Streptomyces thermoviolaceus*, *Streptomyces violaceoruber*, and *Streptomyces* sp.), or bacteria (limited to *Arthrobacter globiformis* and *Bacillus coagulans*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Glucose Isomerase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Glucose Isomerase complies with the Glucose Isomerase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead

Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Solution Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Glucose Isomerase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the sample dilution factor, the buffer solution, and the temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Glucose Isomerase, add water or phosphate buffer (0.05 mol/L) at pH 7.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 3.6 g of D(+)-glucose, dissolve it by adding 25 mL of phosphate buffer (0.4 mol/L) at pH 7.0 and 20 mL of magnesium sulfate (0.1 mol/L), and add water to make 100 mL.

Test Solution Transfer 1 mL of the substrate solution into a test tube, add 0.8 mL of water, and mix. Cover the mouth of the test tube with a glass bead, equilibrate it at 70°C for 5 minutes, and add 0.2 mL of the sample solution. Incubate the mixture at 70°C for 30 minutes with the test tube covered with the glass bead, and cool with ice. To this solution, add 4 mL of diluted perchloric acid (9 in 200), mix, and add water to make 10 mL. Prepare the diluted perchloric acid using perchloric acid with a concentration of 70%. Transfer 0.5 mL of the resulting solution into a test tube, and mix with 0.5 mL of water. In icy water, add 6 mL of 70% (vol) sulfuric acid TS, shake, then add 0.1 mL of L-cysteine hydrochloride, and mix. Warm the solution at 50°C for 10 minutes, and cool to room temperature.

Control Solution Measure 1 mL of the substrate solution into a test tube, add 0.8 mL of water, and mix. Add 4 mL of diluted perchloric acid (9 in 200) and 0.2 mL of the sample solution. Cover the mouth of the test tube with a glass bead, equilibrate the mixture at 70°C for 30 minutes, and add water to make 10 mL. Take 0.5 mL of this solution into a test tube, add 0.5 mL of water, mix, and proceed as directed for the test solution.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 410 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 1.0 g of Glucose Isomerase, add water or maleic acid–magnesium sulfate–cobalt chloride TS to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the TS to the resulting solution.

Substrate Solution Weigh 216.2 g of D(+)-glucose, and dissolve it in maleic acid–magnesium sulfate–cobalt chloride TS to make 500 mL.

Test Solution Equilibrate 1.0 mL of the substrate solution at 60°C for 2 minutes, add 0.25 mL of the sample solution, and mix. Incubate the mixture at 60°C for 30 minutes. Add 0.25 mL of diluted hydrochloric acid (1 in 5), shake, cool the mixture, and filter it through a membrane filter (0.2 µm pore size). Use the filtrate as the test solution.

Control Solution Proceed as directed for the test solution using water or maleic acid–magnesium sulfate–cobalt chloride TS instead of the sample solution.

Standard Solution Dissolve 0.10 g of fructose (for enzyme) in water to make 100 mL.

Procedure Analyze the test solution, the control solution, and the standard solution by liquid chromatography using the operating condition given below. A peak is observed at the retention time of fructose for the test solution. The peak area is larger than that of the peak corresponding to the retention time of fructose in the control solution.

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (8 mm internal diameter and 30 cm length).

Column packing material: About 9-µm Ca-form cation-exchange resin for liquid chromatography.

Column temperature: 80°C.

Mobile phase: Water

Flow rate: 0.6 mL/min.

Method 3

Sample Solution Weigh 1.0 g of Glucose Isomerase, add water or MOPS buffer (0.02 mol/L, pH 7.0, containing magnesium sulfate) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 3.8 g of fructose (for enzyme), and dissolve it in MOPS buffer (0.02 mol/L, pH 7.0, containing magnesium sulfate) to make 25 mL.

Test Solution Add 1.9 mL of the sample solution to 3.1 mL of MOPS buffer (0.04

mol/L, pH 7.0, containing magnesium sulfate–sodium chloride–cobalt chloride), and warm at 37°C for 5 minutes. Add 15 mL of glucose oxidase–peroxidase TS, and warm at 37°C for 8 minutes. To this solution, add 3.7 mL of the substrate solution, and warm at 37°C for 5 minutes.

Control Solution Proceed as directed for the test solution using MOPS buffer (0.02 mol/L, pH 7.0, containing magnesium sulfate) instead of the sample solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 405 nm at 5 minutes after the addition of the substrate solution. The absorbance value of the test solution is higher than that of the control solution.

If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Glucose Oxidase

グルコースオキシダーゼ

Definition Glucose Oxidase includes enzymes that oxidize glucose. It is derived from the culture of filamentous fungi (limited to *Acremonium chrysogenum*, *Aspergillus aculeatus*, *Aspergillus niger,* and sepecies of the genus *Penicillium*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Glucose Oxidase occurs as white to dark brown or white to light yellow granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Glucose Oxidase complies with the Glucose Oxidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count. Use soybean casein digest agar medium, instead of standard agar medium.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Glucose Oxidase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of Glucose Oxidase, add potasium phosphate–sodium hydroxide buffer (0.1 mol/L) at pH 7.0, cooled potasium phosphate–sodium hydroxide buffer (0.1 mol/L) at pH 7.0, or water to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the buffer to the resulting solution.

Substrate Solution Weigh 2.50 g of D-(+)-glucose, and dissolve it in water to make 25 mL.

Test Solution Place 0.5 mL of the substrate solution, 2 mL of potasium phosphate–sodium hydroxide buffer (0.1 mol/L, pH 7.0, containing phenol), 0.5 mL of peroxidase TS (25 units/mL), and 0.1 mL of 4-aminoantipyrine solution (1 in 250) into a quartz cell, and equilibrate at 37°C for 10 minutes. To the mixture, add 0.1 mL of the sample solution, shake well, and incubate at 37°C.

Control Solution Proceed in the same manner as for the test solution using potasium phosphate–sodium hydroxide buffer (0.1 mol/L) at pH 7.0 or water instead of the sample solution.

Procedure Measure the absorbance of the test solution at a wavelength of 500 nm at 2 and 5 minutes after the addition of the sample solution to determine the difference of the two absorbance values. Similarly, measure two absorbance values for the control solution to determine the difference of them. The absorbance difference of the test solution is larger than that of the control solution.

Method 2

Sample Solution Weigh 1.0 g of Glucose Oxidase, add water or acetic acid–sodium hydroxide buffer (0.05 mol/L, pH 5.8, containing sodium chloride) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 2.80 g of D(+)-glucose, and dissolve it in 100 mL of acetic acid–sodium hydroxide buffer (0.05 mol/L, pH 5.8, containing sodium chloride).

Test Solution To 25 mL of the substrate solution, equilibrated at 35°C, add 1 mL of the sample solution, and incubate the mixture at 35°C for 15 minutes while passing air through it using a capillary. Wash the capillary with 10 mL of water, combine the washings with the solution, and remove it. Add 10 mL of sodium hydroxide TS (0.1 mol/L) immediately, and warm at 35°C for 60 minutes.

Control Solution To 25 mL of the substrate solution, add 10 mL of water and 10 mL of sodium hydroxide TS (0.1 mol/L), then add 1 mL of the sample solution, and incubate

the mixture at 35°C for 60 minutes.

Procedure Titrate the test solution and the control solution with hydrochloric acid TS (0.1 mol/L) using 2 drops of phenolphthalein TS as the indicator. The amount of the hydrochloric acid TS consumed by the test solution is less than that consumed by the control solution.

α-Glucosidase

α-グルコシダーゼ

Definition α-Glucosidase includes enzymes that hydrolyze α-D-glucosidic linkages present at the non-reducing end of maltose or oligosaccharides. It is derived from the culture of filamentous fungi (limited to species of the genera *Absidia*, *Acremonium*, and *Aspergillus*), yeasts (limited to species from the genus *Saccharomyces*), actinomycetes (limited to *Streptomyces avermitilis, Streptomyces griseus*, and *Streptomyces violaceoruber*), or bacteria (limited to *Burkholderia ginsengisoli*, *Halomonas aquamarina,* and species of the genera *Bacillus* and *Pseudomonas*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description α-Glucosidase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification α-Glucosidase complies with the α-Glucosidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed in the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

The requirement of total plate count can be disregarded if unsterilized α−Glucosidase

is used for captive consumption in the manufacture of food that will be sterilized or pasteurized before completion of the final product.

α-Glucosidase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of α-Glucosidase, add sodium phosphate buffer (0.05 mol/L) at pH 7.0, McIlvaine buffer (0.02 mol/L) at pH 4.0, or water to dissolve it or disperse it uniformly, and make 10 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding the same buffer or water to the resulting solution.

Substrate Solution Use either of the following preparation methods. Prepare fresh before use.

A. Weigh 2.1 g of D(+)-maltose monohydrate, and dissolve it in a small amount of water by shaking. Add 10 mL of sodium phosphate buffer (0.5 mol/L) at pH 7.0 and water to make 100 mL.

B. Weigh 2.1 g of D(+)-maltose monohydrate, and dissolve it in water by shaking. Add of 10 mL of McIlvaine buffer at pH 4.0 and water to make 100 mL.

Test Solution To 1 mL of the substrate solution, equilibrated at 37°C for 5 minutes, add 1 mL of the sample solution, equilibrated at 37°C, and shake. Incubate the mixture at 37°C for 10 minutes. Add 1 mL of hydrochloric acid TS (0.5 mol/L), mix immediately, and allow to cool, followed by addition of 1 mL of sodium hydroxide TS (0.5 mol/L). To 1 mL of this solution, add 4 mL of TS for D-glucose determination (containing mutarotase), mix, and warm the mixture at 37°C for 20 minutes.

Control Solution To 1 mL of the substrate solution, equilibrated at 37°C for 5 minutes, add 1 mL of hydrochloric acid (0.5 mol/L), shake, and warm the mixture at 37°C for 10 minutes. Add 1 mL of the sample solution, equilibrated at 37°C, and mix. After cooling, proceed as directed for the test solution.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 505 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 1.0 g of α-Glucosidase, add cold water to dissolve it or disperse it uniformly, and make 200 mL. As necessary, prepare a 10-fold or 100-fold dilution by adding cold water to the resulting solution.

Substrate Solution Weigh 2.0 g of α-methyl-D(+)-glucoside, and add water to make 100 mL.

Test Solution To 1 mL of the substrate solution, add 1 mL of acetate buffer (0.02 mol/L) at pH 5.0, and equilibrate at 40°C for 10–15 minutes. Add 0.5 mL of the sample

solution, and shake immediately. Incubate the mixture at 40°C for 60 minutes, heat it for 5 minutes in a water bath, and cool with running water. To 0.1 mL of this solution, add 3 mL of TS for D-glucose determination (containing glucose oxidase–peroxidase), shake well, and warm at 40°C for 20 minutes.

Control Solution To 1 mL of acetate buffer (0.02 mol/L) at pH 5.0, add 0.5 mL of the sample solution, heat the mixture for 5 minutes in a water bath, cool in running water. Add 1 mL of the substrate solution. To 0.1 mL of this solution, add 3 mL of TS for D-glucose determination (containing glucose oxidase–peroxidase), shake well, and warm at 40°C for 20 minutes.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 500 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

β-Glucosidase

β-グルコシターゼ

Definition β-Glucosidase includes enzymes that hydrolyze the β-D-glucosidic linkages in saccharides. It is derived from *Cycas revoluta* Thunb. or the culture of filamentous fungi (limited to *Aspergillus aculeatus*, *Aspergillus niger, Aspergillus oryzae, Aspergillus pulverulentus, Penicillium decumbens, Penicillium multicolor, Trichoderma harzianum, Trichoderma longibrachiatum,* and *Trichoderma reesei*), actinomycetes (limited to *Streptomyces avermitilis, Streptomyces griseus,* and *Streptomyces thermoviolaceus*), or bacteria (limited to species of the genus *Bacillus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description β-Glucosidase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification β-Glucosidase complies with the β-Glucosidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

β-Glucosidase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of β-Glucosidase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold or 100-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Weigh 0.50 g of D(-)-salicin, dissolve it in water, and make 50 mL.

Test Solution Transfer 3 mL of acetate buffer (0.1 mol/L) at pH4.0 into a 50 mL of Nessler tube, add 1 mL of the substrate solution, and equilibrate at 40°C for 10 minutes. Add 1 mL of the sample solution, and shake immediately. Incubate the mixture at 40°C for 30 minutes. To this solution, add 2 mL of Somogyi TS (I), shake, and heat in a water bath for 20 minutes with the test tube plugged loosely. After cooling, add 1 mL of Nelson TS, and shake well until the red precipitate of copper oxide completely dissolves. Allow the solution to stand for about 20 minutes, and add water to make 25 mL.

Control Solution Transfer 3 mL of acetate buffer (0.1 mol/L) at pH4.0 into a 50 mL of Nessler tube, add 1 mL of the substrate solution and 2 mL of Somogyi TS (I), and shake. Add 1 mL of the sample solution, heat in a water bath for 20 minutes with the test tube plugged loosely, and proceed as directed for the test solution.

Procedure Determine the absorbance of the test solution and the control solution at a wavelength of 500 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 0.50 g of β-Glucosidase, add acetate buffer (0.2 mol/L) at pH 5.0 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Weigh 0.151 g of *p*-nitrophenyl-β-D-glucopyranoside, and dissolve it in water to make 100 mL. Prepare fresh before use.

Test Solution To 0.5 mL of the substrate solution, add 1 mL of acetate buffer (0.2 mol/L) at pH 5.0, equilibrate the mixture at 50°C for 5 minutes, add 0.1 mL of the sample solution, and shake immediately. Incubate the mixture at 50°C for 20 minutes, add 1 mL of sodium carbonate solution (53 in 500), and shake immediately.

Control Solution　To 0.5 mL of the substrate solution, add 1 mL of acetate buffer (0.2 mol/L) at pH 5.0 and 1 mL of sodium carbonate solution (53 in 500), and shake. Add 0.1 mL of the sample solution, shake, warm the mixture at 50°C for 20 minutes, and cool.

Procedure　Determine the absorbance of the test solution and the control solution at a wavelength of 400 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 3

Sample Solution　Weigh 1.0 g of β-Glucosidase, add acetate buffer (0.1 mol/L) at pH 5.0 to dissolve it or disperse it uniformly, and make 250 mL. As necessary, prepare a 10-fold or 100-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution　Weigh 0.20 g of D(+)-cellobiose, and dissolve it in acetate buffer (0.1 mol/L) at pH 5.0 to make 100 mL. Prepare fresh before use.

Test Solution　Equilibrate 0.05 mL of the substrate solution at 50°C for 3 minutes, and add 0.025 mL of the sample solution. Incubate the mixture at 50°C for 10 minutes. Add 0.175 mL of TS for D-glucose determination (containing hexokinase), immediately shake, and allow the mixture to stand for 5 minutes.

Control Solution　Proceed as directed for the test solution using 0.025 mL of　acetate buffer (0.1 mol/L) at pH 5.0 instead of the sample solution.

Procedure　Determine the absorbance of the test solution and the control solution at a wavelength of 340 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

α-Glucosyltransferase

4-α-Glucanotransferase
6-α-Glucanotransferase

α-グルコシルトランスフェラーゼ

Definition　α-Glucosyltransferase includes enzymes that transfer glucosyl groups or glucan chains. It is derived from the tubers of the potato *Solanum tuberosum* L. or the culture of actinomycetes (limited t*o Streptomyces avermitilis, Streptomyces cinnamoneus*, *Streptomyces griseus*, *Streptomyces thermoviolaceus*, and *Streptomyces violaceoruber*) or bacteria (limited to *Agrobacterium radiobacter*, *Geobacillus pallidus, Geobacillus stearothermophilus, Gluconobacter oxydans*, *Leuconostoc mesenteroides*, *Paenibacillus alginolyticus, Sporosarcina globispora* , and species of the genera *Arthrobacter*, *Bacillus*, *Erwinia*, *Pimelobacter*, *Protaminobacter*, *Pseudomonas*, *Serratia*, and *Thermus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives

used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description α-Glucosyltransferase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification α-Glucosyltransferase complies with the α-Glucosyltransferase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

The requirement of total plate count can be disregarded if unsterilized α-Glucosyltransferase is used for captive consumption in the manufacture of food that will be sterilized or pasteurized before completion of the final product.

α-Glucosyltransferase Activity Test

Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of α-Glucosyltransferase, add phosphate buffer (0.02 mol/L) at pH 7.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution. Prepare fresh and use within 30 minutes after preparation.

Substrate Solution Use either of the following preparation methods. Prepare fresh before use:

—Add water to 5.0 g of sucrose, dissolve it uniformly by shaking well, and make 100 mL.

—Add hot water to 5.0 g of soluble starch, dissolve it uniformly by shaking well, and

add water to make 100 mL.

Test Solution To 0.1 mL of the substrate solution, add 0.08 mL of phosphate buffer (0.5 mol/L) at pH 7.0, mix them, and equilibrate the mixture at 37°C for 5 minutes. To this solution, add 0.02 mL of the sample solution, incubate the mixture at 37°C for 15 minutes, and heat it for 5 minutes in a water bath. After cooling, add 2.2 mL of Tris buffer (0.05 mol/L) at pH 7.0, and mix. To this solution, add 1.2 mL of TS for α-D-glucose-1-phosphate determination, shake well, and warm at 30°C for 30 minutes.

Control Solution To 0.1 mL of the substrate solution, add 0.08 mL of Tris buffer (0.05 mol/L) at pH 7.0 mix them, and equilibrate the mixture at 37°C for 5 minutes. To this solution, add 0.02 mL of the sample solution, immediately heat for 5 minutes in a water bath. After cooling, add 2.2 mL of Tris buffer (0.05 mol/L) at pH 7.0, and mix. To this solution, add 1.2 mL of TS for α-D-glucose-1-phosphate determination, shake well, and warm at 30°C for 30 minutes.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 340 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 1.0 g of α-Glucosyltransferase, add potassium phosphate buffer (0.05 mol/L) at pH 7.5 to dissolve it or disperse it uniformly, and make 10 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding the same buffer to the resulting solution. Prepare fresh and use within 30 minutes after preparation.

Substrate Solution Mix well 1 mL of amylose TS and 2 mL of potassium phosphate (0.05 mol/L) at pH 7.5, and add water to make 10 mL. Prepare fresh before use.

Test Solution Equilibrate 0.1 mL of the substrate solution at 50°C for 5 minutes, add 0.1 mL of the sample solution, and shake immediately. Incubate the mixture at 50°C for 10 minutes, add 2 mL of hydrochloric acid TS (0.004 mol/L), and shake immediately. To this solution, add 2 mL of iodine TS (for α-glucosyltransferase activity test), and shake.

Control Solution To 0.1 mL of the substrate solution, add 2 mL of hydrochloric acid TS (0.004 mol/L) and 0.1 mL of the sample solution, and immediately shake. Add 2 mL of iodine TS (for α-glucosyltransferase activity test), and shake.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 660 nm. The absorbance value of the test solution is lower than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 3

Sample Solution Weigh 1.0 g of α-Glucosyltransferase, add sodium phosphate buffer (0.1 mol/L) at pH 6.0 to dissolve it or disperse it uniformly, and make 10 mL.

Substrate Solution Weigh 8.6 g of sucrose, dissolve it in water to make 100 mL. Prepare fresh before use.

Test Solution To 1 mL of the sample solution, add 4 mL of the substrate solution, equilibrated at 20°C for 15 minutes, and shake immediately. Incubate the mixture at 20°C for 10 minutes, and heat it for 5 minutes in a water bath. After cooling, filter this solution through a membrane filter (0.45 μm pore size), and use the filtrate as the test solution.

Control Solution Add 1 mL of the sample solution to 4 mL of the substrate solution, immediately heat the mixture for 5 minutes in a water bath, and cool to room temperature. Filter this solution through a membrane filter (0.45 μm pore size), and use the filtrate as the control solution.

Standard Solution Dissolve 0.10 g of isomaltulose in water to make 100 mL.

Procedure Analyze the test solution, the control solution, and the standard solution by liquid chromatography using the operating condition given below. A peak is observed at the retention time of isomaltulose for the test solution. The peak area is larger than that of the peak corresponding to the retention time of isomaltulose in the control solution.

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μm aminopropyl-bonded silica gel for liquid chromatography.

Column temperature: A constant temperature of 20–40°C.

Mobile phase: An 85:15 mixture of acetonitrile/water

Portion of injection: A constant amount of 10–15 μL.

Flow rate: 1 mL/min.

Method 4

Sample Solution Weigh 0.50 g of α-Glucosyltransferase, add water or acetate buffer (0.01 mol/L) at pH 6.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 5.0 g of maltopentaose, dissolve it in 300 mL of water, add 50 mL of acetate acetate buffer (0.2 mol/L) at pH 6.0 and water to make 500 mL.

Test Solution To 5 mL of the substrate solution, equilibrated to 50°C, add 0.2 mL of the sample solution, and mix. Incubate the mixture at 50°C for 60 minutes. To 0.5 mL of this solution, add 5 mL of water, heat immediately for 10 minutes in a water bath, and cool to room temperature. Transfer 0.5 mL of the resulting solution into a test tube containing 2 mL of Somogyi's copper TS, cover the mouth of the test tube with a glass bead, and heat for 10minute in a water bath. After cooling, add 2 mL of Nelson TS, mix well, allow the mixture to stand for 30 minutes, and add 5 mL of water.

Control Solution To 5 mL of the substrate solution, equilibrated at 50°C, add 0.2 mL

of the sample solution, and mix. Add 0.5 mL of this solution to 5 mL of water, heat immediately for 10 minutes in a water bath, and cool to room temperature. Transfer 0.5 mL of the resulting solution into a test tube containing 2 mL of Somogyi's copper TS, cover the mouth of the test tube with a glass bead, and heat for 10minute in a water bath. After cooling, add 2 mL of Nelson TS, mix well, allow the mixture to stand for 30 minutes, and add 5 mL of water.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 520 nm. The absorbance value of the test solution is lower than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 5

Sample Solution Weigh 1.0 g of α-Glucosyltransferase, add water or phosphate buffer (0.01 mol/L) at pH 7.0 to dissolve it or disperse it uniformly, and make 5 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 1.0 g of trehalose dihydrate, and dissolve it in phosphate buffer (0.05 mol/L) at pH 7.0 to make 100 mL.

Test Solution To 2 mL of the substrate solution, equilibrated at 60°C, add 0.2 mL of the sample solution, and mix. Incubate the mixture at 60°C for 30 minutes. Transfer 1.0 mL of the resulting solution into a test tube containing 2 mL of Somogyi's copper TS, cover the mouth of the test tube with a glass bead, heat for 10 minute in a water bath, and cool to room temperature. Add 2 mL of Nelson TS, mix, allow the mixture to stand for 30 minutes, and add 5 mL of water.

Control Solution To 2 mL of the substrate solution, equilibrated at 60°C, add 0.2 mL of the sample solution, and mix. Transfer 1.0 mL of the resulting solution immediately into a test tube containing 2 mL of Somogyi's copper TS, cover the mouth of the test tube with a glass bead, heat for 10 minute in a water bath, and cool to room temperature. Add 2 mL of Nelson TS, mix, allow the mixture to stand for 30 minutes, and add 5 mL of water.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 520 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 6

Sample Solution Weigh 1.0 g of α-Glucosyltransferase, add water or acetate buffer (0.05 mol/L) at pH 6.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 1.0 g of panose, and dissolve it in acetate buffer (0.05 mol/L) at pH 6.0 to make 100 mL.

Test Solution To 2 mL of the substrate solution, equilibrated at 35°C, add 0.2 mL of

the sample solution, and mix. Incubate the mixture at 35°C for 30 minutes, heat 0.5 mL of the resulting solution for 10 minute in a water bath, and cool to room temperature. Add 2 mL of TS for D-glucose determination (containing mutarotase), shake well, and warm the mixture at 37°C for 10 minutes.

Control Solution To 2 mL of the substrate solution, equilibrated at 35°C, add 0.2 mL of the sample solution, and mix. Immediately heat 0.5 mL of the resulting solution for 10 minute in a water bath, and cool to room temperature. To this solution, add 2 mL of TS for D-glucose determination (containing mutarotase), shake well, and warm the mixture at 37°C for 10 minutes.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 505 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 7

Sample Solution Weigh 1.0 g of α-Glucosyltransferase, add water or acetate buffer (0.05 mol/L) at pH 6.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 1.0 g of maltotetraose, and dissolve it in acetate buffer (0.05 mol/L) at pH 6.0 to make 50 mL.

Test Solution To 0.5 mL of the substrate solution, equilibrated at 35°C, add 0.5 mL of the sample solution, and mix. Incubate the mixture at 35°C for 60 minutes. Heat it for 10 minute in a water bath, and cool.

Control Solution Add 0.5 mL of the sample solution to 0.5 mL of the substrate solution, immediately heat for 10 minute in a water bath, and cool.

Standard Solution Dissolve 50 mg of maltotriose in water to make 100 mL.

Procedure Analyze 20 µL each of the test solution, the control solution, and the standard solution by liquid chromatography using the operating condition given below. A peak is observed at the retention time of maltotriose for the test solution. The peak area is larger than that of the maltotriose peak of the control solution. If the maltotriose peak of the test solution cannot be clearly identified in the chromatogram, deproteinize or desalinize the test solution.

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (5–20 mm internal diameter and 20–40 cm length).

Column packing material: 11–25 µm Ag-cation exchange resin for liquid chromatography.

Column temperature: A constant temperature of 50–85°C.

Mobile phase: Water

Flow rate: 0.3–1.0 mL/min. Adjust the retention time of maltotriose to 10–50 minutes.

Method 8

Sample Solution Weigh 0.50 g of α-Glucosyltransferase, add water or acetate buffer (0.05 mol/L, pH 6.0, containing calcium chloride) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 1.0 g of maltotetraose, dissolve it in acetate buffer (0.05 mol/L, pH 6.0, containing calcium chloride) to make 50 mL.

Test Solution To 0.5 mL of the substrate solution, equilibrated at 40°C, add 0.5 mL of the sample solution, and mix. Incubate the mixture at 40°C for 30 minutes. Heat it for 10 minute in a water bath, and cool.

Control Solution Add 0.5 mL of the sample solution to 0.5 mL of the substrate solution, heat the mixture immediately for 10 minute in a water bath, and cool.

Standard Solution Dissolve 50 mg of D(+)-maltose monohydrate in water to make 100 mL.

Procedure Analyze 20 µL each of the test solution, the control solution, and the standard solution by liquid chromatography using the operating condition given below. A peak is observed at the retention time of D(+)-maltose for the test solution. The peak area is larger than that of the D(+)-maltose peak of the control solution. If the D(+)-maltose peak of the test solution cannot be clearly identified in the chromatogram, deproteinize or desalinize the test solution.

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (8 mm internal diameter and 20–50 cm length).

Column packing material: 6-µm Na-form cation exchange resin for liquid chromatography.

Column temperature: A constant temperature of 40–60°C.

Mobile phase: Water

Flow rate: 0.3–1.0 mL/min. Adjust the retention time of D(+)-maltose monohydrate to about 15 minutes.

α-Glucosyltransferase Treated Stevia

α-グルコシルトランスフェラーゼ処理ステビア

Definition α-Glucosyltransferase Treated Stevia is obtained by glucosylating "Stevia Extract" with α-glucosyltransferase. It consists mainly of α-glucosylated steviol

glycosides.

Content α-Glucosyltransferase Treated Stevia, when calculated on the dried basis, contains not less than 80.0% of the total of the four steviol glycosides (stevioside, rebaudioside A, rebaudioside C, and dulcoside A) and α-glucosylated steviol glycosides (the α-glucosylated compounds of the four steviol glycosides), and not less than 65.0% of the total of the α-glucosylated steviol glycosides.

Description α-Glucosyltransferase Treated Stevia occurs as a white to light yellow powder, or as flakes or granules. It is odorless or has a slight characteristic odor. It has a strong sweet taste.

Identification

(1) Dissolve 0.1 g of α-Glucosyltransferase Treated Stevia in 100 mL of a 7 : 3 mixture of water/acetonitrile, and use this solution as the test solution. Analyze 10 μL each of the test solution and Standard Solution A prepared in Assay by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in the Monographs. More than one peak are observed earlier than the retention time of rebaudioside A.

(2) Analyze 10 μL of Test Solution A prepared in Assay by liquid chromatography using the same operating conditions as for Identification (1). The sum of the areas of the peaks observed earlier than the retention time of rebaudioside A is less than that of the peaks from the test solution of Identification (1). Also at least one or each of the peak areas of stevioside and rebaudioside A is larger than that observed in identification (1).

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method)

(2) Arsenic Not more than 1 μg/g as As (1.5 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 6.0% (105°C, 2 hours).

Residue on Ignition Not more than 1.0%.

Assay The total content of four steviol glycosides and α-glucosylated steviol glycosides (α-glucosylated compounds of the four steviol glycosides) is obtained as the sum of the contents of the four steviol glycosides obtained after glucoamylase treatment and the content of α-glucosyl residues liberated by glucoamylase treatment. The content of the α-glucosylated steviol glycosides (four compounds) is obtained as the abovementioned sum from which the total content of the four steviol glycosides (non-glucosylated) is subtracted.

(i) Four steviol glycosides obtained after glucoamylase treatment

Test Solution A Weigh accurately about 0.1 g of α-Glucosyltransferase Treated Stevia,

dissolve it in 20 mL of water, and add exactly 10 mL of acetate buffer (pH 4.5). To this solution, add 2000 units of glucoamylase and allow to stand at 55°C for about 45 minutes. Heat at 95°C for about 30 minutes, cool to room temperature, and add a 7 : 3 mixture of water/acetonitrile to make exactly 100 mL.

Standard Solution A Weigh accurately about 50 mg each of stevioside for assay and rebaudioside A for assay, both previously dried, dissolve each in a 7 : 3 mixture of water/acetonitrile to make exactly 100 mL.

Procedure For Test Solution A and Standard Solution A, proceed as directed in Assay for Stevia Extract in the Monographs to determine the total content of the four steviol glycosides (stevioside, rebaudioside A, rebaudioside C, and dulcoside A)

(ii) α-Glucosyl residues liberated by glucoamylase treatment

Test Solution B Weigh accurately about 1 g of α-Glucosyltransferase Treated Stevia, and dissolve it in 50 mL of water. Pour this solution into a glass tube (25 mm internal diameter) packed with 50 mL of acrylic acid ester resin or styrene-divinylbenzene resin, allow it to flow through at a rate of 3 mL/minute or less, and wash the resin with 250 mL of water. Then pour 250 mL of 50% (vol) ethanol to allow to flow through at a rate of 3 mL/minute or less. Evaporate the collected eluate to about 100 mL, and add exactly 40 mL of acetate buffer (pH 4.5) and water to make about 180 mL. Allow this solution to stand at 55°C for about 5 minutes, add 20,000 units of glucoamylase, and allow to stand at 55°C for about 45 minutes. Heat at 95°C for about 30 minutes, cool to room temperature, and add water to make exactly 200 mL.

Blank Test Solution Measure exactly 40 mL of acetate buffer (pH 4.5), add water to make about 180 mL, and allow to stand at 55°C for about 5 minutes. To this solution, add 20,000 units of glucoamylase, and allow to stand at 55°C for about 45 minutes. Then, heat the solution at 95°C for about 30 minutes, cool to room temperature, and add water to make exactly 200 mL.

Standard Solutions Weigh accurately about 1g of D(+)-glucose, and dissolve it in water to make exactly 100 mL. Transfer exactly 5 mL, 10 mL, 20 mL, and 30 mL of this solution into separate 100-mL volumetric flasks, and dilute each with water to volume.

Procedure Measure 20 μL of Test Solution B, add exactly 3 mL of color fixing TS for D-glucose determination, and shake. Allow to stand at 37°C for exactly 5 minutes, and cool to room temperature. Measure the absorbance of the resulting solution at a wavelength of 505 nm against the reference solution prepared as follows: Measure 20 μL of water instead of the test solution, and proceed as directed for the test solution. Perform a blank test by measuring the absorbance of the blank test solution in the same manner as for the test solution, and make any necessary correction. Prepare a calibration curve by measuring the absorbance of the four standard solutions in the same manner as for the test solution.

Determine the concentration of D(+)-glucose in Test Solution B from the calibration curve and the corrected absorbance of the test solution, and calculate the amount of α-glucosyl residues liberated by glucoamylase treatment by the formula:

$$\text{Amount (\%) of α-glucosyl residues} = \frac{\text{Concentration (mg/mL) of D(+)-glucose in Test Solution B} \times 200}{\text{Dry basis weight (g) of the sample} \times 1000} \times 0.900 \times 100$$

(iii) Four steviol glycosides (non-glucosylated)

Test Solution C Weigh accurately about 0.5 g of α-Glucosyltransferase Treated Stevia and dissolve it in a 7 : 3 mixture of water/acetonitrile to make exactly 100 mL.

Procedure For Test Solution C and Standard Solution A prepared in (i), proceed as directed in Assay for Stevia Extract in the Monographs to determine the total content of the four steviol glycosides (stevioside, rebaudioside A, rebaudioside C, and dulcoside A).

(iv) Four steviol glycosides and four α-glucosylated steviol glycosides

Calculate the total content of the four steviol glycosides and the α-glucosylated compounds of the four steviol glycosides by the formula:

Total content (%) of the four steviol glycosides and four α-glucosylated steviol glycosides

= Total content (%) of the four steviol glycosides obtained after glucoamylase treatment

+ Content (%) of α-glucosyl residues liberated by glucoamylase treatment

(v) α-glucosylated steviol glycosides

Determine the total content of the α-glucosylated compounds of the four steviol glycosides by the formula:

Total content (%) of α-glucosylated steviol glycosides

= Total content (%) of the four steviol glycosides obtained after glucoamylase treatment

+ Content (%) of α-glucosyl residues liberated by glucoamylase treatment

– Total content (%) of the four steviol glycosides

α-Glucosyltransferase Treated Steviol Glycosides

α-グルコシルトランスフェラーゼ処理ステビオール配糖体

Definition α-Glucosyltransferase Treated Steviol Glycosides are obtained by glucosylating “Steviol Glycosides” with α-glucosyltransferase. They consist mainly of α-glucosylated steviol glycosides.

Content α-Glucosyltransferase Treated Steviol Glycosides, when calculated on the dried basis, contain not less than 95.0% of the total of the nine steviol glycosides (stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside, and steviolbiocide) and α-glucosylated steviol glycosides (α-glucosylated compounds of the nine steviol glycosides), and not less than 80.0% of the total of the α-glucosylated steviol glycosides.

Description α-Glucosyltransferase Treated Steviol Glycosides occur as white powders, flakes, or granules. They are odorless or have a slight characteristic odor. They have a strong sweet taste.

Identification Proceed as directed in Identifications (1) and (2) for α-Glucosyltransferase Treated Stevia.

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1 μg/g as As (1.5 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 6.0% (105°C, 2 hours).

Residue on Ignition Not more than 1.0%.

Assay The total content of nine steviol glycosides and α-glucosylated steviol glycosides (α-glucosylated compounds of the nine steviol glycosides) is obtained as the sum of the contents of the nine steviol glycosides obtained after glucoamylase treatment and the content of α-glucosyl residues liberated by glucoamylase treatment. The content of the α-glucosylated steviol glycosides (nine compounds) is obtained as the abovementioned sum from which the total content of the nine steviol glycoside (non-glucosylated) is subtracted.

(i) Nine steviol glycosides and eight steviol glycosides obtained after glucoamylase treatment

Test Solution A Weigh accurately about 0.1 g of the sample, dissolve it in 20 mL of water, and add exactly 10 mL of acetate buffer (pH 4.5). To this solution, add 2000 units of glucoamylase and incubate at 55°C for about 45 minutes. Heat it at 95°C for about 30 minutes, cool to room temperature, and add a 7:3 mixture of water/acetonitrile to make exactly 100 mL.

Standard Solution A Weigh accurately about 50 mg each of stevioside for assay and rebaudioside A for assay, both previously dried, and dissolve them separately in a 7 : 3 mixture of water/acetonitrile to make exactly 100 mL.

Procedure For Test Solution A and Standard Solution A, proceed as directed in the Assay for Steviol Glysocides in the Monographs to separately determine the total content of the nine steviol glycosides (stevioside, rebaudioside A, rebaudiosideB, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside, and steviolbiocide) and the total content of the eight steviol glycosides (stevioside, rebaudioside A, rebaudioside B,

rebaudioside C, rebaudioside F, dulcoside A, rubusoside, and steviolbiocide).

(ii) α-Glucosyl residues liberated by glucoamylase treatment

Proceed as directed in the Assay for α-Glucosyltransferase Treated Stevia to determine α-glucosyl residues liberated by glucoamylase treatment.

(iii) Nine steviol glycosides

Test Solution C Weigh accurately about 0.5 g of the sample and, dissolve it in a 7 : 3 mixture of water/acetonitrile to make exactly 100 mL.

Procedure For Test Solution C and Standard Solution A prepared in (i), proceed as directed in the Assay for Steviol Glucosides in the Monographs to determine the total content of the eight steviol glycosides (stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside F, dulcoside A, rubusoside, and steviolbiocide). Determine the total content of the nine steviol glycosides(stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside, and steviolbiocide) by the formula:

$$\text{Total content (\%) of the nine steviol glycosides} = \text{Total content (\%) of the eight steviol glycosides} \times \frac{\left[\text{Total content (\%) of nine steviol glycoside obtained after glucoamylase treatment}\right]}{\left[\text{Total content (\%) of nine steviol glycoside obtained after glucoamylase treatment}\right]}$$

(iv) Nine steviol glycosides and nine α-glucosylated steviol glycosides

Calculate the total content of the nine steviol glycosides and the α-glucosylated compounds of the nine steviol glycosides by the formula:

Total content (%) of the nine steviol glycosides and nine α-glucosylated steviol glycosides

= Total content (%) of the nine steviol glycosides obtained after glucoamylase treatment

+ Content (%) of α-glucosyl residues liberated by glucoamylase treatment

(v) Nine α-glucosylated steviol glycosides

Determine the total content of the α-glucosylated compounds of the nine steviol glycosides by the formula:

Total content (%) of α-glucosylated steviol glycosides

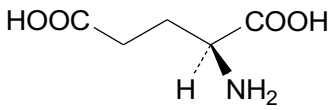
= Total content (%) of the nine steviol glycosides obtained after glucoamylase treatment

+ Content (%) of α-glucosyl residues liberated by glucoamylase treatment

– Total content (%) of the nine steviol glycosides

L-Glutamic Acid

L-グルタミン酸



$C_5H_9NO_4$ Mol. Wt. 147.13

(2*S*)-2-Aminopentanedioic acid [56-86-0]

Content L-Glutamic Acid, when calculated on the dried basis, contains not less than 99.0% of L-glutamic acid ($C_5H_9NO_4$).

Description L-Glutamic Acid occurs as colorless to white crystals or as a white crystalline powder. It has a slight, characteristic and acid taste.

Identification To 5 mL of a solution of L-Glutamic Acid (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

Specific Rotation $[\alpha]_D^{20}$: +31.5 to +32.5° (10 g, hydrochloric acid TS (2 mol/L), 100 mL, on the dried basis).

pH 3.0–3.5 (a saturated solution).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, hydrochloric acid TS (2 mol/L) 10 mL).

(2) Chloride Not more than 0.021% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 2, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.2% (105°C, 3 hours).

Residue on Ignition Not more than 0.2%.

Assay Weigh accurately about 0.2 g of L-Glutamic Acid, dissolve it in 6 mL of formic acid, and proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 14.71 mg of $C_5H_9NO_4$

Glutaminase

グルタミナーゼ

Definition Glutaminase includes enzymes that hydrolyze L-glutamine to produce L-glutamic acid and ammonia. It is derived from the culture of filamentous fungi (limited to species of the genus *Aspergillus*), yeasts (limited to species of the genus *Candida*), or bacteria (limited to *Bacillus amyloliquefaciens, Bacillus circulans,* and *Bacillus subtilis*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Glutaminase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Glutaminase complies with the Glutaminase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Glutaminase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 1.0 g of Glutaminase, add water or acetate buffer (0.01 mol/L, pH 6.0, containing polyoxyethylene(10) octylphenyl ether) to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold or 100-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 2.0 g of L(+)-glutamine, dissolve it in 70 mL of water, and then add 10 mL of acetate buffer (1 mol/L) at pH 6.0 and water to make 100 mL.

Prepare fresh before use.

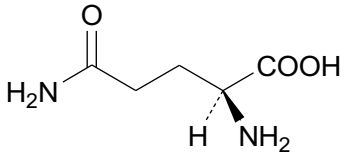
Test Solution Equilibrate 1 mL of the sample solution in a water bath at 37°C for 5 minutes, add 1 mL of the substrate solution, equilibrated at 37°C, and shake immediately. Incubate the mixture at 37°C for 10 minutes, add 1 mL of diluted perchloric acid (83 in 1000), shake, and cool immediately in icy water for at least 1 minute. Prepare the diluted perchloric acid using perchloric acid with a concentration of 60%. To this solution, add 1 mL of sodium hydroxide solution (3 in 100), and shake.

Control Solution To 1 mL of the sample solution, add 1 mL of diluted perchloric acid (83 in 1000), shake, and equilibrate in a water bath at 37°C for 5 minutes. Add 1 mL of the substrate solution, shake, and cool in icy water for at least 1 minute. To this solution, add 1 mL of sodium hydroxide solution (3 in 100), and shake.

Procedure Prepare two test tubes with each containing 3 mL of TS for L-glutamic acid determination. Add 0.2 mL of the test solution into one test tube and 0.2 mL of the control solution into the other. Allow them to stand for 10 minutes. Measure the absorbance of each solution at a wavelength of 600 nm. The absorbance of the solution containing the test solution is higher than that of the solution containing the control solution. The absorbance of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

L-Glutamine

L-グルタミン



$C_5H_{10}N_2O_3$ Mol. Wt.146.14

(2*S*)-2-Amino-4-carbamoylbutanoic acid [56-85-9]

Content L-Glutamine, when calculated on the dried basis, contains 98.0–102.0% of L-glutamine ($C_5H_{10}N_2O_3$).

Description L-Glutamine occurs as white crystals or crystalline powder. It is odorless, and has a very slight characteristic taste.

Identification

(1) To 5 mL of a solution of L-Glutamine (1 in 1000), add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A purple color develops.

(2) Proceed as directed in Identification (2) for L-Asparagine.

Specific Rotation $[\alpha]_D^{20}$: +6.3 to +7.3°.

Weigh accurately about 4 g of L-Glutamine, add water, and dissolve it by warming. Cool rapidly, and add water to make exactly 100 mL. Measure the angular rotation of this solution, and calculate on the dried basis.

pH 4.5–6.0 (1.0 g, water 50 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 50 mL).

(2) Chloride Not more than 0.1% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

Residue on Ignition Not more than 0.1%.

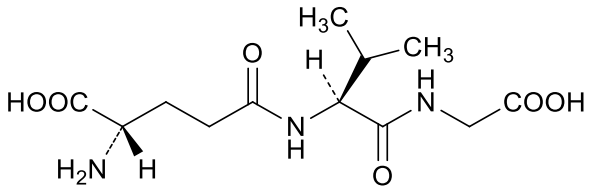
Assay Weigh accurately about 0.3 g of L-Glutamine, and proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 14.61 mg $C_5H_{10}N_2O_3$

Glutamyl-valyl-glycine

L-γ-Glutamyl-L-valyl-glycine

グルタミルバリルグリシン



$C_{12}H_{21}N_3O_6$ Mol. Wt. 303.31

(2*S*)-2-Amino-4-{(1*S*)-1-[(carboxymethyl)carbamoyl]-2-methylpropyl}carbamoylbutanoic acid [38837-70-6]

Content Glutamyl-valyl-glycine, when calculated on the dried basis, contains 95.0–102.0% of glutamyl-valyl-glycine ($C_{12}H_{21}N_3O_6$).

Description Glutamyl-valyl-glycine occurs as a white to light red powder.

Identification Determine the infrared absorption spectrum of Glutamyl-valyl-glycine as directed in the Disk Method under Infrared Spectrophotometry. It exhibits

absorptions at wavenumbers of about 3321 cm^{-1}, 3282 cm^{-1}, 1712 cm^{-1}, 1654 cm^{-1}, 1619 cm^{-1}, and 1541 cm^{-1}.

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method) .

(2) Arsenic Not more than 0.8 μg/g as As (2.5 g, Standard Color: Arsenic Standard Solution 4.0 mL, Apparatus B).

Test Solution Add 20 mL of water to the specified amount of Glutamyl-valyl-glycine, warm it, and sonicate if necessary to dissolve.

Loss on Drying Not more than 1.0% (105°C, 1 hour).

Assay

Test Solution and Standard Solution Weigh accurately about 50 mg each of Glutamyl-valyl-glycine and glutamyl-valyl-glycine for assay, add water to each to make exactly 50 mL. Measure exactly 5 mL each of these solutions, and add water to make exactly 20 ml each.

Procedure Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) for the test solution and the standard solution, and calculate the content by the formula:

$$\text{Content (\%) of Glutamyl-valyl-glycine } (C_{12}H_{21}N_3O_6) = \frac{\text{Dry basis weight (g) of Glutamyl-valyl-glycine for assay}}{\text{Dry basis weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Opereating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 210 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of 30–40°C.

Mobile phase

A: Dissolve 6.8 g of potassium monohydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid.

B: Add 600 mL of acetonitrile to 400 mL of Mobile phase A.

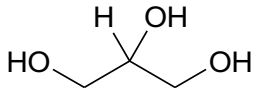
Concentration gradient (A/B): Maintain at 100/0 for 25 minutes, and run a linear gradient from 100/0 to 0/100 in 25 minutes.

Flow rate: 1.0 mL/minute.

Glycerol

Glycerin

グリセリン



$C_3H_8O_3$ Mol. Wt. 92.09

Propane-1,2,3-triol [56-81-5]

Content Glycerol contains not less than 95.0% of glycerol ($C_3H_8O_3$).

Description Glycerol is a colorless, viscous liquid. It is odorless and has a sweet taste.

Identification To 2–3 drops of Glycerol, add 0.5 g of potassium hydrogen sulfate, and heat. An acrolein–like odor is evolved.

Specific Gravity d_{20}^{20}: 1.250–1.264.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (10 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Glycerol, add water to make 100 mL. Use 5 mL of this solution as the test solution.

(3) Chlorinated compounds Not more than 0.003% as Cl.

Test Solution Weigh 5.0 g of Glycerol, transfer into a flask equipped with a reflux condenser, add 15 mL of morpholine, heat, and reflux gently for 3 hours. Cool, rinse the reflux condenser with 10 mL of water, add the rinses to the flask, and acidify the contents with nitric acid. Transfer this solution into a Nessler tube, add 0.5 mL of silver nitrate solution (1 in 50), and add water to make 50 mL.

Procedure The test solution is not more turbid than a control solution prepared as follows: Proceed as directed for the test solution, except for the heating and refluxing operation, using 0.40 mL of 0.01 mol/L hydrochloric acid instead of the sample Glycerol.

(4) Reducing substances Measure 3.0 mL of Glycerol, dissolve it in 5 mL of water, add 0.5 mL of ammonia TS, and warm in a water bath at 60°C for 5 minutes. No yellow color develops. Add 0.5 mL of silver nitrate solution (1 in 10), shake, and allow to stand in a dark place for 5 minutes. The solution is not more turbid than a control solution prepared in the same manner as the sample, using a solution of pyrogallol in glycerol (3 in 100,000) instead of Glycerol.

Residue on Ignition Not more than 0.01% (10 g).

Assay Weigh quickly and accurately about 0.5 g of Glycerol, and add water to make exactly 500 mL. Measure exactly 50 mL of this solution, add about 200 mL of water, and adjust the pH to 7.9 ± 0.1 with diluted sulfuric acid (3 in 1000) or sodium hydroxide solution (1 in 250). Add 50 mL of sodium periodate TS for glycerol, stir gently, cover with a watch glass, and allow to stand in a dark place for 30 minutes. Add 10 mL of a 1:1 mixture of water/ethylene glycol, shake, and allow to stand in a dark place for 20 minutes. Add 5 mL of sodium formate solution (1 in 15), and titrate with 0.1 mol/L sodium hydroxide until the pH becomes 7.9 ± 0.2. Perform a blank test in the same manner. Use only freshly boiled and cooled water for the test.

Each mL of 0.1 mol/L sodium hydroxide = 9.209 mg of $C_3H_8O_3$

Glycerol Esters of Fatty Acids

グリセリン脂肪酸エステル

Definition Glycerol Esters of Fatty Acids are esters of fatty acids and glycerol or polyglycerol and their derivatives. They are categorized into several types: glycerol fatty acid ester, glycerol acetic acid fatty acid ester, glycerol lactic acid fatty acid ester, glycerol citric acid fatty acid ester, glycerol succinic acid fatty acid ester, glycerol diacetyl tartaric acid fatty acid ester, glycerol acetic acid ester, polyglycerol fatty acid ester, and polyglycerol condensed ricinoleic acid ester.

Description Glycerol Esters of Fatty Acids occur as colorless to brown powders, flakes, or granules, as granular or waxy lumps, or as semifluids or liquids. They are odorless or have a characteristic odor.

Identification

(1) To about 5 g of Glycerol Esters of Fatty Acids (1.5 g in case of glycerol acetic acid ester), add 50 mL of 3.5% (w/v) potassium hydroxide–ethanol TS, heat under a reflux condenser in a water bath for 1 hour, and evaporate the ethanol to almost dryness. Add 50 mL of diluted hydrochloric acid (1 in 10), shake well, separate the produced fatty acids by extracting three times with 40 mL of a 7:1 mixture of petroleum ether/2-butanone each time. Stir the aqueous layer well, add sodium hydroxide solution (1 in 9) until it is almost neutral, and concentrate under reduced pressure in a water bath to obtain residue. Use a solution (1 in 10) of the residue in methanol as test solution. Analyze a 5-μL portion of the test solution by thin-layer chromatography, using a 9:1 mixture of methanol/glycerol as the control solution and a 9:1 mixture of acetone/water as the developing solvent. Use a thin layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point 15 cm above the starting line. Air-dry the plate, heat at 110°C for 10 minutes to remove the solvent, and cool. Spray the plate with the thymol–sulfuric acid TS, and heat at 110°C for 20 minutes to develop the color. In the case of glycerol esters, a brown spot is observed at the position corresponding to the spot from the control solution; in the case of polyglycerol ester, a brown spot or a brown band-shaped spot is observed at a position corresponding to or

below the spot from the control solution.

(2) This test applies to esters other than glycerol acetic acid ester. Combine the petroleum ether/2-butanone layers obtained by separation in Identification (1), and evaporate the solvent. An oily substance or white to yellowish white solid remains. Add 5 mL of diethyl ether to 0.1 g of the residue, and shake. It dissolves.

(3) This test applies to esters other than glycerol fatty acid esters and polyglycerol esters.

Test Solution Dissolve 0.1 g of the residue obtained in Identification (1) in 2 mL of sulfuric acid TS (0.005 mol/L).

Standard Solutions Prepare standard solutions by separately dissolving the specified amounts of the substances given below in 2 mL of sulfuric acid TS (0.005 mol/L): 10 mg of acetic acid for glycerol acetic acid fatty acid ester and glycerol acetic acid ester, 20 mg of "Sodium Lactate" for glycerol lactic acid fatty acid ester, 10 mg of citric acid monohydrate for glycerol citric acid fatty acid ester, 10 mg of "Succinic Acid" for glycerol succinic acid fatty acid ester, 10 mg of acetic acid, and 10 mg of L-(+)–tartaric acid for glycerol diacetyl tartaric acid fatty acid ester.

Procedure Analyze 20 μL each of the test solution and the corresponding standard solution by liquid chromatography using the operating conditions given below. The peak of the test solution is observed at the same retention time as that of the standard solution.

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (8 mm internal diameter and 30 cm length).

Column packing material: Strongly acidic cation-exchange resin for liquid chromatography.

Column temperature: 60°C.

Mobile phase: sulfuric acid TS (0.005 mol/L).

Flow rate: 0.7 mL/minute.

(4) In the case of polyglycerol condensed ricinoleic acid ester, combine the petroleum ether/2-butanone layers obtained by separation in Identification (1) above, wash this solution twice with 50 mL of water each time, and dehydrate with sodium sulfate. Filter the dehydrated liquid and remove the solvent by warming under reduced pressure. Weigh accurately about 1 g of the residue, and proceed as directed in the Hydroxyl Value Test in the Fats and Related Substances Tests. Use about 0.5 g of the residue to measure the acid value. The hydroxyl value is 150–170.

Purity

(1) Acid value

Glycerol fatty acid ester: Not more than 6.0 (Fats and Related Substances Tests).

Glycerol acetic acid fatty acid ester: Not more than 6.0 (Fats and Related Substances

Tests).

Glycerol lactic acid fatty acid ester: Not more than 6.0 (Fats and Related Substances Tests).

Glycerol acetic acid ester: Not more than 6.0 (Fats and Related Substances Tests).

Polyglycerol fatty acid ester: Not more than 12 (Fats and Related Substances Tests).

Polyglycerol condensed ricinoleic acid ester: Not more than 12 (Fats and Related Substances Tests).

Glycerol citric acid fatty acid ester: Not more than 100 (Fats and Related Substances Tests).

Glycerol succinic acid fatty acid ester: 60–120 (Fats and Related Substances Tests).

Glycerol diacetyl tartaric acid fatty acid ester: 60–120 (Fats and Related Substances Tests).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

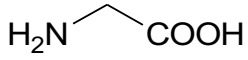
(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Polyoxyethylene Weigh 1.0 g of Glycerol Esters of Fatty Acids, transfer into a 200-mL flask, add 25 mL of 3.5% (w/v) potassium hydroxide–ethanol TS, and heat under a ground-glass reflux condenser on a water bath for 1 hour while shaking. Evaporate the ethanol on a water bath or under reduced pressure until it becomes almost dry, add 20 mL of diluted sulfuric acid (3 in 100), and shake well while warming. Add 15 mL of ammonium thiocyanate–cobalt(II) nitrate TS, shake well, add 10 mL of chloroform, shake again, and allow to stand. The color of the chloroform layer does not change to blue.

Residue on Ignition Not more than 1.5%.

Glycine

グリシン



$C_2H_5NO_2$ Mol. Wt. 75.07

Aminoacetic acid [56-40-6]

Content Glycine, when calculated on the dried basis, contains 98.5–101.5% of glycine ($C_2H_5NO_2$).

Description Glycine occurs as white crystals or crystalline powder having a sweet taste.

Identification

(1) To 5 mL of a solution of Glycine (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) To 5 mL of a solution of Glycine (1 in 10), add 5 drops of diluted hydrochloric acid (1 in 4) and 1 mL of freshly prepared sodium nitrite solution (1 in 10). A colorless gas is evolved. Transfer 5 drops of this solution into a small test tube, boil for a while, and evaporate to dryness on a water bath. After cooling, add 5–6 drops of chromotropic acid TS to the residue, and heat in a water bath for 10 minutes. A deep purple color develops.

pH 5.5–7.0 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.021% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.15 g of Glycine, and proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 7.507 mg of $C_2H_5NO_2$

Grape Seed Extract

ブドウ種子抽出物

Definition Grape Seed Extract is obtained from the seeds of the grapes, *Vitis labrusca* L. or *Vitis vinifera* L., and consists mainly of proanthocyanidin. It may contain dextrin, fructose, or glucose.

Content Grape Seed Extract, when calculated on the dry basis, contains not less than 25% of proanthocyanidin.

Description Grape Seed Extract occurs as a light yellow to deep brown powder.

Identification Mix about 10 mg of Grape Seed Extract well with 10 mL of a 1:1 mixture of water/ethanol (95). To 1 mL of this solution, add 10 mL of a 95:5 mixture of 1-butanol/hydrochloric acid. The resulting solution is colorless to light yellow-brown, which produces a light red to red or red-purple color when heated in a water bath at 95°C or higher.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 8.0% (105°C, 5 hours).

Assay The content of proanthocyanidin is derived from the amounts of total flavanols and total catechins.

(i) Total flavanols

Test Solution Weigh accurately about 0.1 g of Grape Seed Extract, add a 1:1 mixture of water/ethanol (95) to make exactly 100 mL. Refer to this solution as the sample solution. Prepare the sample solution fresh before use. Measure exactly 1.0 mL of the sample solution into a brown test tube, add 6.0 mL of a solution (1 in 25) of vanilline in methanol, and shake well. To the mixture, promptly add 3.0 mL of hydrochloric acid, immediately stopper the test tube tightly, and shake. Allow this solution to stand for 20 to 40 minutes.

Standard Solutions Weigh accurately amounts of (+)-catechin for assay equivalent to about 10 mg, 20 mg, and 30 mg on the anhydrous basis. Add a 1:1 mixture of water/ethanol (95) to make exactly 100 mL of each. Previously, determine the water content of (+)-catechin for assay by the direct titration method or coulometric titration method.

Procedure Measure the absorbance (A_T) of the test solution at a wavelength of 500 nm against a 1:1 mixture of water/ethanol (95) as the reference. Measure the absorbance (A_B) of a solution prepared in the same manner as for the test solution using 1.0 mL of a 1:1 mixture of water/ethanol (95) instead of the sample solution. Measure the absorbance (A_C) of a solution prepared by placing exactly 1.0 mL of the sample solution into a brown test tube, adding 6.0 mL of methanol instead of a solution (1 in 25) of vanilline in methanol, and proceeding as directed for the test solution. Calculate the absorbance (A) corresponding to the total flavanols by the formula:

$$A = A_T - A_B - A_C$$

Prepare a calibration curve as follows: Proceed as directed for the test solution under Procedure using exactly 1.0 mL of each standard solution, and measure the absorbance corresponding to the total flavanols for each solution.

Determine the amount (%) of the total flavanols in the sample on the anhydrous basis from the absorbance (A) and the calibration curve. If the absorbance (A) of the test solution exceeds the range of the calibration curve, dilute the sample solution with a 1:1 mixture of water/ethanol (95) so that it falls within the range. Correct by the dilution factor.

(ii) Total catechins

Test Solution Weigh accurately about 0.1 g of Grape Seed Extract, add dimethyl sulfoxide, dissolve it while stirring to make exactly 10 mL. Refer to this solution as the sample solution. Transfer exactly 0.5 mL of the sample solution into an Erlenmeyer flask, add 10 mL of ethyl acetate, and shake well. Filter the resulting suspension using a glass syringe fitted with a polytetrafluoroethylene membrane filter (0.45-μm pore size), previously washed with 10 mL of ethyl acetate, and collect the filtrate into an eggplant-shaped flask. Wash the Erlenmeyer flask well with 10 mL of ethyl acetate, filter the washings through the membrane filter into the eggplant-shaped flask. Remove the ethyl acetate in the flask under reduced pressure. Add water to the dimethyl sulfoxide remaining in the flask to make exactly 10 mL.

Catechin Standard Solution Weigh accurately about 5 mg of (+)-catechin for assay, add methanol to make exactly 100 mL. Previously, determine the water content of (+)-catechin for assay by the direct titration method or coulometric titration method.

Standard Solutions for Identification Weigh about 2 mg each of (–)-epicatechin, (–)-catechin gallate, and (–)-epigallocatechin gallate, and add methanol separately to make exactly 100 mL of each.

Procedure Analyze 10 μL each of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Measure the peak areas (A_{TC}, A_{TEC}, A_{TCG}, A_{TECG}) of catechin, epicatechin, catechin gallate, and epigallocatechin gallate in the test solution, and also measure the peak area (A_{SC}) for the catechin standard solution. Identify catechin, epicatechin, catechin gallate, and epigallocatechin gallate in the test solution by confirming that the retention times correspond to the main peaks of respective standard solutions. Calculate the amount (%) of the total catechins by the formula:

$$\text{Amount (\%) of the total catechins} = \frac{\left\{ A_{TC} + \frac{A_{TEC}}{0.99} + \frac{442.37}{290.27}\left(\frac{A_{TCG}}{4.03} + \frac{A_{TECG}}{3.58}\right)\right\} \times S_C \times 2}{A_{SC} \times \text{Dry basis weight (mg) of the sample}} \times 100$$

S_C: Weight (mg) of (+)-catechin for assay on the anhydrous basis

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength 280 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C

Mobile phase

A: A 1000:1 mixture of water/formic acid.

B: A 1000:1 mixture of methanol/formic acid.

Concentrate gradient (A/B): Run a linear gradient from 90/10 to 50/50 in 40 minutes.

Flow rate: Adjust the retention time of catechin gallate to about 30 minutes.

Calculate the proanthocyanidin content by the following formula from the total flavanol amount and the total catechin amount determined in (i) and (ii).

Content (%) of proanthocyanidin

= Amount (%) of total flavanols – Amount (%) of total catechin

Grape Skin Extract

Grape Skin Color

ブドウ果皮色素

Definition Grape Skin Extract is obtained from the skins of grapes, *Vitis labrusca* L. or *Vitis vinifera* L., and consists mainly of anthocyanin. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Grape Skin Extract is not less than 50 and is in the range of 90–120% of the labeled value.

Description Grape Skin Extract occurs as a red to dark red powder, as lumps, or as a paste or liquid. It has a slightly characteristic odor.

Identification

(1) Weigh an amount of Grape Skin Extract equivalent to 1 g of grape skin extract with a Color Value 50, and dissolve it in 1000 mL of citrate buffer (pH 3.0). A red to red-purple color develops.

(2) Add sodium hydroxide solution (1 in 25) to the solution obtained in Identification (1) to make alkaline. The solution turns dark green.

(3) A solution of Grape Skin Extract in citrate buffer (pH 3.0) exhibits an absorption maximum at a wavelength of 520–534 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

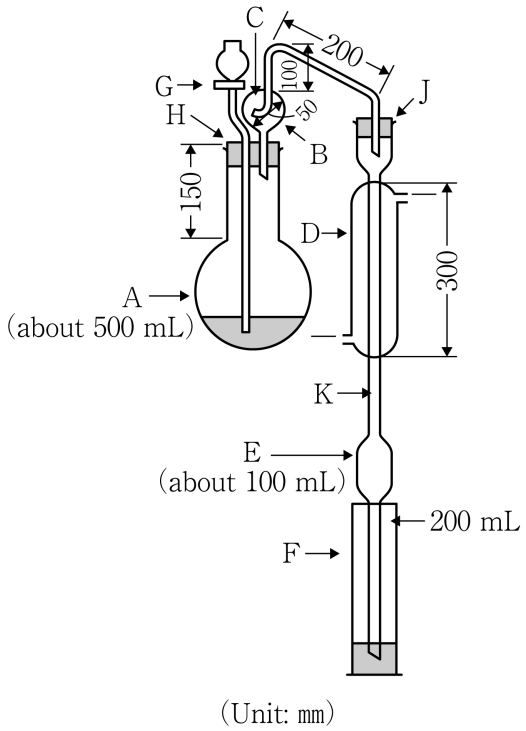
(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Sulfur dioxide Not more than 0.005% per Color Value.

(i) Apparatus Use the apparatus as illustrated in the figure below. Hard glass-made apparatus should be used. Ground-glass may be used for the joint parts.

(ii) Procedure　Weigh accurately 1–3 g of Grape Skin Extract, transfer into a 500 mL-distillation flask A with the Wagner tube, add 100 mL of water, and connect with distillation apparatus. Place 25 mL of a solution of lead(II) acetate trihydrate (1 in 50) in the receiver (measuring cylinder F) as an absorbing solution. In the absorbing solution, immerse the lower end of trap E attached to the condenser. Add 25 mL of diluted phosphoric acid (2 in 7) from a funnel fitted with a stopcock, and distil until the liquid in the receiver reaches 100 mL. Take the lower end of the condenser out of the liquid, rinse the end with a little amount of water into receiver F. To the solution in the receiver add 5 mL of hydrochloric acid, and titrate with 0.005 mol/L iodine (indicator: 1–3 mL of starch TS).

Each mL of 0.005 mol/L iodine = 0.3203 mg of SO_2.



A: Distilling flask

B: Wagner tube with spray trap

C: Small hole

D: Condenser

E: Trap

F: Measuring cylinder

G: Funnel with stopcock

H: Silicon rubber stopper

I: Silicon rubber stopper

J: Silicon rubber tube

Color Value Determination　Proceed as directed under Color Value Determination, using the conditions below.

Operating Conditions

Solvent: Citrate buffer (pH 3.0).

Wavelength: Maximum absorption wavelength of 520–534 nm.

Guar Gum

グァーガム

Definition　Guar Gum is obtained from the seeds of the guar plant *Cyamopsis*

tetragonoloba (L.) Taub. and consists mainly of polysaccharides. It may contain sucrose, glucose, lactose, or dextrin.

Description Guar Gum occurs as a white to slightly yellow-brown powder or as granules. It has slight or no odor.

Identification

(1) Proceed as directed in Identification (1) for Carob Bean Gum, a viscous solution is formed. Heat 100 mL of this solution on the water bath for about 10 minutes, and cool to room temperature, the viscosity of the solution hardly changes after heating.

(2) Proceed as directed in Identification (2) for Carob Bean Gum.

Purity

(1) Protein Not more than 7.0%.

Weigh accurately about 0.15 g of Guar Gum, proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

Each mL of 0.005 mol/L sulfuric acid = 0.8754 mg of protein

(2) Acid-insoluble substances Not more than 7.0%.

Proceed as directed in Purity (4) for Semirefined Carrageenan.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Starch Proceed as directed in Purity (5) for Carob Bean Gum.

(6) 2-Propanol Not more than 1.0%.

Proceed as directed in Purity test (6) for Carob Bean Gum.

Loss on Drying Not more than 14.0% (105°C, 5 hours).

Ash Not more than 1.5% (800°C, 3–4 hours).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid For total plate count and the enumeration of yeasts and molds, prepare as follows: Mix 1 g of Guar Gum with 200 mL of phosphate buffer, 0.1% peptone

solution, or sodium chloride-peptone buffer to disperse uniformly.

Pre-enrichment Culture For the *Escherichia coli* test, prepare as follows: Mix 1 g of Guar Gum with 200 mL of lauryl sulfate broth to disperse uniformly, and incubate at 35 ± 1°C for 48 ± 2 hours. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 1 g of Guar Gum with 200 mL of lactose broth to disperse uniformly, and incubate at 35 ± 1°C for 24 ± 2 hours.

Gum Arabic

Arabic Gum
Acacia Gum

アラビアガム

Definition Gum Arabic is obtained by drying the exudate of *Acacia senegal* (L.) Willd. or *Acacia seyal* Delile or by desalinating the dried exudate. It mainly consists of polysaccharides.

Description Gum Arabic occurs as white to light yellow granules or powder, or as light yellow to brown lumps. It has little or no odor.

Identification

(1) To 1 g of Gum Arabic, previously powdered, add 2 mL of water. It almost dissolves, and the solution is acidic.

(2) To 10 mL of a solution of Gum Arabic (1 in 50), add 0.2 mL of basic lead(II) acetate TS (1 in 50). A white fibrous precipitate is formed immediately.

(3) Dissolve 5 g of Gum Arabic in 100 mL of water. If it is turbid, filter with suction through a 0.45-μm membrane filter or centrifuge to remove the contaminants. Measure the specific rotation of the resulting liquid. Solutions from *Acacia senegal* are levorotatory, and solutions from *Acacia seyal* are dextrorotatory.

Purity

(1) Hydrochloric-acid insoluble substances Not more than 1.0%.

Dry a glass filter (1G3) for 30 minutes at 110°C, cool in a desiccator, and weigh the glass filter accurately. Weigh accurately 5 g of a powder of Gum Arabic, dissolve it in about 100 mL of water, add 10 mL of diluted hydrochloric acid (1 in 4), heat gradually and boil for 15 minutes. Filter the solution while warm, using the glass filter described above under the reduced pressure. Wash the precipitate well by warm water, dry 2 hours at 105°C together with the glass filter. Cool in a desiccator, weigh accurately.

(2) Lead Not more than 2 μg/g as Pb(2.0 g, Method 1, Control Solution: Lead

Standard Solution 4.0 mL, Flame Method).

(3) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Tannin-bearing gums　To 10 mL of a solution of Gum Arabic (1 in 50), add 3 drops of a solution of iron(III) chloride hexahydrate (1 in 10). No dark green color develops.

(5) Starch or Dextrin　To 0.2 g of Gum Arabic , add 10 mL of water, and boil. Cool, and add 1 drop of Iodine TS. No blue or red-purple color develops.

Loss on Drying　Not more than 17.0% (105°C, 6 hours).

Ash　Not more than 4.0%.

Acid-insoluble Ash　Not more than 0.5%.

Microbial Limits　Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 10,000 per gram.

Yeasts and molds: Not more than 1000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid　Prepare as directed in Method 1 for total plate count and the enumeration of yeasts and molds

Pre-enrichment Culture　Prepare as directed in Method 1, respectively, for the *Escherichia coli* test and the *Salmonella* test.

Gum Ghatti

ガティガム

[9000-28-6]

Definition　Gum Ghatti is obtained from the exudate of the ghatti tree (*Anogeissus latifolia* (Roxb. ex DC.) Wall. ex Bedd.) and consists mainly of polysaccharides.

Description　Gum Ghatti occurs as a gray to reddish-gray granules or powder, or as light to dark brown lumps. It is almost odorless.

Identification

(1) To 1 g of Gum Ghatti, add 5 mL of water. A viscous liquid is formed.

(2) To 5 mL of a solution of Gum Ghatti (1 in 100), add 0.2 mL of lead(II) acetate TS (basic) (1 in 5). A little or no precipitate is formed, but a milk white precipitate is formed

on the addition of 0.5 mL of Ammonia TS.

(3) A solution of Gum Ghatti (1 in 50) filtered through diatomaceous earth for chromatography is levorotatory.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 14.0% (105°C, 5 hours).

Ash Not more than 6.0%.

Acid-insoluble Ash Not more than 1.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 10,000 per gram.

Yeasts and molds: Not more than 1000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 1 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture Prepare as directed in Method 1, respectively, for the *Escherichia coli* test and the *Salmonella* test.

Haematococcus Algae Color

ヘマトコッカス藻色素

Definition Haematococcus Algae Color is obtained from the whole algae of *Haematococcus* spp. and consists mainly of astaxanthins. It may contain edible fats or oils.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Haematococcus Algae Color is not less than 600 and is in the range of 95–115% of the labeled value.

Description Haematococcus Algae Color occurs as orange to dark brown lumps, paste, or liquid having a slight characteristic odor.

Identification

(1) Weigh an amount of Haematococcus Algae Color equivalent to 0.4 g of haematococcus algae color with a Color Value 600, and dissolve it in 100 mL of acetone. An orange-yellow to red-orange color develops.

(2) To 0.1 mL of the solution prepared in Identification (1), add 5 mL of sulfuric acid. A blue-green to dark blue color develops.

(3) A solution of Haematococcus Algae Color in acetone exhibits an absorption maximum at a wavelength of 460–480 nm.

(4) Weigh an amount of Haematococcus Algae Color equivalent to 0.4 g of haematococcus algae color with a Color Value 600, dissolve it in 10 mL of acetone, and use this solution as the test solution. Analyze a 5-μL portion of the test solution by thin-layer chromatography using a 7:3 mixture of hexane/acetone as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry. A red-orange spot is observed at an R_f value of about 0.4–0.6. Its color immediately disappears when the spot is sprayed with sodium nitrite solution (1 in 20) followed by sulfuric acid TS (0.5 mol/L).

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination, according to the following operating conditions.

Operating Conditions

Solvent: Acetone.

Wavelength: Maximum absorption wavelength of 460–480 nm.

Heme Iron

ヘム鉄

Definition Heme Iron is obtained by isolation from protease-treated hemoglobin. It consists mainly of heme iron.

Content Heme Iron, when calculated on the dried basis, contains 1.0–2.6% of iron (Fe

= 55.85).

Description Heme Iron occurs as a brown to blackish brown powder or as granules. It is odorless or has a little characteristic odor.

Identification

(1) To 10 mg of Heme Iron, add 1 mL of diluted sulfuric acid (1 in 20) and 1 mL of nitric acid to dissolve, and evaporate on a water bath to dryness. Dissolve the residue in 10 mL of diluted hydrochloric acid (1 in 2), and add ammonium thiocyanate solution (2 in 25). A red color develops.

(2) Dissolve 5 mg of Heme Iron in 10 mL of pyridine-sodium hydroxide TS, and add 0.1 g of sodium dithionite. A red color develops.

(3) To 10 mg of Heme Iron, add 5 mL of nitric acid, and heat. A yellow color develops. After cooling, make the solution alkaline with ammonia solution. The color changes to orange-yellow.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Dryness Not more than 5.0% (105°C, 5 hours).

Residue on Ignition Not more than 12.0%.

Assay Weigh accurately about 10 g of Heme Iron, moisten with 5 mL of diluted sulfuric acid (1 in 20) and 5 mL of nitric acid, heat carefully until white fumes are no longer evolved, and incinerate at 450–550°C. To the residue, add 10 mL of diluted hydrochloric acid (1 in 2), boil until there is almost no insoluble matter, add 20 mL of water, and filter. Wash the insoluble residue on the filter paper, and combine the washing with the filtrate. To the combined solution, add water to make exactly 100 mL. Measure exactly 25 mL of the resulting solution into a stoppered flask, add 2 g of potassium iodine, immediately stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 mL of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Separately, perform a blank test to make any necessary correction, and calculate on the dried basis.

Each mL of 0.1 mol/L sodium thiosulfate = 5.585 mg of Fe

Hemicellulase

ヘミセルラーゼ

Definition Hemicellulase includes enzymes that hydrolyze hemicellulose. It is derived from the culture of basidiomycetes (limited to *Pycnoporus coccineus* and species of the genus *Corticium*), filamentous fungi (limited to *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus usamii*, *Humicola insolens*, *Penicillium multicolor*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, and *Trichoderma viride*), actinomycetes (limited to *Streptomyces avermitilis*, *Streptomyces thermoviolaceus*, and *Streptomyces violaceoruber*), or bacteria (limited to *Bacillus halodurans*, *Bacillus mannanilyticus*, and *Bacillus subtilis*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Hemicellulase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Hemicellulase complies with the Hemicellulase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Hemicellulase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of Hemicellulase, add water or acetate buffer (0.01

mol/L) at pH 4.5 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Suspend 1.0 g of xylan or arabino xylan in 20 mL of water, add 5 mL of sodium hydroxide TS (1 mol/L), and stir for 5 minutes. Then stir the mixture for an additional 30 minutes while warming at 75°C. After cooling, add 20 mL of acetic acid–sodium hydroxide buffer (1 mol/L) at pH 4.5, adjust the pH to 4.5 with hydrochloric acid TS (1 mol/L), add water to make 100 mL. Prepare fresh before use.

Test Solution Transfer 1.9 mL of the substrate solution into a test tube, equilibrate at 45°C for 5 minutes, add 0.1 mL of the sample solution, and shake immediately. Incubate the mixture at 40°C for 10 minutes. To this solution, add 4 mL of 3,5-dinitrosalicylic acid–lactose TS, and mix. Heat the mixture in a water bath for 15 minutes with the mouth of the test tube covered with a glass bead. After cooling, centrifuge at 3000 rpm for 10 minutes. Use the supernatant.

Control Solution Transfer 0.1 mL of the sample solution into a test tube, add 4 mL of 3,5-dinitrosalicylic acid–lactose TS, and mix. Add 1.9 mL of the substrate solution. Heat the mixture in a water bath for 15 minutes with the mouth of the test tube covered with a glass bead, and proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 540 nm. The absorbance value of the test solution is higher than that of control solution.

Method 2

Sample Solution Weigh 0.50 g of Hemicellulase, add water, acetate buffer (0.01 mol/L) at pH 4.5, or acetate buffer (0.02 mol/L) at pH 4.5 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 0.50 g of xylan or arabino xylan, add about 30 mL of water, heat while stirring, and keep boiling for 3 minutes after the start of boiling. After cooling, add 50 mL of water.

Test Solution Transfer 1 mL of the substrate solution into a test tube, add 3 mL of acetate buffer (pH 4.5), and equilibrate the mixture at 40°C for 10 minutes. Add 1 mL of the sample solution, and shake. Incubate the mixture at 40°C for 30 minutes. To this solution, add 2 mL of Somogyi TS (III), and mix. Stopper the test tube, heat it in a water bath for 20 minutes, and cool immediately. Add 1 mL of Nelson TS, and shake well until the red precipitate disappears. Allow to stand at room temperature for about 20 minutes, add water to make 25 mL. Centrifuge at 3000 rpm at 25°C for 10 minutes. Use the supernatant.

Control Solution Transfer 1 mL of the substrate solution into a test tube, add 3 mL of acetate buffer (pH 4.5) and 2 mL of Somogyi TS (III), and mix. Add 1 mL of the sample solution. Stopper the test tube, heat it in a water bath for 20 minutes, and cool immediately. Proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a

wavelength of 500 nm. The absorbance value of the test solution is higher than that of control solution.

Method 3

Sample Solution Weigh 0.50 g of Hemicellulase, add water to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, 10,000-fold, or 100,000-fold dilution by adding water to the resulting solution.

Substrate Solution Add gradually 0.66 g of locust bean gum (for enzyme) into about 240 mL of water while stirring to suspend it, and add water to make 300 mL. Heat the suspension in a water bath for 3 minutes or more to dissolve it. If any insoluble matter is found, filter through a filter paper (5A) using a small amount of diatomaceous earth (flux-calcined) as filtration aid, and use the filtrate. Prepare fresh before use.

Test Solution Transfer 10 mL of the substrate solution into a test tube, add 1 mL of acetic acid–sodium hydroxide buffer (0.5 mol/L) at pH 4.5, and shake. Equilibrate the mixture at 40°C for 5 minutes, add 1 mL of the sample solution, and shake.

Control Solution Proceed as directed for the test solution using water instead of the sample solution.

Procedure Immediately after preparation, transfer the test solution into a Cannon-Fenske viscometer (No. 200), warmed at 40°C for 5 minutes. Measure the flow times (F_2, F_4, and F_6) at 40°C at 2, 4, and 6 minutes after the addition of the sample solution. Similarly, measure the flow time (F_0) of the control solution at 40°C. F_2, F_4, and F_6 are all smaller than F_0.

Method 4

Sample Solution Weigh 50 mg of Hemicellulase, add CHES buffer (0.1 mol/L) at pH 9.0 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Add 60 mL of water to 0.5 g of locust bean gum (for enzyme), stir the solution for 15 minutes, and warm at 80°C for 15 minutes. After cooling, add 1 mL of hydrochloric acid TS (1 mol/L), stir for 15 minutes, and add 20 mL of CHES buffer (0.5 mol/L) at pH 9.0. Adjust the pH to 9.0 with sodium hydroxide TS (1 mol/L), and add water to make 100 mL. Centrifuge this solution at 3000 rpm for 10 minutes, and use the supernatant.

Test Solution Transfer 0.9 mL of the substrate solution into a test tube, equilibrate at 40°C for 3 minutes, add 0.1 mL of the sample solution, and shake immediately. Incubate the mixture at 40°C for 10 minutes, add 3 mL of 3,5-dinitrosalicylic acid–phenol TS, and shake immediately. Heat this solution for 5 minutes in a water bath that enables the test tube to be immersed to a depth of at least 10 cm, and cool immediately in icy water. Allow to stand in running water for 10 minutes, and add 16 mL of water.

Control Solution Transfer 0.1 mL of the sample solution into a test tube, add 3 mL of 3,5-dinitrosalicylic acid–phenol TS, then add 0.9 mL of the substrate solution, and immediately shake. Heat in a water bath for 5 minutes, and cool immediately in icy

water. Allow this solution to stand in running water for 10 minutes, and add 16 mL of water.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 550 nm. The absorbance value of the test solution is higher than that of control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 5

Sample Solution Weigh 0.50 g of Hemicellulase, add water to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water to the resulting solution.

Substrate Solution Add 50 mL of water to 0.20 g of locust bean gum (for enzyme), and stir the solution for 15 minutes. Adjust the pH to 5.0 with sodium hydroxide TS (0.2 mol/L), and add 2 mL of acetate buffer (1 mol/L) at pH 5.0 and water to make 100 mL. Centrifuge this solution at 3000 rpm for 10 minutes, and use the supernatant. Prepare fresh before use.

Test Solution Transfer 4 mL of the substrate solution into a 50-mL Nessler tube, equilibrate the solution at 40°C for 10 minutes, add 1 mL of the sample solution, shake, and incubate this solution at 40°C for 10 minutes. Add 2 mL of Somogyi TS (I), and shake. Loosely stopper the Nessler tube, and heat in a water bath for 30 minutes. After cooling, add 2 mL of Nelson TS, shake, allow to stand for 20 minutes, and add water to make 50 mL. Centrifuge this solution at 3000 rpm for 10 minutes, and use the supernatant.

Control Solution Transfer 1 mL of the sample solution into a 50-mL Nessler tube, add 2 mL of Somogyi TS (I), and shake. Add 4 mL of the substrate solution, and shake. Stopper loosely, and heat for 30 minute in a water bath. Proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 750 nm. The absorbance value of the test solution is higher than that of control solution.

Method 6

Sample Solution Weigh 0.50 g of Hemicellulase, add water to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water to the resulting solution.

Substrate Solution Add 100 mL of water to 1.0 g of galactan or arabinogalactan, and stir for 15 minutes to suspend it. Warm the suspension at 60°C for 30 minutes while stirring to dissolve it. Prepare fresh before use. When using arabinan as the substrate, add 100 mL of water to 1.0 g of arabinan, and stir for 20 minutes to dissolve it. Prepare fresh before use.

Test Solution To 0.1 mL of the substrate solution, add 0.09 mL of phosphate buffer (0.2 mol/L) at pH 7.0 and 0.01 mL of the sample solution, and shake immediately. Incubate this solution at 40°C for 15 minutes, add 0.4 mL of 3,5-dinitrosalicylic acid–potassium sodium tartrate TS, and mix. Heat in a water bath for 5 minutes, allow to cool,

and add 1.8 mL of water.

Control Solution To 0.01 mL of the sample solution, add 0.09 mL of phosphate buffer (0.2 mol/L) at pH 7.0 and add 0.4 mL of 3,5-dinitrosalicylic acid–potassium sodium tartrate TS, immediately shake, and 0.1 mL of the substrate solution, and mix. Heat this solution in a water bath for 5 minutes, allow to cool, and add 1.8 mL of water.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 525 nm. The absorbance value of the test solution is higher than that of control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 7

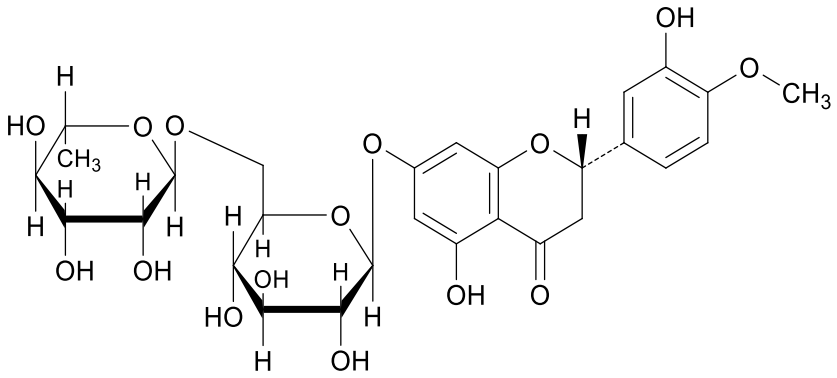
Proceed as directed in Method 1 of the Xylanase Activity Test in the monograph of Xylanase.

Method 8

Proceed as directed in Method 2 of the Xylanase Activity Test in the monograph of Xylanase.

Hesperidin

ヘスペリジン



$C_{28}H_{34}O_{15}$ Mol. Wt. 610.57

(2*S*)-5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxochroman-7-yl

α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside [520-26-3]

Definition Hesperidin is obtained from the peels, juice, or seeds of citrus fruits and consists mainly of hesperidin.

Content Hesperidin, when dried, contains 95.0–110.0% of hesperidin ($C_{28}H_{34}O_{15}$).

Description Hesperidin occurs as colorless to light yellow crystals or as a white to light yellowish white crystalline powder. It is odorless or has a slight characteristic odor.

Identification

(1) Hesperidin dissolves in sodium hydroxide solution (1 in 20) or in heated sodium carbonate solution (1 in 100) producing a reddish yellow to red-yellow color.

(2) To 0.1 g of Hesperidin, add 5 mL of ethanol (95) and 1 mL of sodium hydroxide solution (1 in 20), boil the mixture for 2–3 minutes, cool it, and filter. The filtrate is yellow.

(3) To 0.1 g of Hesperidin, add 5 mL of ethanol (95), heat the mixture, cool it, and filter. To 4 ml of the filtrate, add 1 mL of hydrochloric acid and 10 mg of magnesium powder, and allow it to stand. A red color is produced.

(4) To 0.1 g of Hesperidin, add 10 mL of diluted hydrochloric acid (1 in 9), boil the mixture for 5 minutes, cool it, and filter. Neutralize the filtrate with sodium hydroxide solution (1 in 4), and heat with 4 mL of Fehling's TS. A red precipitate is produced.

Purity

(1) Clarity of solution Reddish yellow to yellow-brown and almost clear. (1.0 g, sodium hydroxide TS (1 mol/L) 10 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 5.0% (105°C, 3 hours).

Residue on Ignition Not more than 0.3%.

Assay Weigh accurately about 50 mg of Hesperidin, previously dried, dissolve it in potassium hydroxide TS (0.01 mol/L) to make exactly 100 mL. To exactly 2 mL of this solution, add potassium hydroxide TS (0.01 mol/L) to make exactly 50 mL. Measure the absorbance (A) of the resulting solution at a wavelength of 286 nm, and calculate the content by the formula:

$$\text{Content (\%) of hesperidin } (C_{28}H_{34}O_{15}) = \frac{A}{\text{Weight (g) of the sample}} \times \frac{25}{251.7} \times 100$$

Hesperidinase

ヘスペリジナーゼ

Definition Hesperidinase includes enzymes that degrade hesperidin. It is derived from the culture of filamentous fungi (limited to *Penicillium decumbens* and species of the genus *Aspergillus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Hesperidinase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Hesperidinase complies with the Hesperidinase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Hesperidinase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 1.0 g of Hesperidinase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 0.125 g of hesperidin, dissolve it by adding 25 mL of water and 12.5 mL of sodium hydroxide TS (1 mol/L), and then add 37.5 mL of McIlvain buffer at pH 3.8. Adjust the pH to 3.8 with hydrochloric acid TS (1 mol/L), and add McIlvain buffer at pH 3.8 to make 100 mL. Use the resulting solution within 60 minutes after preparation.

Test Solution Equilibrate 4 mL of substrate solution at 40°C for 10–15 minutes, add 1 mL of the sample solution, and shake. Incubate the mixture at 40°C for 30 minutes, add 5 mL of Somogyi TS (II), and heat in a water bath for 20 minutes. After cooling, add 1.5 mL of potassium iodide solution (1 in 200) and 3 mL of sulfuric acid TS (1 mol/L), and shake well.

Control Solution Proceed as directed for the test solution using 1 mL of water instead of the sample solution.

Procedure Titrate the test solution and the control solution with 0.01 mol/L sodium thiosulfate (indicator: 3 drops of soluble starch). The amount of 0.01 mol/L sodium thiosulfate consumed by the test solution is less than that of 0.01 mol/L sodium

thiosulfate consumed by the control solution. The endpoint is when the blue color of the solution disappears.

Hexane

ヘキサン

Definition Hexane mainly contains *n*-hexane (C_6H_{14}).

Description Hexane is a clear, colorless, volatile liquid having a characteristic odor.

Refractive Index n_D^{20}: 1.374–1.386

Specific Gravity d_{20}^{20}: 0.659–0.687.

Purity

(1) Distillate Not less than 95% (vol) of Hexane is distilled at 64–70°C (Method 2).

(2) Sulfur compounds Measure 5 mL of Hexane, add 5 mL of silver nitrate–ammonia TS, and heat at 60°C for 5 minutes with protection from light, while shaking well. No brown color develops.

(3) Lead Not more than 1 μg/g as Pb (4.0 g, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Test Solution Heat the specified amount of Hexane to evaporate to dryness. To the residue, add 1 mL of sulfuric acid, and heat until white fumes are no longer evolved. Heat in an electric muffle at 500°C for 3 hours. To the residue, add 10 mL of diluted hydrochloric acid (1 in 4), and heat again to evaporate to dryness. Dissolve the residue in 10 mL of diluted nitric acid (1 in 150) to make 10 mL.

Control Solution To the specified amount of Lead Standard Solution, exactly measured, add diluted nitric acid (1 in 150) to make exactly 10 mL.

(4) Benzene Not more than 0.25% (vol) as benzene.

Test Solution Measure exactly 50 mL of Hexane, add exactly 50 mL of the internal standard solution, and mix. As the internal standard solution, use a solution prepared by adding hexane for ultraviolet absorption spectrum measurement to 0.5 mL of 4-methyl-2-pentanone to make 100 mL.

Control Solution Measure exactly 0.25 mL of benzene, and add hexane for ultraviolet absorption spectrum measurement to make exactly 100 mL. To exactly 50 mL of this solution, add exactly 50 mL of the internal standard solution, and mix.

Procedure Analyze the test solution and the control solution by gas chromatography using the conditions given below. The ratio (Q_T) of the height of the peak corresponding to benzene to the height of the peak of the 4-methyl-2-pentanone in the test solution does not exceed the ratio (Q_S) of the height of the peak corresponding to benzene to the height of the peak of 4-methyl-2-pentanone in the control solution.

Operating Conditions

Detector: Flame ionization detector.

Column: A stainless-steel or glass tube (3–4 mm internal diameter and 2–3 m length).

Column packing material

Liquid phase: 10% polyethylene glycol 6000 of the amount of support.

Support: 177- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of 50–70°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the benzene peak appears about 5 minutes after the injection of the sample.

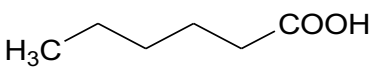
(5) Residue on evaporation Not more than 0.0013% (w/v). Measure 150 mL of Hexane, evaporate carefully, and dry at 105°C for 2 hours. Weigh the residue.

(6) Readily carbonizable substances Perform the test with 5 mL of Hexane, using Matching Fluid B.

Hexanoic Acid

Caproic Acid

ヘキサン酸



$C_6H_{12}O_2$ Mol. Wt. 116.16

Hexanoic acid [142-62-1]

Content Hexanoic Acid contains not less than 98.0% of hexanoic acid ($C_6H_{12}O_2$).

Description Hexanoic Acid is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Hexanoic Acid as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.415–1.418.

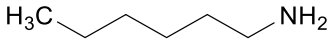
Specific Gravity d_{25}^{25}: 0.923–0.928.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas

Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Hexylamine

ヘキシルアミン



$C_6H_{15}N$ Mol. Wt. 101.19

Hexan-1-amine [111-26-2]

Content Hexylamine contains not less than 95.0% of hexylamine ($C_6H_{15}N$).

Description Hexylamine occurs as a colorless to yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Hexylamine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive index n_D^{20}:1.415–1.421

Specific gravity d_{25}^{25}: 0.761–0.767

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operating conditions (2) except for the column. Use a fused silica tube (0.25–0.53 mm internal diameter and 30–60 m length) coated with a 0.25–1 μm thick layer of dimethylpolysiloxane for gas chromatography.

Higher Fatty Acid (Behenic Acid)

高級脂肪酸（ベヘニン酸）

Definition Higher Fatty Acid (Behenic Acid) is one of the higher fatty acids (substances that are obtained by hydrolyzing animal or vegetable fats or oils or hydrogenated animal or vegetable fats or oils). It consists mainly of behenic acid.

Content Higher Fatty Acid (Behenic Acid) contains not less than 50.0% of behenic acid ($C_{22}H_{44}O_2$ = 340.58).

Description Higher Fatty Acid (Behenic Acid) occurs as white to bright grayish yellow flakes, powder, or granules or as a waxy lump.

Identification Analyze the test solution and the standard solution prepared as directed

in the Assay by gas chromatography using the operating conditions specified in the Assay. The retention time of the main peak from the test solution corresponds to that of the peak of methyl behenate from the standard solution.

Iodine Value Not more than 3.0.

Test Solution Weigh accurately about 1 g of Higher Fatty Acid (Behenic Acid) into a 500-mL stoppered flask, and dissolve it in 20 mL of a 1 : 1 mixture of cyclohexane/chloroform.

Procedure Proceed as directed under Iodine Value in the Fats and Related Substances Tests.

Purity

(1) Acid value 160–175.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.1%.

Assay

Test Solution Weigh 20 mg of Higher Fatty Acid (Behenic Acid), and transfer it into a small conical flask fitted with a reflux condenser. Add 5 mL of boron trifluoride–methanol TS, shake, and heat for about 10 minutes until it dissolves. Add through the condenser 4 mL of hexane, and heat for 10 minutes. After cooling, add 20 mL of a saturate solution of sodium chloride, shake, and leave to stand to allow the solution to separate into two layers. Transfer 2 mL of the separated hexane layer into another flask through about 0.2 g of sodium sulfate previously washed with hexane. To 1 mL of this solution, add hexane to make 10 mL, and shake.

Standard Solution Dissolve 10 mg of methyl behenate by adding 5 mL of hexane.

Procedure Analyze 1 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area (A_A) of methyl behenate and the total peak area (A_T) of all the fatty acid esters (total peak area of all peaks detected) in the test solution, and calculate the behenic acid content in the fatty acid fraction of the sample by the following formula. Identification of methyl behenate should be confirmed by comparing the retention time of the main peak from the test solution with that of methyl behenate in the standard solution. The chromatography should be continued for 1.5 times the retention time of methyl behenate, and the main solvent peak should be excluded from the measurement.

$$\text{Content (\%) of behenic acid} = \frac{A_A}{A_T} \times 100$$

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 50 m length) coated with a 0.2-μm thick layer of 100% cyanopropylpolysiloxane for gas chromatography.

Column temperature: 180°C.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: A constant rate of about 1.0 mL/min.

Injection method: Splitless.

Higher Fatty Acid (Capric Acid)

高級脂肪酸（カプリン酸）

Definition Higher Fatty Acid (Capric Acid) is one of the higher fatty acids (substances that are obtained by hydrolyzing animal or vegetable fats or oils or hydrogenated animal or vegetable fats or oils). It consists mainly of capric acid.

Content Higher Fatty Acid (Capric Acid) contains not less than 50.0% of capric acid ($C_{10}H_{20}O_2$ = 172.26).

Description Higher Fatty Acid (Capric Acid) occurs as white to bright grayish yellow flakes, powder, or granules or as a waxy lump.

Identification Analyze the test solution and the standard solution prepared as directed in the Assay by gas chromatography using the operating conditions specified in the Assay. The retention time of the main peak from the test solution corresponds to that of the peak of methyl caprate from the standard solution.

Iodine Value Not more than 0.5.

Purity

(1) Acid value 321–333.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic

Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.1%.

Assay

Test Solution Weigh 20 mg of Higher Fatty Acid (Capric Acid), and transfer it into a small conical flask fitted with a reflux condenser. Add 5 mL of boron trifluoride–methanol TS, shake, and heat for about 10 minutes until it dissolves. Add through the condenser 4 mL of hexane, and heat for 10 minutes. After cooling, add 20 mL of a saturate solution of sodium chloride, shake, and leave to stand to allow the solution to separate into two layers. Transfer 2 mL of the separated hexane layer into another flask through about 0.2 g of sodium sulfate previously washed with hexane. To 1 mL of this solution, add hexane to make 10 mL, and shake.

Standard Solution Dissolve 10 mg of methyl caprate by adding 5 mL of hexane.

Procedure Analyze 1 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area (A_A) of methyl caprate and the total peak area (A_T) of all the fatty acid esters (total peak area of all peaks detected) in the test solution, and calculate the capric acid content in the fatty acid fraction of the sample by the following formula. Identification of methyl caprate should be confirmed by comparing the retention time of the main peak from the test solution with that of methyl caprate in the standard solution. The chromatography should be continued for 1.5 times the retention time of methyl caprate, and the main solvent peak should be excluded from the measurement.

$$\text{Content (\%) of capric acid} = \frac{A_A}{A_T} \times 100$$

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 50 m length) coated with a 0.2-μm thick layer of 100% cyanopropylpolysiloxane for gas chromatography.

Column temperature: 180°C.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: A constant rate of about 1.0 mL/min.

Injection method: Splitless.

Higher Fatty Acid (Caprylic Acid)

高級脂肪酸（カプリル酸）

Definition Higher Fatty Acid (Caprylic Acid) is one of the higher fatty acids (substances that are obtained by hydrolyzing animal or vegetable fats or oils or hydrogenated animal or vegetable fats or oils). It consists mainly of caprylic acid.

Content Higher Fatty Acid (Caprylic Acid) contains not less than 50.0% of caprylic acid ($C_8H_{16}O_2$ = 144.21).

Description Higher Fatty Acid (Caprylic Acid) occurs as a colorless to light yellow liquid or a white to bright grayish yellow paste.

Identification Analyze the test solution and the standard solution prepared as directed in the Assay by gas chromatography using the operating conditions specified in the Assay. The retention time of the main peak from the test solution corresponds to that of the peak of methyl caprylate from the standard solution.

Iodine Value Not more than 0.5.

Purity

(1) Acid value 380−395.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.1%.

Assay

Test Solution Weigh 20 mg of Higher Fatty Acid (Caprylic Acid), and transfer it into a small conical flask fitted with a reflux condenser. Add 5 mL of boron trifluoride−methanol TS, shake, and heat for about 10 minutes until it dissolves. Add through the condenser 4 mL of hexane, and heat for 10 minutes. After cooling, add 20 mL of a saturate solution of sodium chloride, shake, and leave to stand to allow the solution to separate into two layers. Transfer 2 mL of the separated hexane layer into another flask through about 0.2 g of sodium sulfate previously washed with hexane. To 1 mL of this solution, add hexane to make 10 mL, and shake.

Standard Solution Dissolve 10 mg of methyl caprylate by adding 5 mL of hexane.

Procedure Analyze 1 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area (A_A) of methyl caprylate and the total peak area (A_T) of all the fatty acid esters (total peak area of all peaks detected) in the test solution, and calculate the caprylic acid

content in the fatty acid fraction of the sample by the following formula. Identification of methyl caprylate should be confirmed by comparing the retention time of the main peak from the test solution with that of methyl caprylate in the standard solution. The chromatography should be continued for 1.5 times the retention time of methyl caprylate, and the main solvent peak should be excluded from the measurement.

$$\text{Content (\%) of caprylic acid} = \frac{A_A}{A_T} \times 100$$

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 50 m length) coated with a 0.2-μm thick layer of 100% cyanopropylpolysiloxane for gas chromatography.

Column temperature: 180°C.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: A constant rate of about 1.0 mL/min.

Injection method: Splitless.

Higher Fatty Acid (Lauric Acid)

高級脂肪酸（ラウリン酸）

Definition Higher Fatty Acid (Lauric Acid) is one of the higher fatty acids (substances that are obtained by hydrolyzing animal or vegetable fats or oils or hydrogenated animal or vegetable fats or oils). It consists mainly of lauric acid.

Content Higher Fatty Acid (Lauric Acid) contains not less than 50.0% of lauric acid ($C_{12}H_{24}O_2$ = 200.32).

Description Higher Fatty Acid (Lauric Acid) occurs as white to bright grayish yellow flakes, powder, or granules or as a waxy lump.

Identification Analyze the test solution and the standard solution prepared as directed in the Assay by gas chromatography using the operating conditions specified in the Assay. The retention time of the main peak from the test solution corresponds to that of the peak of methyl laurate from the standard solution.

Iodine Value Not more than 1.0.

Purity

(1) Acid value 275–285.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.1%.

Assay

Test Solution Weigh 20 mg of Higher Fatty Acid (Lauric Acid), and transfer it into a small conical flask fitted with a reflux condenser. Add 5 mL of boron trifluoride–methanol TS, shake, and heat for about 10 minutes until it dissolves. Add through the condenser 4 mL of hexane, and heat for 10 minutes. After cooling, add 20 mL of a saturate solution of sodium chloride, shake, and leave to stand to allow the solution to separate into two layers. Transfer 2 mL of the separated hexane layer into another flask through about 0.2 g of sodium sulfate previously washed with hexane. To 1 mL of this solution, add hexane to make 10 mL, and shake.

Standard Solution Dissolve 10 mg of methyl laurate by adding 5 mL of hexane.

Procedure Analyze 1 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area (A_A) of methyl laurate and the total peak area (A_T) of all the fatty acid esters (total peak area of all peaks detected) in the test solution, and calculate the lauric acid content in the fatty acid fraction of the sample by the following formula. Identification of methyl laurate should be confirmed by comparing the retention time of the main peak from the test solution with that of methyl laurate in the standard solution. The chromatography should be continued for 1.5 times the retention time of methyl laurate, and the main solvent peak should be excluded from the measurement.

$$\text{Content (\%) of lauric acid} = \frac{A_A}{A_T} \times 100$$

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 50 m length) coated with a 0.2-μm thick layer of 100% cyanopropylpolysiloxane for gas chromatography.

Column temperature: 180°C.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: A constant rate of about 1.0 mL/min.

Injection method: Splitless.

Higher Fatty Acid (Myristic Acid)

高級脂肪酸（ミリスチン酸）

Definition Higher Fatty Acid (Myristic Acid) is one of the higher fatty acids (substances that are obtained by hydrolyzing animal or vegetable fats or oils or hydrogenated animal or vegetable fats or oils). It consists mainly of myristic acid.

Content Higher Fatty Acid (Myristic Acid) contains not less than 50.0% of myristic acid ($C_{14}H_{28}O_2$ = 228.38).

Description Higher Fatty Acid (Myristic Acid) occurs as white to bright grayish yellow flakes, powder, or granules or as a waxy lump.

Identification Analyze the test solution and the standard solution prepared as directed in the Assay by gas chromatography using the operating conditions specified in the Assay. The retention time of the main peak from the test solution corresponds to that of the peak of methyl myristate from the standard solution.

Iodine Value Not more than 1.0.

Purity

(1) Acid value 240–250.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.1%.

Assay

Test Solution Weigh 20 mg of Higher Fatty Acid (Myristic Acid), and transfer it into a small conical flask fitted with a reflux condenser. Add 5 mL of boron trifluoride–methanol TS, shake, and heat for about 10 minutes until it dissolves. Add through the condenser 4 mL of hexane, and heat for 10 minutes. After cooling, add 20 mL of a saturate solution of sodium chloride, shake, and leave to stand to allow the solution to separate into two layers. Transfer 2 mL of the separated hexane layer into another flask through about 0.2 g of sodium sulfate previously washed with hexane. To 1 mL of this solution, add hexane to make 10 mL, and shake.

Standard Solution Dissolve 10 mg of methyl myristate by adding 5 mL of hexane.

Procedure Analyze 1 μL each of the test solution and the standard solution by gas

chromatography using the operating conditions given below. Determine the peak area (A_A) of methyl myristate and the total peak area (A_T) of all the fatty acid esters (total peak area of all peaks detected) in the test solution, and calculate the myristic acid content in the fatty acid fraction of the sample by the following formula. Identification of methyl myristate should be confirmed by comparing the retention time of the main peak from the test solution with that of methyl myristate in the standard solution. The chromatography should be continued for 1.5 times the retention time of methyl myristate, and the main solvent peak should be excluded from the measurement.

$$\text{Content (\%) of myristic acid} = \frac{A_A}{A_T} \times 100$$

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 50 m length) coated with a 0.2-μm thick layer of 100% cyanopropylpolysiloxane for gas chromatography.

Column temperature: 180°C.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: A constant rate of about 1.0 mL/min.

Injection method: Splitless.

Higher Fatty Acid (Palmitic Acid)

高級脂肪酸（パルミチン酸）

Definition Higher Fatty Acid (Palmitic Acid) is one of the higher fatty acids (substances that are obtained by hydrolyzing animal or vegetable fats or oils or hydrogenated animal or vegetable fats or oils). It consists mainly of palmitic acid.

Content Higher Fatty Acid (Palmitic Acid) contains not less than 50.0% of palmitic acid ($C_{16}H_{32}O_2$ = 256.42).

Description Higher Fatty Acid (Palmitic Acid) occurs as white to bright grayish yellow flakes, powder, or granules or as a waxy lump.

Identification Analyze the test solution and the standard solution prepared as directed in the Assay by gas chromatography using the operating conditions specified in the Assay. The retention time of the main peak from the test solution corresponds to that of the

peak of methyl palmitate from the standard solution.

Iodine Value Not more than 2.0.

Test Solution Weigh accurately about 1 g of Higher Fatty Acid (Palmitic Acid) into a 500-mL stoppered flask, and dissolve it in 20 mL of a 1 : 1 mixture of cyclohexane/chloroform.

Procedure Proceed as directed under Iodine Value in the Fats and Related Substances Tests.

Purity

(1) Acid value 212–222.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.1%.

Assay

Test Solution Weigh 20 mg of Higher Fatty Acid (Palmitic Acid), and transfer it into a small conical flask fitted with a reflux condenser. Add 5 mL of boron trifluoride–methanol TS, shake, and heat for about 10 minutes until it dissolves. Add through the condenser 4 mL of hexane, and heat for 10 minutes. After cooling, add 20 mL of a saturate solution of sodium chloride, shake, and leave to stand to allow the solution to separate into two layers. Transfer 2 mL of the separated hexane layer into another flask through about 0.2 g of sodium sulfate previously washed with hexane. To 1 mL of this solution, add hexane to make 10 mL, and shake.

Standard Solution Dissolve 10 mg of methyl palmitate by adding 5 mL of hexane.

Procedure Analyze 1 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area (A_A) of methyl palmitate and the total peak area (A_T) of all the fatty acid esters (total peak area of all peaks detected) in the test solution, and calculate the palmitic acid content in the fatty acid fraction of the sample by the following formula. Identification of methyl palmitate should be confirmed by comparing the retention time of the main peak from the test solution with that of methyl palmitate in the standard solution. The chromatography should be continued for 1.5 times the retention time of methyl palmitate, and the main solvent peak should be excluded from the measurement.

$$\text{Content (\%) of palmitic acid} = \frac{A_A}{A_T} \times 100$$

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 50 m length) coated with a 0.2-μm thick layer of 100% cyanopropylpolysiloxane for gas chromatography.

Column temperature: 180°C.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: A constant rate of about 1.0 mL/min.

Injection method: Splitless.

Higher Fatty Acid (Stearic Acid)

高級脂肪酸（ステアリン酸）

Definition Higher Fatty Acid (Stearic Acid) is one of the higher fatty acids (substances that are obtained by hydrolyzing animal or vegetable fats or oils or hydrogenated animal or vegetable fats or oils). It consists mainly of stearic acid.

Content Higher Fatty Acid (stearic Acid) contains not less than 50.0% of stearic acid ($C_{18}H_{36}O_2$ = 284.48).

Description Higher Fatty Acid (Stearic Acid) occurs as white to bright grayish yellow flakes, powder, or granules or as a waxy lump.

Identification Analyze the test solution and the standard solution prepared as directed in the Assay by gas chromatography using the operating conditions specified in the Assay. The retention time of the main peak from the test solution corresponds to that of the peak of methyl stearate from the standard solution.

Iodine Value Not more than 4.0.

Test Solution Weigh accurately about 1 g of Higher Fatty Acid (Stearic Acid) into a 500-mL stoppered flask, and dissolve it in 20 mL of a 1 : 1 mixture of cyclohexane/chloroform.

Procedure Proceed as directed under Iodine Value in the Fats and Related Substances Tests.

Purity

(1) Acid value 194–210.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.1%.

Assay

Test Solution Weigh 20 mg of Higher Fatty Acid (Stearic Acid), and transfer it into a small conical flask fitted with a reflux condenser. Add 5 mL of boron trifluoride–methanol TS, shake, and heat for about 10 minutes until it dissolves. Add through the condenser 4 mL of hexane, and heat for 10 minutes. After cooling, add 20 mL of a saturate solution of sodium chloride, shake, and leave to stand to allow the solution to separate into two layers. Transfer 2 mL of the separated hexane layer into another flask through about 0.2 g of sodium sulfate previously washed with hexane. To 1 mL of this solution, add hexane to make 10 mL, and shake.

Standard Solution Dissolve 10 mg of methyl stearate by adding 5 mL of hexane.

Procedure Analyze 1 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area (A_A) of methyl stearate and the total peak area (A_T) of all the fatty acid esters (total peak area of all peaks detected) in the test solution, and calculate the stearic acid content in the fatty acid fraction of the sample by the following formula. Identification of methyl stearate should be confirmed by comparing the retention time of the main peak from the test solution with that of methyl stearate in the standard solution. The chromatography should be continued for 1.5 times the retention time of methyl stearate, and the main solvent peak should be excluded from the measurement.

$$\text{Content (\%) of stearic acid} = \frac{A_A}{A_T} \times 100$$

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 50 m length) coated with a 0.2-μm thick layer of 100% cyanopropylpolysiloxane for gas chromatography.

Column temperature: 180°C.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: A constant rate of about 1.0 mL/min.

Injection method: Splitless.

High-Test Hypochlorite

高度サラシ粉

Content High-Test Hypochlorite contains not less than 60.0% of available chlorine.

Description High-Test Hypochlorite occurs as a white to whitish powder or as granules. It has an odor of chlorine.

Identification

(1) To 0.5 g of High-Test Hypochlorite, add 5 mL of water, shake, and dip a litmus paper (red) into the solution. Its color changes to blue and then fades.

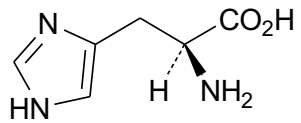
(2) To 0.1 g of High-Test Hypochlorite, add 2 mL of diluted acetic acid (1 in 4). It dissolves with evolution of gas. To the solution, add 5 mL of water, and filter. The solution responds to all the tests for Calcium Salt in the Qualitative Tests.

Assay Weigh accurately an amount of High-Test Hypochlorite equivalent to 0.7–1.3 g of available chlorine. Add about 50 mL of water, grind well in a mortar, and add water to make exactly 500 mL. Shake well, measure exactly 50 mL of the solution, and add 2 g of potassium iodide and 10 mL of diluted acetic acid (1 in 2). Stopper immediately, allow to stand in a dark place for 15 minutes, and titrate liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 3.545 mg of Cl

L-Histidine

L-ヒスチジン



$C_6H_9N_3O_2$ Mol. Wt. 155.15

(2*S*)-2-Amino-3-(1*H*-imidazol-4-yl)propanoic acid [71-00-1]

Content L-Histidine, when calculated on the dried basis, contains 98.0–102.0% of L-histidine ($C_6H_9N_3O_2$).

Description L-Histidine occurs as white crystals or crystalline powder. It is odorless,

and has a slight bitter taste.

Identification

(1) To 5 mL of a solution of L-Histidine (1 in 1000), add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A purple color develops.

(2) To 5 mL of a solution of L-Histidine (1 in 100), add 2 mL of bromine TS. A yellow color develops. When gently heated, the solution turns colorless then reddish brown, and forms a blackish precipitate.

Specific Rotation $[\alpha]_D^{20}$: +11.5 to +13.5° (11 g, hydrochloric acid TS (6 mol/L), 100 mL, on the dried basis).

pH 7.0–8.5 (1.0 g, water 50 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 40 mL).

(2) Chloride Not more than 0.1% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method)

(4) Arsenic Not more than 3 μg/g as As (0.5 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

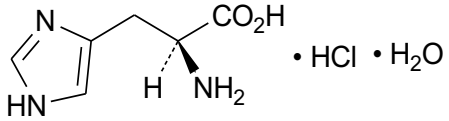
Residue on Ignition Not more than 0.2%.

Assay Weigh accurately about 0.15 g of L-Histidine, and proceed as directed in the Assay for L-Asparagine. The endpoint is when the color of the solution changes to blue.

Each mL of 0.1 mol/L perchloric acid = 15.52 mg of $C_6H_9N_3O_2$

L-Histidine Monohydrochloride

L-ヒスチジン塩酸塩



$C_6H_9N_3O_2 \cdot HCl \cdot H_2O$ Mol. Wt. 209.63

(2*S*)-2-Amino-3-(1*H*-imidazol-4-yl)propanoic acid monohydrochloride monohydrate [7048-02-4]

Content L-Histidine Monohydrochloride, when dried, contains not less than 98.0% of L-histidine monohydrochloride ($C_6H_9N_3O_2 \cdot HCl \cdot H_2O$).

Description L-Histidine Monohydrochloride occurs as white crystals or crystalline powder. It is odorless and has a bitter and slight acid taste.

Identification

(1) To 5 mL of a solution of L-Histidine Monohydrochloride (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) To 5 mL of a solution of L-Histidine Monohydrochloride (1 in 100), add 2 mL of bromine TS. A yellow color develops. When heated gently, the solution turns colorless then red-brown, and forms a blackish precipitate.

(3) To a solution of L-Histidine Monohydrochloride (1 in 10), add sodium hydroxide solution (1 in 5) to make alkaline. The resulting solution is levorotatory. When acidified with hydrochloric acid, it is dextrorotatory.

(4) L-Histidine Monohydrochloride responds to all the tests for Chloride in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +8.5 to +10.5° (5.5 g, hydrochloric acid TS (6 mol/L), 50 mL, on the dried basis).

pH 3.5–4.5 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 10 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

Residue on Ignition Not more than 0.1% .

Assay Weigh accurately about 0.1 g of L-Histidine Monohydrochloride, previously dried, and dissolve it in 2 mL of formic acid. Add exactly 15 mL of 0.1 mol/L perchloric acid, and heat on a water bath for 30 minutes. After cooling, add acetic acid to make 60 mL, and titrate the excess perchloric acid with 0.1 mol/L sodium acetate. The endpoint is usually confirmed by a potentiometer. When 1 mL of crystal violet–acetic acid TS is used as the indicator, the endpoint is when the color of the solution changes from yellow through yellow-green to blue-green. Perform a blank test in the same manner.

Each mL of 0.1 mol/L perchloric acid = 10.48 mg of $C_6H_9N_3O_2 \cdot HCl \cdot H_2O$

Hydrochloric Acid

塩酸

Hydrochloric acid [7647-01-0]

Content Hydrochloric Acid contains 90–120% of the labeled content of hydrogen chloride (HCl = 36.46).

Description Hydrochloric Acid is a colorless to light yellow liquid having a pungent odor.

Identification

(1) A solution of Hydrochloric Acid (1 in 100) is strongly acidic.

(2) Hydrochloric Acid responds to all the tests for Chloride in the Qualitative Tests.

Purity

(1) Sulfate Not more than 0.48% (w/v) as SO_4.

Sample Solution Measure 1.0 mL of Hydrochloric Acid, and add water to make 100 mL. Measure 5.0 mL of this solution, add 20 mL of water, and neutralize with ammonia TS.

Control Solution 0.50 mL of 0.005 mol/L sulfuric acid.

(2) Lead Not more than 1 μg/mL as Pb (4.0 mL, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Test Solution Measure exactly the specified amount of Hydrochloric Acid, evaporate it to dryness. To the residue, add a small amount of diluted nitric acid (1 in 100), and warm. After cooling, add diluted nitric acid (1 in 100) to make exactly 10 mL.

Control Solution Measure exactly the specified amount of Lead Standard Solution, add diluted nitric acid (1 in 100) to make exactly 10 mL.

(3) Iron Not more than 30 μg/mL as Fe (1.0 mL, Method 1, Control Solution: Iron Standard Solution 3.0 mL).

(4) Arsenic Not more than 1.5 μg/mL as As (1.0 mL, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.02% (100 g).

Assay Weigh accurately a ground-glass stoppered flask containing 20 mL of water, add about 3 mL of Hydrochloric Acid, and weigh accurately again. Add 25 mL of water, and titrate with 1 mol/L sodium hydroxide (indicator: 3–5 drops of bromothymol blue TS).

Each mL of 1 mol/L sodium hydroxide = 36.46 mg of HCl

Hydrogen Peroxide

過酸化水素

Hydrogen peroxide [7722-84-1]

Content Hydrogen Peroxide contains 35.0–36.0% of hydrogen peroxide (H_2O_2 = 34.01).

Description Hydrogen Peroxide is a colorless, clear liquid. It is odorless or has a slight odor.

Identification

(1) To 1 mL of a dilute solution of Hydrogen Peroxide (1 in 10), add 5 mL of diluted sulfuric acid (1 in 20) and 1 mL of potassium permanganate solution (1 in 300). The solution effervesces, and the color disappears.

(2) Hydrogen Peroxide responds to all the tests for Peroxide in the Qualitative Tests.

Purity

(1) Free acid Measure exactly 3 mL of Hydrogen Peroxide, add 50 mL of freshly boiled and cooled water and 2 drops of methyl red TS, and titrate with 0.02 mol/L sodium hydroxide. The consumed volume is not more than 1.0 mL.

(2) Phosphate Not more than 62.5 μg/mL as PO_4.

Test Solution Measure exactly 8 mL of Hydrogen Peroxide, add 10 mL of water and 3 mL of hydrochloric acid, and evaporate to dryness while gradually heating on a water bath. Dissolve the residue in about 30 mL of warm water, cool, and add water to make 50 mL. Transfer exactly 5 mL of this solution into a Nessler tube.

Procedure To the test solution, add 4 mL of diluted sulfuric acid (1 in 6) and 1 mL of a solution of hexaammonium heptamolybdate tetrahydrate(1 in 20), shake well, and allow to stand for 3 minutes. Add 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, shake, warm in a water bath at 60°C for 10 minutes, and cool with running water. The blue color of the test solution is not deeper than that of a control solution prepared as follows: Place 5.0 mL of Phosphate Standard Solution into a Nessler tube, and treat in the same manner as the test solution.

(3) Lead Not more than 4 μg/mL as Pb (1.0 mL, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Test Solution To the specified amount of Hydrogen Peroxide, add 10 mL of water, warm gently, add about one-quarter volume of hydrochloric acid, and evaporate to dryness. To the residue, add a small amount of diluted nitric acid (1 in 100), and warm for 5 minutes. After cooling, add diluted nitric acid (1 in 100) to make exactly 10 mL.

Control Solution Measure exactly the specified amount of Lead Standard Solution, and add diluted nitric acid (1 in 100) to make exactly 10 mL.

(4) Arsenic Not more than 3 μg/mL as As (0.50 mL, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Hydrogen Peroxide, add water to make 10 mL, and transfer the solution in small portions into a platinum crucible. Evaporate to dryness by heating gradually on a water bath, and dissolve the residue by adding a small amount of water.

(5) Residue on evaporation Not more than 0.030% (w/v).

Measure 10 mL of Hydrogen Peroxide, add about 20 mL of water, and transfer the solution in small portions into a platinum crucible. Evaporate to dryness while gradually heating on a water bath, and dry the residue at 105°C for 1 hour. Weigh the residue.

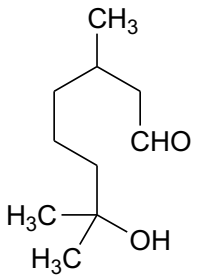
Assay Weigh accurately about 1 g of Hydrogen Peroxide, and add water to make exactly

250 mL. Measure exactly 25 mL of this solution, add 10 mL of diluted sulfuric acid (1 in 20), and titrate with 0.02 mol/L potassium permanganate.

Each mL of 0.02 mol/L potassium permanganate = 1.701 mg of H_2O_2

Hydroxycitronellal

ヒドロキシシトロネラール



$C_{10}H_{20}O_2$ Mol. Wt. 172.26

7-Hydroxy-3,7-dimethyloctanal [107-75-5]

Content Hydroxycitronellal contains not less than 95.0% of hydroxycitronellal ($C_{10}H_{20}O_2$).

Description Hydroxycitronellal is a colorless to light yellow, clear liquid having a lily of the valley-like odor.

Identification Determine the absorption spectrum of Hydroxycitronellal as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.447–1.450.

Specific Gravity d_{25}^{25}: 0.918–0.923.

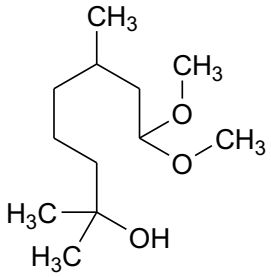
Purity Acid value Not more than 5.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Hydroxycitronellal Dimethylacetal

Hydroxycitronellal Dimethyl Acetal
1,1-Dimethoxy-3,7-dimethyloctan-7-ol

ヒドロキシシトロネラールジメチルアセタール



$C_{12}H_{26}O_3$ Mol. Wt. 218.33

8,8-Dimethoxy-2,6-dimethyloctan-2-ol [141-92-4]

Content Hydroxycitronellal Dimethylacetal contains not less than 95.0% of hydroxycitronellal dimethylacetal ($C_{12}H_{26}O_3$).

Description Hydroxycitronellal Dimethylacetal is a colorless to light yellow, clear liquid having a weak, lily of the valley-like odor.

Identification Determine the absorption spectrum of Hydroxycitronellal Dimethylacetal as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.441–1.444.

Specific Gravity d_{20}^{20}: 0.928–0.934.

Purity

(1) Acid value Not more than 1.0 (Flavoring Substances Tests).

(2) Clarity of solution Clear (2.0 mL, 50% (vol) ethanol 4.0 mL).

(3) Hydroxycitronellal Weigh accurately about 5 g of Hydroxycitronellal Dimethylacetal, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, allow the mixture to stand for 1 hour before titrating. The volume of 0.5 mol/L hydrochloric acid consumed per 1 g of the sample is not more than 0.60 mL.

Assay Weigh accurately about 1.5 g of Hydroxycitronellal Dimethylacetal, and proceed as directed in Method 1 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, boil the mixture for 5 minutes before titrating. Calculate the content by the formula:

$$\text{Content (\%) of hydroxycitronellal dimethylaceta } (C_{12}H_{26}O_3) = \frac{(a - b) \times 109.2}{1000} \times 100$$

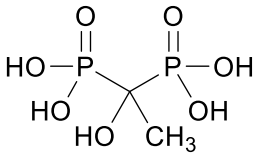
a = the volume (mL) of 0.5 mol/L ethanolic potassium hydroxide consumed per 1 g of the sample,

b = the volume (mL) of 0.5 mol/L hydrochloric acid consumed per 1 g of the sample obtained in Purity (3).

1-Hydroxyethylidene-1,1-diphosphonic Acid

HEDP

1-ヒドロキシエチリデン-1,1-ジホスホン酸



$C_2H_8O_7P_2$ Mol. Wt. 206.03

(1-Hydroxyethane-1,1-diyl)diphosphonic acid [2809-21-4]

Content 1-Hydroxyethylidene-1,1-diphosphonic Acid contains 58.0–62.0% of 1-hydroxyethylidene-1,1-diphosphonic acid ($C_2H_8O_7P_2$).

Description 1-Hydroxyethylidene-1,1-diphosphonic Acid is a colorless to light yellow, clear liquid.

pH Not more than 2.0 (1.0 g, water 100 mL).

Specific Gravity d_{20}^{20}: 1.430–1.471.

Purity

(1) Chlorides Not more than 0.004% as Cl.

Weigh accurately about 25 g of 1-Hydroxyethylidene-1,1-diphosphonic Acid, add about 50 mL of water and 3 mL of nitric acid. Titrate the resulting solution with 0.005 mol/L silver nitrate. Confirm the endpoint by a potentiometer. Use a glass electrode and a silver-silver chloride electrode as the indication and reference electrodes, respectively. Record the amount (a mL) of the silver nitrate solution consumed at the endpoint to determine the amount of chlorides by the following formula. If more than one inflection point exists, the endpoint is the last inflection point.

$$\text{Amount (\%) of chlorides (Cl)} = \frac{a \times 0.005 \times 3.545}{\text{Weight (g) of the sample}}$$

(2) Phosphorous acid Not more than 4.0% as H_3PO_3.

Weigh accurately about 1.5 g of 1-Hydroxyethylidene-1,1-diphosphonic Acid in an iodine flask, add 20 mL of water and 50 mL of phosphate buffer (pH 7.3), and adjust the pH of the mixture to 7.3 with sodium hydroxide solution (1 in 2). Add exactly 25 mL of 0.05 mol/L iodine, immediately stopper tightly, and allow to stand for 15 minutes at a dark place. Add 5 mL of acetic acid, and titrate the excess iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint, when the color of solution changes to light yellow. The endpoint is when the blue color produced disappears. Separately, perform a blank test make any necessary correction.

Each mL of 0.05 mol/L iodine = 4.10 mg of H_3PO_3.

(3) Lead Not more than 5 μg/g as Pb (0.80 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Iron Not more than 10 μg/g as Fe.

Sample Solution Weigh accurately about 0.2 g of 1-Hydroxyethylidene-1,1-diphosphonic Acid in an appropriate container, and add 5 mL of nitric acid. Incinerate the sample in microwave digestion equipment at 230°C. After cooling, transfer the residue in a 50-mL volumetric flask, and make up with water to volume.

Test Solution and Standard Solutions To an appropriate amount of Iron Standard Solution, exactly measured, add diluted nitric acid (1 in 10) to prepare 5 standard stock solutions containing iron (Fe = 55.85) at the concentrations of 10, 25, 50, 100, and 200 ng per mL. Measure 10 mL each of the sample solution and the standard stock solutions in separate flasks, and add 40 μl of the internal standard to each flask to prepare the test solution and the standard solutions, respectively. Prepare the internal standard as follows: To 1.0 mL of Yttrium Standard Stock Solution, add diluted nitric acid (1 in 10) to make 100 mL.

Procedure Proceed as directed in the Internal Standard Method under Inductively Coupled Plasma-Atomic Emission Spectrometry to measure emissions of the test solution and the standard solutions, and prepare a calibration curve. Determine the concentration (ng/mL) of iron in the test solution from the calibration curve, and calculate the iron amount (μg/g) by the formula:

$$\text{Amount (μg/g) of iron (Fe)} = \frac{\text{Iron concentration (ng/mL) in the test solution}}{\text{Weight (g) of the sample} \times 20}$$

(5) Arsenic Not more than 5 μg/g as As (0.30 g, Method 1, Standard Color: Arsenic Standard solution 3.0 mL, Apparatus B).

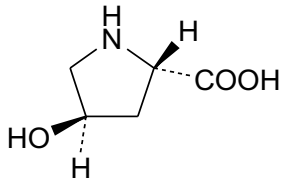
Assay Dissolve about 3 g of 1-Hydroxyethylidene-1,1-diphosphonic Acid, accurately weighed, in 150 mL of water. Titrate this solution with 1 mol/L sodium hydroxide using a potentiometer while stirring. The endpoint is the second inflection point. Record the consumption (a mL) of the sodium hydroxide solution at the endpoint, and calculate the content of 1-hydroxyethylidene-1,1-diphosphonic acid ($C_2H_8O_7P_2$) by the formula:

Content (%) of 1-hydroxyethylidene-1,1-diphosphonic acid ($C_2H_8O_7P_2$)

$$= \frac{a \times 206.0}{\text{Weight (g) of the sample} \times 30} - \text{amount (\%) of phosphorous acid} \times 1.675$$

L-Hydroxyproline

L-ヒドロキシプロリン



$C_5H_9NO_3$ Mol. Wt. 131.13

(2*S*,4*R*)-4-Hydroxypyrrolidine-2-carboxylic acid [51-35-4]

Content L-Hydroxyproline, when calculated on the dried basis, contains 98.0–102.0% of L-hydroxyproline ($C_5H_9NO_3$).

Description L-Hydroxyproline occurs as white crystals or crystalline powder. It is odorless or has a very slight characteristic odor. It has a very slight sweet taste.

Identification To 5 mL of solution of L-Hydroxyproline (1 in 1000), add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A yellow color develops.

Specific Rotation $[\alpha]_D^{20}$: –74.0 to –77.0° (4 g, water, 100 mL, on the dried basis)

pH 5.0–6.5 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.1% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As_2O_3 (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

Residue on Ignition Not more than 0.2%.

Assay Weigh accurately about 0.3 g of L-Hydroxyproline, and proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 13.11 mg of $C_5H_9NO_3$

Hydroxypropyl Cellulose

ヒドロキシプロピルセルロース

2-Hydroxypropyl ether of cellulose [9004-64-2]

Definition Hydroxypropyl Cellulose is hydroxypropyl ether of cellulose.

Content Hydroxypropyl Cellulose, when dried, contains not more than 80.5% of the hydroxypropoxy group ($-OC_3H_6OH$ = 75.09).

Description Hydroxypropyl Cellulose occurs as a white to yellowish white powder or as granules. It is odorless. When water is added, it swells and produces a clear or slightly turbid, viscous liquid.

Identification

(1) Vigorously shake a solution of Hydroxypropyl Cellulose (1 in 1000). Effervescence occurs.

(2) To 5 mL of a solution of Hydroxypropyl Cellulose (1 in 500), add 5 mL of a solution of copper(II) sulfate pentahydrate (1 in 20). No precipitate is produced.

pH 5.0–8.0 (1.0 g, water 100 mL).

Purity

(1) Propylene chlorohydrin Not more than 1.0 μg/g.

Test Solution Weigh 1.0 g of Hydroxypropyl Cellulose, add exactly 5 mL of diethyl ether, and stopper. Perform ultrasonic extraction for 10 minutes. Centrifuge the mixture, and use the supernatant for the test solution.

Standard Solution Weigh 30 mg of propylene chlorohydrin, add diethyl ether to make exactly 100 mL. To exactly 1 mL of the solution, add diethyl ether to make exactly 50 mL. Next, to exactly 1 mL of the diluted solution, add diethyl ether to make exactly 20 mL.

Procedure Analyze 1 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Measure the peak area of propylene chlorohydrin for each solution. The peak area for the test solution is not larger than that for the standard solution.

Operating Conditions

Detector: Flame ionization detector.

Detector temperature: 230°C.

Column: A fused silica tube (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of polyethylene glycol for gas chromatography.

Column temperature: Maintain the temperature at 40°C for 2 minutes, raise at a

rate of 5°C/minute to 80°C, and maintain at 80°C for 8 minutes. Then raise the temperature up to 230°C at a rate of 25°C/minute, and maintain for 5 minutes.

Injection port temperature: 150°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the peak of propylene chlorohydrin appears 15 minute after injection.

Injection method: Splitless.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Loss on Drying Not more than 5.0% (105°C, 4 hours).

Residue on Ignition Not more than 0.5%.

Assay

(i) Apparatus *Reaction flask* A 5-mL screw-cap pressure-tight glass bottle having an inverted conical bottom, a 20 mm external diameter, 50 mm high bottle-neck, and 2 mL capacity at the height of about 30 mm. The cap is made of heat-resistant resin and equipped with a fluoroplastic inside stopper or sealer. Confirm that the contents will not leak when heated.

Heater A 60–80 mm-thick, square-shaped aluminum block with holes 20.6 mm in diameter and 32 mm in depth that is capable of maintaining the inside temperature within ± 1°C.

(ii) Method

Test Solution Weigh accurately about 65 mg of Hydroxypropyl Cellulose, previously dried, transfer it to the reaction flask, add 65 mg of adipic acid, 2.0 mL of the internal standard solution, and 2.0 mL of hydriodic acid. Stopper the flask tightly, and weigh the reaction flask with the mixture accurately. Use a solution (1 in 25) of octane in *o*-xylene as the internal standard solution. Shake the flask for 30 seconds, heat on the heater at 150°C for 30 minute with repeated shaking at 5 minute intervals, and continue heating for an additional 30 minutes. Allow to cool, and then again weigh the flask accurately. Confirm that the weight loss is not more than 10 mg, and use the upper layer of the mixture in the flask as the test solution.

Standard Solution Place 65 mg of adipic acid, 2.0 mL of the internal standard solution, and 2.0 mL of iodine hydriodic acid in another reaction flask. Stopper the flask tightly, and weigh accurately. Add 50 μL of isopropyl iodide for assay, stopper tightly, and weigh accurately. Shake the flask for 30 seconds, and use the upper layer of the mixture as the standard solution.

Procedure Analyze 1 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratio of isopropyl iodide to octane for each of the test solution and the standard solution, and express as Q_T for the test solution and as Q_S for the standard solution. Calculate the hydroxypropoxy group content by the formula:

$$\text{Content (\%) of hydroxypropoxy group } (-OC_3H_6OH) = \frac{M_S}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 44.17$$

Ms = amount (g) of isopropyl iodide in the standard solution.

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube (3 mm internal diameter and 3 m length).

Column packing material

Liquid phase: 20% Methyl silicone polymer of the support.

Support: 180- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium.

Flow rate: Adjust so that the peak of octane appears about 10 minutes after injection.

Column selection: Use a column capable of producing well-resolved peaks of isopropyl iodide and octane, in that order, when 1 μL of the standard solution is chromatographed under the above operating conditions.

Hydroxypropyl Distarch Phosphate

ヒドロキシプロピル化リン酸架橋デンプン

[53124-00-8]

Definition Hydroxypropyl Distarch Phosphate is obtained through esterification of starch with sodium trimetaphosphate or phosphorus oxychloride, followed by etherification with propylene oxide.

Description Hydroxypropyl Distarch Phosphate occurs as a white to off-white powder, or as flakes or granules. It has a faint odor.

Identification

(1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

Purity

(1) Hydroxypropyl groups Not more than 7.0%.

Test Solution Weigh accurately about 0.1 g of Hydroxypropyl Distarch Phosphate, and add 25 mL of diluted sulfuric acid (1 in 36), and heat in a water bath to dissolve. After cooling, add water to make exactly 100 mL. If necessary, dilute it to assure the

presence of less than 4 mg/100 mL of hydroxypropyl groups. Use the resulting solution as the sample solution. Measure exactly 1 mL of the sample solution into a 25-mL graduated test tube, and add dropwise 8 mL of sulfuric acid with the tube immersed in cold water. Mix well, heat in a water bath for exactly 3 minutes, and immediately cool in ice water. Add 0.6 mL of ninhydrin TS for modified starch, carefully allowing the reagent to run down the inside wall of the test tube. Immediately shake well, place in a 25°C water bath for 100 minutes, and add sulfuric acid to make 25 mL. Stopper the tube, and mix slowly by inverting several times. (Do not shake.) Perform the test immediately after the preparation of the test solution.

Standard Solutions Weigh accurately about 25 mg of propylene glycol, add water to make exactly 100 mL. Place exactly 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL of the resulting solution in five separate 50-mL volumetric flasks. To each, add water to make up to the volume. Separately measure exactly 1 mL of these solutions into 25-mL graduated test tubes, and add dropwise 8 mL of sulfuric acid with the tubes immersed in cold water.

Procedure Immediately transfer the test solution into a absorbance measuring cell, and after exactly 5 minutes, measure the absorbance at 590 nm against the reference solution, prepared by treating unmodified starch of the same botanical origin as directed for the test solution. Prepare a calibration curve by measuring the absorbance of the standard solutions in the same manner. Determine the concentration of propylene glycol (μg/mL) in the test solution from the calibration curve, and calculate the content of hydroxypropyl groups by the following formula:

$$\text{Content (\%) of hydroxypropyl groups} = \frac{\text{Propylene glycol concentration (μg/mL) in the test solution} \times 0.7763 \times \text{dilution factor}}{\text{Dry basis weight (g) of the sample} \times 100}$$

(2) Propylene chlorohydrins Not more than 1.0 μg/g.

Test Solution Weigh 50.0 g of Hydroxypropyl Distarch Phosphate into an Erlenmeyer flask, add 125 mL of diluted sulfuric acid (1 in 18), and swirl the flask to disperse the contents. Stopper loosely, heat in a water bath for 10 minutes, mix the contents well, and heat for an additional 30 minutes. For starches that are not easy to hydrolyze, such as wheat starch, heating time should be longer. After cooling, adjust the pH to 7 with sodium hydroxide solution (1 in 4), and filter with suction through a glass-fiber filter paper. Collect the filtrate in a second flask, wash the first flask and the residue on the filter paper with 25 mL of water, and combine the washings with the filtrate. Add 30 g of sodium sulfate, stir for 5–10 minutes, and transfer the solution into a separating funnel. Wash the second flask with 25 mL of water, and add the washings to the funnel. If precipitate remains, stir well with a small amount of water to dissolve it completely, and add the solution to the funnel. Extract five times with five 50-mL portions of diethyl ether. Combine the diethyl ether extracts, add 3 g of sodium sulfate, and filter through a filter paper into a third flask. Wash the flask and filter paper with 25 mL of diethyl ether, and combine the washings with the filtrate. Evaporate to 4 mL in a water bath at about 40°C under atmospheric pressure, cool, and add diethyl ether

to make exactly 5 mL.

Standard Solutions Weigh accurately about 50 mg propylene chlorohydrin, dissolve it in water to make exactly 100 mL. Dilute exactly 10 mL of this solution to exactly 100 mL with water to make a standard stock solution. Place 50.0-g portions of unmodified waxy corn starch in five separate Erlenmeyer flasks, and add 125 mL of diluted sulfuric acid (1 in 18). To the four flasks of them, add exactly 0.5 mL, 1 mL, 2 mL, and 5 mL of the standard stock solution separately. No standard stock solution is added to the remaining flask. Then proceed as directed for the test solution, beginning with "and swirl the flask to disperse the contents," to prepare standard solutions.

Procedure Analyze 1 μL each of the test solution and the standard solutions by gas chromatography, using the operating conditions given below. Prepare a calibration curve from the concentrations of propylene chlorohydrins in the standard solutions and the sums of the peak areas corresponding to 1-chloro-2-propanol and 2-chloro-1-propanol for the individual standard solutions. Then measure the sum of these two peak areas for the test solution, determine the concentration (μg/mL) of propylene chlorohydrins in the test solution from the calibration curve, and calculate the content of propylene chlorohydrins by the following formula:

$$\text{Content (μg/g) of propylene chlorohydrins} = \frac{\text{Propylene chlorohydrins concentration (μg/mL) in the test solution} \times 5}{\text{Dry basis weight (g) of the sample}}$$

Operating conditions

Detector: Flame ionization detector.

Detector temperature: 230°C.

Column: A fused silica tube (0.25 mm internal diameter and 30 m length) coated with a 0.25 μm thick layer of polyethylene glycol for gas chromatography.

Column temperature: Maintain the temperature at 40°C for 2 minutes, raise at 5°C/minute to 80°C, and maintain at 80°C for 8 minutes. Thereafter, raise the temperature at 25°C/minute to 230°C, and maintain the temperature for 5 minutes.

Injection port temperature: 150°C

Carrier gas: Nitrogen or helium

Flow rate: Adjust the retention time of 1-chloro-2-propanolto about 15 minutes.

Injection method: Splitless (purge start: 1 min after injection)

(3) Phosphorous Not more than 0.14% as P.

Proceed as directed in Purity (3) for Acetylated Distarch Phosphate.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(6) Sulfur dioxide　Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying　Not more than 21.0% (not more than 13.3 kPa, 120°C, 4 hours).

Hydroxypropyl Methylcellulose

ヒドロキシプロピルメチルセルロース

A mixed methyl and 2-hydroxypropyl ether of cellulose　[9004-65-3]

Definition　Hydroxypropyl Methylcellulose is a mixed ether of methyl and hydroxypropyl cellulose.

Content　Hydroxypropyl Methylcellulose, when dried, contains 19.0–30.0% of the methoxy group ($-OCH_3$ = 31.03) and 3.0–12.0% of the hydroxypropoxy group ($-OC_3H_6OH$ = 75.09).

Description　Hydroxypropyl Methylcellulose occurs as a white to yellowish white powder or as granules. It is odorless or has a slight characteristic odor. When water is added, it swells and produces a clear or slightly turbid viscous liquid.

Identification

(1) To 1 g of Hydroxypropyl Methylcellulose, add 100 mL of hot water, and cool to room temperature while stirring. Use this solution as the sample solution. Add anthrone TS gently to 5 mL of the sample solution. The boundary surface of both solutions turns blue to blue-green.

(2) To 0.1 mL of the sample solution obtained in Identification (1), add 9 mL of diluted sulfuric acid (9 in 10), shake, heat in a water bath for exactly 3 minutes, and immediately cool in an ice bath. To this solution, carefully add 0.6 mL of a ninhydrin solution (1 in 50), shake, and allow to stand 25°C. A red color develops, and then the color changes to purple within 100 minutes.

(3) Determine the infrared absorption spectrum of Hydroxypropyl Methylcellulose as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3465 cm^{-1}, 2900 cm^{-1}, 1375 cm^{-1}, and 1125 cm^{-1}.

pH　5.0–8.0 (1.0 g, hot water 100 mL).

Purity

(1) Chloride　Not more than 0.28% as Cl.

Test Solution　To 1.0 g of Hydroxypropyl Methylcellulose, add 30 mL of hot water, shake well, heat on a water bath for 10 minutes, and filter by decantation while hot. Wash the residue well with hot water, combine the washings with the filtrate, and cool. To the obtained solution, add water to make 100 mL. To 5 mL of this solution, add 6 mL

of 10% nitric acid and water to make 50 mL.

Control Solution Use 0.40 mL of 0.01 mol/L hydrochloric acid.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 8.0% (105°C, 1 hour).

Residue on Ignition Not more than 1.5 % (on the dried basis).

Assay

(i) Apparatus *Reaction flask* A 5-mL screw-cap pressure-tight glass bottle having an inverted conical bottom, a 20 mm external diameter, 50 mm high bottle-neck, and 2 mL capacity at the height of 30 mm. The cap is made of heat-resistant resin and equipped with a fluoroplastic inside stopper or seal. Confirm that the contents will not leak when heated.

Heater A 60 to 80-mm square-shaped aluminum block with holes 20.6 mm in diameter and 32 mm in depth that is capable of maintaining the inside temperature within ± 1°C.

(ii) Method

Test Solution Weigh accurately about 65 mg of Hydroxypropyl Methylcellulose, previously dried, transfer to a reaction flask, and add 65 mg of adipic acid, 2.0 mL of the internal standard solution, and 2.0 mL of hydriodic acid. Stopper the flask tightly, and accurately weigh the flask with the mixture. Use a solution (1 in 25) of octane in *o*-xylene as the internal standard solution. Shake the flask for 30 seconds, heat on the heater at 150°C for 30 minutes with repeated shaking at 5 minute intervals, and continue heating for an additional 30 minutes. Allow to cool, and then again weigh accurately. Confirm that the weight loss is not more than 10 mg, and use the upper layer of the mixture in the flask as the test solution.

Standard Solution Place 65 mg of adipic acid, 2.0 mL of the internal standard solution, and 2.0 mL of hydriodic acid in another reaction flask. Stopper the flask tightly, and weigh accurately. To the flask, add 15 μL of isopropyl iodide for assay, stopper, and weigh accurately. Add 45 μL of methyl iodide for assay in the same manner, and again weigh accurately. Shake the reaction flask for 30 seconds and use the upper layer of the mixture as the standard solution.

Procedure Analyze 2 μL each of the test solution and the standard solution by gas chromatography using the conditions below. Determine the peak area ratio of each of methyl iodide and isopropyl iodide to octane for the test solution, and express as Q_{Ta} and Q_{Tb}, respectively, and also calculate the peak area ratio for the standard solution in the same manner, and express them as Q_{Sa} and Q_{Sb}, respectively. Calculate the contents of methoxy and hydroxypropoxy groups by the following formulae:

$$\text{Content (\%) of methoy group } (-CH_3O) = \frac{M_{Sa}}{\text{Weight (g) of the sample}} \times \frac{Q_{Ta}}{Q_{Sa}} \times 21.86$$

$$\text{Content (\%) of hydroxypropoxy group } (-C_3H_7O_2) = \frac{M_{Sb}}{\text{Weight (g) of the sample}} \times \frac{Q_{Tb}}{Q_{Sb}} \times 44.17$$

M_{Sa} = amount (g) of methyl iodide in the standard solution,

M_{Sb} = amount (g) of isopropyl iodide in the standard solution.

Operating Conditions

Detector: Flame ionization detector.

Column: A glass column (about 3 mm internal diameter and 3 m length).

Column packing material

Liquid phase: 20% Methyl silicon polymer of the amount of the support.

Support: 180- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium gas.

Flow rate: Adjust so that the peak of octane appears about 10 minutes after injection.

Column selection: Use a column capable of producing well-resolved peaks of methyl iodide, isopropyl iodide, and octane, in that order listed, when 2 μL of the standard solution is chromatographed under the above conditions.

Hydroxypropyl Starch

ヒドロキシプロピルデンプン

[9049-76-7]

Definition Hydroxypropyl Starch is obtained by etherifying starch with propylene oxide.

Description Hydroxypropyl Starch occurs as a white to off-white powder, or as flakes or granules. It has a faint odor.

Identification

(1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

Purity

(1) Hydroxypropyl groups Not more than 7.0%.

Proceed as directed in Purity (1) for Hydroxypropyl Distrach Phosphate.

(2) Propylene chlorohydrins Not more than 1.0 μg/g.

Proceed as directed in Purity (2) for Hydroxypropyl Distrach Phosphate.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (not more than 13.3 kPa, 120°C, 4 hours).

Hypobromous Acid Water

次亜臭素酸水

Definition Hypobromous Acid Water is an aqueous solution consisting mainly of hypobromous acid. It is obtained by hydrolyzing 1,3-dibromo-5,5-dimethylhydantoin or by mixing an aqueous solution of hydrogen bromide with an aqueous solution of sodium hypochlorite, potassium hypochlorite, or calcium hypochlorite.

Content Hypobromous Acid Water contains 75–900 mg/kg of available bromine.

Description Hypobromous Acid Water is a colorless liquid. It is odorless or has a slight characteristic odor.

Identification

(1) To 10 ml of Hypobromous Acid Water, add 0.15 g of potassium iodide. A yellow to brown color is produced.

(2) Prepare a test solution by adding 1 ml of Hypobromous Acid Water to 89 ml of water. To 0.5 ml of DPD–EDTA TS, add 0.5 ml of phosphate buffer (containing disodium dihydrogen ethylenediaminetetraacetate) and 10 ml of the test solution. A light red color is produced.

(3) The solution obtained by adding 1 drop of sodium hydroxide solution (1 in 2) to 10 ml of Hypobromous Acid Water exhibits an absorption maximum at a wavelength of 324–330 nm.

pH 4.0–7.5.

Assay Weigh accurately about 20 g of Hypobromous Acid Water, add 50 ml of water, and then add 1 g of potassium iodide and 5 ml of diluted acetic acid (1 in 4). Immediately stopper tightly and allow to stand in a dark place for 15 minutes. Titrate the liberated iodine with 0.01 mol/L sodium thiosulfate (indicator: 3 ml of starch TS). Add starch TS near the endpoint, when the solution is pale yellow. The endpoint is when the blue color

produced disappears. Separately, perform a blank test to make any necessary correction.

Each ml of 0.01 mol/L sodium thiosulfate = 0.7990 mg of Br

Hypochlorous Acid Water

次亜塩素酸水

Definition Hypochlorous Acid Water is an aqueous solution consisting mainly of hypochlorous acid. It is obtained by electrolyzing hydrochloric acid or an aqueous solution of sodium chloride. There are three types of solutions: Strongly Acidic Hypochlorous Acid Water, Weakly Acidic Hypochlorous Acid Water, and Slightly Acidic Hypochlorous Acid Water. Strongly Acidic Hypochlorous Acid Water is produced from the anode by electrolyzing sodium chloride solution of not more than 0.2% in an electrolytic cell with a septum ("electrolytic cell with a septum" refers to a cell consisting of an anode and a cathode separated by a septum). Weakly Acidic Hypochlorous Acid Water is produced from the anode by electrolyzing sodium chloride solution of an appropriate concentration in an electrolytic cell with a septum or obtained by adding a solution produced from the cathode through electrolysis to a solution produced from the anode. Slightly Acidic Hypochlorous Acid Water is produced by electrolyzing hydrochloric acid of an appropriate concentration or a solution of an appropriate concentration prepared by adding sodium chloride solution to hydrochloric acid of an appropriate concentration in an electrolytic cell without a septum.

Content

Strongly Acidic Hypochlorous Acid Water includes 20–60 mg/kg of available chlorine.
Weakly Acidic Hypochlorous Acid Water includes 10–60 mg/kg of available chlorine.

Slightly Acidic Hypochlorous Acid Water includes 10–80 mg/kg of available chlorine.

Description Hypochlorous Acid Water is a colorless liquid. It has little or no odor of chlorine.

Identification

(1) To 5 mL of Hypochlorous Acid Water, add 1 mL of a sodium hydroxide solution (1 in 2500) and 0.2 mL of potassium iodide TS. A yellow color is produced, which changes to deep blue on the addition of 0.5 mL of starch TS.

(2) To 5 mL of Hypochlorous Acid Water, add 0.1 mL of a potassium permanganate solution (1 in 300) and then 1 mL of diluted sulfuric acid (1 in 20). A reddish purple color of the solution does not fade.

(3) To 90 mL of Hypochlorous Acid Water, add 10 mL of a sodium hydroxide solution (1 in 5). The solution exhibits an absorption maximum at a wavelength of 290–294 nm.

pH

Strongly Acidic Hypochlorous Acid Water: Not more than 2.7.

Weakly Acidic Hypochlorous Acid Water: 2.7–5.0.

Slightly Acidic Hypochlorous Acid Water: 5.0–6.5.

Purity

Residue on drying (evaporating) Not more than 0.25 %.

Weigh and evaporate 20.0 g of Hypochlorous Acid Water. Then dry it at 110°C for 2 hours, and weigh the residue.

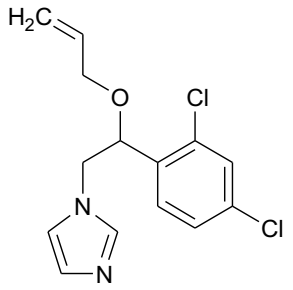
Assay

Weigh accurately about 200 g of Hypochlorous Acid Water, add 2 g of potassium iodide and 10 mL of diluted acetic acid (1 in 4), immediately stopper the container, and allow to stand in a dark place for 15 minutes. Titrate the liberated iodine with 0.01 mol/L sodium thiosulfate, using 1 mL of starch TS as the indicator. Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Separately, perform a blank test in the same manner to make any necessary correction.

Each mL of 0.01 mol/L sodium thiosulfate = 0.3545 mg of Cl

Imazalil

イマザリル



$C_{14}H_{14}Cl_2N_2O$ Mol. Wt. 297.18

1-[(2*RS*)-2-(Allyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole [35554-44-0]

Content Imazalil contains not less than 97.5% of imazalil ($C_{14}H_{14}Cl_2N_2O$).

Description Imazalil occurs as a light yellow to light brown powder or as granules. It is odorless.

Identification Dissolve 40 mg of Imazalil in 10 mL of hydrochloric acid (0.1 mol/L), add 2-propanol to make 100 mL. This solution exhibits absorption maxima at wavelengths of 263–267 nm, 270–274 nm, and 278–282 nm.

Melting Point 49–54°C.

Purity Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

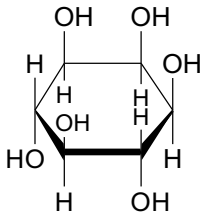
Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.7 g of Imazalil, and dissolve it in a 7:3 mixture of 2-butanone/acetic acid. Titrate this solution with 0.1 mol/L of perchloric acid (indicator: 10 drops of *p*-naphtholbenzein TS). The endpoint is when the color of solution changes from orange to green. Perform a blank test to make any necessary correction.

Each mL of 0.1 mol/L of perchloric acid = 29.72 mg of $C_{14}H_{14}Cl_2N_2O$

myo-Inositol

***myo*-イノシトール**



$C_6H_{12}O_6$ Mol. Wt. 180.16

(1*R*,2*S*,3*S*,4*R*,5*R*,6*S*)-Cyclohexane-1,2,3,4,5,6-hexol [87-89-8]

Definition *myo*-Inositol is one of the inositol isomers and consists mainly of *myo*-inositol. It is produced by decomposing phytic acid obtained from the seed bran of the rice plant *Oryza sativa* L. or the seeds of the corn plant *Zea mays* L. It is also produced by isolation from the juice of the sugar beet, *Beta vulgaris* L., or molasses.

Content *myo*-Inositol, when dried, contains not less than 97.0% of *myo*-inositol ($C_6H_{12}O_6$).

Description *myo*-Inositol occurs as white crystals or crystalline powder. It is odorless and has a sweet taste.

Identification Determine the absorption spectrum of *myo*-Inositol as directed in the Disk Method under Infrared Spectrophotometry. The spectrum exhibits absorption bands at about 3380 cm^{-1}, 3220 cm^{-1}, 1446 cm^{-1}, 1147 cm^{-1}, 1114 cm^{-1}, and 1049 cm^{-1}.

Melting Point 223–227°C.

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.005% as Cl (2.0 g, Control Solution: 0.01 mol/L Hydrochloric acid 0.30 mL).

(3) Sulfate Not more than 0.006% as SO_4 (4.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Iron Not more than 5.0 μg/g as Fe (1.0 g, Method 1, Control Solution: Iron Standards Solution 0.5 mL).

(6) Calcium Dissolve 1.0 g of *myo*-Inositol in 10 mL of water, add 1 mL of a solution of ammonium oxalate monohydrate (1 in 30), and allow to stand for 1 minute. The solution is clear.

(7) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(8) Reducing substance Dissolve 0.50 g of *myo*-Inositol in 10 mL of water, add 5 mL of Fehling's TS, heat for 3 minutes, and allow to stand for 30 minutes. A yellowish orange to red precipitate is not formed.

Loss on Drying Not more than 0.5% (105°C, 4 hours).

Residue on Ignition Not more than 0.1%.

Assay

Test Solution and Standard Solution Weigh accurately about 0.2 g each of *myo*-Inositol and *myo*-inositol for assay, previously dried. To each, add exactly 30 mL of water and exactly 5 mL of 1-propanol solution (3 in 25), and then add water to make 2 solutions of exactly 50 mL each. Use these solutions as the test solution and standard solution, respectively.

Procedure Analyze 10 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions below. Determine the peak area ratios (Q_T and Q_S) of *myo*-inositol to 1-propanol for the test solution and the standard solution, and calculate the *myo*-inositol content by the formula:

$$\text{Content (\%) of } myo\text{-inositol } (C_6H_{12}O_6) = \frac{\text{Weight (g) of } myo\text{-inositol for assay}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (8 mm internal diameter and 30 cm length).

Column packing material: 6- to 8-μm strongly acidic cation-exchange resin for liquid chromatography.

Column temperature: A constant temperature of about 65°C.

Mobile phase: Water.

Flow rate: Adjust the retention time of *myo*-inositol to about 9 minutes.

Inulinase

イヌリナーゼ

Definition Inulinase includes enzymes that hydrolyze inulin. It is derived from the culture of filamentous fungi (limited to *Aspergillus aculeatus*, *Aspergillus niger, Aspergillus phoenicis, Penicillium purpurogenum,* and species of the genus *Trichoderma*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Inulinase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Inulinase complies with the Inulinase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* and Method 2 for the *Salmonella* test.

Inulinase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by any given methods, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Inulinase, add acetate buffer (0.1 mol/L) at pH 5.0 or water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer or water to the resulting solution.

Substrate Solution Weigh 1.50 g of inulin (chicory-derived), and dissolve it in acetate buffer (0.1 mol/L) at pH 5.0 by stirring while heating in a water bath. Add the buffer again to make 100 mL.

Test Solution　Transfer 0.2 mL of the substrate solution in a test tube, equilibrate at 50°C for 5 minutes, add 0.2 mL of the sample solution, and shake immediately. Incubate the mixture at 50°C for 30 minutes. To this solution, add 1.2 mL of 3,5-dinitrosalicylic acid–phenol TS, and mix. Cover the mouth of the test tube with a glass bead, heat it in a water bath for 5 minutes, and cool. To this solution, add 8.4 mL of water, and shake.

Control Solution　Transfer 1.2 mL of 3,5-dinitrosalicylic acid–phenol TS in a test tube, add 0.2 mL of the substrate solution and 0.2 mL of the sample solution, and shake immediately. Cover the mouth of the test tube with a glass bead, heat it in a water bath for 5 minutes, and cool. To this solution, add 8.4 mL of water, and shake.

Procedure　Measure the absorbance of the test solution and the control solution at a wavelength of 550 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution　Weigh 1.0 g of Inulinase, add acetate buffer (0.1 mol/L) at pH 4.5 or water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer or water to the resulting solution.

Substrate Solution　Add 0.56 g of inulin (dahlia-derived) to 70 mL of water gradually while stirring, and dissolve it by heating in a water bath. Add 10 mL of acetate buffer (1 mol/L) at pH 4.5 and water to make 100 mL.

Test Solution　Transfer 1.8 mL of the substrate solution into a test tube, equilibrate at 40°C for 5 minutes, add 0.2 mL of the sample solution, and shake immediately. Incubate the mixture at 40°C for 20 minutes. To this solution, add 4 mL of 3,5-dinitrosalicylic acid–lactose TS, and shake immediately. Cover the mouth of the test tube with a glass bead, heat in a water bath for 15 minutes, and cool.

Control Solution　Transfer 0.2 mL of the sample solution into a test tube, equilibrate at 40°C for 5 minutes, add 4 mL of 3,5-dinitrosalicylic acid–lactose TS, and shake immediately. To this solution, add 1.8 mL of the substrate solution, and mix. Cover the mouth of the test tube with a glass bead, heat in a water bath for 15 minutes, and cool.

Procedure　Measure the absorbance of the test solution and the control solution at 540 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Invertase

インベルターゼ

Definition　Invertase includes enzymes that hydrolyze non-reducing terminal residues

of β-D-fructofuranoside. It is produced through the refinement of the culture of filamentous fungi (limited to *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus niger,* and *Aspergillus japonicus*), yeasts (limited to *Kluyveromyces lactis* and *Saccharomyces cerevisiae*), or bacteria (limited to species of the genera *Arthrobacter* and *Bacillus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Invertase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Invertase complies with the Invertase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

The requirement of total plate count can be disregarded if unsterilized Invertase is used for captive consumption in the manufacture of food that will be sterilized or pasteurized before the completion of the final product.

Invertase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Invertase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 20.0 g of sucrose, and dissolve it in water to make 100 mL.

Test Solution To 5 mL of the substrate solution, add 4 mL of acetate buffer (0.1

mol/L) at pH 5.0, and allow the mixture to equilibrate at 30°C for 5 minutes. Add 1 mL of the sample solution, mix, and allow to incubate at 30°C for 10 minute. To this solution, add 10 mL of sodium hydroxide TS (0.1 mol/L), shake well, add 20 mL of Fehling's TS, heat in a water bath for 5 minutes, and cool. Then, add 5 mL of potassium iodide TS (for β-amylase/invertase activity test) and 10 mL of diluted sulfuric acid (4 in 25), and shake well.

Control Solution To 5 mL of the substrate solution, add 4 mL of acetate buffer (0.1 mol/L) at pH 5.0 and 1 mL of water, allow the mixture to stand at 30°C for 15 minutes. To this solution, add 10 mL of sodium hydroxide TS (0.1 mol/L), shake well, then proceed as directed for the test solution.

Procedure Titrate each of the test solution and the control solution with 0.1 mol/L sodium thiosulfate (indicator: 2-3 drops of soluble starch). The amount of 0.1 mol/L sodium thiosulfate consumed by the test solution is less than that of 0.1 mol/L sodium thiosulfate consumed by the control solution.

Method 2

Sample Solution Weigh 1.0 g of Invertase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 11.2 g of sucrose, dissolve it in 70 mL of water, add 10 mL of acetate buffer (1 mol/L) at pH 4.5 and water to make 100 mL.

Test Solution Transfer 1.8 mL of the substrate solution into a test tube, allow it to equilibrate at 30°C for 5 minutes, add 0.2 mL of the sample solution, and shake immediately. Incubate the mixture at 30°C for 10 minutes. To this solution, add 4 mL of 3,5-dinitrosalcylic acid–lactose TS, and shake immediately. Cover the mouth of the test tube with a glass bead, heat in a water bath for 15 minutes, and cool.

Control Solution Proceed as directed for the test solution, using 0.2 mL of water instead of the sample solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 540 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Ion Exchange Resins

イオン交換樹脂

Definition Ion Exchange Resins occur as granules, powders, and suspensions called Ion Exchange Resin (granule), Ion Exchange Resin (powder), and Ion Exchange Resin (suspension), respectively.

Ion Exchange Resin (granule)

イオン交換樹脂（粒状）

Description Ion Exchange Resin (granule) occurs as a black, brown, light red-brown, or white, spherical, massive, or granular substance. It is almost odorless.

Identification Perform test (I) or (II) whichever is appropriate to identify cation exchange resin or anion exchange resin.

(I) Cation exchange resin Prepare a resin column by pouring 5 mL of Ion Exchange Resin (granule) with water into a glass tube for chromatography (about 1-cm internal diameter). Run 25 mL of diluted hydrochloric acid (1 in 10) through the column at a rate of about 5 mL per minute, and wash the resin by running 100 mL of water through at the same rate. Then run 25 mL of potassium hydroxide solution (1 in 15) through at the same rate, and wash again by running 75 mL of water through at the same rate. To 5 mL of the last washing, add 2 mL of diluted acetic acid (1 in 20) and then 3 drops of sodium hexanitrocobaltate(III) TS. No yellow turbidity appears. Transfer 2 mL of the resin in the column to a test tube, add 5 mL of diluted hydrochloric acid (1 in 10), shake well for 5 minutes, and filter. Wash the resin on the filter paper with water, and combine the washings with the filtrate to make about 5 mL. Add 4 mL of sodium hydroxide solution (1 in 25) to the solution, shake, add 2 mL of diluted acetic acid (1 in 20), and add 3 drops of sodium hexanitrocobaltate(III) TS. A yellow precipitate is formed.

(II) Anion exchange resin Prepare a resin column pouring 5 mL of Ion Exchange Resin (granule) with water into a glass tube for chromatography (about 1-cm internal diameter). Run 25 mL of diluted hydrochloric acid (1 in 10) through the column at a rate of about 5 mL per minute, and wash the resin by running 100 mL of water through at the same rate. To 5 mL of the last washing, add 1 mL of diluted nitric acid (1 in 10) and 3 drops of silver nitrate solution (1 in 50). No white turbidity appears. Transfer 1 mL of the resin in the column to a test tube, add 3 mL of sodium hydroxide solution (1 in 25), and shake well for 5 minutes, and filter. Wash the resin on the filter paper with water, and combine the washings with the filtrate to make about 5 mL. To this solution, add 3 mL of diluted nitric acid (1 in 10) and then 3 drops of silver nitrate solution (1 in 50). A white precipitate is formed.

Purity Prepare the sample by procedure (I) for cation exchange resin or procedure (II) for anion exchange resin, given below, immerse thoroughly in water, and blot the adhering water with a filter paper. Use the prepared resin as the test sample.

(I) Cation exchange resin Prepare the sample (H form) as follows: Measure 30 mL of Ion Exchange Resin (granule), transfer into a glass tube for chromatography (about 3 cm in internal diameter), and run 1000 mL of diluted hydrochloric acid (1 in 10) through at a rate of 15–20 mL per minute. Then wash the resin by running water through at the same rate. Measure 10 mL of the washings, and perform the test for Chloride. Wash the resin repeatedly with water until the chloride amount is not more than the equivalent of 0.3 mL of 0.01 mol/L hydrochloric acid.

(II) Anion exchange resin Prepare the sample (OH form) as follows: Measure 30 mL of Ion Exchange Resin (granule), transfer it into a glass tube for chromatography (about

3 cm in internal diameter), run 1000 mL of sodium hydroxide solution (1 in 25) through at a rate of 15–20 mL per minute, and wash the resin by running water through at the same rate. Wash the resin with water until the washings become neutral with phenolphthalein TS.

(1) Solids Not less than 25%.

Weigh 10.0 g of the test sample. Dry it at 100°C for 12 hours in the case of the cation exchange resin or at 40°C for 12 hours in a vacuum desiccator at 4 kPa in the case of the anion exchange resin. Then weigh it again.

(2) Water-soluble substances Not more than 0.50%.

Weigh 10.0 g of the test sample, transfer into a cylindrical filter (28 mm in internal diameter, 10 cm in length), suspend the filter with the sample in 1000 mL of water, and extract for 5 hours with occasional shaking. Measure 50 mL of the extract, evaporate carefully, and dry at 110°C for 3 hours. Weigh the residue. Perform a blank test in the same manner, and make any necessary correction.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Total Ion Exchange Capacity Perform the test for cation exchange resin using procedure (I) or for anion exchange resin using procedure (II).

(I) Cation exchange resin Not less than 1.0 milliequivalent/g.

Weigh accurately about 5 g of the test sample prepared for the Purity Tests. Add exactly 500 mL of 0.2 mol/L sodium hydroxide, and allow to stand for 12 hours with occasional shaking. Measure exactly 10 mL of the supernatant, and titrate with 0.05mol/L sulfuric acid (indicator: 3 drops of methyl orange TS). Perform a blank test in the same manner, and calculate the total ion exchange capacity by the formula:

$$\text{Total ion exchange capacity (milliequivalent/g)} = \frac{\left[\begin{array}{c}\text{Volume (mL) of}\\ \text{0.05 mol/L sulfuric acid}\\ \text{consumed in the blank test}\end{array}\right] - \left[\begin{array}{c}\text{Volume (mL) of}\\ \text{0.05 mol/L sulfuric acid}\\ \text{consumed in the test}\end{array}\right]}{\text{Weight (g) of the sample} \times \text{solid (\%)}/100} \times 5$$

(II) Anion exchange resin Not less than 1.0 milliequivalent/g.

Weigh accurately about 5 g of the test sample prepared for the Purity Tests. Add exactly 500 mL of 0.2 mol/L hydrochloric acid, and allow to stand for 12 hours with occasional shaking. Measure exactly 10 mL of the supernatant, and titrate with 0.1 mol/L sodium hydroxide (indicator: 3 drops of phenolphthalein TS). Perform a blank test in the same manner, and calculate the total ion exchange capacity by the formula:

Total ion exchange capacity (milliequivalent/g)

$$= \frac{\left[\begin{array}{c}\text{Volume (mL) of}\\ \text{0.1 mol/L sodium hydroxide}\\ \text{consumed in the blank test}\end{array}\right] - \left[\begin{array}{c}\text{Volume (mL) of}\\ \text{0.1 mol/L sodium hydroxide}\\ \text{consumed in the test}\end{array}\right]}{\text{Weight (g) of the sample} \times \text{solid (\%)}/100} \times 5$$

Ion Exchange Resin (powder)

イオン交換樹脂（粉状）

Description Ion Exchange Resin (powder) occurs as a black, brown, light red-brown, or white, powdery substance. It is almost odorless.

Identification Perform test (I) or (II) whichever is appropriate to identify cation exchange resin or anion exchange resin.

(I) Cation exchange resin Prepare a resin layer by pouring 2 g of Ion Exchange Resin (powder) with water into a pressure filter (about 7.5 cm internal diameter) equipped with a membrane filter (1 μm pore size). Run 25 mL of diluted hydrochloric acid (1 in 10) through at a rate of about 5 mL per minute, and wash the resin by running 100 mL of water through at the same rate. Next, run 25 mL of potassium hydroxide solution (1 in 15) through at the same rate, and wash the resin again by running 75 mL of water through at the same rate. To 5 mL of the last washings, add 2 mL of diluted acetic acid (1 in 20) and then 3 drops of sodium hexanitrocobaltate(III) TS. No yellow turbidity appears. Transfer 0.5 g of the resin layer to a test tube, add 5 mL of diluted hydrochloric acid (1 in 10), shake well for 5 minutes, and filter. Wash the resin on the filter paper with water, and combine the washings with the filtrate to make about 5 mL. Add 4 mL of sodium hydroxide solution (1 in 25) to the solution, and shake. Add 2 mL of diluted acetic acid (1 in 20) and then 3 drops of sodium hexanitrocobaltate(III) TS. A yellow precipitate is formed.

(II) Anion exchange resin Prepare a resin layer by pouring 2 g of Ion Exchange Resin (powder) with water into a pressure filter (about 7.5 cm internal diameter) equipped with a membrane filter (1 μm pore size). Run 25 mL of diluted hydrochloric acid (1 in 10) through at a rate of about 5 mL per minute, and wash the resin by running 100 mL of water through at the same rate. To 5 mL of the last washing, add 1 mL of diluted nitric acid (1 in 10) and then 3 drops of silver nitrate solution (1 in 50). No white turbidity appears. Transfer 0.5 g of the resin layer to a test tube, add 3 mL of sodium hydroxide solution (1 in 25), shake well for 5 minutes, and filter. Wash the resin on the filter paper with water, and combine the washings with the filtrate to make about 5 mL. To the solution, add 3 mL of diluted nitric acid (1 in 10) and then 3 drops of silver nitrate solution (1 in 50). A white precipitate is formed.

Purity Prepare the sample by procedure (I) for cation exchange resin or by procedure (II) for anion exchange resin, given below, immerse thoroughly in water, and blot the

adhering water with a filter paper. Use the prepared resin as the test sample.

(I) Cation exchange resin Prepare the sample (H form) as follows: Weigh 30 g of Ion Exchange Resin (powder), transfer into a pressure filter (about 7.5 cm internal diameter) equipped with a membrane filter (1 μm pore size), run 1000 mL of diluted hydrochloric acid (1 in 10) through at a rate of 15–20 mL per minute, and wash the resin by running water through at the same rate. Measure 10 mL of the washings, and perform the test for Chloride. Wash the resin repeatedly with water until the chloride amount is not more than the equivalent of 0.3 mL of 0.01 mol/L hydrochloric acid.

(II) Anion exchange resin Prepare the sample (OH form) as follows: Weigh 30 g of Ion Exchange Resin (powder), transfer into a pressure filter (about 7.5 cm in internal diameter) equipped with a membrane filter (1 μm pore size), run 1000 mL of sodium hydroxide solution (1 in 25) through at a rate of 15–20 mL per minute, and wash the resin by running water through at the same rate. Wash the resin with water until the washings become neutral with phenolphthalein TS.

(1) Solids Not less than 25%.

Proceed as directed in Purity (1) in Ion Exchange Resin (granule).

(2) Water-soluble substances Not more than 0.50%.

Weigh 10.0 g of the test sample, make a suspension by adding 1000 mL of water, and extract for 5 hours with occasional stirring. Filter the suspension through a pressure filter (about 7.5 cm internal diameter) equipped with a membrane filter (1 μm pore size). Measure 50 mL of the filtrate, evaporate carefully, and dry at 110°C for 3 hours. Weigh the residue.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As_2O_3 (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Total Ion Exchange Capacity Perform the test for cation exchange resin using procedure (I) or for anion exchange resin using procedure (II).

(I) Cation exchange resin Not less than 1.0 milliequivalent/g.

Weigh accurately about 5 g of the test sample prepared for the Purity Tests. Add exactly 500 mL of 0.2 mol/L sodium hydroxide, and allow to stand for 12 hours with occasional shaking. Filter the suspension through a pressure filter (7.5 cm internal diameter) equipped with a membrane filter (1 μm pore size). Measure exactly 10 mL of the filtrate, and titrate with 0.05 mol/L sulfuric acid (indicator: 3 drops of methyl orange TS). Perform a blank test in the same manner, and calculate the total ion capacity by the formula:

Total ion exchange capacity (milliequivalent/g)

$$= \frac{\left[\begin{array}{c}\text{Volume (mL) of}\\ \text{0.05 mol/L sulfuric acid}\\ \text{consumed in the blank test}\end{array}\right] - \left[\begin{array}{c}\text{Volume (mL) of}\\ \text{0.05 mol/L sulfuric acid}\\ \text{consumed in the test}\end{array}\right]}{\text{Weight (g) of the sample} \times \text{solid (\%)}/100} \times 5$$

(II) Anion exchange resin Not less than 1.0 milliequivalent/g.

Weigh accurately about 5 g of the test sample prepared for the Purity Tests. Add exactly 500 mL of 0.2 mol/L hydrochloric acid, and allow to stand for 12 hours with occasional shaking. Filter the suspension through a pressure filter (about 7.5 cm internal diameter) equipped with a membrane filter (1 μm pore size). Measure exactly 10 mL of the filtrate, and titrate with 0.1 mol/L sodium hydroxide (indicator: 3 drops of phenolphthalein TS). Perform a blank test in the same manner, and calculate the total ion capacity by the formula:

Total ion exchange capacity (milliequivalent/g)

$$= \frac{\left[\begin{array}{c}\text{Volume (mL) of}\\ \text{0.1 mol/L sodium hydroxide}\\ \text{consumed in the blank test}\end{array}\right] - \left[\begin{array}{c}\text{Volume (mL) of}\\ \text{0.1 mol/L sodium hydroxide}\\ \text{consumed in the test}\end{array}\right]}{\text{Weight (g) of the sample} \times \text{solid (\%)}/100} \times 5$$

Ion Exchange Resin (suspension)

イオン交換樹脂（懸濁液）

Description Ion Exchange Resin (suspension) is a brown, light red-brown, or white suspension. It is almost odorless.

Identification Perform test (I) or (II) whichever is appropriate to identify whether the resin is cation exchange resin or anion exchange resin.

(I) Cation exchange resin To 0.5 mL of Ion Exchange Resin (suspension), add 5 mL of water and 1 mL of a strongly acidic cation-exchange resin, react for 1 hour with occasional shaking, and filter through absorbent cotton on a funnel. To the filtrate, add 0.3 g of sodium chloride, shake for 3 minutes, add 1 drop of methyl red TS, and shake. A red color develops.

(II) Anion exchange resin To 0.5 mL of Ion Exchange Resin (suspension), add 5 mL of water and 1 mL of a strongly basic anion-exchange resin, react for 1 hour with occasional shaking, and filter through absorbent cotton on a funnel. To the filtrate, add 0.3 g of sodium chloride, shake for 3 minutes, add 1 drop of phenolphthalein TS, and shake. A red color develops.

Purity

(1) Solids Not less than 4.0%.

Weigh 1.0 g of Ion Exchange Resin (suspension), dry at 105°C for 5 hours, and weigh again.

(2) Water-soluble substances Not more than 0.50% (w/v).

Measure 100 mL of Ion Exchange Resin (suspension), and filter through a pressure filter (about 7.5 cm internal diameter) equipped with a membrane filter (0.05 μm pore size). Measure 10 mL of the filtrate, evaporate carefully, and dry at 105°C for 3 hours. Weigh the residue.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Total Ion Exchange Capacity Perform the test for cation exchange resin using procedure (I) or for anion exchange resin using procedure (II).

(I) Cation exchange resin Not less than 1.0 milliequivalent/g.

Weigh accurately an amount of Ion Exchange Resin (suspension) equivalent to about 0.2 g of the solid. Pour it into a glass tube for chromatography (about 1 cm internal diameter) previously packed with 10 mL of strongly acidic cation-exchange resin to allow the liquid in the suspension to run through at a rate of about 2 mL per minute. Then run about 20 mL of water through at the same rate. Wash the resin again by running about 80 mL of water through at a rate of 15–20 mL per minute. Collect all of the effluent and washings into a beaker, and add about 1 g of sodium chloride. Titrate with 0.1 mol/L sodium hydroxide to a pH of 7.0, using a pH meter. Perform a blank test in the same manner, make any necessary correction, and calculate the total ion exchange capacity by the formula:

$$\text{Total ion exchange capacity (milliequivalent/g)} = \frac{\left[\begin{array}{c}\text{Volume (mL) of}\\ \text{0.1 mol/L sodium hydroxide}\\ \text{consumed in the test}\end{array}\right] - \left[\begin{array}{c}\text{Volume (mL) of}\\ \text{0.1 mol/L sodium hydroxide}\\ \text{consumed in the blank test}\end{array}\right]}{\text{Weight (g) of the sample} \times \text{solid (\%)}/100} \times 0.1$$

(II) Anion exchange resin Not less than 1.0 milliequivalent/g.

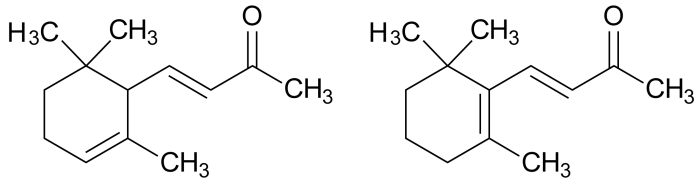
Weigh accurately an amount of Ion Exchange Resin (suspension) equivalent to about 0.2 g of the solid. Pour it into a glass tube for chromatography (about 1 cm in internal diameter) packed with 10 mL of a strongly basic anion-exchange resin to allow the liquid in the suspension to run through at a rate of about 2 mL per minute, and run about 20 mL of water through at the same rate. Wash the resin again by running about 80 mL of water through at a rate of 15–20 mL per minute. Collect all of the effluent and the

washings into a beaker, and add about 1 g of sodium chloride. Titrate with 0.1 mol/l hydrochloric acid to a pH of 7.0, using a pH meter. Perform a blank test in the same manner, make any necessary correction, and calculate the total ion exchange capacity by the formula:

$$\text{Total ion exchange capacity (milliequivalent/g)} = \frac{\left[\begin{array}{c}\text{Volume (mL) of}\\ \text{0.1 mol/L hydrochloric acid}\\ \text{consumed in the test}\end{array}\right] - \left[\begin{array}{c}\text{Volume (mL) of}\\ \text{0.1 mol/L hydrochloric acid}\\ \text{consumed in the blank test}\end{array}\right]}{\text{Weight (g) of the sample} \times \text{solid (\%)}/100} \times 0.1$$

Ionone

イオノン



α-Ionone β-Ionone

$C_{13}H_{20}O$ Mol. Wt. 192.30

Mixture of (3*E*)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one (α-ionone) and (3*E*)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one (β-ionone) [8013-90-9]

Content Ionone contains not less than 90.0% of ionone ($C_{13}H_{20}O$).

Description Ionone is a colorless to light yellow clear liquid having a characteristic odor.

Identification Proceed as directed in the Liquid Film Method under Infrared Spectrophotometry. Ionone exhibits absorption bands at about 2960 cm^{-1}, 1696 cm^{-1}, 1674 cm^{-1}, 1363 cm^{-1}, 1255 cm^{-1}, and 982 cm^{-1}.

Refractive Index n_D^{20}: 1.497–1.522.

Specific Gravity d_{20}^{20}: 0.930–0.948.

Purity Clarity of solution Clear (1.0 mL, 70% (vol) ethanol 4.0 mL).

Assay Weigh accurately about 1.3 g of Ionone, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, boil the mixture for 1 hour before titrating.

Each mL of 0.5 mol/L hydrochloric acid = 96.15 mg of $C_{13}H_{20}O$

Iron Lactate

乳酸鉄

Content Iron Lactate contains 15.5–20.0% of iron (Fe = 55.85).

Description Iron Lactate occurs as a greenish white to yellow-brown powder or as lumps. It has a slight, characteristic odor.

Identification

(1) Ignite 0.5 g of Iron Lactate at 450–550°C for 1 hour. To the residue, add 3 mL of diluted hydrochloric acid (1 in 2), and dissolve it by heating. The solution responds to all the tests for Iron(III) Salt in the Qualitative Tests.

(2) Iron Lactate responds to all tests for Lactate in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear.

Test Solution Weigh 1.0 g of Iron Lactate, add 20 mL of water, and dissolve it by heating in a water bath.

(2) Chloride Not more than 0.071% as Cl (0.10 g, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Sulfate Not more than 0.48% as SO_4.

Sample Solution Weigh 0.20 g of Iron Lactate, dissolve it in 5 mL of water, and add water to make 10 mL. Use 2.0 mL of this solution as the sample solution.

Control Solution 0.40 mL of 0.005 mol/L sulfuric acid.

(4) Lead Not more than 1 μg/g as Pb (4.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (1.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Iron Lactate in 25 mL of water, add 1 mL of sulfuric acid and 10 mL of sulfurous acid solution, evaporate to about 2 mL, and add water to make 10 mL. Use 5 mL of this solution as the test solution.

(6) Readily carbonizable substances and butyrate

Weigh 0.5 g of powdered Iron Lactate, and mix with 1 mL of sulfuric acid. No color develops, and no butyric acid-like odor is evolved.

Assay Weigh accurately about 1 g of Iron Lactate, carbonize by heating gradually, add 1 mL of nitric acid, evaporate to dryness, taking care to prevent the solution from splattering, and ignite. To the residue, add 10 mL of hydrochloric acid (1 in 2), boil until the insoluble substance almost disappears, add 20 mL of water, and filter. Wash the insoluble substance with water, combine the washings with the filtrate, and add water to make exactly 100 mL. Measure exactly 25 mL of this solution, transfer into a ground-glass stoppered flask, add 2 g of potassium iodide, immediately stopper tightly, and allow

to stand in a dark place for 15 minutes. Add 100 mL of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner.

Each mL of 0.1 mol/L sodium thiosulfate = 5.585 mg of Fe

Iron Sesquioxide

Diiron Trioxide
Iron Oxide Red

三二酸化鉄

Fe_2O_3 Mol. Wt. 159.69

Iron(III) oxide [1309-37-1]

Content Iron Sesquioxide contains not less than 98.0% of iron(III) oxide (Fe_2O_3).

Description Iron Sesquioxide occurs as a red to yellow-brown powder.

Identification To 1 g of Iron Sesquioxide, add 3 mL of diluted hydrochloric acid (1 in 2), and dissolve it by heating. The solution responds to all the tests for Iron(III) Salt in the Qualitative Tests.

Purity

(1) Water-soluble substances Not more than 0.75%.

Weigh 5.0 g of Iron Sesquioxide, add 200 mL of water, and boil for 5 minutes. Cool, add water to make 250 mL, and filter. Discard about 50 mL of the initial filtrate, measure exactly 100 mL of the subsequent filtrate, and evaporate to dryness on a water bath. Dry the residue at 105–110°C for 2 hours, and weigh.

(2) Lead Not more than 10 μg/g as Pb (0.40 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Iron Sesquioxide, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(3) Arsenic Not more than 1.5 μg/g as As (1.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Iron Sesquioxide, add 30 mL of diluted hydrochloric acid (1 in 2) and 1 mL of nitric acid, dissolve it while heating, evaporate to about 5 mL on a water bath, add 15 mL of water, and filter. Wash the insoluble substances on the filter paper three times with 5 mL of hot water each time, and combine the washings with the filtrate. Add 1 mL of sulfuric acid to this solution, and evaporate

until white fumes are no longer evolved. Add 10 mL of sulfurous acid solution, evaporate to about 2 mL, and add water to make 5 mL.

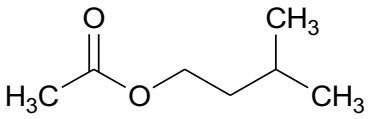
Assay Weigh accurately about 0.2 g of Iron Sesquioxide in an iodine flask, add 5 mL of hydrochloric acid, and heat on the water bath to make a solution. Add 25 mL of water and 3 g of potassium iodate, put a stopper tightly, and allow to stand for 15 minutes in a dark place. Add 100 mL of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate. Add 3 mL of starch TS near the endpoint when the color of the solution changes to a light yellow color. The endpoint is when the blue color produced by the addition of starch TS fades. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 7.984 mg of Fe_2O_3

Isoamyl Acetate

Isopentyl Acetate

酢酸イソアミル



$C_7H_{14}O_2$ Mol. Wt. 130.18

3-Methylbutyl acetate [123-92-2]

Content Isoamyl Acetate contains not less than 95.0% of isoamyl acetate ($C_7H_{14}O_2$).

Description Isoamyl Acetate is a colorless, clear liquid having a banana-like odor.

Identification Determine the absorption spectrum of Isoamyl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.399–1.403.

Specific Gravity d_{25}^{25}: 0.868–0.878.

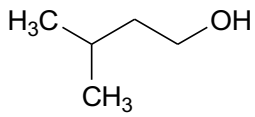
Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (2).

Isoamyl Alcohol

Isopentanol

イソアミルアルコール



$C_5H_{12}O$ Mol. Wt. 88.15

3-Methylbutan-1-ol [123-51-3]

Content Isoamyl Alcohol contains not less than 98.0% of isoamyl alcohol ($C_5H_{12}O$).

Description Isoamyl Alcohol is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isoamyl Alcohol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.404–1.410.

Specific Gravity d_{25}^{25}: 0.806–0.813.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2).

Isoamylase

イソアミラーゼ

Definition Isoamylase includes enzymes that hydrolyze α-1,6-glucosidic linkages in starch-based polysaccharides. It is derived from the culture of bacteria (limited to *Flavobacterium odoratum*, *Naxibacter* sp., *Pseudomonas amyloderamosa*, and species of the genus *Bacillus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Isoamylase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Isoamylase complies with the Isoamylase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

The requirement of total plate count can be disregarded if unsterilized Isoamylase is used for captive consumption in the manufacture of food that will be sterilized or pasteurized before completion of the final product.

Isoamylase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given methods, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Isoamylase, add acetate buffer (0.05 mol/L, pH 6.0, containing calcium chloride) or water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Suspend 0.50 g of waxy cornstarch in 50 mL of water, and dissolve it by stirring while heating. Add water to this solution to make 100 mL. Prepare fresh before use and keep at 45°C.

Test Solution Add 0.35 mL of the substrate solution and 0.1 mL of the sample solution to 0.1 mL of acetate buffer (0.05 mol/L, pH 6.0, containing calcium chloride), equilibrated at 45°C, shake immediately. Incubate the mixture at 45°C for 15 minutes. To this solution, add 0.5 mL of iodine TS (for isoamylase activity test), allow the mixture to stand at room temperature for 15 minutes, add 10 mL of water, and mix.

Control Solution Add 0.35 mL of the substrate solution to 0.1 mL of acetate buffer (0.05 mol/L, pH 6.0, containing calcium chloride), equilibrate the mixture at 45°C for 15 minutes, and add 0.5 mL of iodine TS (for isoamylase activity test). To this solution, add 0.1 mL of the sample solution, shake immediately, allow the mixture to incubate at room

temperature for 15 minutes, add 10 mL of water, and mix.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 610 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 1.0 g of Isoamylase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 0.40 g of branched dextrin, dissolve it in 40 mL of acetate buffer (0.05 mol/L) at pH 5.0 , and add the same buffer to make 100 mL.

Test Solution Equilibrate 6 mL of the substrate solution at 50°C for 5 minutes, add 1 mL of the sample solution, shake well, incubate the mixture at 50°C for 30 minutes, add 2 mL of trichloroacetic acid–sulfuric acid TS, and shake well. To this solution, add 1 mL of iodine TS (2.75 mmol/L), shake well, and allow the mixture to stand at room temperature for 15 minutes.

Control Solution To 1 mL of the sample solution, add 2 mL of trichloroacetic acid–sulfuric acid TS, and mix. Add 6 mL of the substrate solution, shake well, and allow to incubate at room temperature for 15 minutes.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 610 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them and use the supernatants.

Method 3

Sample Solution Weigh 1.5 g of Isoamylase, add acetate buffer (0.01 mol/L) at pH 4.5 to dissolve it or disperse it uniformly, and make 500 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Suspend 4.2 g of waxy cornstarch (Lintner soluble) in 300 mL of water, heat the suspension while stirring, boil it for 5 minutes, and cool. To this solution, add 50 mL of acetate buffer (1 mol/L) at pH 3.5 and water to make 500 mL. Prepare fresh before use and keep at 40°C.

Test Solution To 3 mL of the substrate solution, equilibrated at 40°C, add 0.5 mL of the sample solution, and shake immediately. Incubate the mixture at 40°C for 30 minutes. To 15 mL of diluted sulfuric acid (1 in 1800), add 0.5 mL of this solution and 0.5 mL of iodine TS (0.005 mol/L), allow the mixture to stand at 25°C for 15 minutes.

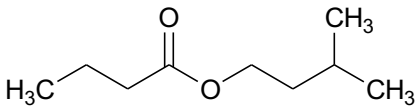
Control Solution To 3 mL of the substrate solution, equilibrated at 40°C, add 0.5 mL of the sample solution, and shake. Immediately add 0.5 mL of the resulting solution to 15 mL of diluted sulfuric acid (1 in 1800), and proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a

wavelength of 610 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them and use the supernatants.

Isoamyl Butyrate

酪酸イソアミル



$C_9H_{18}O_2$ Mol. Wt. 158.24

3-Methylbutyl butanoate [106-27-4]

Content Isoamyl Butyrate contains not less than 98.0% of isoamyl butyrate ($C_9H_{18}O_2$).

Description Isoamyl Butyrate is a colorless to light yellow, clear liquid having a fruity odor.

Identification Determine the absorption spectrum of Isoamyl Butyrate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.409–1.413.

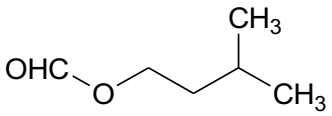
Specific Gravity d_{25}^{25}: 0.859–0.864.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Isoamyl Formate

ギ酸イソアミル



$C_6H_{12}O_2$ Mol. Wt. 116.16

3-Methylbutyl formate [110-45-2]

Content Isoamyl Formate contains not less than 92.0% of isoamyl formate ($C_6H_{12}O_2$).

Description Isoamyl Formate is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isoamyl Formate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.396–1.400.

Specific Gravity d_{25}^{25}: 0.876–0.884.

Purity Acid value Not more than 3.0 (Flavoring Substances Tests).

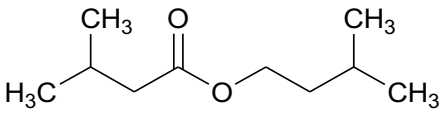
In the test, titrate to the first light pink color that persists for 10 seconds while cooling in ice water.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (2).

Isoamyl Isovalerate

3-Methylbutyl 3-Methylbutyrate

イソ吉草酸イソアミル



$C_{10}H_{20}O_2$ Mol. Wt. 172.26

3-Methylbutyl 3-methylbutanoate [659-70-1]

Content Isoamyl Isovalerate contains not less than 98.0% of isoamyl isovalerate ($C_{10}H_{20}O_2$).

Description Isoamyl Isovalerate is a colorless to light yellow, clear liquid having a fruity odor.

Identification Determine the absorption spectrum of Isoamyl Isovalerate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.411–1.414.

Specific Gravity d_{25}^{25}: 0.851–0.857.

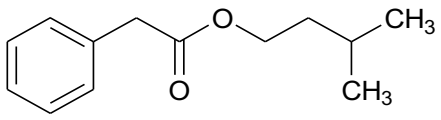
Purity Acid value Not more than 2.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas

Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Isoamyl Phenylacetate

フェニル酢酸イソアミル



$C_{13}H_{18}O_2$ Mol. Wt. 206.28

3-Methylbutyl 2-phenylacetate [102-19-2]

Content Isoamyl Phenylacetate contains not less than 97.0% of isoamyl phenylacetate ($C_{13}H_{18}O_2$).

Description Isoamyl Phenylacetate is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isoamyl Phenylacetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.483–1.490.

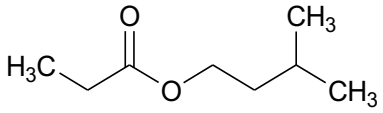
Specific Gravity d_{25}^{25}: 0.975–0.981.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Isoamyl Propionate

プロピオン酸イソアミル



$C_8H_{16}O_2$ Mol. Wt. 144.21

3-Methylbutyl propanoate [105-68-0]

Content Isoamyl Propionate contains not less than 98.0% of isoamyl propionate

($C_8H_{16}O_2$).

Description Isoamyl Propionate is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isoamyl Propionate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.405–1.409

Specific Gravity d_{25}^{25}: 0.864–0.869

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Iso-*α*-bitter Acids

イソアルファー苦味酸

Definition Iso-*α*-bitter Acids are obtained by the flowers of the hops (*Humulus lupulus* L.). They consist mainly of isohumulones.

Content Iso-*α*-bitter Acids contain not less than 20.0% of iso-*α*-bitter acids.

Description Iso-*α*-bitter Acids occur as yellow-brown liquids. They have a characteristic odor and a strongly bitter taste.

Identification

Analyze the test solution and the standard solution prepared as directed in the Assay by liquid chromatography using the operating conditions specified in the Assay. Three of the peaks from the test solution correspond to the retention times of main peaks from the standard solution, respectively.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 4, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay

Test Solution Weigh accurately about 0.1 g of the sample, and dissolve it in a 500 : 1 mixture of methanol/phosphoric acid TS (0.1 mol/L) to make exactly 100 mL. To exactly 10 mL of this solution, add a 500 : 1 mixture of methanol/phosphoric acid TS (0.1 mol/L) to make exactly 50 mL. If the solution is turbid, filter it through with a membrane filter

(0.45 μm pore size).

Standard Solution Weigh accurately about 50 mg of iso-α-bitter acids for assay. Add a 500 : 1 mixture of methanol/phosphoric acid TS (0.1 mol/L) to make exactly 100 mL. To exactly 10 mL of this solution, add a 500 : 1 mixture of methanol/phosphoric acid TS (0.1 mol/L) to make exactly 50 mL.

Procedure Analyze 10 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. For the standard solution, three main peaks appear in the order of isocohumulone, isohumulone, and isoadhumulone. Sum up the peak areas of all the peaks between the retention times of isocohumulone and isoadhumulone in the test solution, and determine the content of iso-α-bitter acids by the formula:

$$\text{Content (\%) of iso-}\alpha\text{-bitter acids} = \frac{a \times b \times A_A}{M \times A_S \times 1000}$$

a = weight (mg) of iso-α-bitter acids for assay.

b = content (%) of iso-α-bitter acids in iso-α-bitter acids for assay.

A_A = total area of all the peaks between the retention times of isocohumulone and isoadhumulone in the test solution.

A_S = total area of the three main peaks from the standard solution.

M = weight (g) of the sample.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 270 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 35°C.

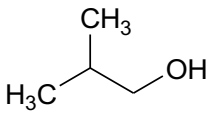
Mobile phase: A 75 : 24 : 1 mixture of methanol/water/phosphoric acid.

Flow rate: 1 mL/min.

Isobutanol

Isobutyl Alcohol
2-Methylpropan-1-ol

イソブタノール



$C_4H_{10}O$ Mol. Wt. 74.12

2-Methylpropan-1-ol [78-83-1]

Content Isobutanol contains not less than 98.0% of isobutanol ($C_4H_{10}O$).

Description Isobutanol is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isobutanol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.392–1.398.

Specific Gravity d_{25}^{25}: 0.799–0.801.

Purity Acid value Not more than 2.0 (Flavoring Substances Tests).

Assay Proceed as directed in Method 1 in the Gas Chromatographic Assay in the Flavoring Substances Tests, using operating conditions (2).

Isobutylamine

イソブチルアミン

$C_4H_{11}N$ Mol. Wt. 73.14

2-Methylpropan-1-amine [78-81-9]

Content Isobutylamine contains not less than 95.0% of isobutylamine ($C_4H_{11}N$).

Description Isobutylamine occurs as a colorless to yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Isobutylamine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive index n_D^{20}: 1.391–1.400

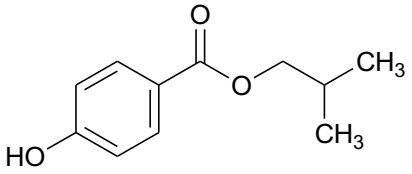
Specific gravity d_{25}^{25}: 0.724–0.737

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operating conditions (2) except for the column. Use a fused silica tube (0.25–0.53 mm internal diameter and 30–60 m length) coated with a 0.25–1 μm thick layer of

dimethylpolysiloxane for gas chromatography.

Isobutyl *p*-Hydroxybenzoate

パラオキシ安息香酸イソブチル



$C_{11}H_{14}O_3$ Mol. Wt. 194.23

2-Methylpropyl 4-hydroxybenzoate [4247-02-3]

Content Isobutyl *p*-Hydroxybenzoate, when dried, contains not less than 99.0% of isobutyl *p*-hydroxybenzoate ($C_{11}H_{14}O_3$).

Description Isobutyl *p*-Hydroxybenzoate occurs as colorless crystals or as a white crystalline powder. It is odorless.

Identification

(1) Boil 0.5 g of Isobutyl *p*-Hydroxybenzoate with 10 mL of sodium hydroxide solution (1 in 25) for 30 minutes, evaporate it to about 5 mL, and allow to cool. Acidify this solution with diluted sulfuric acid (1 in 20). Collect the precipitate produced by filtration, wash it well with water, and dry at 105°C for 1 hour. The melting point of the residue is 213–217°C.

(2) To 50 mg of Isobutyl *p*-Hydroxybenzoate, add 2 drops of acetic acid and 5 drops of sulfuric acid, and warm for 5 minutes. An odor of isobutyl acetate is evolved.

Melting Point 75–78°C.

Purity

(1) Free acid Not more than 0.55% as *p*-hydroxybenzoic acid.

Heat 0.75 g of Isobutyl *p*-Hydroxybenzoate with 15 mL of water in a boiling water bath for 1 minute, cool, and filter. The filtrate is acidic or neutral. To 10 mL of the filtrate, and add 0.20 mL of 0.1 mol/L sodium hydroxide and 2 drops of methyl red TS. The solution is yellow.

(2) Sulfate Not more than 0.024% as SO_4.

Sample Solution To 1.0 g of Isobutyl *p*-Hydroxybenzoate, add 100 mL of hot water, and heat for 5 minutes while shaking well. After cooling, add water to make 100 mL, and filter. Use 40 mL of the filtrate.

Control Solution 0.20 mL of 0.005 mol/L sulfuric acid.

(3) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more than 0.5% (5 hours).

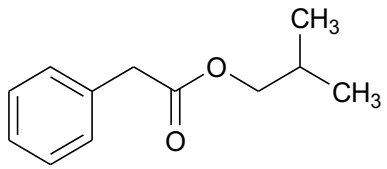
Residue on Ignition　Not more than 0.1%.

Assay　Weigh accurately about 2 g of Isobutyl *p*-Hydroxybenzoate, previously dried, add exactly 40 mL of 1 mol/L sodium hydroxide, boil for 30 minutes, and cool. Titrate the excess alkali with 0.5 mol/L sulfuric acid (indicator: 5 drops of bromothymol blue). The endpoint is when the solution produces the same color as that produced when the indicator is added to phosphate buffer (pH 6.5). Perform a blank test.

Each mL of 1 mol/L sodium hydroxide = 194.2 mg of $C_{11}H_{14}O_3$

Isobutyl Phenylacetate

フェニル酢酸イソブチル



$C_{12}H_{16}O_2$　Mol. Wt. 192.25

2-Methylpropyl 2-phenylacetate　[102-13-6]

Content　Isobutyl Phenylacetate contains not less than 98.0% of isobutyl phenylacetate ($C_{12}H_{16}O_2$).

Description　Isobutyl Phenylacetate is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isobutyl Phenylacetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index　n_D^{20}: 1.484–1.488.

Specific Gravity　d_{25}^{25}: 0.984–0.988.

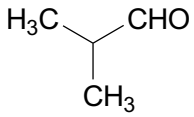
Purity　Acid value　Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Isobutyraldehyde

Isobutanal

イソブチルアルデヒド



C_4H_8O Mol. Wt. 72.11

2-Methylpropanal [78-84-2]

Content Isobutyraldehyde contains not less than 98.0% of isobutyraldehyde (C_4H_8O).

Description Isobutyraldehyde is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isobutyraldehyde, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.369–1.379.

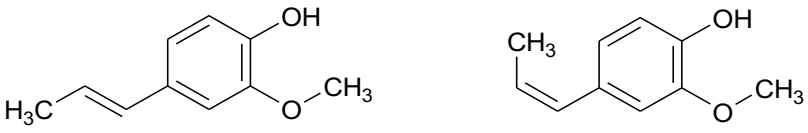
Specific Gravity d_{25}^{25}: 0.783–0.791.

Purity Acid value Not more than 5.0 (Flavoring Substance Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (3).

Isoeugenol

イソオイゲノール



$C_{10}H_{12}O_2$ Mol. Wt. 164.20

2-Methoxy-4-(prop-l-en-1-yl)phenol [97-54-1]

Content Isoeugenol contains not less than 98.5% (vol) of isoeugenol ($C_{10}H_{12}O_2$).

Description Isoeugenol is a colorless to light yellow-brown, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isoeugenol as directed in the

Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

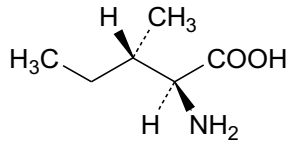
Refractive Index n_D^{20}: 1.572–1.577.

Specific Gravity d_{25}^{25}: 1.081–1.087.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

L-Isoleucine

L-イソロイシン



$C_6H_{13}NO_2$ Mol. Wt. 131.17

(*2S,3S*)-2-Amino-3-methylpentanoic acid [73-32-5]

Content L-Isoleucine, when calculated on the dried basis, contains 98.0–102.0% of L-isoleucine ($C_6H_{13}NO_2$).

Description L-Isoleucine occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and has a slightly bitter taste.

Identification To 5 mL of L-Isoleucine solution (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

Specific Rotation $[\alpha]_D^{20}$: +38.0 to +41.5° (2 g, hydrochloric acid TS (6 mol/L), 50 mL, on the dried basis).

pH 5.5–7.0 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution Colorless and clear (0.50 g, hydrochloric acid TS (1 mol/L)10 mL).

(2) Chloride Not more than 0.021% as Cl (0.50 g, 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 2, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.25 g of L-Isoleucine, and proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 13.12 mg of $C_6H_{13}NO_2$

Isomaltodextranase

イソマルトデキストラナーゼ

Definition Isomaltodextranase includes enzymes that degrade dextran. It is derived from the culture of bacteria (limited to the genus *Arthrobacter*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Isomaltodextranase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Isomaltodextranase complies with the Isomaltodextranase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution specified in Method 1, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

The requirement of total plate count can be disregarded if unsterilized Isomaltodextranase is used for captive consumption in the manufacture of food that will be sterilized or pasteurized before the completion of the final product.

Isomaltodextranase Activity Test Perform the test using either of the methods given below. If the activity test cannot be performed by the given method, appropriate

replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Isomaltodextranase, add water or acetate buffer (0.05 mol/L) at pH 4.5 to dissolve it or disperse it uniformly, and make 10 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 1.25 g of dextran (molecular weight: 150,000), and dissolve it in acetate buffer (0.05 mol/L) at pH 4.5 to make 100 mL.

Test Solution To 5 mL of the substrate solution, equilibrated at 40°C, add 0.2 mL of the sample solution, and mix. Incubate the mixture at 40°C for 20 minutes. Transfer 1 mL of this solution into a test tube containing 2 mL of Somogyi copper TS. Cover the mouth of the test tube with a glass bead, heat in a water bath for 10 minutes, and cool to room temperature. Add 2 mL of Nelson TS, mix, allow to stand for 30 minutes, and add 5 mL of water.

Control Solution To 5 mL of the substrate solution, equilibrated at 40°C, add 0.2 mL of the sample solution and mix. Transfer 1 mL of this solution into a test tube containing 2 mL of Somogyi copper TS, and mix immediately. Cover the mouth of the test tube with a glass bead, heat in a water bath for 10 minutes, and cool to room temperature. Add 2 mL of Nelson TS, mix, allow to stand for 30 minutes, and add 5 mL of water.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 520 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 1.0 g of Isomaltodextranase, add water or acetate buffer (0.05 mol/L) at pH 4.5 to dissolve it or disperse it uniformly, and make 10 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 1.25 g of dextran (molecular weight: 150,000), and dissolve it in acetate buffer (0.05 mol/L) at pH 4.5 to make 50 mL.

Test Solution Add 500 µL of the sample solution to 500 µL of the substrate solution and mix. Incubate the mixture at 40°C for 4 hours, heat in a water bath for 10 minutes, and cool to room temperature.

Standard Solution Dissolve 0.13 g of isomaltose in 10 mL of water.

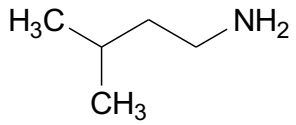
Control Solution Add 500 µL of the sample solution to 500 µL of the substrate solution, and mix. Heat immediately in a water bath for 10 minutes and cool.

Procedure Analyze 2 µL each of the test solution, standard solution, and control solution by thin-layer chromatography using a 6 : 4 : 1 mixture of 1-butanol/pyridine/water as the developing solvent. Use a thin-layer plate coated with silica gel for thin-

layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 15 cm above the starting line, and air-dry the plate. Spray with 15% sulfuric acid–methanol TS, heat at 100°C for 10 minutes, and examine. One of the spots from the test solution has the same R_f value as that from the standard solution and is darker in color than the spot with the same R_f value from the control solution.

Isopentylamine

イソペンチルアミン



$C_5H_{13}N$ Mol. Wt. 87.16

Isopentylamine [107-85-7]

Content Isopentylamine contains not less than 98.0% of isopentylamine ($C_5H_{13}N$).

Description Isopentylamine occurs as a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Isopentylamine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.405–1.411.

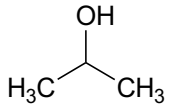
Specific Gravity d_{20}^{20}: 0.747–0.753.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2). For the column, use a fused silica tube (0.25–0.53 mm internal diameter and 30–60 m length) coated with a 0.25–1 μm thick layer of dimethylpolysiloxane for gas chromatography.

Isopropanol

2-Propanol

イソプロパノール



C_3H_8O Mol. Wt. 60.10

Propan-2-ol [67-63-0]

Content Isopropanol contains not less than 99.7% of isopropanol (C_3H_8O).

Description Isopropanol is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of isopropanol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.374–1.380.

Specific Gravity d_{20}^{20}: 0.784–0.788.

Purity

(1) Free acids To 15.0 mL of Isopropanol, add 50 mL of freshly boiled and cooled water and 2 drops of phenolphthalein TS, then 0.40 mL of 0.01 mol/L sodium hydroxide solution. The solution develops red color.

(2) Lead Not more than 1 μg/g as Pb (4.0 g, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Test Solution Evaporate the specified amount of Isopropanol to dryness by heating. Add 1 mL of sulfuric acid to the residue, and heat until white fumes are no longer evolved. Heat it at 500°C for 3 hours in an electric furnace. Add 10 mL of diluted hydrochloric acid (1 in 4), and evaporate to dryness by heating. Dissolve the residue by adding diluted nitric acid (1 in 150) to make 10 mL.

Control Solution To the specified amount of Lead Standard Solution, add diluted nitric acid (1 in 150) to make 10 mL.

(3) Residue on evaporation Not more than 0.002 %(w/v)

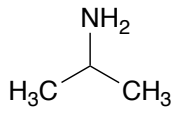
Heat an evaporating dish at 105°C for 30 minutes, cool it in a desiccator, and weigh accurately. Measure 100 mL of Isopropanol into the tared evaporating dish, and evaporate to dryness on a water bath. Heat the dish at 105°C for 30 minutes or to constant weight, and weigh it.

Water Content Not more than 0.20% (10 g, Volumetric Titration, Direct Titration).

Assay Proceed as directed in Method 1 in the Gas Chromatographic Assay in the Flavoring Substances Tests, using operating conditions (2).

Isopropylamine

イソプロピルアミン



C_3H_9N Mol. Wt. 59.11

Propan-2-amine [75-31-0]

Content Isopropylamine contains not less than 95.0% of isopropylamine (C_3H_9N).

Description Isopropylamine occurs as a colorless to yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Isopropylamine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive index n_D^{20}: 1.367–1.378

Specific gravity d_{25}^{25}: 0.681–0.693

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operating conditions (2) except for the column. Use a fused silica tube (0.25–0.53 mm internal diameter and 30–60 m length) coated with a 0.25–1 μm thick layer of dimethylpolysiloxane for gas chromatography.

Isopropyl Citrate

クエン酸イソプロピル

Mixture of 1-methylethyl esters of 2-hydroxypropane-1,2,3-tricarboxylic acid and glycerol esters of fatty acids

Definition Isopropyl Citrate is a mixture of isopropyl citrate and glycerol esters of fatty acids.

Description Isopropyl Citrate occurs as a colorless to white, oily or waxy substance. It

is odorless. When it is allowed to stand, crystals may be deposited.

Identification

(1) Heat 2 g of Isopropyl Citrate with 50 mL of sodium hydroxide solution (1 in 25), distill it to collect 20 mL of distillate, and refer to it as Solution A. After cooling, neutralize the residue solution with diluted sulfuric acid (1 in 20). The resulting solution responds to the test (2) for Citrate in the Qualitative Tests.

(2) Use Solution A prepared in Identification (1) as the test solution. Use a diluted solution of 2-propanol (1 in 5) as the standard solution. Analyze 1.0 μL each of the test solution and standard solution by gas chromatography using the operating conditions given below. The retention time of the main peak of the test solution corresponds to that of the 2-propanol peak of the standard solution.

Operation Condition

Detector: Flame ionization detector.

Column: A fused silica tube (0.25mm internal diameter and 60 m length) coated with a 1.40-μm thick layer of 25% dipheny/75% dimethyl polysiloxane.

Column temperature: Maintain the temperature at 40°C for 6 minutes, raise at a rate of 5°C/minute to 110°C, and maintain for 10 minutes.

Injection port temperature: 200°C.

Detector temperature: 250°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention time of 2-propanol to about 10 minutes.

Injection method: Split.

Split ratio: 1:100.

Purity

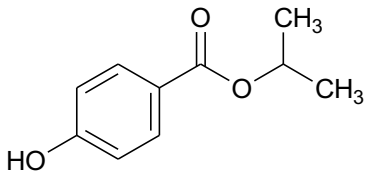
(1) Lead Not more than 2 μg/g as Pb. (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1 μg/g as As (1.5 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.3%.

Isopropyl *p*-Hydroxybenzoate

パラオキシ安息香酸イソプロピル



$C_{10}H_{12}O_3$ Mol. Wt. 180.20

1-Methylethyl 4-hydroxybenzoate [4191-73-5]

Content Isopropyl *p*-Hydroxybenzoate, when dried, contains not less than 99.0% of isopropyl *p*-hydroxybenzoate ($C_{10}H_{12}O_3$).

Description Isopropyl *p*-Hydroxybenzoate occurs as colorless crystals or as a white crystalline powder. It is odorless.

Identification

(1) Proceed as directed in Identification (1) for Isobutyl *p*-Hydroxybenzoate.

(2) To 50 mg of Isopropyl *p*-Hydroxybenzoate, add 2 drops of acetic acid and 5 drops of sulfuric acid, and warm for 5 minutes. An odor of isopropyl acetate is evolved.

Melting Point 84–86°C.

Purity

(1) Free acid Not more than 0.55% as *p*-hydroxybenzoic acid.

Proceed as directed in Purity (1) for Isobutyl *p*-Hydroxybenzoate.

(2) Sulfate Not more than 0.024% as SO_4.

Proceed as directed in Purity (2) for Isobutyl *p*-Hydroxybenzoate.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.5% (5 hours).

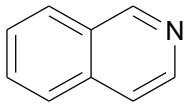
Residue on Ignition Not more than 0.1% .

Assay Proceed as directed in the Assay for Isobutyl *p*-Hydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide = 180.2 mg of $C_{10}H_{12}O_3$

Isoquinoline

イソキノリン



C_9H_7N Mol. Wt. 129.16

Isoquinoline [119-65-3]

Content Isoquinoline contains not less than 97.0% of isoquinoline (C_9H_7N).

Description Isoquinoline occurs as a colorless to light yellow liquid or a white to light yellow solid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Isoquinoline, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers. If the test sample is solid, warm in a water bath at 40°C to melt before using.

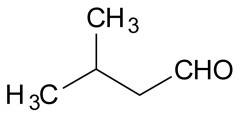
Refractive Index n_D^{30}: 1.618–1.624.

Specific Gravity d_{30}^{30}: 1.093–1.099.

Assay Use a solution (1 in 10) of Isoquinoline in ethanol (95) as the test solution. Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (1), except for the column temperature. Inject the sample at 150°C, raise the temperature to 230°C at a rate of 5°C/minute, and maintain at 230°C for 24 minutes.

Isovaleraldehyde

イソバレルアルデヒド



$C_5H_{10}O$ Mol. Wt. 86.13

3-Methylbutanal [590-86-3]

Content Isovaleraldehyde contains not less than 95.0% of isovaleraldehyde ($C_5H_{10}O$).

Description Isovaleraldehyde occurs as a colorless to pale yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isovaleraldehyde, as directed in

the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.387–1.408.

Specific Gravity d_{20}^{20}: 0.795–0.815.

Purity Acid value Not more than 10.0 (Flavoring Substance Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (3).

Japanese Persimmon Color

カキ色素

Definition

Japanese Persimmon Color is obtained from the fruits of the *Diospyros kaki* Thunb. It is produced from fermented and roasted fruits through extraction with hydrous ethanol or through extraction with an alkaline solution and neutralization. It may contain dextrin or lactose.

Color Value The Color Value $\left(E_{1cm}^{10\%}\right)$ of Japanese Persimmon Color is not less than 20 and is in the range of 90-110% of the labeled value.

Description Japanese Persimmon Color occurs as red-brown to dark brown lumps, powder, paste, or liquid. It has a slight characteristic odor.

Identification

(1) Weigh an amount of Japanese Persimmon Color equivalent to 2.5 g of Japanese persimmon color with a Color Value 20, and dissolve it in 100 mL of citrate buffer (pH 7.0). The resulting solution is red-brown to dark brown.

(2) To 5 mL of the solution prepared in Identification (1), add 2–3 drops of hydrochloric acid and allow to stand. A red-brown to dark brown precipitate is produced.

(3) To 5 mL of the solution prepared in Identification (1), add 2 mL of iron(III) chloride hexahydrate solution (1 in 50). A gray to dark brown precipitate is produced.

(4) Weigh an amount of Japanese Persimmon Color equivalent to 1 g of Japanese persimmon color with a Color Value 20, and dissolve it in 100 mL of sodium hydroxide solution (1 in 250). To 5 mL of this solution, add 10 mL of diluted hydrochloric acid (9 in 1000) and 0.1 mL of zinc chloride TS (pH 3.0), and stir. Stopper it, and warm at 50°C for 20 minutes. If necessary, centrifuge at 3000 rpm for 10 minutes. A yellow-brown to dark brown precipitate is produced.

Purity

(1) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination　Proceed as directed under Color Value Determination, according to the following operating conditions:

Operating Conditions

Solvent: Citrate buffer (pH 7.0).

Wavelength: 500 nm.

Kansui

かんすい

Definition　Kansui contains one or more of the following food additives: "Potassium Carbonate, Anhydrous," "Sodium Carbonate," "Sodium Hydrogen Carbonate," and "Potassium or Sodium Salts of Metaphosphoric Acid, Phosphoric Acids, Polyphosphoric Acid, and Pyrophosphoric Acid."*

There are three types of Kansui: Solid Kansui, Liquid Kansui, and Diluted Powder Kansui, which is diluted with flour.

Solid Kansui

固形かんすい

Description　Solid Kansui occurs as colorless to white crystals, powder, or lumps, or as a mixture of these.

Identification

(1) A solution of Solid Kansui (1 in 10) is alkaline.

(2) A solution of Solid Kansui (1 in 10) responds to test (1) for Potassium Salt or to test (1) for Sodium Salt in the Qualitative Tests.

(3) If Solid Kansui contains a carbonate or hydrogen carbonate salt, a solution of it (1

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* "Potassium or Sodium Salts of Metaphosphoric Acid, Phosphoric Acids, Polyphosphoric Acid, and Pyrophosphoric Acid" refers to the following 13 substances: Dipotassium Hydrogen Phosphate, Disodium Dihydrogen Pyrophosphate, Disodium Hydrogen Phosphate, Potassium Dihydrogen Phosphate, Potassium Metaphosphate, Potassium Polyphosphate, Potassium Pyrophosphate, Sodium Dihydrogen Phosphate, Sodium Metaphosphate, Sodium Polyphosphate, Sodium Pyrophosphate, Tripotassium Phosphate, Trisodium Phosphate.

in 10) responds to test (1) for Carbonate in the Qualitative Tests,

(4) If Solid Kansui contains a phosphate salt, a solution of it (1 in 10) responds to test (2) for Phosphate in the Qualitative Tests, when made acidic with the addition of diluted nitric acid (1 in 10).

Purity Weigh 10 g of Solid Kansui, and dissolve it in water to make 200 mL. Refer to this solution as Solution A.

(1) Clarity of solution Very slightly turbid.

Test Solution Use 20 mL of Solution A.

(2) Alkali hydroxide Measure 40 mL of Solution A, add 50 mL of a solution of barium chloride dihydrate (3 in 25) and water to make 100 mL, shake vigorously, and filter. Measure 50 mL of the filtrate, and add 3 drops of 0.1 mol/L hydrochloric acid and 3 drops of phenolphthalein TS. No pink color develops.

(3) Chloride Not more than 0.35% as Cl (Solution A 1.0 mL, Control Solution: 0.01 mol/L hydrochloric acid 0.50 mL).

(4) Silicate Measure 10 mL of Solution A, add 1 drop of phenolphthalein TS, then add diluted hydrochloric acid (1 in 4) until the pink color disappears, and heat in a water bath for 15 minutes. Cool, and if the solution is pink in color, add diluted hydrochloric acid (1 in 4) until the pink color disappears. To this solution, add 1 drop of methylene blue TS and 10 mL of ammonium chloride saturated solution, and allow to stand for 2 hours. No colored precipitate or colored turbidity appears.

(5) Heavy metals Not more than 40 μg/g as Pb.

Test Solution Measure 10 mL of Solution A, add 3 mL of diluted hydrochloric acid (1 in 4), and evaporate to dryness on a water bath. Dissolve the residue by adding 2 mL of diluted acetic acid (1 in 20) and 20 mL of water, and add water to make 50 mL.

Control Solution Measure exactly 2 mL of Lead Standard Solution (for heavy metals limit test), and add 2 mL of diluted acetic acid (1 in 20) and water to make 50 mL.

(6) Arsenic Not more than 3 μg/g as As (Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution 10 mL of Solution A.

Liquid Kansui

液状かんすい

Description Liquid Kansui is a colorless, clear liquid.

Identification Proceed as directed in Identification (1) through (4) for Solid Kansui.

Specific Gravity d_{20}^{20}: 1.20–1.33.

Purity For the tests (i), (ii), (iii), (iv), and (v), use Solution B prepared as follows: Measure the volume of Liquid Kansui, indicated in Table 1, that corresponds to its

specific gravity, and add water to make 200 mL.

(i) Alkali hydroxide Measure 40 mL of Solution B, and proceed as directed in Purity (2) for Solid Kansui.

(ii) Chloride Not more than 0.35% as Cl (Solution B 1.0 mL, Control Solution: 0.01 mol/L hydrochloric acid 0.50 mL).

(iii) Silicate Measure 10 mL of Solution B, and proceed as directed in Purity (4) for Solid Kansui.

(iv) Heavy metals Not more than 40 μg/g of solid as Pb.

Measure 10 mL of Solution B, and proceed as directed in Purity (5) for Solid Kansui.

(v) Arsenic Not more than 3 μg/g of solid as As (Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Use 10 mL of Solution B.

Table 1

Specific gravity	Sample volume (mL)	Specific gravity	Sample volume (mL)	Specific gravity	Sample volume (mL)
1.20	39.8	1.25	31.0	1.30	25.4
1.21	37.6	1.26	29.8	1.31	24.4
1.22	35.6	1.27	28.6	1.32	23.6
1.23	34.0	1.28	27.4	1.33	22.8
1.24	32.4	1.29	26.4		

Diluted Powder Kansui

希釈粉末かんすい

Description Diluted Powder Kansui occurs as a homogeneous, white to light yellow powder.

Identification

(1) To 1 g of Diluted Powder Kansui, add 1 drop of iodine TS. A purple color develops.

(2) To 10 g of Diluted Powder Kansui, add 50 mL of water, shake well, filter, and proceed as directed in Identification (1) through (4) for Solid Kansui, using the filtrate.

Specific Gravity Weigh 60 g of Diluted Powder Kansui, add water to make 200 mL, shake well, and filter. The specific gravity (d_{20}^{20}) of the filtrate is 1.12–1.17.

Purity

(1) Insoluble substances Not more than 2.0%.

Weigh 0.50 g of Diluted Powder Kansui, add 100 mL of sodium hydroxide solution (1 in 100), boil for 15 minutes, and allow to stand for 30 minutes. No precipitate is observed. If a precipitate is observed, filter through a filter paper for quantitative analysis (5C), wash with water until the washings are no longer alkaline, and ignite the residue together with the filter paper at about 550°C to constant weight. Weigh the residue.

(2) For the tests (i), (ii), and (iii), use Solution C prepared as follows: Measure the volume of the filtrate obtained in Purity (1), indicated in Table 2, that corresponds to the specific gravity obtained in Purity (1), and add water to make 100 mL.

(i) Alkali hydroxide Measure 40 mL of Solution C, and proceed as directed in Purity (2) for Solid Kansui.

(ii) Chloride Not more than 0.35% of water-soluble solid as Cl (Solution C 1.0 mL, Control Solution: 0.01 mol/L hydrochloric acid 0.50 mL).

(iii) Silicate Measure 10 mL of Solution C, and proceed as directed in Purity (4) for Solid Kansui.

Table 2

Specific gravity	Filtrate volume (mL)	Specific gravity	Filtrate volume (mL)	Specific gravity	Filtrate volume (mL)
1.12	34.3	1.14	29.2	1.16	25.4
1.13	31.7	1.15	27.2	1.17	23.7

(3) Heavy metals Not more than 30 μg/g as Pb (1.0 g, Method 2, Control Solution: Lead Standard Solution (for heavy metals limit test) 3.0 mL).

(4) Arsenic Not more than 1.9 μg/g as As (0.79 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Kaoliang Color

コウリャン色素

Definition Kaoliang Color is obtained from the seeds and husks of the sorghum plant *Sorghum bicolor* (L.) Moench (*Sorghum nervosum* Besser ex Schult. & Schult. f., *Sorghum vulgare* Pers.) by extraction with water, hydrous ethanol, or acidic hydrous ethanol or by extraction with an alkaline solution and neutralization. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Kaoliang Color is not less than 50 and is in the range of 90–110% of the labeled value.

Description Kaoliang Color occurs as a brown to black powder, as lumps , or as a paste

or liquid. It has a slight, characteristic odor.

Identification

(1) Weigh an amount of Kaoliang Color equivalent to 1 g of kaoliang color with a Color Value 50, and add 500 mL of a 3:2 mixture of water/ethanol (95). The resulting solution is yellow-brown to red-brown.

(2) To 10 mL of the solution obtained in Identification (1), add 1 mL of iron(III) chloride hexahydrate solution (1 in 10). A brown to dark brown color is produced.

(3) Weigh an amount of Kaoliang Color equivalent to 0.4 g of kaoliang color with a Color Value 50, and dissolve it in 100 mL of sodium hydroxide solution (1 in 250). To 5 mL of this solution, add 10 mL of diluted hydrochloric acid (9 in 1000), then add 0.1 mL of zinc chloride TS (pH 3.0), and shake. Stopper, warm at 50°C for 20 minutes, and centrifuge at 3000 rpm for 10 minutes if necessary. A yellow-brown to dark brown precipitate is produced.

(4) Weigh an amount of Kaoliang Color equivalent to 0.2 g of kaoliang color with a Color Value 50, and add 100 mL of a 3:2 mixture of water/ethanol (95). Centrifuge this solution at 3000 rpm for 10 minutes. To 5 mL of the supernatant, add 5 mL of a solution (1 in 20) of hydrochloric acid in 1-butanol, and shake. Stopper, and heat in a water bath for 30 minutes. After cooling, centrifuge at 3000 rpm for 10 minutes. The resulting supernatant exhibits an absorption maximum at a wavelength of 475–500 nm.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination. Prepare a test solution as directed below. Weigh accurately a specified amount of Kaoliang Color, dissolve it in 10 mL of sodium hydroxide TS (0.1 mol/L), and then add water to make exactly 100 mL. Refer to this as the sample solution. Dilute the sample solution if necessary. Centrifuge or filter the sample solution or diluted sample solution if necessary. Use the supernatant or filtrate as the test solution. Determine the color value of the test solution.

Operating Conditions

Reference: Water.

Wavelength: 500 nm.

Kaolin

カオリン

Definition Kaolin is a refined product of natural hydrated aluminum silicate.

Description Kaolin occurs as a white or whitish powder.

Identification

(1) Mix 0.2 g of Kaolin with 1.5 g of a 1:1 mixture of sodium carbonate/potassium carbonate, transfer to a platinum or nickel crucible, and heat until completely fused. After cooling, add 5 mL of water, allow to stand for about 3 minutes, and heat gently the bottom of the crucible to remove the fused mixture like a lump. Transfer the fused lump together with water into a beaker, and add hydrochloric acid in small portions until effervescence ceases. Add another 10 mL of hydrochloric acid, and evaporate to dryness on a water bath. Add 200 mL of water, boil, and filter. Transfer the gelatinous residue to a platinum dish, and add 5 mL of hydrofluoric acid. The residue dissolves. Then heat the solution. It almost completely evaporates.

(2) The filtrate obtained in Identification (1) responds to all the tests for Aluminum Salt in the Qualitative Tests.

(3) To 8 g of Kaolin, add 5 mL of water, and mix well. The resulting mixture has a plasticity.

pH 6.0–8.0.

Test Solution Weigh 10.0 g of Kaolin into an appropriate container, add 100 mL of water, and heat on a water bath for 2 hours with occasional shaking while replenishing the evaporated water. After cooling, filter by suction, using a filter holder equipped with a 47-mm diameter membrane filter (0.45 μm pore size). If the filtrate is turbid, repeat the suction filtration through the same filter. Wash the container and the residue on the filter with water, combine the washings with the filtrate, and add water to make 100 mL.

Purity

(1) Water-soluble substances Not more than 0.30 %.

Measure exactly 50 mL of the test solution prepared for the pH test, evaporate to dryness, and dry the residue at 105°C for 2 hours. Weigh the residue.

(2) Sulfuric acid-soluble substances Not more than 2.0%.

Weigh 1.0 g of Kaolin, add 20 mL of diluted sulfuric acid (1 in 15), shake for 15 minutes, and filter. Wash the container and the residue on the filter paper with a small amount of water, combine the washings with the filtrate, and add water to make 20 mL. Measure 10 mL of this solution, evaporate to dryness, and ignite at 550°C to constant weight. Weigh the residue.

(3) Lead Not more than 5 μg/g as Pb (0.80 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Kaolin, add 20 mL of diluted

hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, filter the supernatant to remove the insoluble matter, and wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water. Combine the washings with the filtrate.

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution　To the specified amount of Kaolin, add 2.5 mL of water and 0.5 mL of sulfuric acid, and heat on a hot plate until white fumes are evolved. Cool, and add water to make 5 mL.

(5) Foreign matter　Weigh 5 g of Kaolin, add 300 mL of water, shake, and allow to stand for 30 seconds. Discard most of the solution containing fine particles by decantation, and press the portion remaining on the bottom of the container using a glass rod with a flat tip. There is no sound of sand.

Loss on Ignition　Not more than 15.0% (550°C, constant weight).

Karaya Gum

カラヤガム

[9000-36-6]

Definition　Karaya Gum is obtained from the exudate of the karaya tree *Sterculia urens* Roxb. or the silk cotton tree *Cochlospermum religiosum* (L.) Alston and consists mainly of polysaccharides.

Description　Karaya Gum occurs as a light gray to light red-brown powder or as light yellow to light red-brown lumps. It has an acetic acid odor.

Identification

(1) To 1 g of a powder of Karaya Gum, add 50 mL of water, and mix it. A viscous liquid is produced, and it is acidic.

(2) Suspend 0.4 g of a powder of Karaya Gum in 6 mL of ethanol (95), and add 4 mL of water while stirring. The powder swells.

Purity

(1) Hydrochloric acid-insoluble substances　Not more than 3.0 %.

Weigh accurately about 5 g of Karaya Gum, and transfer into a Erlenmeyer flask containing 100 mL of diluted hydrochloric acid (1 in 10) to dissolve. Cover with a watch dish, heat gradually until the gum dissolves, and boil. While warming, filter with suction through a glass filter (1G3), previously dried at 105°C for 1 hour and weighed. Wash the residue well with warm water. Dry the residue with the glass filter at 105°C for 1 hour, and weigh.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Starch and dextrin Add 0.2 g of Karaya Gum to 10 mL of water, and boil. Allow to cool, and add 2 drops of Iodine TS. No dark blue or red purple color develops.

Loss on Drying Not more than 20.0% (105°C, 5 hours).

Ash Not more than 8.0%.

Acid-insoluble Ash Not more than 1.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 10,000 per gram.

Yeasts and molds: Not more than 3000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 2 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture Prepare as directed in Method 2 for the *Escherichia coli* test. For the *Salmonella* test, prepare by mixing 1 g of Karaya Gum with 100 mL of lactose broth to disperse uniformly and incubating at 35 ± 1°C for 24 ± 2 hours.

Lac Color

ラック色素

Definition Lac Color is obtained from the secretion of lac scale insects, *Laccifer* spp., and consists mainly of laccaic acids.

Color Value The color value of Lac Color ($E_{1cm}^{10\%}$) is not less than 1000 and is in the range of 95–115% of the labeled value.

Description Lac Color occurs as a red to dark red powder or as granules. It has a slight characteristic odor.

Identification

(1) Weigh an amount of Lac Color equivalent to 50 mg of lac color with a Color Value 1000, and dissolve it in 500 mL of sodium hydroxide TS (0.1 mol/L). A purplish red color develops.

(2) To 10 mL of the solution prepared in Identification (1), add 20 mL of hydrochloric acid TS (0.1 mol/L). The solution turns orange and exhibits an absorption maximum at

a wavelength of 485–495 nm.

(3) Weigh an amount of Lac Color equivalent to 0.1 g of lac Color with a Color Value 1000, and dissolve it in 10 mL of ethanol (95). Centrifuge this solution, and use the supernatant as the test solution. Analyze a 2-μL portion of the test solution by paper chromatography using a 4:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use No. 2 filter paper for chromatography. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry. A yellowish red to red spot is observed at an R_f value of about 0.4. An additional spot may be observed at an R_f value of about 0.2. When sprayed with ammonia solution, these spots turn dark red-purple.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Weigh accurately an appropriate amount of Lac Color so that its absorbance is in the range of 0.3–0.7. Dissolve it in 20 mL of sodium carbonate solution (1 in 200), and add water to make exactly 100 mL. Measure exactly 5 mL of this solution, and add hydrochloric acid TS (0.1 mol/L) to make exactly 50 mL. Use the last solution as the test solution. Centrifuge the solution if necessary, and use the supernatant for the test. Perform the test as directed under Color Value Determination according to the following operating conditions.

Operating Conditions

Reference solution: hydrochloric acid TS (0.1 mol/L).

Wavelength: Maximum absorption wavelength of 485–495 nm.

Lactic Acid

乳酸

Definition Lactic Acid is a mixture of lactic acid and condensation polymers of lactic acid.

Content Lactic Acid contains the equivalent of not less than 40.0% of lactic acid ($C_3H_6O_3$ = 90.08), and the equivalent of 95–105% of the labeled content of lactic acid.

Description Lactic Acid occurs as a white to light yellow solid or as a colorless to light yellow, clear liquid. It is odorless or has little or no unpleasant odor. It has an acid taste.

Identification

(1) A solution of Lactic Acid (1 in 10) is acidic.

(2) Lactic Acid responds to the test for Lactate in the Qualitative Tests.

Purity

(1) Clarity of solution Dissolve Lactic Acid in water, heating in a water bath if necessary, to prepare a solution with a concentration of 80%. Weigh 10 g of this solution, add 12 mL of diethyl ether, and mix. The resulting solution is clear, or passes the following test:

Filter the solution through a glass filter (G3), wash the residue three times with 10 mL of diethyl ether each time, then once with 10 mL of acetone, dry the residue together with the filter under reduced pressure at 50°C for 14 hours. The residue weighs not more than70 mg. (Diethyl ether-insoluble substances: Not more than 0.7% for 80% lactic acid)

(2) Citric acid, oxalic acid, tartaric acid, and phosphoric acid Dissolve Lactic Acid in water, heating in a water bath if necessary, to prepare a solution with a concentration of 40.0%. Refer to the resulting solution as Solution A. Weigh 2.0 g of Solution A, add 8 mL of water and 40 mL of calcium hydroxide TS, and boil for 2 minutes. No turbidity appears.

(3) Sulfate Not more than 0.010% as SO_4 on the basis of 80% lactic acid (2.0 g of Solution A, Control Solution: 0.005 mol/L sulfuric acid 0.20 mL).

(4) Cyanide Weigh 2.0 g of Solution A, and add water to make 100 mL. Measure 10 mL of this solution, transfer into a Nessler tube, add 1 drop of phenolphthalein TS, and add sodium hydroxide solution (1 in 10) until the solution is pink. Add another 1.5 mL of sodium hydroxide solution (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. Cool, neutralize with diluted acetic acid (1 in 20), and after the pink color of the solution disappears, add another drop. Add 10 mL of phosphate buffer (pH 6.8) and 0.25 mL of sodium *p*-toluenesulfonchloramide TS, stopper tightly, shake gently, and allow to stand for 3–5 minutes. Add 15 mL of pyridine–pyrazolone TS and water to make 50 mL, and allow to stand at about 25°C for 30 minutes. The solution does not turn blue.

(5) Lead Not more than 2 μg/g as Pb on the basis of 80% lactic acid (4.0 g of Solution A, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(6) Iron Not more than 10 μg/g as Fe on the basis of 80% lactic acid (2.0 g of Solution A, Method 1, Control Solution: Iron Standard Solution 1.0 mL).

(7) Arsenic Not more than 3 μg/g as As on the basis of 80% lactic acid (Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To 2.0 g of Solution A, add water to make 10 mL. Use 5 mL of this solution.

(8) Volatile fatty acids Weigh 5.0 g of Solution A, and heat on a water bath. This solution emits no butyric acid-like odor.

(9) Methanol Not more than 0.20% (v/w) as CH_3OH on the basis of 80% lactic acid.

Tests Solution Weigh 10 g of Solution A, add 8 mL of water and 5 g of calcium carbonate, and distill the solution. To about 5 mL of the initial distillate, add water to make 100 mL.

Control Solution To 1.0 mL of methanol, add water to 100 mL. Then add water to 1.0 mL of the resulting solution to make 100 mL.

Procedure Measure 1.0 mL of the test solution, add 0.1 mL of phosphoric acid (1 in 20) and 0.2 mL of potassium permanganate solution (1 in 300), allow to stand for 10 minutes, add 0.4 mL of sodium sulfite solution (1 in 5) and 3 mL of sulfuric acid, and then add 0.2 mL of chromotropic acid TS. The resulting solution is not darker in color than the control solution treated in the same manner as the test solution.

(10) Readily carbonizable substances Weigh 5.0 g of Solution A, and adjust to 15°C. Place gradually this solution on the surface of 5-mL sulfuric acid pre-heated to 15°C, and keep at 15°C. No circular band is formed on the boundary surface within 15 minutes. Or, a circular band formed on the boundary surface within 15 minutes is not dark gray.

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately an amount equivalent to about 1.2 g of lactic acid, add exactly 20 mL of 1 mol/L sodium hydroxide, and add water to make 100 mL. Heat on a water bath for 20 minutes, and while hot, titrate the excess alkali with 0.5 mol/L sulfuric acid (indicator: 1–2 drops of phenolphthalein TS). Perform a blank test in the same manner.

Each mL of 1 mol/L sodium hydroxide = 90.08 mg of $C_3H_6O_3$

Lactoferrin Concentrates

ラクトフェリン濃縮物

Definition Lactoferrin Concentrates are obtained from mammalian milk and consist mainly of lactoferrin.

Content Lactoferrin Concentrates, when calculated on the dried basis, contain 14.0–16.5% of nitrogen (N = 14.01) and not less than 85.0% of lactoferrin.

Description Lactoferrin Concentrates occur as light red-orange to dark red-brown powders. They are odorless.

Identification

(1) To 10 mL of a solution of the sample (1 in 100), add 1 mL of sodium hydroxide solution (1 in 10) and 1 drop of copper(II) sulfate pentahydrate (1 in 8), and shake. A blue precipitate is produced and the solution is purple.

(2) To 1 g of the sample, add 20 mL of water gradually to dissolve, and add 1 mL of 10% hydrochloric acid TS. The red color of the solution disappears.

pH 5.2–7.2 (1.0 g, potassium chloride TS (0.2 mol/L) 50 mL).

Purity

(1) Iron Not more than 0.050% as Fe.

Test Solution Weigh 0.50 g of the sample, dissolve it in water, and add 1 mL of hydrochloric acid and water to make 100 mL.

Control Solution Exactly measure 25 mL of Iron Standard Solution, add 1 mL of hydrochloric acid and water to make 100 mL.

Procedure Measure the atomic absorbance of the test solution and the control solution using the operating conditions given below. The absorbance of the test solution does not exceed that of the control solution.

Operating Conditions

Light Source: Iron hollow cathode lamp.

Wavelength: 248.3 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 6.0% (105°C, 5 hours).

Residue on Ignition Not more than 2.5%.

Assay

(1) Nitrogen Weigh accurately about 20 mg of the sample, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination. Determine the nitrogen content on the dried basis.

(2) Lactoferrin

Test Solution Weigh accurately about 0.1 g of the sample, and dissolve it in sodium chloride solution (3 in 100) to make exactly 50 mL.

Standard Solutions Prepare three standard solutions with different concentrations. Weigh accurately about 0.2 g of lactoferrin for assay, and dissolve it in sodium chloride solution (3 in 100) to make exactly 50 mL. Measure exactly 5 mL each of this solution into two separate volumetric flasks, and add sodium chloride solution (3 in 100) to make exactly 10 mL and 20 mL, respectively. Use the resulting two solutions and the first solution as the standard solutions.

Procedure Analyze 25 μL each of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Measure the peak areas of lactoferrin in the standard solutions to prepare a calibration curve. Determine the amount (g) of lactoferrin in the test solution from the calibration curve and the peak area of lactoferrin in the test solution, and calculate the content by the formula:

Content (%) of lactoferrin

$$= \frac{\text{Dry basis weight (g) of lactoferrin in the test solution}}{\text{Dry basis weight of the sample}} \times \text{content (\%) of lactoferrin for assay}$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength 280 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 15 cm length).

Column packing material: 5-μm butylated polyvinyl alcohol polymer gel for liquid chromatography.

Column temperature: A constant temperature of 30–40°C

Mobile phase

A. A 9000:1000:3 mixture of sodium chloride solution (3 in 100)/acetonitrile forHPLC/trifluoroacetic acid.

B. A 5000:5000:3 mixture of sodium chloride solution (3 in 100)/acetonitrile for HPLC/trifluoroacetic acid.

Concentrate gradient (A/B): Run a linear gradient from 50/50 to 0/100/ in 25 minutes.

Flow rate: 0.8 mL/minute.

Lactoperoxidase

ラクトパーオキシダーゼ

Definition Lactoperoxidase includes enzymes that reductively degrade hydrogen peroxide. It is derived from the milk of mammals. It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Lactoperoxidase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Lactoperoxidase complies with the Lactoperoxidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the

preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Lactoperoxidase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 0.50 g of Lactoperoxidase, add water to dissolve it or disperse it uniformly, and make 300 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water to the resulting solution.

Substrate Solution Add water to 70 μL of hydrogen peroxide to make 50 mL. Prepare fresh before use.

Procedure To 3 mL of citrate buffer (0.1 mol/L) at pH 5.5, add 0.05 mL of the substrate solution and 0.2 mL of ABTS TS, and mix. Equilibrate the mixture at 37°C for 10 minutes, then add 0.1 mL of the sample solution, shake well, and incubate at 37°C. Measure the absorbance of this solution at a wavelength of 413 nm. The absorbance at 1 minute after the addition of the sample solution is lower than that measured at 3 minutes after its addition.

Lanolin

ラノリン

Definition Lanolin is a waxy substance obtained from the wool of sheep, *Ovis aries* Linnaeus, and consists mainly of esters of higher alcohols and α-hydroxy acids.

Description Lanolin occurs as a slightly yellow to pale yellow-brown viscous paste. It has a slight characteristic odor.

Identification Pour carefully 1 mL of a solution (1 in 50) of Lanolin in cyclohexane on the surface of 2 mL of sulfuric acid. The boundary surface of the two liquids turns red-brown. The sulfuric acid phase has a green fluorescence.

Melting Point 37–44°C (Method 2).

Iodine Value 18–36.

Test Solution Weigh accurately about 0.8 g of Lanolin in a 500-mL stoppered flask, and dissolve it in 10 mL of cyclohexane.

Procedure Proceed as directed in the Iodine Value Test in the Fats and Related Substances Tests.

Purity

(1) Acid value Not more than 1.0.

Test Solution Weigh accurately about 5 g of Lanolin, and dissolve it in 80 mL of a 1:1 mixture of ethanol (95)/xylene.

Procedure Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests. Titration should be done while warm.

(2) Lead Not more than 2 μg/g as Pb (4.0 g, Method 2, Control Solution: Lead Standard Solution 8.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 0.1%.

Lecithin

レシチン

Definition Lecithin is obtained from oil seeds or animal materials. It consists mainly of phospholipids.

Description Lecithin occurs as white to brown granules or powder, as light yellow to dark brown lumps, or as a light yellow to dark brown viscous fluid. It has a slight, characteristic odor.

Identification

(1) Proceed as directed in Identification (1) for Enzymatically Decomposed Lecithin.

(2) To 0.5 g of Lecithin, add 5 mL of diluted hydrochloric acid (1 in 2), heat in a water bath for 2 hours, and filter. Use this solution as the test solution. Analyze 10 μL of the test solution by paper chromatography, using choline chloride solution (1 in 200) as the control solution and a 4:2:1 mixture of butanol/water/acetic acid as the developing solvent. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the developing solvent ascends to a point about 25 cm above the base line, air-dry, spray with Dragendorff TS to let color develop, and observe in daylight. A red-orange spot corresponding to the spot obtained from the control solution is observed.

Purity

(1) Acid value　Not more than 40.

Test Solution　Weigh accurately about 2 g of Lecithin, dissolve it in 50 mL of petroleum ether, and add 50 mL of ethanol (95).

Procedure　Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests, using this solution as the test solution.

(2) Toluene-insoluble substances　Not more than 0.30%.

Weigh accurately about 10 g of Lecithin, dissolve it in 100 mL of toluene, and filter the insoluble substances through a crucible-type glass filter (1G4). Wash several times with 25 mL of toluene, dry together with the glass filter at 105°C for 1 hour, and allow to cool in a desiccator. Weigh accurately the glass filter containing the residue.

(3) Acetone-soluble substances　Not more than 40%.

Weigh accurately about 2 g of Lecithin into a scaled, a 50-mL ground-glass stoppered centrifuge tube, dissolve it by adding 3 mL of petroleum ether, add 15 mL of acetone, and stir well, and allow to stand in ice water for 15 minutes. Then proceed as directed in Purity (2) for Enzymatically Decomposed Lecithin.

(4) Peroxide value　Not more than 10.

Weigh accurately about 5 g of Lecithin, transfer it into a 250-mL ground-glass stoppered Erlenmeyer flask, add 35 mL of a 2:1 mixture of chloroform/acetic acid, and dissolve to a transparent state while shaking gently. Then proceed as directed in Purity (3) for Enzymatically Decomposed Lecithin.

(5) Lead　Not more than 2 μg/g as Pb (1.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Test Solution　Proceed as directed in Method 2, and make up to exactly 5 mL with diluted nitric acid (1 in 100).

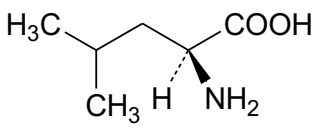
(6) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more than 2.0%.

Proceed as directed in the Loss on Drying Test for Enzymatically Decomposed Lecithin.

L-Leucine

L-ロイシン



$C_6H_{13}NO_2$ Mol. Wt. 131.17

(2*S*)-2-Amino-4-methylpentanoic acid [61-90-5]

Content L-Leucine, when calculated on the dried basis, contains 98.0–102.0% of L-leucine ($C_6H_{13}NO_2$).

Description L-Leucine occurs as white crystals or crystalline powder. It is odorless or has a very slight characteristic odor. It has a very slight bitter taste.

Identification

(1) To 5 mL of a solution of L-Leucine (1 in 1000), add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A bluish purple color develops.

(2) Dissolve 0.3 g of L-Leucine in 10 mL of water while warming, and add 10 drops of diluted hydrochloric acid (1 in 4) and 2 mL of sodium nitrite solution (1 in 10). A colorless gas emitted while bubbles are formed.

Specific Rotation $[\alpha]_D^{20}$: +14.5 to +16.5° (4 g, hydrochloric acid TS (6 mol/L), 100mL, on the dried basis).

pH 5.5–6.5 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution Colorless and clear (0.5 g, hydrochloric acid TS (1 mol/L) 10 mL).

(2) Chloride Not more than 0.1% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.3 g of L-Leucine, and proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 13.12 mg of $C_6H_{13}NO_2$

Licorice Extract

カンゾウ抽出物

Definition Licorice Extract is obtained from the roots or rhizomes of *Glycyrrhiza uralensis* Fisch. ex DC., *Glycyrrhiza inflata* Batalin, or *Glycyrrhiza glabra* L., or their allied plants. It consists mainly of glycyrrhizic acid. There are two types of products: Licorice Extract, Crude and Licorice Extract, Purified.

Licorice Extract, Crude

粗製物

Content Crude Licorice Extract, when calculated on the dried basis, contains not less than 5.0% and less than 50.0% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$ = 822.93).

Description Crude Licorice Extract occurs as a yellow to blackish brown powder, as flakes, granules, or lumps, or as a paste or liquid.

Identification Prepare a test solution by dissolving 0.01–0.10 g of Crude Licorice Extract in 10 mL of 50% (vol) ethanol. Prepare a control solution by dissolving 5 mg of glycyrrhizic acid for thin-layer chromatography in 10 mL of 50% (vol) ethanol. Analyze a 2-μL portion each of both solutions by thin-layer chromatography using a 7:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. Use a thin-layer plate coated with fluorescent silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm) in a dark place. One of the spots in the test solution corresponds in color tone and R_f value to the dark purple spot of glycyrrhizic acid in the control solution.

pH 2.5–7.0 (1.0 g of solid sample or previously dried paste or liquid sample, a 1:1 mixture of water/ethanol (95) 100 mL).

Purity

(1) Insoluble substances Dissolve 5.0 g of Crude Licorice Extract, previously dried, in 100 mL of 50% (vol) ethanol, filter through a filter paper whose weight is known, wash the residue on the filter paper with 50% (vol) ethanol, and dry the residue with the filter paper at 105°C for 5 hours. The residue weighs not more than 1.25 g.

(2) Lead Not more than 10 μg/g as Pb (0.50 g of a solid sample or previously dried paste or liquid sample, Method 1, Control Solution: Lead Standard Solution 5.0 mL, Flame Method).

(3) Arsenic Not more than 1.5 μg/g as As (1.0 g of a solid sample or previously dried paste or liquid sample, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying

Solid sample: Not more than 8.0% (105°C, 2 hours).

Paste or liquid sample: Not more than 60.0% (105°C, 5 hours).

Residue on Ignition Not more than 15.0% (dry the sample before use if it is paste or liquid).

Assay

Test Solution Weigh accurately 40 mg–0.4 g of Crude Licorice Extract, and dissolve it in 50% (vol) ethanol to make exactly 100 mL.

Standard Solution Weigh accurately about 20 mg of Glycyrrhizic Acid Reference Standard—the water content should be measured previously—and dissolve it in 50% (vol) ethanol to make exactly 100 mL.

Procedure Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of glycyrrhizic acid for the test solution and the standard solution, and calculate the content by the formula:

$$\text{Content (\%) of glycyrrhizic acid } (C_{42}H_{62}O_{16}) = \frac{\text{Anhydrous basis weight (g) of Glycyrrhizic Acid Reference Standard}}{\text{Dry basis weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 254 nm).

Column: A stainless steel tube (4–6 mm internal diameter and 15–30 cm length).

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 3:2 mixture of diluted acetic acid (1 in 50)/acetonitrile.

Flow rate: Adjust the retention time of glycyrrhizic acid to about 10 minutes.

Column selection: Use a column capable of eluting glycyrrhizic acid and propyl *p*-hydroxybenzoate in that order and completely separating their peaks when 20 μL of the solution (prepared by dissolving 5 mg of Glycyrrhizic Acid Reference Standard and 1 mg of propyl *p*-hydroxybenzoate in 20 mL of 50% (vol) ethanol) is chromatographed according to the above operating conditions.

Licorice Extract, Purified

精製物

Content Purified Licorice Extract, when calculated on the dried basis, contains 50.0–80.0% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$ = 822.93).

Description Purified Licorice Extract occurs as white to yellow crystals or powder.

Identification Weigh 5–10 mg of Purified Licorice Extract, proceed as directed in Identification for Crude Licorice Extract.

pH 2.5–5.0 (1.0 g, a 1:1 mixture of 50% ethanol 100 mL).

Purity

(1) Lead Not more than 10 μg/g as Pb (0.50 g, Method 1, Control Solution: Lead Standard Solution 5.0 mL, Flame Method).

(2) Arsenic　Not more than 1.5 μg/g as As　(1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

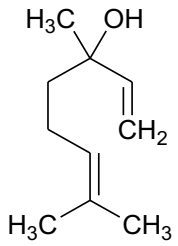
Loss on Drying　Not more than 8.0% (105°C, 2 hours).

Residue on Ignition　Not more than 15.0%.

Assay　Weigh accurately 20–40 mg of Purified Licorice Extract, and proceed as directed in the Assay for Crude Licorice Extract.

Linalool

リナロオール



$C_{10}H_{18}O$　Mol. Wt. 154.25

3,7-Dimethylocta-1,6-dien-3-ol　[78-70-6]

Content　Linalool contains not less than 95.0% of linalool ($C_{10}H_{18}O$).

Description　Linalool is a colorless, clear liquid having a characteristic odor.

Identification　Determine the absorption spectrum of Linalool as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum of Linalool. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index　n_D^{20}: 1.461–1.465.

Specific Gravity　d_{25}^{25}: 0.858–0.867.

Assay　Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Linalyl Acetate

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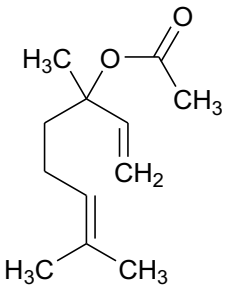


$C_{12}H_{20}O_2$ Mol. Wt. 196.29

3,7-Dimethylocta-1,6-dien-3-yl acetate [115-95-7]

Content Linalyl Acetate contains not less than 95.0% of linalyl acetate ($C_{12}H_{20}O_2$).

Description Linalyl Acetate is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Linalyl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum of Linalyl Acetate. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.448–1.452.

Specific Gravity d_{25}^{25}: 0.895–0.914.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Lipase

リパーゼ

Definition Lipase includes enzymes that hydrolyze fats and oils. It is derived from the internal organs of terrestrial animals or fishes, hypoglottis, or the culture of filamentous fungi (limited to *Aspergillus awamori, Aspergillus japonicas*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus phoenicis*, *Aspergillus usamii*, *Geotrichum candidum*, *Mucor circinelloides* f. *circinelloides*, *Mucor javanicus*, *Mucor miehei*, *Penicillium camemberti*, *Penicillium chrysogenum*, *Penicillium roqueforti*, *Rhizomucor miehei*, *Rhizopus arrhizus*, *Rhizopus delemar*, *Rhizopus japonicus*, *Rhizopus miehei*, *Rhizopus niveus*, *Rhizopus oryza*, and species of the genus *Humicola*), yeasts (limited to species of the genus *Candida*), actinomycetes (limited to species of genus *Streptomyces*) or bacteria

(limited to *Bacillus subtilis*, *Burkholderia plantarii*, *Burkholderia pyrrocinia*, *Burkholderia ubonensis*, *Chromobacterium viscosum*, *Geobacillus thermocatenulatus*, *Serratia marcescens,* and species of the genera *Alcaligenes*, *Arthrobactor*, *and Pseudomonas*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Lipase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Lipase complies with the Lipase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Lipase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of Lipase, add water, cold water, phosphate buffer (0.1 mol/L) at pH 7.0 cooled by icy, or sodium chloride solution (1 in 100) cooled by icy, to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the corresponding buffer or solution to the resulting solution.

Substrate Solution Place 75 mL of olive oil and 225 mL of emulsifying solution (polyvinyl alcohol I TS or polyvinyl alcohol I– polyvinyl alcohol II TS) into the vessel of an emulsifying device, stir continuously or intermittently the mixture at 12,000–16,000 rpm for 10 minutes while cooling at 10°C or lower to emulsify it. Before use, allow to

stand for 1 hour in a dark place at 5–10°C to confirm that the oil layer is not separated.

Test Solution To 5 mL of the substrate solution, add 4 mL of an appropriate buffer (phosphate buffer (0.1 mol/L) at pH 6.0, phosphate buffer (0.1 mol/L) at pH 7.0, phosphate buffer (0.1 mol/L) at pH 8.0, or McIlvain buffer at pH 7.0), shake, and equilibrate at 37°C for 10 minutes. Add 1 mL of the sample solution, shake immediately, and incubate at 37°C for 20 minutes. To this solution, add 10 mL of a 1:1 mixture of ethanol (95)/acetone, and shake. Add 10 mL of 0.05 mol/L sodium hydroxide and 10 mL of a 1:1 mixture of ethanol (95)/acetone, and shake.

Control Solution To 5 mL of the substrate solution, add 4 mL of the same buffer used to prepare the test solution, equilibrate at 37°C for 30 minutes, add 10 mL of a 1:1 mixture of ethanol (95)/acetone and 1 mL of the sample solution, and shake. Add 10 mL of 0.05 mol/L sodium hydroxide and 10 mL of a 1:1 mixture of ethanol (95)/acetone, and shake.

Procedure Titrate the test solution and the control solution with hydrochloric acid TS (0.05 mol/L) (indicator: 2 to 3 drops of phenolphthalein TS). If a pH meter is used, the endpoint is pH 10.0. The amount of hydrochloric acid TS (0.05 mol/L) consumed by the test solution is less than that of hydrochloric acid TS (0.05 mol/L) consumed by the control solution.

Method 2

Sample Solution Weigh 0.50 g of Lipase, add an appropriate diluent (cold water, phosphate buffer (0.02 mol/L) at pH 7.0 cooled, or sodium dodecyle sulfate–bovine serum albumin TS) to dissolve it or disperse it uniformly, and make 5 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding the same diluent to the resulting solution.

Substrate Solution To 15 mL of tributyrin, add 235 mL of water and 50 mL of Arabic gum TS, and using an emulsifying device, stir the mixture at 11,000–13,000 rpm for 2.5 minutes to emulsify. Prepare fresh before use.

Test Solution Equilibrate 30 mL of the substrate solution at 30°C for 15 minutes, add 0.05 mol/L sodium hydroxide while stirring, adjust the pH to 7.00 ± 0.05 at 30°C, and add 2 mL of the sample solution.

Control Solution Using 2 mL of the diluent used for the preparation of the sample solution instead of the sample solution, proceed as directed for the test solution.

Procedure To each of the test solution and control solution, continuously add 0.05 mol/L sodium hydroxide dropwise so that the pH is kept at 7.00 ± 005 at 30°C for 5 minutes. The consumption by the test solution is more than that by the control solution.

Method 3

Sample Solution Weigh 1.0 g of Lipase, add potassium phosphate buffer (0.02 mol/L) at pH 7.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution To 50 mL of a solution of polysorbate 20 (1 in 1000), add 0.05 g

of *p*-nitrophenyl butyrate or *p*-nitrophenyl palmitate, and disperse it uniformly by ultrasonic irradiation for 1 minute in an ice-cooled state.

Test Solution　Mix 0.2 mL of potassium phosphate buffer (0.02 mol/L) at pH 7.0　and 0.75 mL of the substrate solution, equilibrate the mixture at 37°C for 5 minutes, add 0.05 mL of the sample solution, and shake. Incubate the mixture at 37°C for 30 minutes. To this solution, add 0.05 mL of trichloroacetate (1 in 20), shake, add 1.4 mL of polyoxyethylene(10) octylphenyl ether TS, and shake.

Control Solution　Mix 0.2 mL of potassium phosphate buffer (0.02 mol/L) at pH 7.0 with 0.75 mL of the substrate solution, equilibrate the mixture at 37°C for 5 minutes, and add 0.05 mL of trichloroacetate (1 in 20).　Add 0.05 mL of the sample solution, shake, then add 1.4 mL of polyoxyethylene(10) octylphenyl ether TS, and shake.

Procedure　Measure the absorbance of the test solution and the control solution at a wavelength of 400 nm. The absorbance value of the test solution is higher than that of control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Lipoxygenase

リポキシゲナーゼ

Definition　Lipoxygenase includes oxidation-reduction enzymes that add molecular oxygen to unsaturated fatty acids with the cis, cis-1,4-pentadien structure to introduce hydroperoxide groups. It is derived from vegetable oil meals or the culture of filamentous fungi (limited to species of the genus *Rhizopus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description　Lipoxygenase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification　Lipoxygenase complies with the Lipoxygenase Activity Test.

Purity

(1) Lead　Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits　Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid　Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture　Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Lipoxygenase Activity Test　Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution　Weigh 1.0 g of Lipoxygenase, add water or sodium borate–hydrochloric acid buffer (0.1 mol/L) at pH 9.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution　Dissolve 1.4 mL of ammonia solution and 2.8 g of linoleic acid in sodium borate–hydrochloric acid buffer (0.1 mol/L) at pH 9.0, warmed to 30°C, and make exactly 100 mL. Dilute this solution to 500 times its original volume using sodium borate–hydrochloric acid buffer (0.1 mol/L) at pH 9.0.

Procedure　Place the substrate solution into a Erlenmeyer flask, equilibrate it at 25°C, blow oxygen into the solution for 5 minutes through a glass tube with an fine-drawn end that is immersed into the solution. Measure exactly 3 mL of the substrate solution saturated by dissolved oxygen, allow it to equilibrate at 25°C for 5 minutes, add 0.3 mL of the sample solution, and immediately shake. Transfer this solution into a quartz cell, kept at 25°C, measure the absorbance at a wavelength of 234 nm. The absorbance at 3 minutes after the addition of the sample solution is lower than that at 5 minutes after the addition of the sample solution. Use the substrate solution as a reference for absorbance measurement.

Liquid Paraffin

流動パラフィン

Definition　Liquid Paraffin is a mixture of hydrocarbons obtained from petroleum.

Description　Liquid Paraffin is a colorless, clear, and viscous liquid having almost no fluorescence. It is odorless and tasteless.

Identification　Determine the absorption spectrum of Liquid Paraffin as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Free acid and free alkali Measure 10 mL of Liquid Paraffin, add about 10 mL of boiling water and 1 drop of phenolphthalein TS, and shake vigorously. No pink color develops. To the resulting solution, add 0.20 mL of 0.02 mol/L sodium hydroxide, and shake. A pink color develops.

(2) Lead Not more than 1 μg/g as Pb (4.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Sulfur compounds Measure 4.0 mL of Liquid Paraffin, add 2 mL of ethanol (99.5), add 2 drops of a transparent solution of sodium hydroxide solution (1 in 5) saturated with lead(II) oxide, warm at 70°C for 10 minutes with occasional shaking, and allow to cool. The color of the solution does not change to dark brown.

(5) Polycyclic aromatic hydrocarbons

Test Solution Place 25 mL of Liquid Paraffin into a 25-mL measuring cylinder, and transfer into a 100-mL separating funnel. Place 25 mL of hexane for ultraviolet absorption spectrum measurement into the same measuring cylinder, transfer into the separating funnel, and shake well. Add 5 mL of dimethylsulfoxide for ultraviolet spectrum measurement, shake vigorously for 2 minutes, and allow to stand for 15 minutes. Transfer the lower layer into a 50-mL separating funnel, add 2 mL of hexane for ultraviolet absorption spectrum measurement, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer into a 10-mL stoppered centrifuge tube, and centrifuge at 2500–3000 rpm for about 10 minutes. Use the supernatant as the test solution.

Procedure Transfer the test solution into a cell with a tight stopper, and immediately measure the absorbance at a wavelength of 260–350 nm against the reference solution prepared as follows: To 25 mL of hexane for ultraviolet absorption spectrum measurement, add 5 mL of dimethylsulfoxide for ultraviolet spectrum measurement, and proceed in the same manner as test solution. The absorbance is not more than 0.10.

(6) Readily carbonizable substances Measure 5 mL of Liquid Paraffin, transfer into a Nessler tube, add 5 mL of sulfuric acid for the readily carbonizable substances test (94.5–94.9%), heat in a water bath for 2 minutes, and immediately shake up and down vigorously for 5 seconds. Repeat this procedure four times. The color of liquid paraffin layer does not change. In addition, the color of the sulfuric acid layer is not deeper than that of the solution prepared as follows: Mix 3.0 mL of Iron(III) Chloride CSSS, 1.5 mL of Cobalt(II) Chloride CSSS, and 0.5 mL of Copper(II) Sulfate CSSS together in a Nessler tube.

Luohanguo Extract

ラカンカ抽出物

Definition Luohanguo Extract is obtained from the fruits of the luohanguo plant *Siraitia grosvenorii* (Swingle) C. Jeffrey ex A. M. Lu & Zhi Y. Zhang (*Momordica grosvenori* Swingle) and consists mainly of mogrosides.

Content Luohanguo Extract, when dried, contains not less than 20% of mogroside V ($C_{60}H_{102}O_{29}$ = 1287.43).

Description Luohanguo Extract occurs as a light yellow to light brown powder having a sweet taste.

Identification

(1) To 5–10 mg of Luohanguo Extract, previously dried, add 2 mL of acetic anhydride, warm for 2 minutes, and slowly add 0.5 mL of sulfuric acid. The boundary surface turns red-brown.

(2) Prepare a test suspension containing 50 mg–0.1 g of Luohanguo Extract in 1–3 mL of 70% (vol) methanol. Separately, prepare a control solution by dissolving 5–10 mg of mogroside V for assay in 1–3 mL of 70% (vol) methanol. Analyze 2 μL each of the test suspension and the control solution by thin-layer chromatography using a 15:15:4 mixture of methanol/butyl acetate/water as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line. Air-dry the plate, spray uniformly with diluted sulfuric acid (1 in 10), and heat at 105°C for 10 minutes. One of the spots in the test solution corresponds in color tone and R_f value to the dark purple spot of mogroside V in the control solution.

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 0.8 μg/g as As (2.5 g, Method 3, Standard Color: Arsenic Standard Solution 4.0 mL, Apparatus B).

Loss on Drying Not more than 6.0% (105°C, 2 hours).

Residue on Ignition Not more than 2.0%.

Assay

Test Solution Weigh accurately about 0.2 g of Luohanguo Extract, previously dried, suspend in 70% (vol) methanol to make exactly 100 mL, and filter through a membrane filter (0.45 μm pore size).

Standard Solution Weigh accurately about 5 mg of mogroside V for assay, previously dried, and dissolve it in 70% (vol) methanol to make exactly 10 mL.

Procedure Analyze 20 μL each of the test solution and the standard solution by

liquid chromatography according to the operating conditions given below. Measure the peak areas (A_T and A_S) of mogroside V for the test solution and the standard solution. Calculate the content by the formula:

$$\text{Content (\%) of mogroside V } (C_{60}H_{102}O_{29}) = \frac{\text{Weight (g) mogroside V for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 10 \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength 203 nm).

Column: A stainless steel tube (4–6 mm internal diameter and 25–30 cm length).

Column packing material: 5-μm of aminated polyvinyl alcohol gel for liquid chromatography.

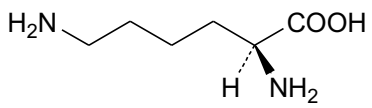
Column temperature: 40°C.

Mobile phase: A 37:13 mixture of acetonitrile/water.

Flow rate: Adjust the retention time of mogroside V to 15–20 minutes.

L-Lysine

L-リシン



$C_6H_{14}N_2O_2$ Mol. Wt. 146.19

(2*S*)-2,6-Diaminohexanoic acid [56-87-1]

Content L-Lysine, when calculated on the anhydrous basis, contains 97.0–103.0% of L-lysine ($C_6H_{14}N_2O_2$).

Description L-Lysine occurs as white crystals or crystalline powder. It has a characteristic odor, and has a characteristic taste.

Identification

(1) To 5 mL of a solution of L-Lysine (1 in 1000), add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A reddish purple color develops.

(2) A solution of L-Lysine is alkaline.

Specific Rotation $[\alpha]_D^{20}$: +23.3 to +29.3° (2 g, hydrochloric acid TS (6 mol/L), 100 mL, on the anhydrous basis).

Purity

(1) Clarity of solution　Colorless and almost clear (1.0 g, water 40 mL).

(2) Chloride　Not more than 0.1% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Water Content　Not more than 8.0% (0.20 g, Volumetric Titration, Back Titration).

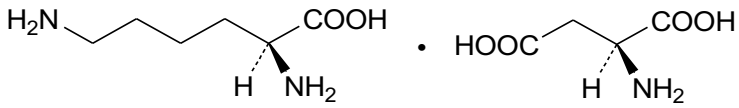
Residue on Ignition　Not more than 0.2%.

Assay　Weigh accurately about 0.2 g of L-Lysine, and proceed as directed in the Assay in L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 7.310 mg of $C_6H_{14}N_2O_2$

L-Lysine L-Aspartate

L-リシン L-アスパラギン酸塩



$C_{10}H_{21}N_3O_6$　Mol. Wt. 279.29

(2*S*)-2,6-Diaminohexanoic acid mono[(2*S*)-2-aminobutanedioate]

Content　L-Lysine L-Aspartate, when calculated on the dried basis, contains 98.0–102.0% of L-lysine L-aspartate ($C_{10}H_{21}N_3O_6$).

Description　L-Lysine L-Aspartate occurs as a white powder. It is odorless or has a slight odor, and a characteristic taste.

Identification

(1) To 5 mL of a solution of L-Lysine L-Aspartate (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) Use a solution of L-Lysine L-Aspartate (1 in 500) as the test solution. Prepare a control solution by dissolving together 0.1 g of sodium L(+)-aspartate monohydrate and 0.1 g of L-lysine monohydrochloride in water to make exactly 100 mL. Analyze 5 μL each of the test solution and the control solution by paper chromatography using a 5:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. Use a No. 2 filter paper for chromatography. Stop the development when the developing solvent has ascended to a point about 30 cm above the starting line. Air-dry the filter paper, then dry at 100°C for 20 minutes, and spray with a solution (1 in 50) of ninhydrin in acetone. Heat at 100°C

for 5 minutes to allow a color to develop. Examine in daylight. Two major spots in the test solution correspond to the spots in the control solution.

Specific Rotation $[\alpha]_D^{20}$: +24.0 to +26.5° (4.0 g, diluted hydrochloric acid (1 in 2), 50 mL, on the dried basis).

pH 5.0–7.0 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.041% as Cl (0.30 g, Control Solution: 0.01 mol/L hydrochloric acid 0.35 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.5% (reduced pressure, 5 hours).

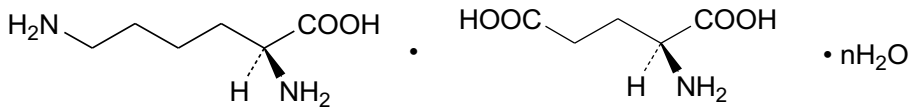
Residue on Ignition Not more than 0.3%.

Assay Proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 9.310 mg of $C_{10}H_{21}N_3O_6$

L-Lysine L-Glutamate

L-リシン L-グルタミン酸塩



n = 2 or 0

$C_{11}H_{23}N_3O_6 \cdot nH_2O$ (n = 2 or 0) Mol. Wt. dihydrate 329.35

anhydrous 293.32

(2*S*)-2,6-Diaminohexanoic acid mono[(2*S*)-2-aminopentanedioate] dehydrate

(2*S*)-2,6-Diaminohexanoic acid mono[(2*S*)-2-aminopentanedioate]

Content L-Lysine L-Glutamate, when calculated on the dried basis, contains 98.0–102.0% of L-lysine L-glutamate ($C_{11}H_{23}N_3O_6$).

Description L-Lysine L-Glutamate occurs as a white powder. It is odorless or has a slight odor, and a characteristic taste.

Identification

(1) To 5 mL of a solution of L-Lysine L-Glutamate (1 in 1000), add 1 mL of ninhydrin

solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) Proceed as directed in Identification (2) for L-Lysine L-Aspartate. Prepare a control solution by dissolving 0.1 g of sodium L-glutamate monohydrate and 0.1 g of L-lysine monohydrochloride in water to make 100 mL.

Specific Rotation $[\alpha]_D^{20}$: +27.5 to +29.5° (4.0 g, hydrochloric acid TS (6 mol/L), 50 mL, on the dried basis).

pH 6.0–7.5 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.041% as Cl (0.30 g, Control Solution: 0.01 mol/L hydrochloric acid 0.35 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 11.4% (105°C, 5 hours).

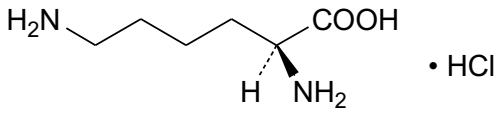
Residue on Ignition Not more than 0.3%.

Assay Proceed as directed in the Assay in DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 9.777 mg of $C_{11}H_{23}N_3O_6$

L-Lysine Monohydrochloride

L-リシン塩酸塩



$C_6H_{14}N_2O_2 \cdot HCl$ Mol. Wt. 182.65

(2*S*)-2,6-Diaminohexanoic acid monohydrochloride [657-27-2]

Content L-Lysine Monohydrochloride, when dried, contains not less than 98.0% of L-lysine monohydrochloride ($C_6H_{14}N_2O_2 \cdot HCl$).

Description L-Lysine Monohydrochloride occurs as a white powder. It is odorless or has a slight, characteristic odor, and has a slight, characteristic taste.

Identification

(1) To 5 mL of a solution of L-Lysine Monohydrochloride (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) L-Lysine Monohydrochloride responds to all the tests for Chloride in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +19.0 to +21.5° (4 g, hydrochloric acid TS (6 mol/L), 50 mL, on the dried basis).

pH 5.0–6.0 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 1.0% (105°C, 3 hours).

Residue on Ignition Not more than 0.3%.

Assay Proceed as directed in the Assay for L-Histidine Monohydrochloride.

Each mL of 0.1 mol/L perchloric acid = 9.132 mg of $C_6H_{14}N_2O_2 \cdot HCl$

L-Lysine Solution

L-リシン液

Content L-Lysine Solution contains not more than 80% of L-lysine ($C_6H_{14}N_2O_2$ = 146.19) and contains 95.0–110.0% of the labeled content of L-lysine.

Description L-Lysine Solution is a yellow liquid. It has a characteristic odor and has a characteristic taste.

Identification

(1) To 5 mL of diluted L-Lysine Solution (1 in 200), add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A reddish purple color develops.

(2) To 5 g of L-Lysine Solution, add 50 mL of diluted hydrochloric acid (1 in 2), and mix. It shows dextrorotatory.

Purity

(1) Lead Not more than 2 μg/g of $C_6H_{14}N_2O_2$ as Pb (an amount equivalent to 2.0 g of L-lysine ($C_6H_{14}N_2O_2$), Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g of $C_6H_{14}N_2O_2$ as As (an amount equivalent to 0.50 g of L-lysine ($C_6H_{14}N_2O_2$), Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of L-Lysine Solution in 5 mL of water

while heating if necessary.

Residue on Ignition Not more than 0.2% per L-lysine ($C_6H_{14}N_2O_2$).

Assay Weigh accurately an amount of L-Lysine Solution equivalent to about 0.2 g of L-lysine ($C_6H_{14}N_2O_2$), and proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 7.310 mg of $C_6H_{14}N_2O_2$

Lysozyme

リゾチーム

Definition Lysozyme is an enzyme that dissolves the cell wall substances of bacteria. It is obtained from hen egg white by resin purification process after treatment with an alkaline aqueous solution and a saline solution or by column purification or re-crystallization after resin or salting treatment.

Enzyme Activity Lysozyme has an enzyme activity equivalent to not less than 0.9 mg (potency) per mg, when dried.

Description Lysozyme occurs as an odorless white powder.

Identification Lysozyme has activity when tested as directed under Enzyme Activity Determination.

pH Not less than 5.0 (3.0 g, water 200 mL).

Purity

(1) Clarity of solution Adjust 5 mL of a solution of Lysozyme (1 in 100) to pH 3.0 by adding 10% hydrochloric acid TS if necessary. It has a transmittance of not less than 80.0% at 660 nm.

(2) Chloride Not more than 4.5% as Cl.

Weigh accurately about 0.5 g of Lysozyme, and dissolve it in 50 mL of water. To this solution, add 0.1 mL of potassium chromate solution (1 in10), and titrate with 0.1 mol/L silver nitrate solution. The endpoint is when the solution turns light red-brown.

Each mL of 0.1 mol/L silver nitrate = 3.545 mg of Cl

(3) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3 under the Lead Limit Test.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 6.0% (1.0 g, reduced pressure, 2 hours).

Microbial Limits Proceed as directed in the Microbial Limit Tests.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Enzyme Activity Determination

(i) *Test Solution* Weigh accurately an amount of Lysozyme equivalent to about 50 mg potency, previously dried, and add phosphate buffer (pH 6.2) to make exactly 100 mL. Measure exactly 2 mL of this solution, and add phosphate buffer (pH 6.2) to make exactly 100 mL of solution. Then measure exactly 2 mL of the second solution, and add phosphate buffer (pH 6.2) to make exactly 50 mL.

(ii) *Standard Solution* Dry about 0.1 g of Lysozyme Reference Standard in a vacuum desiccator for about 2 hours. Weigh accurately an amount of the dried Standard equivalent to about 50 mg potency, and add phosphate buffer (pH 6.2) to make exactly 100 mL. Measure exactly 2 mL of this solution, and add phosphate buffer (pH 6.2) to make exactly 100 mL. Then measure exactly 2 mL of the second solution, and add phosphate buffer (pH 6.2) to make exactly 50 mL.

(iii) *Procedure* Place exactly 3 mL of the substrate solution for lysozyme, into each of three test tubes, and warm the tubes at 35°C for 3 minutes. Warm the standard solution, test solution, and phosphate buffer (pH 6.2) at 35°C for 3 minutes. To the test tubes, separately add exactly 3 mL each of the warmed three solutions, and allow to react at 35°C for 10 ± 0.1 minutes. Immediately measure the absorbance at a wavelength of 640 nm, using water as the reference. Express the absorbance values of the standard solution, the test solution, and the phosphate buffer as A_S, A_T, and A_0, respectively. Repeat the whole procedure three times, obtain the average of the three absorbance values, and calculate the enzyme activity by the formula:

$$\text{Enzyme activity of dried Lysozyme sample (mg (potency)/mg)} = \frac{\text{Weight (mg (potency)) of dried Lysozyme Reference Standard}}{\text{Weight (mg) of the dried sample}} \times \frac{(A_O - A_T)}{(A_O - A_S)}$$

Macrophomopsis Gum

マクロホモプシスガム

Definition Macrophomopsis Gum is obtained from the culture fluid of *Macrophomopsis* (*Fusicoccum*) and consists mainly of polysaccharides. It may contain sucrose, glucose,

lactose, dextrin, or maltose.

Description Macrophomopsis Gum occurs as a light yellow to light brown powder having a slight characteristic odor.

Identification

(1) To 100 mL of hot water, gradually add 0.5 g of Macrophomopsis Gum while stirring, and cool to room temperature. A viscous liquid is produced.

(2) To 100 mL of hot water, gradually add 0.1 g of Macrophomopsis Gum while stirring, and dissolve by homogenizing at 8000 rpm for 15 minutes. Use this solution as the test solution. After cooling, measure 5 mL of the test solution into a test tube, add 1 mL of 2-propanol, mix well, and heat for 10 minutes in a water bath. Stir well, and allow to stand at room temperature for 2 hours. A gel is formed.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Total nitrogen Not more than 1.0% (on the dried basis).

Weigh accurately about 0.3 g of Macrophomopsis Gum, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

(4) 2-Propanol Not more than 0.50%.

(i) Apparatus Use the apparatus illustrated in Purity (7) for Semirefined Carrageenan.

(ii) Method Prepare a test solution and an internal standard solution as directed in Purity (7) for Semirefined Carrageenan.

Standard Solution Weigh accurately about 0.5 g of 2-propanol, and add water to make exactly 50 mL. Measure exactly 5 mL of this solution, and add water to make 50 mL. To exactly 10 mL of the second solution, add exactly 4 mL of the internal standard solution and water to make exactly 100 mL.

Procedure Analyze 2.0 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratios of 2-propanol to 2-methyl-2-propanol for the test solution and the standard solution, and express as Q_T and Q_S, respectively. Calculate the amount of 2-propanol by the formula:

Amount (%) of 2-propanol

= (Weight (g) of 2-propanol/Weight (g) of the sample) × (Q_T/Q_S) × 2

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube (3 mm internal diameter and 2 m length).

Column packing material: 180- to 250-μm styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Injection port temperature: A constant temperature of about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention time of 2-propanol to 10 minutes.

Loss on Drying Not more than 15.0% (105°C, 2.5 hours).

Ash Not more than 10.0% (on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 10,000 per gram.

Yeasts and molds Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 2 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture Prepare as directed in Method 2 for the *Escherichia coli* test. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 5 g of Macrophomopsis Gum with 500 mL of lactose broth to disperse completely, and incubate it at 35 ± 1°C for 24 ± 2 hours.

Magnesium Carbonate

炭酸マグネシウム

Content Magnesium Carbonate contains the equivalent of 40.0–44.0% of magnesium oxide (MgO = 40.30).

Description Magnesium Carbonate occurs as a white, bulky powder or as brittle lumps.

Identification To 0.2 g of Magnesium Carbonate, add gradually 3 mL of diluted hydrochloric acid (1 in 4). It dissolves with effervescence. Add ammonia TS to make the solution alkaline. The solution responds to the test for Magnesium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution　Very slightly turbid.

Test Solution　Weigh 1.0 g of Magnesium Carbonate, dissolve it in 10 mL of diluted hydrochloric acid (2 in 3), and add 10 mL of water.

(2) Water-soluble substances　Not more than 1.0%.

Weigh 2.0 g of Magnesium Carbonate, add 100 mL of freshly boiled and cooled water, and boil for 5 minutes while stirring. Cool and filter it. Combine the washings with the filtrate, add water to make 100 mL. Measure 50 mL of this solution, evaporate to dryness in a water bath, and dry the residue at 105°C for 1 hour. Weigh the residue.

(3) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution　To the specified amount of Magnesium Carbonate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool.

(4) Calcium oxide　Not more than 0.60% as CaO.

Weigh 0.600g of Magnesium Carbonate, dissolve it by adding 35 mL of water and 6 mL of diluted hydrochloric acid (1 in 4), and add 250 mL of water and 5 mL of L(+)-tartaric acid solution (1 in 5). Add 10 mL of 2,2′,2″-nitrilotriethanol solution (3 in 10) and 10 mL of potassium hydroxide solution (1 in 2), and allow to stand for 5 minutes. Titrate with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 0.1 g of NN indicator), and calculate the content of calcium oxide. The endpoint is when the color of the solution changes from red-purple to blue. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate = 0.5608 mg of CaO

(5) Arsenic　Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution　Weigh the specified amount of Magnesium Carbonate, moisten with 1.5 mL of water, and dissolve it by adding 3.5 mL of diluted hydrochloric acid (1 in 4).

Assay　Weigh accurately about 0.4g of Magnesium Carbonate, dissolve it by adding 10 mL of water and 3.5 mL of diluted hydrochloric acid (1 in 4), and add water to make exactly 500 mL. Measure exactly 25 mL of this solution, add 50 mL of water and 5 mL of ammonium buffer (pH 10.7), and titrate with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 40 mg of eriochrome black T–sodium chloride indicator). Perform a blank test in the same manner, make any necessary correction, and calculate the volume (a mL) of the disodium dihydrogen ethylenediaminetetraacetate solution consumed. Refer to the volume of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetat consumed by the titration in Purity (4) as b mL, and calculate the content by the formula:

$$\text{Content (\%) of magnesium oxide (MgO)} = \frac{(a - 0.33\,b) \times 0.8061}{\text{Weight (g) of the sample}}$$

Magnesium Chloride

塩化マグネシウム

$MgCl_2 \cdot 6H_2O$ Mol. Wt. 203.30

Magnesium chloride hexahydrate [7791-18-6]

Content Magnesium Chloride contains 95.0–103.0% of magnesium chloride ($MgCl_2 \cdot 6H_2O$).

Description Magnesium Chloride occurs as colorless to white crystals, powder, flakes, granules, or lumps.

Identification Magnesium Chloride responds to all the tests for Magnesium Salt and for Chloride in the Qualitative Tests.

Purity

(1) Clarity of solution Slightly turbid (1.0 g, water 10 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Magnesium Chloride, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Gently boil for 5 minutes, and allow to cool.

(3) Zinc Not more than 70 μg/g as Zn.

Sample Solution Weigh 4.0 g of Magnesium Chloride, dissolve it in water to make 40 mL, and use it as the sample solution.

Procedure Measure 30 mL of the sample solution, add 5 drops of acetic acid and 2 mL of a solution of potassium hexacyanoferrate(II) trihydrate (1 in 20), shake, and allow to stand for 10 minutes. The turbidity of this solution does not exceed that of the solution prepared as follows: Measure 14 mL of Zinc Standard Solution, and add 10 mL of the sample solution and water to make 30 mL. Add 5 drops of acetic acid and 2 mL of a solution of potassium hexacyanoferrate(II) trihydrate (1 in 20), shake, and allow to stand for 10 minutes.

(4) Calcium Not more than 0.5%.

Measure exactly 50 mL of Solution A prepared in Assay, add 0.6 mL of L(+)–tartaric acid solution (1 in 5), 10 mL of 2,2′,2″-nitrilotriethanol (3 in 10), and 10 mL of potassium hydroxide solution (1 in 10), and allow to stand for 5 minutes. Titrate the resulting solution with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator:

0.1 g of NN indicator) using a microburet. The endpoint is when the red-purple color of the solution completely disappears and changes to blue. Calculate by the formula:

$$\text{Content (\%) of calcium (Ca)} = \frac{\left[\begin{array}{c}\text{Volume (mL) of 0.01 mol/L disodium dihydrogen} \\ \text{ethylenediaminetetraacetate consumed}\end{array}\right] \times 0.08016}{\text{Weight (g) of the sample}}$$

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay Weigh accurately about 0.3 g of Magnesium Chloride, and dissolve it in water to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 20 mL of Solution A, add 50 mL of water and 5 mL of ammonium buffer (pH 10.7), and titrate with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 2 drops of eriochrome black T TS) until the red color of the solution changes to blue. Calculate the content by the formula:

$$\text{Content (\%) of magnesium chloride } (MgCl_2 \cdot 6H_2O) = \frac{\left[\begin{array}{c}\text{Volume (mL) of 0.01 mol/L disodium dihydrogen} \\ \text{ethylenediaminetetraacetate consumed}\end{array}\right] \times 1.017}{\text{Weight (g) of the sample}}$$

Magnesium Hydroxide

水酸化マグネシウム

$Ma(OH)_2$ Mol. Wt. 58.32

Magnesium hydroxide [1309-42-8]

Content Magnesium hydroxide, when dried, contains not less than 95.0% of magnesium hydroxide ($Mg(OH)_2$).

Description Magnesium hydroxide occurs an odorless, white powder.

Identification

(1) To 0.1 g of Magnesium Hydroxide, add 10 mL of water, and shake. The resulting liquid is alkaline.

(2) A solution of 1 g of Magnesium Hydroxide in 20 mL of 10% hydrochloric acid TS responds to the test for Magnesium Salt as directed in the Qualitative Tests.

Purity

(1) Free alkali and soluble salts Weigh 2.0 g of Magnesium Hydroxide into a beaker,

and add 100 mL of water. Cover the beaker with a watch dish, and heat in a water bath for 5 minutes. Immediately filter it, and cool. To 50 mL of the filtrate, add 2 drops of methyl red TS, and titrate with 0.05 mol/L sulfuric acid. The volume of the sulfuric acid consumed is not more than 2.0 mL. Evaporate 25 mL of the filtrate, measured exactly, to dryness, and dry the residue at 105°C for 3 hours. The mass of the residue is not more than 10 mg.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Magnesium Hydroxide, add 40 mL of diluted hydrochloric acid (1 in 2), cover with a watch glass, and boil gently for 5 minutes. Allow to cool.

(3) Calcium oxide Not more than 1.5%.

Weigh accurately about 0.35 g of Magnesium Hydroxide, previously dried, add 6 mL of 10% hydrochloric acid TS, and dissolve it by warming. Cool, and add 300 mL of water, 3 mL of L-(+)-tartaric acid solution (1 in 5), 10 mL of 2,2′,2″-nitrilotriethanol solution (3 in 10), and 10 mL of potassium hydroxide (1 in 2), and allow to stand for 5 minutes. Titrate with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: about 0.1 g of NN indicator). The endpoint is when the solution turns from red-violet to blue. Perform a blank test to make necessary correction, and determine the content of calcium oxide.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate = 0.5608 mg of CaO

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Magnesium Hydroxide in 8 mL of 10% hydrochloric acid TS.

Loss on Drying Not more than 2.0% (105°C, 2 hours).

Loss on Ignition 30.0–33.0% (800°C, constant weight).

Assay Weigh accurately about 0.3 g of Magnesium Hydroxide, previously dried, add 10 mL of water and 4.0 mL of 10% hydrochloric acid TS, and dissolve it by warming. Cool, and add water to make exact 100 mL. To 25 mL of the resulting solution, measured exactly, add 50 mL of water and 5 mL of ammonium buffer (pH 10.7), and titrate with 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 40 mg of eriochrome black T–sodium chloride indicator). Perform a blank test. Determine the content by the following formula using the content of CaO determined in Purity (3).

$$\text{Content (\%) of magnesium hydroxide } (Mg(OH)_2) = \frac{(a - b - c \times \text{weight (g) of the sample} \times 0.9) \times 1.1664}{\text{Weight (g) of the sample}}$$

a = volume (mL) of 0.05 mol/L disodium dihydrogen ethylenediamin- tetraacetate

consumed in the test,

b = volume (mL) of 0.05 mol/L disodium dihydrogen ethylenediamine- tetraacetate consumed in the blank,

c = content (%) of calcium oxide (CaO) determined in Purity (3).

Magnesium Monohydrogen Phosphate

リン酸一水素マグネシウム

$MgHPO_4 \cdot 3H_2O$ Mol. Wt 174.33

Magnesium monohydrogen phosphate trihydrate [7782-75-4]

Content Magnesium Monohydrogen Phosphate, when ignited, contains not less than 96.0% of magnesium monohydrogen phosphate ($Mg_2P_2O_7$).

Description Magnesium Monohydrogen Phosphate occurs as a white crystalline powder.

Identification

(1) To 0.1 g of Magnesium Monohydrogen Phosphate, add 0.5 mL of acetic acid TS (1 mol/L) and 20 mL of water, and then add 1 mL of iron(III) chloride TS. Allow to stand for 5 minutes, and filter. The filtrate responds to all the tests for Magnesium Salt in the Qualitative Tests.

(2) A solution of 0.2 g of Magnesium Monohydrogen Phosphate in 10 mL of 10% nitric acid TS produces a yellow precipitate when ammonium molybdate TS is added dropwise. The resulting precipitate dissolves on the addition of ammonia TS.

Purity

(1) Fluoride Not more than 25 μg/g as F.

Test Solution Weigh 0.20 g of Magnesium Monohydrogen Phosphate into a beaker, and add 10 mL of diluted hydrochloric acid (1 in 10) to dissolve it. Boil the resulting solution for 1 minute, transfer into a polyethylene beaker, and immediately cool with ice. Add 15 mL of a solution of trisodium citrate dihydrate (1 in 4) and 10 mL of a solution of disodium dihydrogen ethylenediaminetetraacetate dihydrate (1 in 40), and mix. Adjust the pH of the solution to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5). Transfer this solution into a 100-mL volumetric flask, and dilute with water to volume. Place about 50 mL of this solution in a polyethylene beaker.

Control Stock Solution Weigh 2.210 g of sodium fluoride, previously dried at 110°C for 2 hours, transfer into a polyethylene beaker, add 200 mL of water, and dissolve it while stirring. Transfer this solution into a 1000-mL volumetric flask, and dilute with water to volume. Store the resulting solution a polyethylene container.

Control Solution Prepare before use. Transfer exactly 5 mL of the control stock solution into a 1000-mL volumetric flask, and add water to volume. Transfer exactly 1 mL of this solution into a polyethylene beaker, add 15 mL of a solution of trisodium

citrate dihydrate (1 in 4) and 10 mL of a solution of disodium dihydrogen ethylenediaminetetraacetate dihydrate (1 in 40), and mix. Adjust the pH of the resulting solution to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5). Transfer this solution into a 100-mL volumetric flask, and add water to volume. Place about 50 mL of the solution into a polyethylene beaker. Use this solution as the control solution.

Procedure Measure the electric potential of both solutions, using a potentiometer with a fluorine-ion indicator electrode and a silver/silver chloride reference electrode. The electric potential of the test solution is not lower than that of the control solution.

(2) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Magnesium Monohydrogen Phosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Magnesium Monohydrogen Phosphate in 5 mL of 10% hydrochloric acid TS.

Loss on Ignition 29–36% (800 ± 25°C, 3 hours).

Assay Weigh accurately about 0.5 g of Magnesium Monohydrogen Phosphate, previously ignited, add 50 mL of water and 2 mL of hydrochloric acid, and dissolve it by heating. After cooling, make exactly 100 mL with water. Transfer 50 mL of this solution into a beaker, add 100 mL of water, and heat to 55–60°C. Add 15 mL of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate using a buret, and adjust its pH to 10 with sodium hydroxide TS (1 mol/L) while agitating using a magnetic stirrer. Add 10 mL of ammonium buffer (pH 10.7) solution, and titrate with 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 12 drops of eriochrome black T TS). The endpoint is when the color of the solution changes to blue.

Each mL of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate = 11.13 mg of $Mg_2P_2O_7$

Magnesium Oxide

酸化マグネシウム

MgO Mol. Wt. 40.30

Magnesium oxide [1309-48-4]

Content Magnesium Oxide, when ignited, contains not less than 96.0% of magnesium

oxide (MgO).

Description Magnesium Oxide occurs as a white or whitish powder or as granules.

Identification Dissolve 1 g of Magnesium Oxide in 25 mL of diluted hydrochloric acid (1 in 4). The resulting solution responds to the test for Magnesium Salt in the Qualitative Tests.

Purity

(1) Water-soluble substances Not more than 2.0%.

Weigh 2.0 g of Magnesium Oxide, add 100 mL of water, heat in a water bath for 5 minutes, and immediately filter. Cool, measure 25 mL of the filtrate, evaporate to dryness in a water bath, and dry at 105°C for 1 hour. Weigh the residue.

(2) Hydrochloric acid-insoluble substances Not more than 1.0%.

Weigh 2.0 g of Magnesium Oxide, and add 75 mL of water. Add dropwise hydrochloric acid to the suspension while shaking until the substance no longer dissolves, and boil for 5 minutes. Cool, filter through a filter paper for quantitative analysis (5C), and wash the residue on the filter paper with hot water until the washings are free of chlorides. Heat the residue with the filter paper by gradually raising the temperature until it is carbonized, and ignite it at 450–550°C for 3 hours. Weigh the residue.

(3) Free alkali Measure 50 mL of the filtrate obtained in test (1), add 2 drops of methyl red TS, and add 2.0 mL of 0.05 mol/L sulfuric acid. A red color develops.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Magnesium Oxide, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(5) Calcium oxide Not more than 1.5%.

Measure exactly 50 mL of Solution A prepared in the Assay below, and add water to make 300 mL. Add 0.6 mL of L(+)-tartaric acid (1 in 5), then 10 mL of 2,2′,2″-nitrilotriethanol (3 in 10) and 10 mL of potassium hydroxide solution (1 in 2). Allow to stand for 5 minutes, titrate with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate using a microburet (indicator: about 0.1 g of NN indicator), and express the volume consumed as b (mL). The endpoint is observed when the red-purple color of the solution completely disappears and the solution becomes blue. Calculate the content by the formula:

$$\text{Content (\%) of calcium oxide (CaO)} = \frac{\text{b (mL)} \times 0.5608}{\text{Weight (g) of the sample}}$$

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of Magnesium Oxide, and dissolve it in 5 mL of diluted hydrochloric acid (1 in 4).

Loss on Ignition Not more than 10.0% (1000°C, 30 minutes).

Assay Weigh accurately about 0.5 g of Magnesium Oxide, previously ignited, moisten with 5 mL of water, add 10 mL of hydrochloric acid and 10 mL of perchloric acid, cover with a watch glass, and heat gradually. After thick white fumes are evolved, heat for another 10 minutes. After cooling, add about 50 mL of hot water and 5 mL of diluted hydrochloric acid (1 in 2), heat slightly, and immediately filter through a filter paper for quantitative analysis (5C), and add water to the filtrate to make exactly 500 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, add water to make 100 mL, add 5 mL of ammonium buffer (pH 10.7) and 2 drops of eriochrome black T TS, and immediately titrate with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate until the red color of the solution changes to blue. Determine the consumed volume (a mL). From the consumed volume (b mL) obtained in Purity (5), calculate the content by the formula:

$$\text{Content (\%) of magnesium oxide (MgO)} = \frac{(a - 0.2b) \times 2.015}{\text{Weight (g) of the sample}}$$

Magnesium Silicate

ケイ酸マグネシウム

Magnesium silicate [1343-88-0]

Definition Magnesium Silicate is a synthetic compound manufactured by the precipitation reaction between sodium silicate and a soluble magnesium salt. Its molar ratio of magnesium oxide to silicon dioxide is approximately 2 to 5.

Content Magnesium Silicate, when calculated on the ignited basis, contains the equivalent of not less than 15.0% of magnesium oxide (MgO = 40.30) and the equivalent of not less than 67.0% of silicon dioxide (SiO_2 = 60.08).

Description Magnesium Silicate occurs as a white, odorless, fine powder.

Identification

(1) Mix 0.5 g of Magnesium Silicate with 10 mL of 10% hydrochloric acid TS, and filter the mixture. The filtrate neutralized with ammonia TS responds to the test for Magnesium Salt in the Qualitative Tests.

(2) Prepare a bead by fusing a few crystals of ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop in the flame of a Bunsen burner. Place the hot, transparent bead in contact with Magnesium Silicate, and fuse again. An unfused lump floats about in the bead. When cooled, the bead becomes opaque producing a web-like pattern.

pH 7.0–11.0 (10% suspension).

Purity

(1) Water-soluble substances Not more than 3.0%.

Weigh about 10.0 g of Magnesium Silicate in a beaker, and add 150 mL of water. Cover the beaker with a watch glass, and boil gently for 15 minutes. Cool, replenish the lost water, and allow the mixture to stand for 15 minutes. Filter through a filter paper for quantitative analysis (5C) by suction. If the filtrate is turbid, repeat the filtration until the clear filtrate is obtained. Add water to exactly 75 mL of the filtrate to make exactly 100 mL. Refer to the resulting solution as Solution A. Transfer exactly 50 mL of Solution A into a tared platinum dish, evaporate to dryness, and ignite for 3 hours at 450–550°C. Cool, and weigh the dish with the residue. Determine the amount of the residue. The residue shall not exceed 75 mg.

(2) Free alkali Not more than 1.0% as NaOH.

Add 2 drops of phenolphthalein TS to 20 mL of Solution A prepared in Purity (1), and add 0.1 mol/L hydrochloric acid until the color of the solution disappears. The consumption of the hydrochloric acid is not more than 2.5 mL.

(3) Fluoride Not more than 10 μg/g as F.

Test Solution Weigh 2.0 g of Magnesium Silicate in a polyethylene beaker, add 60 mL of water, and stir for 15 minutes. Transfer the suspension into a 100-mL volumetric flask, and add water to volume. Centrifuge 50 mL of the resulting suspension at 5000 rpm for 15 minutes, transfer exactly 20 mL of the supernatant into a polyethylene beaker, and add 10 mL of disodium dihydrogen ethylenediaminetetraacetate–Tris TS.

Control Solution Weigh 2.210 g of sodium fluoride, previously dried at 110°C for 2 hours, in a polyethylene beaker, add 200 mL of water, and dissolve it while stirring. Transfer this solution into a 1000-mL polyethylene volumetric flask, and add water to volume. Use the resulting solution as the control stock solution. Store this solution in a polyethylene bottle. Prepare a control solution fresh before use by the following procedure. Place exactly 2 mL of the control stock solution in a 1000-mL volumetric flask, and dilute with water to volume. Transfer exactly 5 mL of this solution into a 50-mL volumetric flask, and dilute with water to volume. Place exactly 20 mL of the second solution in a polyethylene beaker, and add l0 mL of disodium dihydrogen ethylenediaminetetraacetate–Tris TS.

Procedure Measure the electric potential using a potentiometer with a fluorine-ion indicator electrode and a silver-silver chloride reference electrode. The potential of the test solution is not less than that of the control solution.

(4) Lead Not more than 5 μg/g as Pb (5.0 g, Control Solution: Lead Standard Solution 10.0 mL, Flame Atomic Absorption Spectrophotometry).

Test Solution Transfer the specified amount of Magnesium Silicate into a beaker, add 50 mL of diluted hydrochloric acid solution (1 in 4), and stir. Cover the beaker with a watch dish, and boil gently for 15 minutes. Filter this solution by suction through a quantitative filter paper (5C) into a 50-mL volumetric flask. Rinse the beaker and the residue on the filter paper with hot water, and add the washings to the flask. Cool, and add diluted hydrochloric acid solution (1 in 4) to make exactly 50 mL.

Control Solution Measure exactly the specified amount of Lead Standard Solution, add diluted hydrochloric acid (1 in 4) to make 20 mL.

Procedure Determine the absorbance of the test solution and the control solution by atomic absorption spectrophotometry using the operating conditions given below. The absorbance of the test solution is not more than that of the control solution.

Operating conditions

Light source: Lead hollow cathode lamp.

Analytical line wavelength: 217 nm.

Combustion-Supporting gas: Air.

Combustible gas: Acetylene.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Add 5 mL of diluted hydrochloric acid (1 in 4) to the specified amount of Magnesium Silicate, and heat gently while agitation until boiling. Cool immediately, centrifuge the mixture at 3000 rpm for 5 minutes, and collect the supernatant. To the residue, add 5 mL of diluted hydrochloric acid (1 in 4), agitate, and centrifuge. Combine the washings with the collected supernatant. Add 10 mL of water to the residue, and repeat the procedure. Combine the washings with the supernatant, and evaporate to 5 mL by heating on a water bath.

Loss on Drying Not more than 15% (105°C, 2 hours).

Loss on Ignition Not more than 15% (dried sample, 900–1000°C, 20 minutes).

Assay

(1) Magnesium oxide To accurately weighed about 1.5 g of Magnesium Silicate, add exactly 50 mL of 0.5 mol/L sulfuric acid, and heat on a water bath for 1 hour. Cool to room temperature, add methyl orange TS, and titrate the excess acid with 1 mol/L sodium hydroxide. Perform a blank test, and calculate the content by the formula:

$$\text{Content (\%) of magnesium oxide (MgO)} = \frac{(a - b) \times 2.015}{\left[\begin{matrix}\text{Weight (g) of}\\ \text{the sample}\end{matrix}\right] \times \left(1 - \frac{\text{Loss on drying (\%)}}{100}\right) \times \left(1 - \frac{\text{Loss on ignition (\%)}}{100}\right)} \times 100$$

a = volume (mL) of 1 mol/L sodium hydroxide consumed in the blank test,

b = volume (mL) of 1 mol/L sodium hydroxide consumed in this test.

(2) Silicon dioxide Weigh accurately about 0.7 g of Magnesium Silicate in a beaker, add 20 mL of diluted sulfuric acid (3 in 100), and heat on a water bath for 90 minutes. Filter the supernatant by suction using a filter holder equipped with a membrane filter (0.1 μm pore size). Add 10 mL of hot water to the residue in the beaker containing, stir, and filter the supernatant by decantation through the membrane filter. Wash the residue

twice with 10 mL of hot water each time, and decant each supernatant onto the filter. To the residue, add 25 mL of water, heat on a water bath for 15 minutes, and transfer the residue onto the membrane filter. Wash the residue with hot water until the washings no longer respond to test (1) for Sulfate in the Qualitative Tests. Heat the residue with the membrane filter in a platinum crucible to dryness, incinerate, and ignite for 30 minutes. Cool, and weigh the crucible with residue (M_1 g). Moisten the residue with water, add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid, evaporate to dryness, and ignite for 5 minutes. Cool, and weigh the crucible with residue (M_2 g). Determine the silicon dioxide content by the formula:

$$\text{Content (\%) of silicon dioxide } (SiO_2) = \frac{M_1 - M_2}{\left[\begin{matrix}\text{Weight (g) of}\\ \text{the sample}\end{matrix}\right] \times \left(1 - \frac{\text{Loss on drying (\%)}}{100}\right) \times \left(1 - \frac{\text{Loss on ignition (\%)}}{100}\right)} \times 100$$

Magnesium Stearate

ステアリン酸マグネシウム

Definition Magnesium Stearate is a mixture of magnesium salts consisting principally of stearic acid and palmitic acid.

Content Magnesium Stearate, when calculated on the dried basis, contains 4.0%–5.0% of magnesium (Mg = 24.31).

Description Magnesium Stearate occurs as a white, light, bulky powder. It has no odor or a faint, characteristic odor.

Identification

(1) Place 5.0 g of Magnesium Stearate in a round-bottom flask, and add 50 mL of peroxide-free diethyl ether, 20 mL of 10% nitric acid TS, and 20 mL of water. Heat under a reflux condenser until the sample dissolves completely. After cooling, transfer the contents of the flask into a separating funnel, shake, and allow to stand. Transfer the aqueous layer into a flask. Extract twice from the diethyl ether layer with 4 mL of water each time, and combine these extracts with the aqueous layer. Wash the extract with 15 mL of peroxide-free diethyl ether, and add water to make exactly 50 mL. Shake this solution and use as the test solution. This solution responds to the test for Magnesium Salt in the Qualitative Tests.

(2) Prepare a test solution and standard solutions as directed in Purity (5). Analyze equal portions of the solutions by gas chromatography using the conditions given in Purity (5). The chromatogram from the test solution shows peaks at the retention times of methyl stearate and methyl palmitate for the corresponding standard solutions.

Purity

(1) Acid or alkali To 1.0 g of Magnesium Stearate, add 20 mL of freshly boiled and cooled water, heat on a water bath for 1 minute with shaking, and filter after cooling. To 10 mL of the filtrate, add 50 μL of bromothymol blue TS, then exactly 50 μL of 0.1 mol/L hydrochloric acid or 0.1 mol/L sodium hydroxide. The color of the solution changes.

(2) Chloride Not more than 0.10% as Cl.

Perform the test on 10.0 mL of the test solution obtained in Identification (1). Prepare a control solution, using 1.40 mL of 0.02 mol/L hydrochloric acid.

(3) Sulfate Not more than 1.0% as SO_4.

Perform the test on 10.0 mL of the test solution obtained in Identification (1). Prepare a control solution, using 10.2 mL of 0.01 mol/L sulfuric acid.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Relative content ratio of stearic acid and palmitic acid

Test Solution Weigh 0.10 g of Magnesium Stearate, and transfer into a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride–methanol TS, shake, and heat for about 10 minutes to dissolve. Add 4.0 mL of heptane through the condenser, and heat for about 10 minutes. After cooling, add 20 mL of a saturated solution of sodium chloride, shake, and allow the layers to separate. Transfer the heptane layer into another flask through about 0.1 g of sodium sulfate, previously rinsed with heptane. Transfer 1.0 mL of this solution into a 10-mL volumetric flask, add heptane to volume, and mix.

Standard Solutions Weigh 50 mg each of stearic acid and palmitic acid in separate small conical flasks fitted with a reflux condenser. Add 5.0 mL of boron trifluoride–methanol TS to each, and shake. Prepare methyl stearate standard solution and methyl palmitate standard solution, respectively, in the same manner as for the test solution.

Procedure Analyze 1 μL each of the test solution and the standard solutions by gas chromatography using the conditions given below. Determine the peak areas (A_A and A_B) of methyl stearate and methyl palmitate for the test solution, respectively. Also, determine the total peak area (A_T) of all the peaks of fatty acid esters (all peaks detected) for the test solution. The main solvent peak is excluded from measurement. The chromatography should be continued for about 1.5 times the retention time of methyl stearate, and solvent peaks should be excluded from the measurement. Determine the percentages of stearic acid and the sum of stearic acid and palmitic acid in the fatty acid fraction of magnesium stearate using the following formula:.

$$\text{Ratio (\%) of stearic acid} = \frac{A_A}{A_T} \times 100$$

$$\text{Ratio (\%) the sum of stearic acid and palmitic acid} = \frac{A_A + A_B}{A_T} \times 100$$

The area of the methyl stearate peak and the total area of the methyl stearate peak and methyl palmitate peak are not less than 40% and not less than 90%, respectively, of the total area of all the peaks of fatty acid esters obtained.

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (about 0.32 mm internal diameter and 30 m length) coated with a 0.5-μm thick layer of polyethylene glycol 15,000–diepoxide for gas chromatography.

Column temperature: Maintain the temperature at 70°C for 2 minutes, raise at a rate of 5°C/minute to 240°C, and then maintain at 240°C for 5 minutes.

Injection port temperature: A constant temperature of about 220°C.

Carrier gas: Helium.

Flow rate: Adjust the retention time of methyl stearate to about 32 minutes.

Injection method: Splitless.

Loss on Drying Not more than 6.0 % (105°C, 2 hours).

Assay Weigh accurately about 0.5 g of Magnesium Stearate, and add 50 mL of a 1:1 mixture of ethanol (99.5)/1-butanol, 5 mL of ammonia solution (28), and 3 mL of ammonium buffer (pH10.0). To this, add exactly 30.0 mL of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate, and shake, and heat at 45–50°C until the solution is clear. After cooling, titrate with 0.1 mol/L zinc sulfate. The endpoint is when the solution turns from blue to red-purple. Use 1 to 2 drops of eriochrome black T TS as the indicator. Perform a blank test and make any necessary correction.

Each mL of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.431 mg of Mg

Magnesium Sulfate

硫酸マグネシウム

$MgSO_4 \cdot nH_2O$ (n = 7 or 3)

Mol. Wt. heptahydrate 246.47

trihydrate 174.41

Magnesium sulfate heptahydrate [10034-99-8]

Magnesium sulfate trihydrate

Definition Magnesium Sulfate occurs in crystalline form (heptahydrate) called Magnesium Sulfate (crystal), and in dried form (trihydrate) called Magnesium Sulfate (dried).

Content Magnesium Sulfate, when ignited, contains not less than 99.0% of magnesium sulfate ($MgSO_4$ = 120.37).

Description Magnesium Sulfate (crystal) occurs as colorless prisms or needles having a salty and bitter taste. Magnesium Sulfate (dried) occurs as a white powder having a salty and bitter taste.

Identification Magnesium Sulfate responds to all the tests for Magnesium Salt and for Sulfate in the Qualitative Tests.

Purity

(1) Clarity of solution

Crystal: Colorless, almost clear (1.0 g, water 10 mL).

Dried: Colorless, very slightly turbid (1.0 g, water 10 mL).

(2) Chloride Not more than 0.014% as Cl (1.0 g, Control Solution: 0.01 mol/L hydrochloric acid 0.40 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Magnesium Sulfate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate the solution to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Ignition

Crystal: 40.0–52.0% (100°C for 2 hours, then 300–400°C for 4 hours).

Dried: 25.0–35.0% (300–400°C, 4 hours).

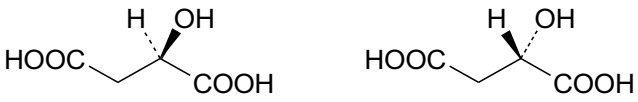
Assay Weigh accurately about 0.6 g of Magnesium Sulfate, previously ignited, and dissolve it in 2 mL of diluted hydrochloric acid (1 in 4) and water to make exactly 100 mL. Measure exactly 25 mL of this solution, add 50 mL of water and 5 mL of ammonium buffer (pH 10.7), and titrate with 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 5 drops of eriochrome black T TS) until the color of the solution changes from the red-purple to blue. Perform a blank test, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 6.018 mg of $MgSO_4$

DL-Malic Acid

***dl*-Malic Acid**
Malic Acid

DL-リンゴ酸



$C_4H_6O_5$ Mol. Wt. 134.09

(2*RS*)-2-Hydroxybutanedioic acid [6915-15-7]

Content DL-Malic Acid contains not less than 99.0% of DL-malic acid ($C_4H_6O_5$).

Description DL-Malic Acid occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and a characteristic acid taste.

Identification

(1) Place 1 mL of a solution of DL-Malic Acid (1 in 20) into a porcelain dish, neutralize with ammonia TS, add 10 mg of sulfanilic acid, and heat on a water bath for a few minutes. Add 5 mL of sodium nitrite solution (1 in 5), warm slightly, and make the solution alkaline with sodium hydroxide solution (1 in 25). A red color develops.

(2) Place 1 mL of DL-Malic Acid solution (1 in 20) into a test tube, add 2–3 mg of resorcinol and 1 mL of sulfuric acid, shake, heat at 120–130°C for 5 minutes, cool, and add water to make 5 mL. Make the solution alkaline by adding sodium hydroxide solution (3 in 10) dropwise while cooling, and add water to make 10 mL. A light blue fluorescence is observed under ultraviolet light.

Melting Point 127–132°C.

Purity

(1) Clarity of solution Clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.004% as Cl (1.0 g, Control Solution: 0.01 mol/L hydrochloric acid 0.10 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Readily oxidizable substances Weigh 0.10 g of DL-Malic Acid, dissolve it in 25 mL of water and 25 mL of diluted sulfuric acid (1 in 20), keep at 20°C, and add 1.0 mL of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 3 minutes.

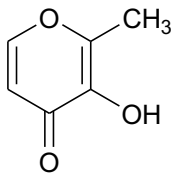
Residue on Ignition Not more than 0.05% (5 g).

Assay Weigh accurately about 1.5 g of DL-Malic Acid, and dissolve it in water to make exactly 250 mL. Measure exactly 25 mL of this solution, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide = 6.704 mg of $C_4H_6O_5$

Maltol

マルトール



$C_6H_6O_3$ Mol. Wt. 126.11

3-Hydroxy-2-methyl-4*H*-pyran-4-one [118-71-8]

Content Maltol contains not less than 99.0% of maltol ($C_6H_6O_3$).

Description Maltol occurs as white to light yellow needles or crystalline powder having a sweet odor.

Identification Determine the absorption spectrum of Maltol as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum of Maltol. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 160–164°C.

Assay Using a solution (1 in 100) of Maltol in ethanol (95), proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Maltose Phosphorylase

マルトースホスホリラーゼ

Definition Maltose Phosphorylase includes enzymes that phosphorolyze maltose. It is derived from the culture of bacteria (limited to *Paenibacillus* sp. and species of the genus *Plesiomonas*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Maltose phosphorylase occurs as white to dark brown granules, powder, or

paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Maltose Phosphorylase complies with the Maltose Phosphorylase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Maltose Phosphorylase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 1.0 g of Maltose Phosphorylase, add phosphate buffer (0.05 mol/L) at pH 7.0 or water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer or water to the resulting solution.

Substrate Solution Weigh 3.60 g of D(+)-maltose monohydrate, and dissolve it in phosphate buffer (0.05 mol/L) at pH 7.0 to make 500 mL.

Test Solution To 0.5 mL of the substrate solution, equilibrated at 50°C for 5 minutes, add 0.01 mL of the sample solution, and shake immediately. Incubate the mixture at 50°C for 15 minutes, then heat it in a water bath for 3 minutes, and allow to cool. Add 2 mL of TS for D-glucose determination (containing mutarotase), mix, and warm at 37°C for 10 minutes.

Control Solution To 0.5 mL of the substrate solution, add 0.01 mL of the sample solution, and immediately heat the mixture in a water bath for 3 minutes. After cooling, add 2 mL of TS for D-glucose determination (containing mutarotase), mix, and warm at 37°C for 10 minutes.

Procedure Measure the absorbance of the test solution and the control solution at a

wavelength of 505 nm. The absorbance value of the test solution is higher than that of control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Maltotriohydrolase

マルトトリオヒドロラーゼ

Definition Maltotriohydrolase includes enzymes that hydrolyze polysaccharides, such as starch, to produce maltotriose. It is derived from the culture of filamentous fungi (limited to species of the genus *Penicillium*), actinomycetes (limited to *Streptomyces avermitilis*, *Streptomyces cinnamonensis*, *Streptomyces griseus*, *Streptomyces thermoviolaceus*, *Streptomyces violaceoruber*), or bacteria (limited to *Bacillus subtilis*, *Cellulosimicrobium cellulans*, and species of the genus *Microbacterium*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Maltotriohydrolase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Maltotriohydrolase complies with the Maltotriohydrolase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

The requirement of total plate count can be disregarded if unsterilized α-Glucosyltransferase is used for captive consumption in the manufacture of food that will be sterilized or pasteurized before completion of the final product.

Maltotriohydrolase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by any given method, appropriate

replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of Maltotriohydrolase, add Tris buffer (0.005 mol/L, pH 7.0, containing calcium chloride) to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Test Solution To 30 mL of dextrin TS, add 0.1 mL of pullulanase TS (100 units/mL) and 0.1 mL of the sample solution, and mix. Incubate the mixture at 50°C for 24 hours. Heat 10 mL of this solution in a water bath for 10 minutes, and allow to cool.

If the test solution is turbid, use the filtrate collected by filtration or ultrafiltration or the supernatant collected by centrifugation.

Standard Solution Dissolve 0.25 g of maltotriose in water to make 50 mL

Procedure Analyze 10 μL each of the test solution and the standard solution by liquid chromatography using operating condition given below. The retention time of the main peak of the test solution corresponds to that of the peak of the maltotoriose standard solution.

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (5–20 mm internal diameter and 20–40 cm length).

Column packing material: 11–25 μm Ag-form cation exchange resin for liquid chromatography.

Column temperature: A constant temperature of 50–85°C.

Mobile phase: Water

Flow rate: 0.3–1.0 mL/min. Adjust the retention time of maltototriose to10–50 minutes.

Method 2

Sample Solution Weigh 0.50 g of Maltotriohydrolase, add cooled acetate buffer (0.1 mol/L, pH 6.0, containing calcium chlorid) or water to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer or water to the resulting solution.

Substrate Solution Suspend 1.0 g of soluble starch in a small amount of water. Add this suspension to about 50 mL of boiling water, keep boiling for an addition 5 minutes, allow to cool, and add water to make 100 mL. Prepare fresh before use.

Test Solution To 0.5 mL of the substrate solution, add 0.4 mL of acetate buffer (0.1 mol/L, pH 6.0, containing calcium chloride), mix, and equilibrate the mixture at 40°C for 15 minutes. Add 0.1 mL of the sample solution, shake immediately, and incubate the mixture at 40°C for 15 minutes. To this solution, add 1 mL of cupper TS (for maltotriohydrolase activity test), and mix. Heat this solution in a water bath for 20

minutes. After cooling, add 1 mL of Nelson TS, shake well, allow to stand at room temperature for 20 minutes. Dilute this solution to 25 mL with water.

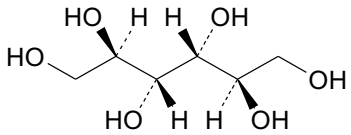
Control Solution To 0.5 mL of the substrate solution, add 0.4 mL of acetate buffer (0.1 mol/L, pH 6.0, containing calcium chloride), and mix. Add 1 mL of cupper TS (for maltotriohydrolase activity test), and shake. Add 0.1 mL of the sample solution, and mix. Heat this solution for 20 minutes in a water bath, allow to cool, and then proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 520 nm. The absorbance value of the test solution is higher than that of control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

D-Mannitol

Mannitol
D-Mannite

D-マンニトール



$C_6H_{14}O_6$ Mol. Wt. 182.17

D-Mannitol [69-65-8]

Content D-Mannitol, when dried, contains not less than 96.0% of D-mannitol ($C_6H_{14}O_6$).

Description D-Mannitol occurs as white crystals or powder. It is odorless and has a cool, sweet taste.

Identification

(1) Transfer 3 mL of a solution of D-Mannitol (1 in 5) to a test tube containing 1 mL of iron(III) chloride hexahydrate (1 in 10), and add 1.5 mL of sodium hydroxide solution (1 in 25). A yellow precipitate is formed. Shake vigorously. The precipitate dissolves, and the liquid is yellow and transparent. Even when sodium hydroxide solution (1 in 25) is added, no precipitate is formed.

(2) To 0.5 g of D-Mannitol, add 3 mL of acetic anhydride and 1 mL of pyridine, and dissolve completely by heating in a water bath with occasional shaking. Continue heating for 5 minutes, and cool. To this solution, add 20 mL of water, mix well, and allow to stand for 5 minutes. Collect the deposited crystals by filtration, wash with water, and recrystallize from diethyl ether. The melting point of crystals is 120–125°C.

Melting Point 165–169°C.

Purity

(1) Free acid Weigh 5 g of D-Mannitol, dissolve it in 50 mL of freshly boiled and cooled water, add 1 drop of phenolphthalein TS and 0.5 mL of 0.01 mol/L sodium hydroxide, and shake. The color of the solution changes to pink that persists for not less than 30 seconds.

(2) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Nickel Weigh 0.5 g of D-Mannitol, dissolve it in 5 mL of water, add 3 drops of a solution (1 in 100) of dimethylglyoxime in ethanol (95) and 3 drops of ammonia TS, and allow to stand for 5 minutes. The color of the solution does not change to pink.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Saccharide Weigh 0.5 g of D-Mannitol, add 10 mL of water and 2 mL of diluted hydrochloric acid (1 in 4), boil for 2 minutes, and cool. Add 5 mL of sodium carbonate solution (1 in 8), allow to stand for 5 minutes, add 2 mL of Fehling's TS, and boil for 1 minute. An orange-yellow to red precipitate is not immediately formed.

Loss on Drying Not more than 0.3% (105°C, 4 hours).

Residue on Ignition Not more than 0.02% (5 g).

Assay

Test Solution and Standard Solution Weigh accurately about 1 g each of D-Mannitol and D-mannitol for assay, previously dried, separately dissolve them in water to make two solutions of exactly 50 mL each. Use these solutions as the test solution and the standard solution, respectively.

Procedure Analyze 10 μL each of these solutions by liquid chromatography using the conditions given below. Measure the peak areas (A_T and A_S) of D-mannitol for the test solution and the standard solution, and determine the content by the formula:

$$\text{Content (\%) of D-mannitol } (C_6H_{14}O_6) = \frac{\text{Weight (g) of D-mannitol for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (4–8 mm internal diameter and 20–50 cm length).

Column packing material: 5- to 12-μm strongly acidic cation-exchange resin for liquid chromatography.

Column temperature: A constant temperature of 40–85°C.

Mobile phase: Water.

Flow rate: A constant rate of 0.5–1.0 mL/minute.

Marigold Color

マリーゴールド色素

Definition Marigold Color is obtained from the flowers of the marigold plant, *Tagetes patula* L., *Tagetes erecta* L., or their interspecific hybrids. It consists mainly of xanthophylls.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Marigold Color is not less than 2500 and is in the range of 95–115% of the labeled value.

Description Marigold Color is a dark brown solid or liquid having a characteristic odor.

Identification

(1) Weigh an amount of Marigold Color equivalent to 0.1 g of marigold color with a Color Value 2500, and dissolve it in 100 mL of a 1:1 mixture of ethanol (95)/hexane. A deep yellow color develops.

(2) A solution of Marigold Color in a 1:1 mixture of ethanol (95)/hexane exhibits absorption maxima at wavelengths of 469–475 nm and 441–447 nm. It may, in addition, exhibit an absorption maximum at a wavelength of 420–426 nm.

(3) Weigh an amount of Marigold Color equivalent to 0.1 g of marigold color with a Color Value 2500, and dissolve it in 10 mL of a 1:1 mixture of ethanol (95)/hexane. Use the solution obtained as the test solution. Analyze a 5-μL portion of the test solution by thin-layer chromatography using a 15:4:1 mixture of toluene/ethyl acetate/ethanol (95) as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry. One yellow spot is observed at both or either of the R_f values of approximately 0.8 (fatty acid esters of lutein) and approximately 0.35 (lutein). The spot is decolorized immediately when sprayed with sodium nitrite solution (1 in 20) followed by with sulfuric acid TS (0.5 mol/L).

Purity

(1) Lead Not more than 3 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 6.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination.

Operating Conditions

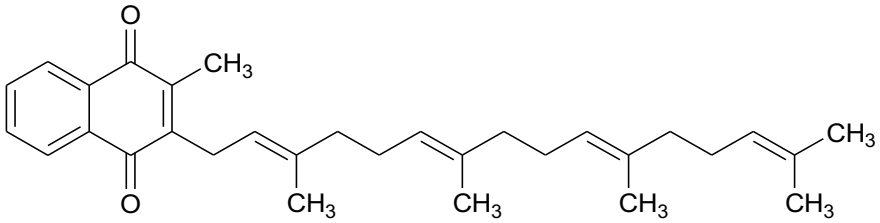
Solvent: A 1:1 mixture of Ethanol (95)/hexane.

Wavelength: Maximum absorption wavelength of 441–447 nm.

Menaquinone (Extract)

Vitamin K_2 (Extract)

メナキノン(抽出物)



$C_{31}H_{40}O_2$ Mol. Wt. 444.65

2-Methyl-3-[(2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl]naphthalene-1,4-dione [863-61-6]

Definition Menaquinone (Extract) is obtained from the culture fluid of *Arthrobacter nicotianae* and consists mainly of menaquinone-4.

Content Menaquinone (Extract), when calculated on the anhydrous basis, contains 98.0–102.0% of menaquinone-4 ($C_{31}H_{40}O_2$).

Description Menaquinone (Extract) occurs as yellow crystals, crystalline powder, wax-like lumps, or oil-like liquid.

Identification Determine the absorption spectrum of Menaquinone (Extract), previously kept in a vacuum desiccator containing phosphorous(V) oxide at 40°C for 24 hours, as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Menadion To 0.20 g of Menaquinone (Extract), add 5 mL of diluted ethanol (99.5) (1 in 2), shake well, and filter. To 0.5 mL of the filtrate, add 1 drop of a solution (1 in 20) of 3-methyl-1-phenyl-5-pyrazoron in ethanol (99.5) and 1 drop of aqueous ammonia, and allow to stand for 2 hours. A blue-purple color does not develop.

Water Content Not more than 0.50% (0.50 g, Volumetric Titration, Direct Titration).

Residue on Ignition Not more than 0.1%.

Assay Throughout this assay, all procedures should be protected from direct light and the apparatus used should be light-resistant. Before the assay, determine the water content of menaquinone-4 in the same manner as Menaquinone (Extract).

Test Solution and Standard Solution Weigh accurately about 0.1 g each of Menaquinone (Extract) and menaquinone-4 for assay, dissolve them separately in 50 mL of 2-propanol, and add ethanol (99.5) to make 2 solutions, exactly 100 mL each. Measure exactly 10 mL each of these solutions, and add ethanol (99.5) to each to make exactly 100 mL. To exactly 2 mL each of the resulting solutions, add 4 mL of a solution (1 in 20,000) of phytonadion in 2-propanol. Use these solutions as the test solution and the standard solution, respectively.

Procedure Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Determine the peak area ratios (Q_T and Q_S) of menaquinone-4 to phytonadion for the test solution and the standard solution, and calculate the content of menaquinone-4 by the formula:

$$\text{Content (\%) of menaquinone-4 } (C_{31}H_{40}O_2) = \frac{\text{Anhydrous basis weight (g) of menaquinone-4 for assay}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 270 nm).

Column: A stainless steel tube (about 5 mm internal diameter and about 15 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of about 40°C.

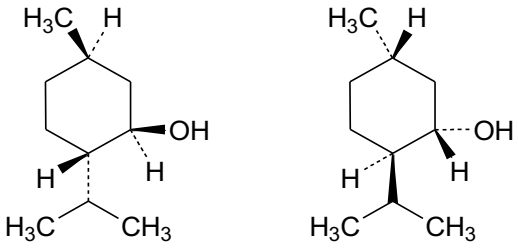
Mobile phase: Methanol.

Flow rate: Adjust the retention time of menaquinone-4 to about 7 minutes.

dl-Menthol

dl-Peppermint Camphor

dl-メントール



$C_{10}H_{20}O$ Mol. Wt. 156.27

(1*RS*,2*SR*,5*RS*)-5-Methyl-2-(1-methylethyl)cyclohexan-1-ol [89-78-1]

Content *dl*-Menthol contains not less than 95.0% of *dl*-menthol ($C_{10}H_{20}O$).

Description *dl*-Menthol occurs as colorless prisms or needles, or as a white crystalline powder. It has a peppermint-like odor.

Identification Determine the absorption spectrum of *dl*-Menthol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers. For a solid sample, melt it by heating before use.

Congealing Point 27–28°C.

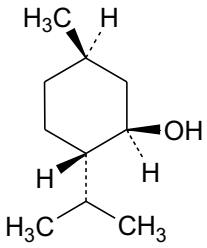
Specific Rotation $[\alpha]_D^{20}$: −2.0 to +2.0° (2.5 g, ethanol (95), 25 mL).

Assay Using a solution (1 in 10) of *dl*-Menthol in ethanol (95) as the test solution, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

l-Menthol

Peppermint Camphor

l-メントール



$C_{10}H_{20}O$ Mol. Wt. 156.27

(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)cyclohexan-1-ol [2216-51-5]

Content *l*-Menthol contains not less than 95.0% of *l*-menthol ($C_{10}H_{20}O$).

Description *l*-Menthol occurs as colorless prisms or needles, or as a white crystalline powder. It has a peppermint-like odor and a cool taste.

Identification Determine the absorption spectrum of *l*-Menthol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers. For a solid sample, melt it by heating before use.

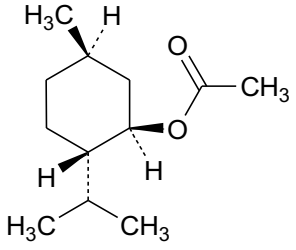
Specific Rotation $[\alpha]_D^{20}$: −40.0 to –52.0° (2.5 g, ethanol (95), 25 mL).

Melting Point 41–44°C.

Assay Using a solution (1 in 10) of *l*-Menthol in ethanol (95), proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

l-Menthyl Acetate

酢酸 *l*-メンチル



$C_{12}H_{22}O_2$ Mol. Wt. 198.30

(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)cyclohexyl acetate [2623-23-6]

Content *l*-Menthyl Acetate contains not less than 98.0% of *l*-menthyl acetate ($C_{12}H_{22}O_2$).

Description *l*-Menthyl Acetate is a colorless to light yellow, clear liquid having a cool odor.

Identification Determine the absorption spectrum of *l*-Menthyl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.445–1.449.

Angular Rotation α_D^{20}: Not more than –69°.

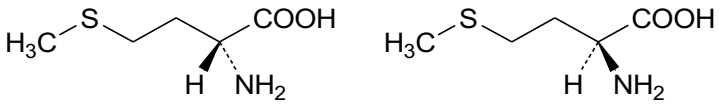
Specific Gravity d_{25}^{25}: 0.921–0.926.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

DL-Methionine

DL-メチオニン



$C_5H_{11}NO_2S$ Mol. Wt. 149.21

(*2RS*)-2-Amino-4-(methylsulfanyl)butanoic acid [59-51-8]

Content DL-Methionine, when dried, contains not less than 98.5% of DL-methionine ($C_5H_{11}NO_2S$).

Description DL-Methionine occurs as white plates or crystalline powder having a characteristic odor and a slightly sweet taste.

Identification

(1) Determine the absorption spectrum of DL-Methionine, previously dried as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of DL-Methionine (1 in 100) exhibits no optical rotation.

pH 5.6–6.1 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution　Colorless and clear (0.50 g, water 20 mL).

(2) Chloride　Not more than 0.021% as Cl.

Test Solution　Weigh 0.50 g of DL-Methionine, dissolve it by adding 6 mL of diluted nitric acid (1 in 10) and water, and make 40 mL.

Control Solution　To 0.30 mL of 0.01 mol/L hydrochloric acid, add 6 mL of diluted nitric acid (1 in 10) and water to make 40 mL.

Procedure　In the test, use 10 mL of silver nitrate solution (1 in 50).

(3) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Proceed as directed in Purity (3) for L-Cysteine Monohydrochloride.

Loss on Drying　Not more than 0.5% (105°C, 3 hours).

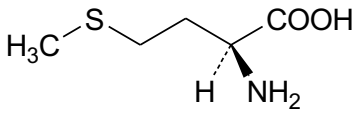
Residue on Ignition　Not more than 0.1% .

Assay　Weigh accurately about 0.3 g of DL-Methionine, and proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 14.92 mg of $C_5H_{11}NO_2S$

L-Methionine

L-メチオニン



$C_5H_{11}NO_2S$　Mol. Wt. 149.21

(2*S*)-2-Amino-4-(methylsulfanyl)butanoic acid　[63-68-3]

Content　L-Methionine, when dried, contains not less than 98.5% of L-methionine ($C_5H_{11}NO_2S$).

Description　L-Methionine occurs as white plates or crystalline powder having a characteristic odor and a slight bitter taste.

Identification

(1) To 5 mL of a solution of L-Methionine (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) To 25 mg of L-Methionine, add 1 mL of　sulfuric acid saturated with copper(II)

sulfate. A yellow color develops.

(3) To 2 mL of a solution of L-Methionine (1 in 100), add 2 mL of sodium hydroxide solution (1 in 25), shake, add 0.3 mL of a solution of sodium pentacyanonitrosylferrate(III) dihydrate (1 in 20), and shake again. Allow to stand for 1–2 minutes, and add 4 mL of diluted hydrochloric acid (1 in 10). A red-purple color develops.

Specific Rotation $[\alpha]_D^{20}$: +21.0 to +25.0° (1 g, hydrochloric acid TS (6 mol/L), 50 mL, on the dried basis).

pH 5.6–6.1 (0.5 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and clear (0.50 g, water 20 mL).

(2) Chloride Not more than 0.021% as Cl.

Proceed as directed in Purity (2) for DL-Methionine.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Proceed as directed in Purity (3) for L-Cysteine Monohydrochloride.

Loss on Drying Not more than 0.5% (105°C, 3 hours).

Residue on Ignition Not more than 0.1% .

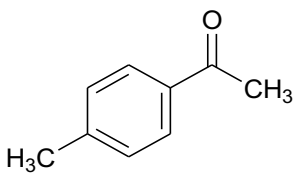
Assay Weigh accurately about 0.3 g of L-Methionine, and proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 14.92 mg of $C_5H_{11}NO_2S$

p-Methylacetophenone

4-Methyl Acetophenone

パラメチルアセトフェノン



$C_9H_{10}O$ Mol. Wt. 134.18

1-(4-Methylphenyl)ethanone [122-00-9]

Content *p*-Methylacetophenone contains not less than 95.0% of *p*-methylacetophenone ($C_9H_{10}O$).

Description *p*-Methylacetophenone is a colorless to light yellow, clear liquid having a characteristic odor.

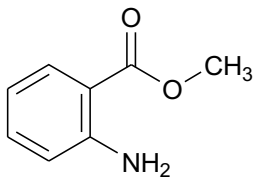
Identification Determine the absorption spectrum of *p*-Methylacetophenone as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Gravity d_{25}^{25}: 0.999–1.010.

Assay Using a solution (1 in 10) of *p*-Methylacetophenone in acetone as the test solution, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Methyl Anthranilate

アントラニル酸メチル



$C_8H_9NO_2$ Mol. Wt. 151.16

Methyl 2-aminobenzoate [134-20-3]

Content Methyl Anthranilate contains not less than 98.0% of methyl anthranilate ($C_8H_9NO_2$).

Description Methyl Anthranilate occurs as colorless to light yellow lumps or as a colorless to light yellow, clear liquid. It has a grape-like odor. The liquid fluoresces a blue-purple color.

Identification Determine the absorption spectrum of Methyl Anthranilate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.581–1.585.

Specific Gravity d_{25}^{25}: 1.161–1.169.

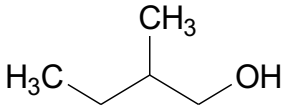
Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Using a solution (1 in 10) of Methyl Anthranilate in acetone as the test solution, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic

Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

2-Methylbutanol

2-メチルブタノール



$C_5H_{12}O$ Mol. Wt. 88.15

2-Methylbutan-1-ol [137-32-6]

Content 2-Methylbutanol contains not less than 99.0% of 2-methylbutanol ($C_5H_{12}O$).

Description 2-Methylbutanol is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of 2-Methylbutanol, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.409–1.412.

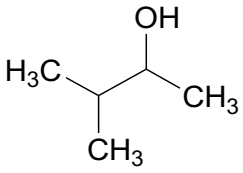
Specific Gravity d_{25}^{25}: 0.815–0.820

Purity Acid value Not more than 1.0 (Flavoring Substance Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2).

3-Methyl-2-butanol

3-メチル-2-ブタノール



$C_5H_{12}O$ Mol. Wt. 88.15

3-Methylbutan-2-ol [598-75-4]

Content 3-Methyl-2-butanol contains not less than 98.0% of 3-methyl-2-butanol

($C_5H_{12}O$).

Description 3-Methyl-2-butanol occurs as a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of 3-Methyl-2-butanol, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.406–1.412.

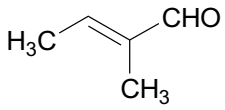
Specific Gravity d_{25}^{25}: 0.815–0.821.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2).

trans-2-Methyl-2-butenal

(*E*)-2-Methyl-2-butenal

***trans*-2-メチル-2-ブテナール**



C_5H_8O Mol. Wt. 84.12

(2*E*)-2-Methylbut-2-enal [497-03-0]

Content *trans*-2-Methyl-2-butenal contains not less than 97.0% of *trans*-2-methyl-2-butenal (C_5H_8O).

Description *trans*-2-Methyl-2-butenal occurs as a colorless, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of *trans*-2-Methyl-2-butenal as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.445–1.450.

Specific Gravity d_{20}^{20}: 0.866–0.873.

Purity Acid value Not more than 3.0 (Flavoring Substances Tests).

Assay Using a solution (1 in 10) of *trans*-2-Methyl-2-butenal in acetone as the test solution, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions

(3). For the column, column temperature, and flow rate, follow the conditions given below.

Operating conditions

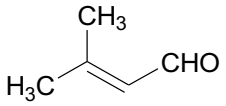
Column: Use a fused silica tube (0.25–0.53 mm in internal diameter and 50–60 m in length) coated with a 0.5–1 μm thick layer of polyethylene glycol for gas chromatography.

Column temperature: Maintain the temperature at 50°C for 15 minutes, raise at a rate of 10°C/minute to 230°C, and maintain for 27 minutes.

Flow rate: Adjust so that the peak of the component to be determined appears between 10–30 minutes after injection.

3-Methyl-2-butenal

3-メチル-2-ブテナール



C_5H_8O Mol. Wt. 84.12

3-Methylbut-2-enal [107-86-8]

Content 3-Methyl-2-butenal contains not less than 97.0% of 3-methyl-2-butenal (C_5H_8O).

Description 3-Methyl-2-butenal occurs as a colorless, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 3-Methyl-2-butenal, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.458–1.464.

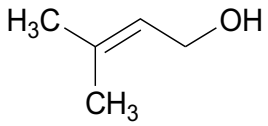
Specific Gravity d_{25}^{25}: 0.870–0.875.

Purity Acid value Not more than 5.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (3). For the column, use a fused silica tube (0.25–0.53 mm in internal diameter and 30–60 m in length) coated with a 0.25–1 μm thick layer of polyethylene glycol.

3-Methyl-2-butenol

3-メチル-2-ブテノール



$C_5H_{10}O$ Mol. Wt. 86.13

3-Methylbut-2-en-1-ol [556-82-1]

Content 3-Methyl-2-butenol contains not less than 98.5% of 3-methyl-2-butenol ($C_5H_{10}O$).

Description 3-Methyl-2-butenol occurs as a colorless, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 3-Methyl-2-butenol, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.438–1.448.

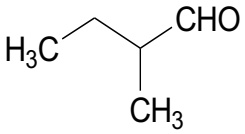
Specific Gravity d_{25}^{25}: 0.855–0.863.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2). For the column, use a fused silica tube (0.25–0.53 mm in internal diameter and 30–60 m in length) coated with a 0.25–1 μm thick layer of polyethylene glycol.

2-Methylbutyraldehyde

2-メチルブチルアルデヒド



$C_5H_{10}O$ Mol. Wt. 86.13

2-Methylbutanal [96-17-3]

Content 2-Methylbutyraldehyde contains not less than 95.0% of 2- methylbutyraldehyde ($C_5H_{10}O$).

Description 2-Methylbutyraldehyde occurs as a colorless to light yellow, clear liquid

having a characteristic odor.

Identification Determine the absorption spectrum of 2-Methylbutyraldehyde, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.388–1.396.

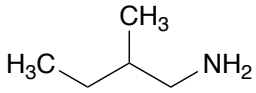
Specific Gravity d_{25}^{25}: 0.799–0.815.

Purity Acid value Not more than 10.0 (Flavoring Substance Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (3).

2-Methylbutylamine

2-メチルブチルアミン



$C_5H_{13}N$ Mol. Wt. 87.16

Chemical name [CAS number] 2-Methylbutan-1-amine [96-15-1]

Content 2-Methylbutylamine contains not less than 95.0% of 2-methylbutylamine ($C_5H_{13}N$).

Description 2-Methylbutylamine occurs as a colorless to yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 2-Methylbutylamine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive index n_D^{20}: 1.408–1.423

Specific gravity d_{25}^{25}: 0.752–0.779

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operating conditions (2) except for the column. Use a fused silica tube (0.25–0.53 mm internal diameter and 30–60 m length) coated with a 0.25–1 μm thick layer of dimethylpolysiloxane for gas chromatography.

Methyl Cellulose

メチルセルロース

Methyl ether of cellulose [9004-67-5]

Content Methyl Cellulose, when dried, contains 25.0–33.0% of methoxy group (-OCH_3 = 31.03).

Description Methyl Cellulose occurs as a white to whitish powder or fibrous substance. It is odorless.

Identification Add 1.0 g of Methyl Cellulose to 100 mL of water at about 70°C, stir well, cool while shaking, and allow to stand in a cold place until it becomes a homogeneous paste. Use the resulting solution as the test solution.

(1) Heat about 10 mL of the test solution in a water bath. White turbidity or precipitate is formed. Cool it. The white turbidity or precipitate dissolves and becomes a homogeneous pasty again.

(2) On the top of about 2 mL of the test sample, superimpose 1 mL of anthrone TS gently along the tube wall. The junction between the two solutions turns blue to green.

Kinematic Viscosity When the viscosity is declared, perform the following test. The kinematic viscosity is 80–120% of the declared amount when the declared amount is not more than 100 mm^2/s, and 70–140% when it exceeds 100 mm^2/s.

Weigh an amount of Methyl Cellulose equivalent to 2 g, calculated on the dried basis, add 50 mL of water of 85°C, and stir for 10 minutes using a stirrer. Add 40 mL of water, dissolve the sample in ice water while stirring for 40 minutes, and add water to make exactly 100 mL. Remove the effervescence by centrifuging if necessary, and measure the viscosity at 20 ± 0.1°C.

Purity

(1) Chloride Not more than 0.57% as Cl.

Sample Solution Weigh 0.50 g of Methyl Cellulose, transfer into a beaker, add 30 mL of hot water, stir well, and filter while hot with a warmed funnel. Wash the beaker and the residue on filter paper three times, using 15 mL of hot water each time, combine the washings with the filtrate, and add water to make 100 mL. Refer to this solution as Solution A. Use 5 mL of Solution A as the sample solution.

Control Solution Use 0.40 mL of 0.01 mol/L hydrochloric acid.

(2) Sulfate Not more than 0.096% as SO_4.

Sample Solution Use exactly 40 mL of Solution A obtained in Purity (1).

Control Solution Use 0.40 mL of 0.005 mol/L hydrochloric acid.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic

Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 8.0% (105°C, 1 hour)

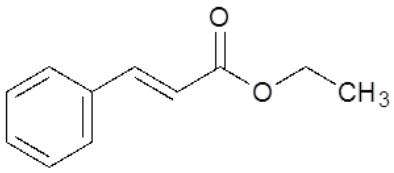
Residue on Ignition Not more than 1.5% (calculated on the dried basis).

Assay Weigh accurately about 25 mg of Methyl Cellulose, previously dried, and proceed as directed under Methoxy Determination.

$$\text{Content (\%) of methoxy group } (-OCH_3) = \frac{(\text{Volume (mL) of 0.01 mol/L sodium thiosulfate consumed}) \times 0.0517}{\text{Weight (mg) of the sample}} \times 100$$

Methyl Cinnamate

ケイ皮酸メチル



$C_{10}H_{10}O_2$ Mol. Wt. 162.19

Methyl (2*E*)-3-phenylprop-2-enoate [1754-62-7]

Content Methyl Cinnamate contains not less than 98.0% of methyl cinnamate ($C_{10}H_{10}O_2$).

Description Methyl Cinnamate occurs as a white solid having a *matsutake* mushroom-like odor.

Identification Determine the absorption spectrum of Methyl Cinnamate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

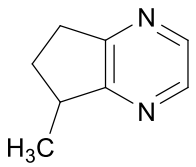
Melting Point Not less than 33°C.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Using a solution (1 in 10) of Methyl Cinnamate in acetone, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

5-Methyl-6,7-dihydro-5*H*-cyclopentapyrazine

5-メチル-6,7-ジヒドロ-5*H*-シクロペンタピラジン



$C_8H_{10}N_2$ Mol. Wt. 134.18

5-Methyl-6,7-dihydro-5*H*-cyclopenta[*b*]pyrazine [23747-48-0]

Content 5-Methyl-6,7-dihydro-5*H*-cyclopentapyrazine contains not less than 97.0% of 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine ($C_8H_{10}N_2$).

Description 5-Methyl-6,7-dihydro-5*H*-cyclopentapyrazine occurs as a light yellow to brown, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 5-Methyl-6,7-dihydro-5*H*-cyclopentapyrazine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.525–1.535.

Specific Gravity d_{25}^{25}: 1.048–1.059.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (1).

Methyl Hesperidin

Soluble Vitamin P

メチルヘスペリジン

Content Methyl Hesperidin, when dried, contains 97.5–103.0% of methyl hesperidin.

Description Methyl Hesperidin occurs as a yellow to orange-yellow powder. It is odorless or has a slight odor.

Identification

(1) To 10 mg of Methyl Hesperidin, add 2 mL of sulfuric acid. A red color develops. Add 1–2 drops of hydrogen peroxide TS. A dark red color develops.

(2) To 0.1 g of Methyl Hesperidin, add 5 mL of ethanol (95) and 1 mL of sodium hydroxide solution (1 in 25), boil for 3 minutes, cool, and filter. The color of the filtrate is

yellow to orange-yellow. To the filtrate, add 1 mL of hydrochloric acid and about 10 mg of magnesium powder, and allow to stand. A red color develops.

(3) To 0.1 g of Methyl Hesperidin, add 10 mL of diluted hydrochloric acid (1 in 4), boil for 5 minutes, cool, and filter. Neutralize the filtrate with sodium hydroxide solution (1 in 5), add 2 mL of Fehling's TS, and heat. A red precipitate is formed.

Purity

(1) Clarity of solution Almost clear (1.0 g, water 10 mL).

(2) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Loss on Drying Not more than 3.0% (reduced pressure, 24 hours).

Residue on Ignition Not more than 0.5%.

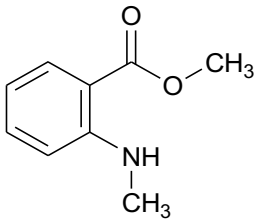
Assay Weigh accurately about 0.3 g of Methyl Hesperidin, previously dried, and dissolve it in water to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add water to make exactly 100 mL. Measure the absorbance (A) of the resulting solution at a wavelength of 300 nm, and calculate the content of methyl hesperidin by the formula:

$$\text{Content (\%) of methyl hesperidin} = \frac{A \times 0.75}{\text{Weight (g) of the sample}} \times 100$$

Methyl *N*-Methylanthranilate

Dimethyl Anthranilate

***N*-メチルアントラニル酸メチル**



$C_9H_{11}NO_2$ Mol. Wt. 165.19

Methyl 2-(methylamino)benzoate [85-91-6]

Content Methyl *N*-Methylanthranilate contains not less than 98.0% of methyl *N*-methylanthranilate ($C_9H_{11}NO_2$).

Description Methyl *N*-Methylanthranilate occurs as colorless to light yellow, clear

crystalline lumps or liquid. It has a grape-like odor. A liquid product generates a blue-purple fluorescence.

Identification Determine the absorption spectrum of Methyl *N*-Methylanthranilate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Congealing Point Not less than 11°C.

Refractive Index n_D^{20} : 1.578–1.581.

Specific Gravity d_{20}^{20}: 1.129–1.135.

Purity

(1) Acid value Not more than 1.0 (Flavoring Substances Tests).

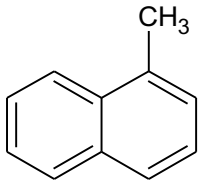
(2) Clarity of solution Clear (1.0 mL, 70% (vol) ethanol 10 mL).

Assay Weigh accurately about 1 g of Methyl *N*-Methylanthranilate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each mL of 0.5 mol/L ethanolic potassium hydroxide = 82.60 mg of $C_9H_{11}NO_2$

1-Methylnaphthalene

1-メチルナフタレン



$C_{11}H_{10}$ Mol.Wt. 142.20

1-Methylnaphthalene [90-12-0]

Content 1-Methylnaphthalene contains not less than 96.0% of 1-methylnaphthalene ($C_{11}H_{10}$).

Description 1-Methylnaphthalene is a colorless to pale yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 1-Methylnaphthalene as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.612–1.618.

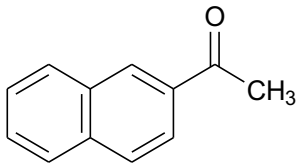
Specific Gravity d_{25}^{25}: 1.017–1.025.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1) except for the column temperature. Raise the column temperature from 150°C to 230°C at a rate of 5°C/minute, and maintain at 230°C for 24 minutes.

Methyl β-Naphthyl Ketone

メチルβ-ナフチルケトン



$C_{12}H_{10}O$ Mol. Wt. 170.21

1-(Naphthalen-2-yl)ethanone [93-08-3]

Content Methyl β-Naphthyl Ketone contains not less than 97.0% of methyl β-naphthyl ketone ($C_{12}H_{10}O$).

Description Methyl β-Naphthyl Ketone occurs as white to light yellow crystals or crystalline powder having a characteristic odor.

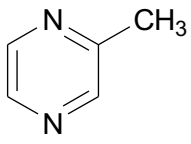
Identification Determine the absorption spectrum of Methyl β-Naphthyl Ketone as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 52–56°C.

Assay Using a solution (1 in 10) of Methyl β-Naphthyl Ketone in ethanol (95) as the test solution, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

2-Methylpyrazine

2-メチルピラジン



$C_5H_6N_2$ Mol. Wt. 94.11

2-Methylpyrazine [109-08-0]

Content 2-Methylpyrazine contains not less than 98.0% of 2-ethylpyrazine ($C_5H_6N_2$).

Description 2-Methylpyrazine occurs as a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of 2-Methylpyrazine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

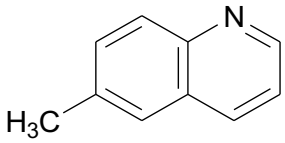
Refractive Index n_D^{20}: 1.501–1.509.

Specific Gravity d_{25}^{25}: 1.007–1.033.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2).

6-Methylquinoline

6-メチルキノリン



$C_{10}H_9N$ Mol. Wt. 143.19

6-Methylquinoline [91-62-3]

Content 6-Methylquinoline contains not less than 98.0% of 6-methylquinoline ($C_{10}H_9N$).

Description 6-Methylquinoline occurs as a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of 6-Methylquinoline, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

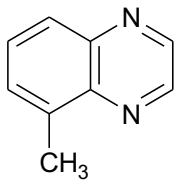
Refractive Index n_D^{20}: 1.611–1.617.

Specific Gravity d_{25}^{25}: 1.060–1.066.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (4).

5-Methylquinoxaline

5-メチルキノキサリン



$C_9H_8N_2$ Mol. Wt. 144.17

5-Methylquinoxaline [13708-12-8]

Content 5-Methylquinoxaline contains not less than 98.0% of 5-methylquinoxaline ($C_9H_8N_2$).

Description 5-Methylquinoxaline occurs as a colorless to orange-color liquid or as crystalline lumps. It has a characteristic odor.

Identification Determine the infrared absorption spectrum of 5-Methylquinoxaline as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

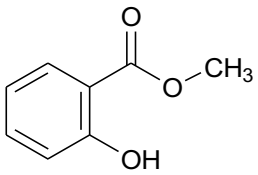
Refractive Index n_D^{20}: 1.615–1.625.

Specific Gravity d_{25}^{25}: 1.102–1.132.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavor Substance Tests. Use operating conditions (4).

Methyl Salicylate

サリチル酸メチル



$C_8H_8O_3$ Mol. Wt. 152.15

Methyl 2-hydroxybenzoate [119-36-8]

Content Methyl Salicylate contains not less than 98.0% of methyl salicylate ($C_8H_8O_3$).

Description Methyl Salicylate is a colorless to light yellow, clear liquid having a cool odor.

Identification Determine the absorption spectrum of Methyl Salicylate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.534–1.538.

Specific Gravity d_{25}^{25}: 1.176–1.185.

Purity Acid value Not more than 2.0 (Flavoring Substances Tests).

Use Phenol Red TS as the indicator.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Microcrystalline Cellulose

微結晶セルロース

Definition Microcrystalline Cellulose is obtained from pulp and consists mainly of crystalline cellulose. There are two forms of products: the desiccated form and the hydrated form.

Description A desiccated product occurs as a white or almost white, odorless, free-flowing crystalline powder. A hydrated product occurs as white or almost white, odorless, moist, cotton-like lumps.

Identification

(1) *Sample Preparation* In the case of a desiccated product: Confirm the percentage of residue by sieving 20 g of Microcrystalline Cellulose through a 38-μm sieve for 5 minutes, using a vacuum suction-type sieving machine. If not less than 5% of residue remains on the sieve, add 270 mL of water to 30 g of Microcrystalline Cellulose, and if less than 5% of residue remains, add 255 mL of water to 45 g of Microcrystalline Cellulose. Then lightly stir the mixture with a spatula.

In the case of a hydrated product: To an amount equivalent to 30 g of the sample on the dried basis, add water to make a 300-g mixture, and lightly stir with a spatula.

Procedure Mix the corresponding mixture in a high-speed (18,000 rpm) blender for 5 minutes, transfer 100 mL of the mixture to a 100-mL measuring cylinder, allow to stand for 3 hours. A white opaque, bubble-free dispersion is obtained. No separation is observed.

(2) Proceed as directed in the Disk Method under Infrared Spectrometry. Compare the spectrum obtained with the Reference Spectrum of Microcrystalline Cellulose. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH 5.0–7.5.

To an amount equivalent to 5.0 g of Microcrystalline Cellulose on the dried basis, add 40 mL of freshly boiled and cooled water, shake for 20 minutes, and centrifuge. Use the supernatant for pH determination.

Purity

(1) Water-soluble substances　Not more than 0.26%.

Weigh accurately an amount equivalent to 5.0 g of Microcrystalline Cellulose on the dried basis, add water to make 85 g, shake for 10 minutes, and filter by suction through a filter paper (5C). Transfer the filtrate to a beaker, previously dried and weighed, evaporate to dryness, taking care not to scorch. Dry at 105°C for 1 hour, cool in a desiccator, and weigh accurately. Separately, perform a blank test for correction.

(2) Lead　Not more than 2 μg/g as Pb (an amount equivalent to 2.0 g on the dried basis, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic　Not more than 3 μg/g as As (an amount equivalent to 0.50 g on the dried basis, Standard Color: Arsenic Standard Solution 3.0 mL, Method 3, Apparatus B)

(4) Starch　To 20 mL of the liquid obtained under the Identification (1), add a few drops of iodine TS, and mix. No bluish-purple or blue color develops.

Loss on Drying

Desiccated sample　Not more than 7.0% (105°C, 3 hours).

Hydrated sample　40.0–70.0% (4 g, 105°C, 3 hours).

Residue on ignition　Not more than 0.05% (an amount equivalent to 2 g on the dried basis).

Microcrystalline Wax

マイクロクリスタリンワックス

Definition　Microcrystalline Wax is a mixture of solid hydrocarbons obtained from petroleum vacuum distillation residues or heavy distillates. It consists mainly of branched and linear saturated hydrocarbons.

Description　Microcrystalline Wax is a colorless or white to yellow, partially translucent solid at room temperature. It has a slight characteristic odor.

Identification　Determine the absorption spectrum of Microcrystalline Wax as directed in the Thin Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 70–95°C (Method 2).

Purity

(1) Lead Not more than 3 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 6.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Polycyclic aromatic hydrocarbons

Proceed as directed in Purity (4) for Paraffin Wax in the Monographs.

Residue on Ignition Not more than 0.1%.

Microfibrillated Cellulose

微小繊維状セルロース

Definition Microfibrillated Cellulose is obtained by microfibrillating pulp or cotton and consists mainly of cellulose.

Description Microfibrillated Cellulose is a white, wet-cotton-like substance.

Identification

(1) Dry Microfibrillated Cellulose to produce a thin film-form sample, and cut into small pieces or break into flakes. Determine the absorption spectrum of the prepared sample as directed in the Disk Method under Infrared Spectrophotometry. The sample disk should be prepared so that the transmittances in the main absorption bands are in a range of 30–80%. Compare the obtained spectrum with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Weigh an amount of Microfibrillated Cellulose equivalent to 5.0 g on the dried basis, and add water to make 100 g. Mix it at 10,000–12,000 rpm for 3 minutes, using a homogenizer with a rotor blade of 35 mm in diameter and a cup of 150 mL in volume (59 mm in upper internal diameter, 44 mm in lower internal diameter, and 75 mm in depth). The mixture is in a white opaque dispersed-form and remains the same even after 3 hours without separating.

(3) Weigh an amount of Microfibrillated Cellulose equivalent to 1.0 g on a dry basis, and add water to make 100 g. Homogenize for 3 minutes as specified in Identification (1). Place the white turbid liquid obtained on a 25-μm standard sieve (20 cm diameter) with a receiver, and gently vibrate horizontally for 10 seconds. Evaporate the liquid passing through the sieve to dryness. The weight of the residue is not more than 0.30 g.

pH 5.0–8.0 (2.0 g, water 100 mL, suspension).

Purity

(1) Lead Not more than 2 μg/g as Pb (an amount equivalent to 2.0 g on the dried basis, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic　Not more than 1.5 μg/g as As　(an amount equivalent to 1.0 g on the dried basis, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Water-soluble substance　Not more than 0.50%.

Weigh an amount of Microfibrillated Cellulose equivalent to 4.0 g on the dried basis, add 200 mL of water, and disperse using a high-speed disperser (composed of 4 blades about 13 mm in length and about 16 mm in maximum width) at 5000 rpm for 5 minutes. Filter the dispersed liquid by suction through a 5C filter paper. Evaporate 50 mL of the filtrate to dryness on a water bath, dry the residue at 120°C for 1 hour, and allow to stand in a desiccator.　Weigh the residue accurately.

Loss on Drying　60.0–92% (5 g, 120°C, 5hours).

Ash　Not more than 0.5 % (sample amount: equivalent of 2.0 g on the dried basis).

Microbial Limits　Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid　Prepare as directed in Method 1 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture　Prepare as directed in Method 1, respectively, for the *Escherichia coli* test and *the Salmonella* test.

Milt Protein

しらこたん白抽出物

Definition　Milt Protein is obtained from the testes of the ainame *Hexagrammos otakii* Jordan et Starks, the humpback salmon *Oncorhynchus gorbuscha* (Walbaum), the chum salmon *Oncorhynchus keta* (Walbaum), the sockeye salmon *Oncorhynchus nerka* (Walbaum), the skipjack tuna *Katsuwonus pelamis* (Linnaeus), or the Pacific herring *Clupea pallasii pallasii* Valenciennes, and consists mainly of basic proteins.

Content　Milt Protein, when calculated on the dried basis, contains the equivalent of not less than 50% of protamine.

Description Milt Protein occurs as a white to light yellow powder having a slight characteristic odor.

Identification

(1) Dissolve 1 mg of Milt Protein in 2 mL of water, and add 5 drops of a solution of 0.1 g of 1-naphthol in 100 mL of a solution (7 in 10) of ethanol (95) and 5 drops of sodium hypochlorite TS. When the solution is made alkaline with sodium hydroxide solution (1 in 20), a bright red color develops.

(2) Dissolve 5 mg of Milt Protein in 1 mL of water while warming. To this solution, add 1 drop of sodium hydroxide solution (1 in 10) and 2 drops of a solution of copper(II) sulfate pentahydrate (1 in 7). A blue-purple color develops.

Purity

(1) Clarity of solution Colorless to light yellow and turbid (0.5 g, water 50 mL, 5 minutes of stirring).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 7.0% (100°C, 3 hours).

Ash Not more than 15.0%.

Assay Determine the amount of nitrogen in about 0.1–0.15 g of Milt Protein, accurately weighed, as directed in the Kjeldehl Method under Nitrogen Determination. Calculate the content of protamine by the formula given below.

Each mL of 0.05 mol/L sulfuric acid = 1.401 mg of N

$$\text{Content (\%) of protamine} = \frac{\text{Amount (mg) of nitrogen} \times 3.19}{\text{Dry basis weight (g) of the sample} \times 1000} \times 100$$

Mixed Tocopherols

ミックストコフェロール

Definition Mixed Tocopherols are obtained from vegetable fats or oils and consist mainly of *d*-α-tocopherol, *d*-β-tocopherol, *d*-γ-tocopherol, and *d*-δ-tocopherol. They may contain edible fats or oils.

Content Mixed Tocopherols contain not less than 34% of tocopherols as the total tocopherols.

Description Mixed Tocopherols are light yellow to red-brown, clear viscous liquids having a characteristic odor.

Identification Proceed as directed under Identification for *d*-α-Tocopherol.

Specific Rotation $[\alpha]_D^{20}$: not less than +20°C.

Proceed as directed in Specific Rotation for *d*-α-Tocopherol.

Purity

(1) Acid value　Not more than 5.0.

Proceed as directed in Purity (1) for Tocotrienol.

(2) Lead　Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 10.0 mL, Apparatus B).

(4) Antioxidation value　Not less than 40.

Test Solution　Weigh accurately an amount of the sample equivalent to about 30 mg of total tocopherols, transfer to a 200-mL brown volumetric flask, dissolve it in ethanol (99.5) to make 200 mL. Place 2 mL each of this solution and ethanol (99.5) in a 25-mL brown volumetric flask, add 1 mL of a solution (1 in 500) of iron(III) chloride hexahydrate in ethanol (99.5), immediately add 1 mL of a solution (1 in 200) of2,2′ bipyridyl in ethanol (99.5), shake mildly, and add ethanol (99.5) to make exactly 25 mL.

Control Solution　Place 2 mL of ethanol (99.5) in a 25-mL brown volumetric flask, and then proceed as directed for the test solution.

Procedure　Exactly 10 minutes after the addition of the solution of iron(III) chloride hexahydrate in ethanol (99.5), measure the absorbance (A and A′) of the test solution and the control solution at 520 nm against ethanol (99.5). Calculate the antioxidation value by the formula:

$$\text{Antioxidation value} = \frac{A - A'}{\text{Weight (g) of the sample}} \times 2.82 \times 2$$

Assay　Proceed as directed in the Assay for *d*-α-Tocopherol.

Monascus Color

ベニコウジ色素

Definition　Monascus Color is obtained from the culture fluid of *Monascus pilosus* or *Monascus purpureus* and consists mainly of ankaflavins and monascorubrins.

Color Value　The Color Value ($E_{1cm}^{10\%}$) of Monascus Color is not less than 50 and is in the range of 90–110% of the labeled value.

Description　Monascus Color is a dark red powder, paste, or liquid having a slight, characteristic odor.

Identification

(1) Weigh an amount of Monascus Color equivalent to 1 g of monascus color with a

Color Value 50, and dissolve it in 100 mL of a 1:1 mixture of water/ethanol (95). A red-orange to dark red color develops.

(2) To 1 mL of the solution obtained in Identification (1), add 1 mL of ammonia solution and 1 mL of acetone, and heat at 45–55°C for 1 minute. A yellow-orange color develops. Allow it to stand for 10 minutes. A yellow-green fluorescence is emitted.

(3) To 0.1 mL of the solution obtained in Identification (1), add 3 mL of nitric acid, and shake immediately. A yellow color develops.

(4) A solution of Monascus Color in a 1:1 mixture of water/ethanol (95) exhibits an absorption maximum at a wavelength of 480–520 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Citrinine Not more than 0.2 μg/g (on the basis of a Color Value 50).

Test Solution As a packing material, use styrene–divinylbenzene resin for adsorption or acrylic ester resin for adsorption. Rinse the resin with methanol to replace water, pack a glass column with a 1 cm internal diameter to the height of 10 cm. Check the adsorption resin to confirm that citrinine runs off in the first 20 mL. Weigh accurately the equivalent of about 1 g of Monascus Color with a Color Value 50, and place on the surface of resin-layer in the glass column. Allow a 7:3 mixture of methanol/water to flow through the column at a flow rate of 2–3 mL/minute, and collect the first 20-mL effluent. Filter the effluent through a membrane filter with a pore size of not more than 0.5 μm.

Standard Solutions Weigh accurately 10 mg of citrinine, and dissolve it in methanol to make exactly 100 ml. To exactly 1 mL of this solution, add a 7:3 mixture of methanol/water to make exactly 100 mL. Then transfer exactly 1.0 mL, 5.0 mL, and 10.0 mL of the second solution into separate 100-mL volumetric flasks, and dilute each with a 7:3 mixture of methanol/water to volume.

Procedure Analyze 5 μL potions of the test solution and the standard solutions by liquid chromatography using the conditions below. The procedure should be promptly carried out. Measure the peak areas of citrinine for the standard solutions, and prepare a calibration curve. To determine citrinine in the test solution, correct the peak area as the peak overlapping on the tailing because the peak of citrinine is interfered with tailing of another peak.

Operating Conditions

Detector: Fluorescence spectrophotometer (excitation wavelength 330 nm; fluorescence wavelength 500 nm).

Column: A stainless steel tube (3.9–4.6 mm internal diameter and 25–30 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: Ordinary temperature.

Mobile phase: A 1000:1000:1 mixture of water/acetonitril/trifluoroacetic acid.

Flow rate: 1 mL/minute.

Color Value Determination Proceed as directed under Color Value Determination.

Operating Conditions

Solvent: A 1:1 mixture of water/ethanol (95).

Wavelength: Maximum absorption wavelength of 480–520 nm.

Monascus Yellow

ベニコウジ黄色素

Definition Monascus Yellow is derived from the culture fluid of *Monascus pilosus* or *Monascus purpures* and consists mainly of xanthomonasins.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Monascus Yellow is not less than 70 and is in the range of 90–110% of the labeled value.

Description Monascus Yellow occurs as a yellow to yellow-brown powder, as lumps, or as a paste or liquid. It has a slight, characteristic odor.

Identification

(1) Weigh an amount of Monascus Yellow equivalent to 1 g of monascus yellow with a Color Value 70, and dissolve it in 100 mL of ethanol (95). The resulting solution is yellow emitting green fluorescence.

(2) Weigh an amount of Monascus Yellow equivalent to 1 g of monascus yellow with a Color Value 70, dissolve it in 5 mL of water, add 1 mL of sodium hydroxide solution (1 in 25), and shake. The solution turns red-brown.

(3) Weigh an amount of Monascus Yellow equivalent to 1 g of monascus yellow with a Color Value 70, and dissolve it in 5 mL of water, add 0.1 mL of sulfuric acid, and shake. A yellow to yellow brown turbidity is produced.

(4) A solution of Monascus Yellow in 50% (vol) ethanol exhibits an absorption maximum at a wavelength of 458–468 nm.

(5) Weigh an amount of Monascus Yellow equivalent to 1 g of monascus yellow with a Color Value 70, and dissolve it in 10 mL of ethanol (95). Centrifuge it at 3000 rpm 10 for minutes, and use the supernatant as the test solution. Analyze a 5-μL portion of the test

solution by thin-layer chromatography using a 4:4:2:1 mixture of ethanol (95)/3-methyl-1-butanol/water/ammonia solution. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. A yellow spot with fluorescence is observed at R_f of about 0.8. When irradiated with ultraviolet (366 nm), the spot emits yellow-green fluorescence.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination, using the operating conditions given below.

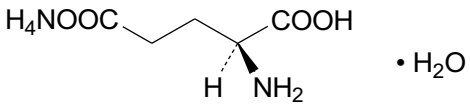
Operating Conditions

Solvent: 50% (vol) ethanol.

Wavelength: A maximum absorption wavelength at 458–468 nm.

Monoammonium L-Glutamate

グルタミン酸アンモニウム



$C_5H_{12}N_2O_4 \cdot H_2O$ Mol. Wt. 182.18

Monoammonium monohydrogen (2*S*)-2-aminopentanedioate monohydrate [139883-82-2]

Content Monoammonium L-Glutamate contains not less than 99.0% of monoammonium L-glutamate ($C_5H_{12}N_2O_4 \cdot H_2O$), when calculated on the dried basis.

Description Monoammonium L-Glutamate occurs as colorless to white crystals or as a white crystalline powder.

Identification

(1) Use a solution of Monoammonium L-Glutamate (1 in 200) as the test solution. Use a solution of monosodium L-glutamate monohydrate (1 in 200) as the control solution. Analyze 1 μL each of the test solution and the control solution by thin-layer chromatography using a 2:1:1 mixture of 1-butanol/water/acetic acid as the developing solution. Use a thin-layer plate coated with silica gel for thin-layer chromatography as

the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front ascends to a point about 10 cm above the starting line, air-dry the plate, and then dry for an additional 30 minutes at 80°C. Spray evenly with ninhydrin solution (1 in 500), and dry at 80°C for 10 minutes to develop color. Examine in daylight. The spot from the test solution is the same as the red-purple spot from the control solution in tone of color and R_f value.

(2) Monoammonium L-Glutamate responds to the test for Ammonium Salt in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +25.4 to +26.4° (10 g, hydrochloric acid (1 in 6), 100 mL, on the dried basis).

pH 6.0–7.0 (1.0 g, water 20 mL).

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Atomic Absorption Spectrophotometry).

(2) Arsenic Not more than 1.9 μg/g as As (0.79 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Pyrrolidone carboxylic acid

Test Solution Dissolve 0.50 g of Monoammonium L-Glutamate, weighed exactly, in 100 mL of water.

Control Solution Dissolve 0.50 g of monosodium L-glutamate monohydrate and 2.5 mg of DL-2-pyrrolidone 5-carboxylic acid in water to make exactly 100 mL. Analyze 2 μL each of the test solution and the control solution by thin-layer chromatography using a 2:1:1 mixture of 1-butanol/water/acetic acid as the developing solution. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and dried at 110°C for 1 hour. Stop the development when the solvent front ascends to a point about 10 cm above the starting line, air-dry the plate, and then dry for an additional 30 minutes at 120°C to remove the solvent.

Place the plate and a 50-mL beaker containing 5 mL of sodium hypochlorite into another developing chamber with the glass surface of the plate facing the beaker. Add gently about 2 mL of hydrochloric acid to the beaker to generate chlorine gas. Cover the developing chamber, and allow to stand for 20 minutes. Put the plate out of the chamber, and allow to stand for 10 minutes. Spray evenly with ethanol (95), and air-dry. Then spray with potassium iodide–starch TS, and examine in daylight. No spot is observed for the test solution at the position of the spot corresponding to pyrrolidone carboxylic acid from the control solution.

Loss on Drying Not more than 0.5% (50°C, 4 hours).

Residue on Ignition Not more than 0.1% (800°C, 15 minutes).

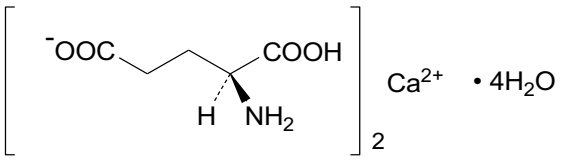
Assay Weigh accurately about 0.15 g of Monoammonium L-Glutamate, and proceed as directed in the Assay for L-Asparagine in the Monographs.

Each mL of 0.1 mol/L perchloric acid = 9.109 mg of $C_5H_{12}N_2O_4 \cdot H_2O$

Monocalcium Di-L-Glutamate

Calcium Diglutamate

L-グルタミン酸カルシウム



$C_{10}H_{16}N_2CaO_8 \cdot 4H_2O$ Mol. Wt. 404.38

Monocalcium bis[monohydrogen (2*S*)-2-aminopentanedioate] tetrahydrate [69704-19-4]

Content Monocalcium Di-L-Glutamate, when calculated on the anhydrous basis, contains 98.0–102.0% of monocalcium di-L-glutamate ($C_{10}H_{16}N_2CaO_8$ = 332.32).

Description Monocalcium Di-L-Glutamate occurs as colorless to white prismatic crystals or as white crystals. It has a characteristic taste.

Identification

(1) To 5 mL of a solution of Monocalcium Di-L-Glutamate (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000) and heat for 3 minutes. A purple color develops.

(2) Monocalcium Di-L-Glutamate responds to all the tests for Calcium Salt in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +27.4 to +29.2° (10 g, diluted hydrochloric acid (1 in 4), 100 mL, on the anhydrous basis).

pH 6.7–7.3 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.10% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 1 μg/g as Pb (4.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Monocalcium Di-L-Glutamate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, and add 30 mL of water. Use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, allow to cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen

citrate (1 in 2), 1mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green

(4) Arsenic Not more than 1.9 μg/g as As (0.79 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Water Content Not more than 19% (0.3 g, Volumetric Titration, Direct Titration).

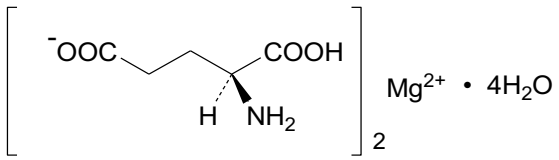
Assay Weigh accurately about 0.2 g of Monocalcium Di-L-Glutamate, dissolve it in 50 mL of water, add about 2 mL of ammonium buffer (pH10.7), and titrate with 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 3 drops of Eriochrome Black T TS) until the color of the solution changes from red to blue. Perform a blank test in the same manner, make any necessary correction, and calculate on the anhydrous basis.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate = 6.646 mg of $C_{10}H_{16}N_2CaO_8$

Monomagnesium Di-L-Glutamate

Magnesium Diglutamate

L-グルタミン酸マグネシウム



$C_{10}H_{16}N_2MgO_8 \cdot 4H_2O$ Mol. Wt. 388.61

Monomagnesium bis[monohydrogen (2*S*)-2-aminopentanedioate] tetrahydrate [129160-51-6]

Content Monomagnesium Di-L-Glutamate, when calculated on the anhydrous basis, contains 95.0–105.0% of monomagnesium di-L-glutamate ($C_{10}H_{16}N_2MgO_8$ = 316.55).

Description Monomagnesium Di-L-Glutamate occurs as colorless to white prismatic crystals or as white crystals. It has a characteristic taste.

Identification

(1) To 5 mL of a solution of Monomagnesium Di-L-Glutamate (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000) and heat for 3 minutes. A purple color develops.

(2) Monomagnesium Di-L-Glutamate responds to the test for Magnesium Salt in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +28.8 to +30.7° (10 g, diluted hydrochloric acid (1 in 4), 100 mL,

on the anhydrous basis).

pH 6.5–7.5 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.10% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 1 μg/g as Pb (4.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Monomagnesium Di-L-Glutamate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(4) Arsenic Not more than 1.9 μg/g as As (0.79 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

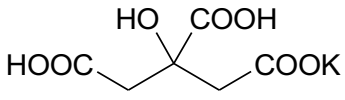
Water Content Not more than 24% (0.2 g, Volumetric Titration, Direct Titration).

Assay Weigh accurately about 0.2 g of Monomagnesium Di-L-Glutamate, dissolve it in 50 mL of water, add about 2 mL of ammonium buffer solution (pH10.7), and titrate with 0.02 mol/L disodum dihydrogen ethylenediaminetetraacetae (indicator: 3 drops of Eriochrome Black T TS) until the color of the solution changes from red to blue. Perform a blank test in the same manner, make any necessary correction, and calculate on the anhydrous basis.

Each mL of 0.02 mol/L disodum dihydrogen ethylenediaminetetraacetae = 6.331 mg of $C_{10}H_{16}N_2MgO_8$

Monopotassium Citrate

クエン酸一カリウム



$C_6H_7KO_7$ Mol. Wt. 230.21

Monopotassium dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate [866-83-1]

Content Monopotassium Citrate, when calculated on the dried basis, contains not less than 99.0% of monopotassium citrate ($C_6H_7KO_7$).

Description Monopotassium Citrate occurs as colorless crystals or crystalline powder. It is odorless.

Identification Monopotassium Citrate responds to all the tests for Potassium Salt and to test (2) for Citrate in the Qualitative Tests.

pH 3.0–4.2 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 20 mL).

(2) Sulfate Not more than 0.024% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.5% (105°C, 3 hours).

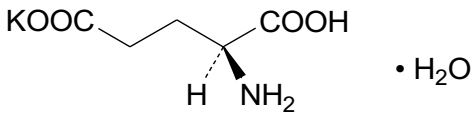
Assay Weigh accurately about 0.4 g of Monopotassium Citrate, add 30 mL of acetic acid for nonaqueous titration, and dissolve it by warming. Cool, and titrate with 0.1 mol/L perchloric acid. Usually, a potentiometer is used to confirm the endpoint. When crystal violet–acetic acid TS (1 mL) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Perform a blank test in the same manner to make any necessary correction, and calculate on the dried basis.

Each mL of 0.1 mol/L perchloric acid = 23.022 mg of $C_6H_7KO_7$

Monopotassium L-Glutamate

Monopotassium Glutamate

L-グルタミン酸カリウム



$C_5H_8NKO_4 \cdot H_2O$ Mol. Wt. 203.23

Monopotassium monohydrogen (2*S*)-2-aminopentanedioate monohydrate [6382-01-0]

Content Monopotassium L-Glutamate, when calculated on the dried basis, contains not less than 99.0% of monopotassium L-glutamate ($C_5H_8NKO_4 \cdot H_2O$).

Description Monopotassium L-Glutamate occurs as colorless to white prismatic crystals or as a white crystalline powder. It is hygroscopic and has a characteristic taste.

Identification

(1) To 5 mL of a solution of Monopotassium L-Glutamate (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000) and heat for 3 minutes. A purple color develops.

(2) Monopotassium L-Glutamate responds to all the tests for Potassium Salt in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +22.5 to +24.0° (10 g, diluted hydrochloric acid (1 in 4), 100 mL, on the dried basis).

pH 6.7–7.3 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.10% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 1 μg/g as Pb (4.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 1.9 μg/g as As (0.79 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

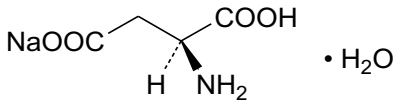
Loss on Drying Not more than 0.5% (80°C, 5 hours).

Assay Weigh accurately about 0.15 g of Monopotassium L-Glutamate, dissolve it in 3 mL of formic acid, add 50 mL of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid. The endpoint is usually confirmed by using a potentiometer. When crystal violet–acetic acid TS (1 mL) is used as the indicator, the endpoint is when the color of the solution changes from brown to green. Perform a blank test in the same manner, make any necessary correction, and calculate on the dried basis.

Each mL of 0.1 mol/L perchloric acid = 10.16 mg of $C_5H_8NKO_4 \cdot H_2O$

Monosodium L-Aspartate

L-アスパラギン酸ナトリウム



$C_4H_6NNaO_4 \cdot H_2O$ Mol. Wt. 173.10

Monosodium (2*S*)-2-aminobutanedioate monohydrate [3792-50-5]

Content Monosodium L-Aspartate, when calculated on the dried basis, contains not less than 98.0% of monosodium L-aspartate ($C_4H_6NNaO_4 \cdot H_2O$).

Description Monosodium L-Aspartate occurs as colorless to white prisms or as a white crystalline powder. It has a characteristic taste.

Identification

(1) To 5 mL of a solution of Monosodium L-Aspartate (1 in 1000), add 1 mL of

ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) Monosodium L-Aspartate responds to all the tests for Sodium Salt in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +18.0 to +21.0° (4 g, hydrochloric acid TS (6 mol/L), 50 mL, on the dried basis).

pH 6.0–7.5 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.041% as Cl (0.30 g, Control Solution: 0.01 mol/L hydrochloric acid 0.35 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (reduced pressure, 5 hours).

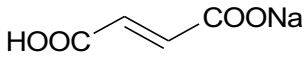
Assay Weigh accurately about 0.1g of Monosodium L-Aspartate, add 3 mL of formic acid and 100 mL of acetic acid, and titrate with 0.1 mol/L perchloric acid. Proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 8.655 mg of $C_4H_6NNaO_4 \cdot H_2O$

Monosodium Fumarate

Sodium Fumarate

フマル酸一ナトリウム



$C_4H_3NaO_4$ Mol. Wt. 138.05

Monosodium monohydrogen (2*E*)-but-2-enedioate [5873-57-4]

Content Monosodium Fumarate, when dried, contains 98.0–102.0% of monosodium fumarate ($C_4H_3NaO_4$).

Description Monosodium Fumarate occurs as a white crystalline powder. It is odorless and has a characteristic acidic taste.

Identification

(1) Proceed as directed in Identification (3) and (4) for Fumaric Acid.

(2) Monosodium Fumarate responds to all the tests for Sodium Salt in the Qualitative

Tests.

pH 3.0–4.0 (1.0 g, water 30 mL).

Purity

(1) Clarity of solution Colorless and clear.

Test Solution Weigh 0.50 g of Monosodium Fumarate, add 10 mL of water, warm to 40°C, and dissolve it by shaking for 10 minutes.

(2) Sulfate Not more than 0.010% as SO_4.

Proceed as directed in Purity (2) for Fumaric Acid.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Monosodium Fumarate, add 10 mL of water, dissolve it by warming, and cool.

Procedure Use 10 mL of tin(II) chloride TS (acidic) and 3 g of zinc for arsenic analysis.

Loss on Drying Not more than 0.5% (120°C, 4 hours).

Residue on Ignition 50.5–52.5% (dried sample).

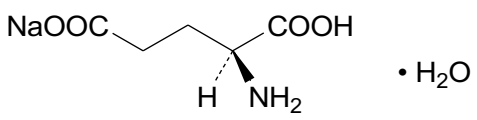
Assay Weigh accurately about 0.3 g of Monosodium Fumarate, previously dried, dissolve it in 30 mL of water, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide = 13.81 mg of $C_4H_3NaO_4$

Monosodium L-Glutamate

Monosodium Glutamate
Soda Glutamate

L-グルタミン酸ナトリウム



$C_5H_8NNaO_4 \cdot H_2O$ Mol. Wt. 187.13

Monosodium monohydrogen (2*S*)-2-aminopentanedioate monohydrate [6106-04-3]

Content Monosodium L-Glutamate, when calculated on the dried basis, contains not less than 99.0% of monosodium L-glutamate ($C_5H_8NNaO_4 \cdot H_2O$).

Description Monosodium L-Glutamate occurs as colorless to white prisms or as a white crystalline powder. It has a characteristic taste.

Identification

(1) To 5 mL of a solution of Monosodium L-Glutamate (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) Monosodium L-Glutamate responds to all the tests for Sodium Salt in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +24.8 to +25.3° (10 g, hydrochloric acid TS (2 mol/L), 100 mL, on the dried basis).

pH 6.7–7.2 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.041% as Cl (0.30 g, Control Solution: 0.01 mol/L sulfuric acid 0.35 mL).

(3) Lead Not more than 1 μg/g as Pb (4.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 1.9 μg/g as As (0.79 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

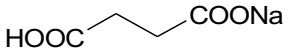
Loss on Drying Not more than 0.5% (97–99°C, 5 hours).

Assay Weigh accurately about 0.15 g of Monosodium L-Glutamate, and proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 9.356 mg of $C_5H_8NNaO_4 \cdot H_2O$

Monosodium Succinate

コハク酸一ナトリウム



$C_4H_5NaO_4$ Mol. Wt. 140.07

Monosodium monohydrogen butanedioate [2922-54-5]

Content Monosodium Succinate contains 98.0–102.0% of monosodium succinate ($C_4H_5NaO_4$).

Description Monosodium Succinate occurs as colorless to white crystals or as a white crystalline powder. It is odorless and has a characteristic taste.

Identification Monosodium Succinate responds to all the tests for Sodium Salt and for Succinate in the Qualitative Tests.

pH 4.3–5.3 (1.0 g, water 20 mL).

Purity

(1) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Readily oxidizable substances Weigh 2.0 g of Monosodium Succinate, dissolve it in 25 mL of water and 25 mL of diluted sulfuric acid (1 in 20), and add 4.0 mL of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 3 minutes.

Residue on Ignition 49.5–51.5%.

Assay Weigh accurately about 0.3 g of Monosodium Succinate, dissolve it in 30 mL of water, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide = 14.01 mg of $C_4H_5NaO_4$

Monostarch Phosphate

リン酸化デンプン

[63100-01-6]

Definition Monostarch Phosphate is obtained by esterifying starch with orthophosphoric acid, its sodium or potassium salt, or sodium tripolyphosphate.

Description Monostarch Phosphate occurs as a white to off-white powder, or as flakes or granules. It is odorless.

Identification

(1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

Purity

(1) Phosphorous Not more than 0.5% as P.

Proceed as directed in Purity (3) for Acetylated Distrach Phosphate.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Sulfur dioxide　Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying　Not more than 21.0% (not more than 13.3 kPa, 120°C, 4 hours).

Morpholine Salts of Fatty Acids

モルホリン脂肪酸塩

Description　Morpholine Salts of Fatty Acids occur as light yellow to yellow-brown waxy or oily substances.

Identification

(1) To 2 g of the sample, add 10 mL of diluted hydrochloric acid (3 in 5), heat in a water bath for 10 minutes with occasional stirring, and allow to cool. Remove the oily or solid portions deposited, and make the resulting solution alkaline with sodium hydroxide solution (1 in 25). Prepare a metanolic solution (1 in 3) of this solution, and use as the test solution.　Separately, prepare a solution (1 in 200) of morpholine in methanol, and use as the standard solution.

Analyze 1.0 μL each of the test solution and the standard solution by gas chromatography using the conditions below. The retention time of the main peak from the test solution corresponds to that of the peak of morpholine from the standard solution.

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of a mixture of 5% diphenyl/95% dimethylpolysiloxane.

Column temperature: Maintain the temparture at 50°C for 1 minute, and raise at a rate of 10°C/minute to 250°C and then at a rate of 5°C/minute to 325°C.

Carrier gas: Nitrogen.

Flow rate: A constant rate of about 1.2 mL/minute.

(2) Dissolve 1 g of the sample in 2 mL of ethanol (95) while heating, add 5 mL of diluted sulfuric acid (1 in 20), heat in a water bath for 30 minutes, and cool. Oil drops or white to yellow-white solids are deposited.

Collect the oil drops or solids by separation, add 5 mL of diethyl ether, and shake. The oil drops or solids dissolve.

Purity

(1) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic　Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard

Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of the sample, add 5 mL of diluted sulfuric acid (1 in 20), heat in a water bath for 30 minutes, and cool. Remove the deposited fatty acid by extraction with diethyl ether, and heat the resulting solution on a water bath to remove the diethyl ether.

Residue on Ignition Not more than 1.0%.

Muramidase

ムラミダーゼ

Definition Muramidase includes enzymes that hydrolyze mucopolysaccharides. It is derived from the culture of actinomycetes (limited to species of the genera *Actinomyces* and *Streptomyces*) or bacteria (limited to species of the genus *Bacillus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Muramidase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Muramidase complies with the Muramidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Preincubation Fluids Prepare as directed in Method 3 and Method 2, respectively, for the *Escherichia coli* and *Salmonella* tests.

Muramidase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 1.0 g of Muramidase, add water or phosphate buffer (1/15 mol/L) at pH 6.2 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to

the resulting solution.

Substrate Solution Uniformly disperse or suspend 30 mg of dried bacterial cells in phosphate buffer (1/15 mol/L) at pH 6.2 so that the absorbance of the suspension or dispersion at a wavelength of 640 nm is 1.2–1.4. In addition, the substrate solution for lysozyme can be also used as the substrate solution. Prepare fresh before use. Cool by icy, and use within 30 minutes.

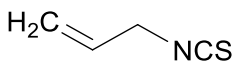
Test Solution Equilibrate 3.8 mL of the substrate solution at 35°C for 3 minutes, add 0.2 mL of the sample solution, and shake.

Control Solution Proceed as directed for the test solution using 0.2 mL of water or phosphate buffer (1/15 mol/L) at pH6.2 instead of the sample solution.

Procedure After preparation, transfer the test solution into a quartz cell immediately, and warm at 35°C. Measure the absorbance at a wavelength of 640 nm both at 3 minutes and at 10 minutes after the addition of the sample solution. Similarly, transfer the control solution into a quartz cell, and proceed as directed for the test solution. Measure the absorbance values at 3 minutes and at 10 minutes after the addition of the sample solution. The difference of the values at 10 minutes between both solutions is smaller than that of the values at 3 minutes between both solutions.

Mustard Extract

カラシ抽出物



C_4H_5NS Mol. Wt. 99.16

Allyl isothiocyanate [57-06-7]

Definition Mustard Extract is obtained from the seeds of *Brassica juncea* (L.) Czern. It consists mainly of allyl isothiocyanate.

Content Mustard Extract contains not less than 93.0% of allyl isothiocyanate (C_4H_5NS).

Description Mustard Extract occurs as a colorless to light yellow, transparent liquid having a strong, pungent odor like mustard.

Identification

Test Solution To 0.15 g of Mustard Extract, add 20 mL of cyclohexane.

Standard Solutions Separately weigh 0.15 g of allyl isothiocyanate for assay, *sec*-butyl isothiocyanate, and 3-butenyl isothiocyanate, and add 20 mL of cyclohexane to each of them. Designate them as Standard Solutions A, B, and C, respectively.

Procedure Analyze 0.5 μL each of the test solution and Standard Solution A by gas chromatography using the operating conditions specified in Assay. Inject the solutions

at a column temperature of 80°C, and raise the temperature at a rate of 4°C/minutes to 250°C. The retention time of the main peak of the test solution corresponds to that of Standard Solution A. Similarly, analyze 0.5 μL each of the test solution and Standard Solutions B and C by gas chromatography. The test solution gives two separate peaks whose retention times correspond to those of Standard Solutions B and C, respectively.

Purity

(1) Lead Not more than 2.0 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 4, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay

Test Solution Weigh accurately about 0.15 g of Mustard Extract, add exactly 10 mL of internal standard solution, and add cyclohexane to make exactly 20 mL.

Internal Standard Solution Use a solution (1 in 100) of decane in cyclohexane.

Standard Solution To about 0.15 g of allyl isothiocyanate for assay, accurately weighed, add exactly 10 mL of internal standard solution and cyclohexane to make exactly 20 mL.

Procedure Analyze 1 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Measure the peak area ratios (Q_T and Q_S) of allyl isothiocyanate to decane for the test solution and the standard solution, respectively, and calculate the content of allyl isothiocyanate by the formula:

$$\text{Content (\%) of allyl isothiocyanate} = \frac{\text{Weight (g) of allyl isothiocyanate for assay}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 60 m length) coated with a 0.25-μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Inject at 80°C, and raise the temperature at a rate of 4°C/minutes to 180°C.

Injection port temperature: 100°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: Adjust the retention time of allyl isothiocyanate to 7–8 minutes.

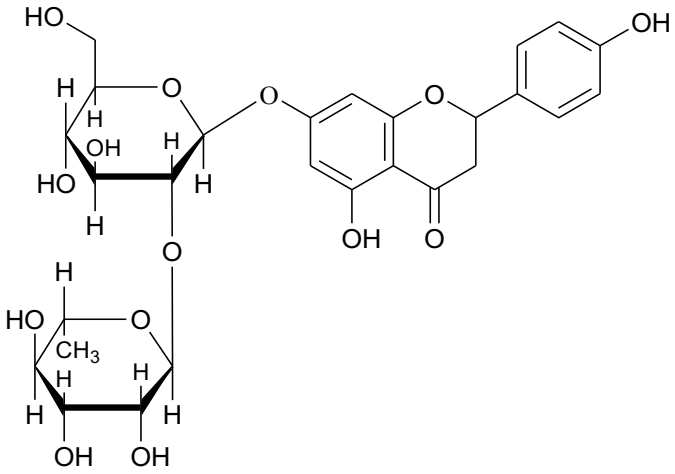
Injection method: Split.

Split ratio: 1:50

Measurement time: 30 minutes.

Naringin

ナリンジン



$C_{27}H_{32}O_{14}$ Mol. Wt. 580.53

5-Hydroxy-2-(4-hydroxyphenyl)-4-oxochroman-7-yl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside [10236-47-2]

Definition Naringin is obtained from the peels, juice, or seeds of the grapefruit *Citrus paradisi* Macfad. by extraction and separation with water, ethanol (95), or methanol. It consists of naringin ($C_{27}H_{32}O_{14}$).

Content Naringin, when dried, contains 90–110% of naringin ($C_{27}H_{32}O_{14}$ = 580.53).

Description Naringin occurs as white to pale yellow crystals.

Identification

(1) Dissolve 5 mg of Naringin in 10 mL of 50% (vol) ethanol, and add 1 to 2 drops of a solution of iron(III) chloride hexahydrate (1 in 500). A brown color develops.

(2) Dissolve 5 mg of Naringin in 5 mL of sodium hydroxide TS (1 mol/L). A yellow to orange color develops.

(3) Dissolve 10 mg of Naringin in 500 mL of water. The solution has a bitter taste, and exhibits an absorption maximum at a wavelength of 280–285 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead

Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Methanol Not more than 50 μg/g.

(i) Apparatus Use the apparatus specified in Purify (3) for Enju Extract in the Monographs.

(ii) Method

Test Solution Weigh accurately about 5 g of Naringin into eggplant-shaped flask A, add 100 mL of water, a few boiling chips, and 3–4 drops of silicon resin, and stir well. Place exactly 2 mL of the internal standard solution in volumetric flask E, and assemble the apparatus. Moisten the joint parts with water. Distill at a rate of 2 to 3 mL/minute while adjusting so that bubbles do not enter into delivery tube C, and collect about 45 mL of the distillate. To the distillate, add water to make exactly 50 mL. Use 2-methyl-2-propanol solution (1 in 1000) as the internal standard solution.

Standard Solution Weigh accurately about 0.5 g of methanol, and add water to make exactly 100 mL. Measure exactly 5 mL of this solution, and add water to make exactly 100 mL. Next, measure exactly 2 mL of the second solution and 4 mL of the internal standard solution into a 100-mL volumetric flask, and add water exactly to volume.

Procedure Analyze 2.0 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratios (Q_T and Q_S) of methanol to 2-methyl-2-propanol for the test solution and the standard solution, and calculate the methanol amount by the formula:

$$\text{Amount (μg/g)} = \frac{\text{Weight (g) of methanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 500$$

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube (3 mm internal diameter and 2 m length).

Column packing material: 180- to 250-μm styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Injection port temperature: A constant temperature of about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention time of methanol to about 2 minutes.

Loss on Drying Not more than 10 % (105°C, 3 hours).

Assay Weigh accurately about 0.2 g of Naringin, previously dried at 105°C for 3 hours, and dissolve it in 50% (vol) ethanol to make exactly 100 mL. Filter this solution through a membrane filter (0.45 μm pore size), measure exactly 1 mL of the filtrate, and add water to make exactly 100 mL. Measure the absorbance (A) of this solution at a wavelength of 280 nm, using water as the control. Calculate the content by the formula:

$$\text{Content (\%) of naringin } (C_{27}H_{32}O_{14}) = \frac{A}{28.0} \times \frac{10}{\text{Weight (g) of the sample}} \times 100$$

Naringinase

ナリンジナーゼ

Definition Naringinase includes enzymes that degrade naringin. It is derived from the culture of filamentous fungi (limited to *Aspergillus usamii* and *Penicillium decumbens*).

It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Naringinase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Naringinase complies with the Naringinase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Naringinase Activity Test Perform the test using the method given below. If the

identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 0.50 g of Naringinase, add water to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 0.125 g of naringin *n*-hydrate, and dissolve it by adding 25 mL of water and 12.5 mL of sodium hydroxide TS (1 mol/L). Add 37.5 mL of McIlvain buffer at pH 3.5, adjust the pH of the solution to 3.5 with hydrochloric acid TS (1 mol/L), and add McIlvain buffer to make 100 mL. Use immediately after preparation.

Test Solution Equilibrate 4 mL of the substrate solution at 40°C for 10–15 minutes, add 1 mL of the sample solution, and shake. Incubate the mixture at 40°C for 30 minutes, and add 5 mL of Somogyi TS (II), and heat in a water bath for 20 minutes. After cooling, add 1.5 mL of potassium iodide solution (1 in 200) and 3 mL of sulfuric acid TS (1 mol/L), and shake well.

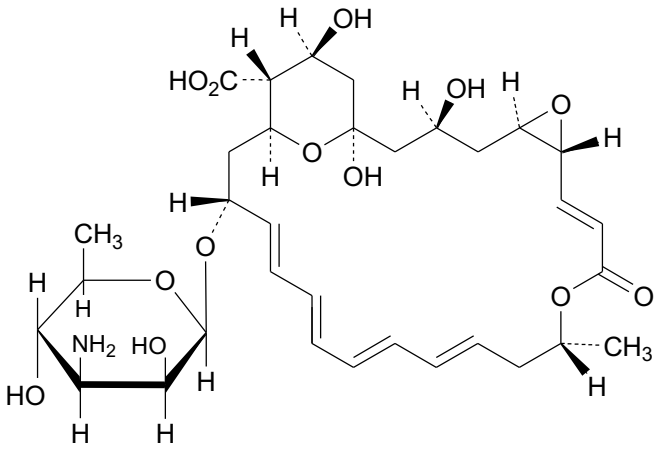
Control Solution Proceed as directed for the test solution using 1 mL of water, instead of the sample solution.

Procedure Titrate the test solution and the control solution with 0.01 mol/L sodium thiosulfate (indicator: 3 drops of soluble starch). The amount of 0.01 mol/L sodium thiosulfate consumed by the test solution is less than that of 0.01 mol/L sodium thiosulfate consumed by the control solution. The endpoint is when the blue color of the solution disappears. If a red precipitate of copper oxide forms even when diluted sample solution is used and the titration with 0.01 mol/L sodium thiosulfate is impossible, treat the sample solution by dialysis or ultrafiltration.

Natamycin

Pimaricin

ナタマイシン



$C_{33}H_{47}NO_{13}$ Mol. Wt. 665.73

(1*R**,3*S**,5*R**,7*R**,8*E*,12*R**,14*E*,16*E*,18*E*,20*E*,22*R**,24*S**,25*R**,26*S**)-22-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid [7681-93-8]

Content Natamycin, when calculated on the anhydrous basis, contains not less than 95.0% of natamycin ($C_{33}H_{47}NO_{13}$).

Description Natamycin occurs as a white to creamy-white crystalline powder.

Identification

(1) To 1 mg of Natamycin, add 1 mL of hydrochloric acid, and shake. A blue-purple color develops.

(2) Dissolve 5 mg of Natamycin in 1000 mL of a solution (1 in 1000) of acetic acid in methanol. The solution exhibits absorption maximum at wavelengths of approximately 290, 303, and 318 nm.

(3) Determine the infrared absorption spectrum of Natamycin as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Rotation $[\alpha]_D^{20}$: +250 to +295° (1 g, acetic acid, 100 mL, on the anhydrous basis).

pH 5.0–7.5 (1% suspension).

Purity Lead Not more than 2 μg/g (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Water Content 6.0–9.0% (30 mg, Coulometric Titration).

Residue on Ignition Not more than 0.5%.

Assay Throughout this assay, all procedures should be protected from direct light and the apparatus used should be light-resistant.

Test Solution and Standard Solution Weigh accurately about 20 mg each of Natamycin and Natamycin Reference Standard (the water content should be previously determined in the same manner as for the sample). Add 5 mL of tetrahydrofuran to each, sonicate for 10 minutes, and add 60 mL of methanol to dissolve. Then add 25 mL of water, and allow to cool to room temperature. Add water to make two solutions of exactly 100 mL each. Use them as the test solution and the standard solution, respectively.

Procedure Analyze 20 μL each of both solutions by liquid chromatography using the operation conditions given below. Measure the peak areas (A_T and A_S) of natamycin for the test solution and the standards solution, and calculate on the anhydrous basis, and then calculate the natamycin content in the sample by the formula:

Content (%) of natamycin ($C_{33}H_{47}NO_{13}$)

$$= \frac{\text{Anhydrous basis weight (g) of Natamycin and Natamycin Reference Standard}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 303 nm).

Column: A stainless steel (4.6 mm internal diameter and 25 cm length).

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: Room temperature.

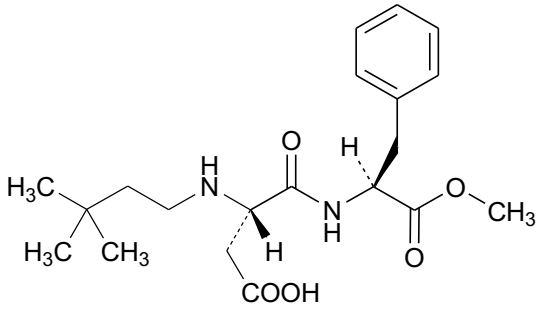
Mobile phase: Use the mobile phase prepared by dissolving 3.0 g of ammonium acetate and 1.0 g of ammonium chloride in 760 mL of water, and adding 5.0 mL of tetrahydrofuran and 240 mL of acetonitrile.

Flow rate: 2 mL/minute.

Storage standard Store in a light-resistant bottle in a cold place.

Neotame

ネオテーム



$C_{20}H_{30}N_2O_5$ Mol. Wt. 378.46

Methyl *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalaninate [165450-17-9]

Content Neotame contains 97.0–102.0% of neotame ($C_{20}H_{30}N_2O_5$), when calculated on the anhydrous basis.

Description Neotame occurs as a white or off-white powder.

Identification Determine the absorption spectrum of Neotame, as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Rotation $[\alpha]_D^{20}$: −40.0° to −43.4° (0.25 g, water, 50 mL, on the anhydrous basis).

pH 5.0–7.0 (1.0g, water 200 mL).

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine Not more than 1.5%.

Test Solution Use Solution A prepared in the Assay.

Standard Solutions Weigh accurately about 30 mg of *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine (the water content should be determined previously in the same manner as for the sample), and dissolve it in the mobile phase prepared as directed in the operating conditions in the Assay to make exactly 50 mL. To 10 mL of the solution obtained, measured exactly, add the mobile phase to make exactly 100 mL. Use the resulting solution as the standard stock solution. Place exactly 2 mL, 10 mL, 25 mL, and 50 mL of the standard stock solution in separate 100-mL volumetric flasks, and dilute each to volume with the mobile phase.

Procedure Analyze 25 μL each of the test solution, the standard solutions, and the standard stock solution by liquid chromatography using the operation conditions below. Prepare a calibration curve from the peak areas of *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine in the standard solutions and standard stock solution. Obtain the concentration (M mg/mL) of *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine in the test solution from the calibration curve and the peak area of *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine for the test solution. Determine the amount of *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine from the formula:

$$\text{Amount (\%) of } N\text{-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine} = \frac{\text{M}}{\text{Anhydrous basis weight (g) of the sample}}$$

Operating conditions

Use the conditions given in the Assay except for the flow rate. Adjust the flow rate so that the retention time of *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine is about 4 minutes.

(4) Other impurities Not more than 2.0%.

Test Solution Use Solution A prepared in the Assay.

Standard Solution Use the standard solution prepared in the Assay.

Procedure Analyze 25 μL each of the test solution and the standard solution by liquid chromatography. Continue the chromatography for 1.5 times the retention time of

neotame. Measure the sum (A_{sum}) of the peak areas of all peaks other than the peaks of neotame, *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine, and the solvent in the test solution. Then, measure the peak area (A_S) of neotame for the standard solution. Determine the amount of other impurities by the formula:

$$\text{Amount (\%) of other impurities} = \frac{\text{Anhydrous basis weight (g) neotame for assay}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_{sum}}{A_S}$$

Operating conditions

Use the conditions given in the Assay.

Water Content Not more than 5.0% (0.25 g, Volumetric Titration, Direct Titration).

Residue on ignition Not more than 0.2% (1 g, 800°C, 1 hour).

Assay

Test Solution Weigh accurately about 0.1 g of Neotame, and dissolve it in the mobile phase directed in the operating conditions to make exactly 50 mL. Refer to the resulting solution as Solution A. To 25 mL of Solution A, measured exactly, add the mobile phase solution to make exactly 50 mL.

Standard Solution Dissolve about 50 mg of neotame for assay (the water content should be determined previously in the same manner as for the sample), accurately weighed, in the mobile phase to make exactly 50 mL.

Procedure Analyze 25 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions below, and measure the peak areas (A_T and A_S) of neotame for both solutions. Determine the neotame content by the formula:

$$\text{Content (\%) of neotame } (C_{20}H_{30}N_2O_5) = \frac{\text{Anhydrous basis weight (g) neotame for assay}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T}{A_S} \times 200$$

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 210 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 10 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of about 45°C.

Mobile phase: Dissolve 3.0 g of sodium 1-heptanesulfonate in 740 mL of water, add 3.8 mL of triethylamine, adjust the pH to 3.5 with phosphoric acid, and add water to make 750 mL. To this solution, add 250 mL of acetonitrile, and adjust

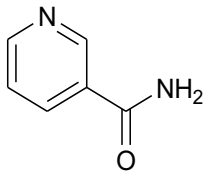
the pH to 3.7 with phosphoric acid.

Flow rate: Adjust the retention time of neotame to about 12 minutes.

Nicotinamide

Niacinamide

ニコチン酸アミド



$C_6H_6N_2O$ Mol. Wt. 122.12

Pyridine-3-carboxamide [98-92-0]

Content Nicotinamide, when calculated on the dried basis, contains not less than 98.5% of nicotinamide ($C_6H_6N_2O$).

Description Nicotinamide occurs as a white crystalline powder. It is odorless and has a bitter taste.

Identification

(1) Proceed as directed in Identification (1) for Nicotinic Acid.

(2) To 20 mg of Nicotinamide, add 5 mL of sodium hydroxide solution (1 in 25), and boil gently. An odor of ammonia is evolved.

pH 6.0–7.5.

Measure the pH of a solution prepared by dissolving 1.0 g of Nicotinamide in water to make 20 mL.

Melting Point 128–131°C.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Readily carbonizable substances Perform the test for 0.20 g of Nicotinamide, using Matching Fluid A.

Loss on Drying Not more than 0.5% (4 hours).

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.2 g of Nicotinamide, dissolve it in 30 mL of acetic acid, and titrate with 0.1 mol/L perchloric acid (indicator: 1 mL of crystal violet–acetic acid

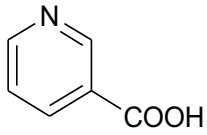
TS) until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, make any necessary correction, and calculate on the dried basis.

Each mL of 0.1 mol/L perchloric acid = 12.21 mg of $C_6H_6N_2O$

Nicotinic Acid

Niacin

ニコチン酸



$C_6H_5NO_2$ Mol. Wt. 123.11

Pyridine-3-carboxylic acid [59-67-6]

Content Nicotinic Acid, when calculated on the dried basis, contains not less than 99.5% of nicotinic acid ($C_6H_5NO_2$).

Description Nicotinic Acid occurs as white crystals or crystalline powder. It is odorless and has a slightly acidic taste.

Identification

(1) To 5 mg of Nicotinic Acid, add 10 mg of 1-chloro-2,4-dinitrobenzene, mix, and fuse by heating for several seconds. Cool, and add 4 mL of 3.5% (w/v) potassium hydroxide–ethanol TS. A dark purple color develops.

(2) To 20 mL of a solution of Nicotinic Acid (1 in 400), add sodium hydroxide solution (1 in 250) to neutralize it, and add 3 mL of a solution of copper(II) sulfate pentahydrate (1 in 8). A blue precipitate is gradually formed.

Melting Point 234–238°C.

Purity

(1) Chloride Not more than 0.021% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(2) Sulfate Not more than 0.019% as SO_4 (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.20 mL).

(3) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

Loss on Drying Not more than 1.0% (105°C, 1 hour).

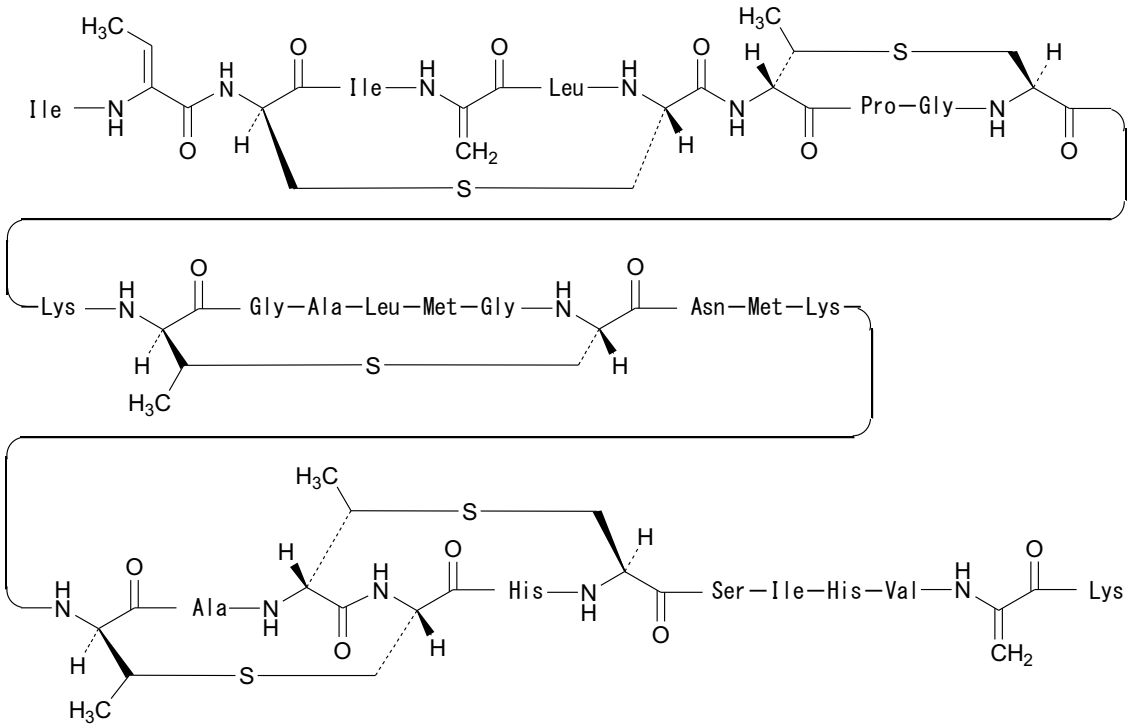
Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.3 g of Nicotinic Acid, dissolve it in 50 mL of water, and titrate with 0.1 mol/L sodium hydroxide (indicator: 5 drops of phenolphthalein TS). Calculate on the dried basis.

Each mL of 0.1 mol/L sodium hydroxide = 12.31 mg of $C_6H_5NO_2$

Nisin

ナイシン



$C_{143}H_{230}N_{42}O_{37}S_7$ Mol. Wt. 3354.07

[1414-45-5]

Definition A mixture of sodium chloride and antimicrobial polypeptides obtained from a culture fluid of *Lactococcus lactis* subsp. *lactis*. It contains components derived from nonfat milk medium or sugar medium. The main antimicrobial polypeptide is nisin A ($C_{143}H_{230}N_{42}O_{37}S_7$).

Activity and content Nisin has an activity of not less than 900 units per milligram. One unit of activity is equivalent to 0.025 μg of antimicrobial polypeptides including nisin A ($C_{143}H_{230}N_{42}O_{37}S_7$). The product contains not less than 50% of sodium chloride.

Description Nisin occurs as a white to pale yellow-red powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Suspend 0.100 g of Nisin, exactly weighed, in 80 mL of diluted hydrochloric acid

(1 in 600). Allow to stand for 2 hours at room temperature, and add a diluted hydrochloric acid (1 in 600) to make exactly 100 mL. Use the resulting sample solution for tests (i) and (ii).

(i) Heat the sample solution in a water bath for 5 minutes. To exactly 1 mL of the heated sample solution, add hydrochloric acid (1 in 600) to make exactly 200 mL. Use the resulting solution as the test solution. Determine the activity of the test solution as directed under the Assay. It is 100 ± 5% of that of the test solution prepared in the Assay.

(ii) Adjust the pH of the remaining sample solution to 11 by adding sodium hydroxide solution (1 in 5), and heat at 65°C for 30 minutes. After cooling, adjust the pH to 2.0 by adding hydrochloric acid, and dilute 1 mL of this solution with diluted hydrochloric acid (1 in 600) to 200 mL. Use the resulting solution as the test solution. Determine the activity as directed under the Assay. Complete loss of the antimicrobial activity of Nisin is observed.

(2) Prepare a test culture by incubating *Lactococcus lactis* (ATCC 11454 or NCIMB 8586) in a 1 in 10 suspension of sterile powdered skim milk at 30°C for 18 hours. Sterilize a flask containing 100 mL of litmus milk in an autoclave at 121°C for 15 minutes, add 0.1 g of Nisin, and allow to stand for 2 hours at room temperature. To the resulting solution, add 0.1 mL of the test culture, and incubate at 30°C for 24 hours. The growth of *Lactococcus lactis* is observed.

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 3.0% (105°C, 2 hours).

Microbial Limit Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 100 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Individual tests should be done according to the following procedures.

Total Plate Count Use the Membrane Filter Method. Prepare a sample fluid by mixing 1 g of Nisin with sodium chloride–peptone buffer to make 1000 mL. Filter 100 mL of the sample solution through a cellulose ester membrane filter, and wash the filter with sodium chloride–peptone buffer. Place the filter on soybean-casein digest agar medium, and incubate for at least 5 days at 30–35°C.

Escerichia coli

Confirmation test for *Escherichia coli*—To 1 g of Nisin, add lactose broth to make 100 mL, and incubate the mixture at 30–35°C for 24–72 hours. When microbial growth is observed, shake the culture fluid lightly, inoculate an appropriate portion of the fluid on

MacConkey agar medium using a platinum loop, and incubate at 30–35ºC for 18–24 hours. Examine the plate for suspicious colonies. If red-brick colonies of Gram-negative rod-shaped bacteria surrounded by a reddish precipitation zone are not found, the sample is determine to be *E. coli* negative. If colonies with these characteristics are found, then transfer the suspect colonies individually onto the surface of EMB agar medium and incubate at 30–35°C for 18–24 hours. Upon examination, if no colonies exhibit a metallic sheen or a blue-black color under transmitted light, the sample is determined to be negative. Confirm suspect colonies on the plate by conducting the IMViC tests (Indole production test, Methyl red reaction test, Voges-Proskauer test, and Citrate utilization test) and the growth test at 44.5°C. When the colonies exhibit the pattern [+ + – –] for these four IMViC tests (in the order listed above) and a positive response for the growth test, they are determined to be *Escherichia coli*. Rapid detection kits for *Escherichia coli* may be used. If uncertain or vague results are obtained, conduct the test again using an amount of the sample equivalent to 2.5-times the amount specified. In this case, increase the amount of medium in proportion to the mount of the sample used.

Effectiveness of culture media (media growth promotion test)—Mix lactose broth and 0.1 mL of the test suspension, instead of Nisin, prepared as directed in (1) of "Effectiveness of Culture Media and Method Suitability" under "3. Tests for Coliforms and *Escherichia coli*" in the Microbial Limit Tests in the General Tests. Proceed as directed in the procedure for the *Escerichia coli* test above. Incubation should be done for the shortest time specified.

Medium

MacConkey agar medium

Peptone (gelatin)	17.0 g
Peptone (casein)	1.5 g
Peptone (meat)	1.5 g
Lactose	10.0 g
Sodium de(s)oxycholate	1.5 g
Sodium chloride	5.0 g
Agar	13.5 g
Neutral red	30 mg
Crystal violet	1.0 mg
Water	1000 mL

Mix all the ingredients, boil for 1 minute, and autoclave at 121°C for15–20 minutes. The pH should be 6.9–7.3 after sterilization

Salmonella

Confirmation of *Salmonella* colonies—To 10 g of Nisin, add soybean-casein digest agar medium to make 500 mL, and incubate at 30–35ºC for 24–72 hours. When microbial growth is observed, shake the culture fluid lightly, and inoculate 1-mL portions

separately in 10 mL each of fluid tetrathionate medium and fluid rappaport medium. Incubate them at 30–35ºC for 18–24 hours. Inoculate appropriate portions from both fluid media onto each of brilliant green agar medium and XLD agar medium, and incubate at 30–35ºC for 42–48 hours. *Salmonella* cultures typically produce small, transparent, colorless colonies or opaque, white to pink colonies on brilliant green medium, or red colonies on XLD agar medium. Colonies on brilliant green agar medium are frequently surrounded by a pink to red zone, and colonies on XLD agar medium have dark centers. If typical colonies are not present, *salmonella* is determined to be absent. If colonies of Gram-negative rods with the typical characteristics are observed, inoculate suspect colonies into TSI slant agar medium by streaking slant and stabbing butt by means of a platinum inoculating needle, and incubate for 18–24 hours at 35–37ºC. In the presence of *salmonella*, the color changes from red to yellow deep in the medium, and the slant surface remains unchanged (red). Usually, there is a formation of gas with or without the production of hydrogen sulfide, deep in the medium. It is desirable to conduct biochemical and serological tests (including use of an identification kit) for definitive identification and serotyping. If uncertain or vague results are obtained, conduct the test again using an amount of the sample equivalent to 2.5-times the amount specified. In this case, increase the amount of medium in proportion to the mount of the sample used.

Effectiveness of culture media (media growth promotion test)—Mix soybean-casein digest agar medium and 0.1 mL of the test suspension, instead of Nisin, prepared as directed in (1) of "Effectiveness of Culture Media and Method Suitability" under "4. *Salmonella*" in the Microbial Limit Tests. Proceed as directed in the procedure for the *Escerichia coli* test above. Incubation should be done for the shortest time specified.

Meida

(i) Rappaport liquid medium

Peptone (soybean)	5.0 g
Potassium dihydrogen phosphate	1.6 g
Sodium chloride	8.0 g
Malachite green oxalate	0.12 g
Magnesium chloride hexahydrate	40.0 g
Water	1000 mL

Dissolve malachite green oxalate, magnesium chloride hexahydrate, and the remaining three solid ingredients separately in water, and autoclave them at 121ºC for 15–20 minutes, and mix. The pH should be 5.4–5.8.

(ii) Brilliant green agar medium

Peptone (meat or casein)	10.0 g
Yeast extract	3.0 g
Lactose	10.0 g
Sucralose	10.0 g

Sodium chloride	5.0 g
Phenol red	80 mg
Brilliant green	12.5 mg
Agar	20.0 g
Water	1000 mL

Mix the ingredients, and boil for 1 minute. Immediately before use, autoclave at 121°C for 15–20 minutes. The pH should be 6.7–7.1 after sterilization. Cool to about 50°C, and dispense to Petri dishes.

Assay

(1) Activity

Antimicrobial activity is determined based on the index "the size of growth inhibition zone of test organism" that is obtained on perforated agar plates. As necessary, sterilize water, regents, solutions, and other equipment used for the test.

(i) Test Organism Use *Micrococcus luteus* (ATCC 10240, NCIMB 8166)

(ii) Media Adjust the pH of each medium using sodium hydroxide TS (1 mol/L) or diluted hydrochloric acid (1 in 10) so that it becomes the specified value after sterilization. Sterilization should be done by high-pressure steam. Media other than those given below may be used as well if they consist of similar ingredients to the specified media and they are comparable or superior in efficiency of microbial growth to the specified media.

a. Assay medium

Tryptone	10 g
Meat extract	3 g
Sodium chloride	3 g
Yeast extract	1.5 g
Sucrose	1 g
Agar	15 g
Water	1000 mL

Mix the ingredients, and sterilize at 121°C for 15 minutes. The pH must be 7.4–7.6 after sterilization. Add 2 mL of a 50% solution of polysorbate 20 at the same temperature as the medium.

b. Slant agar medium (for test organism incubation)

Brain heart infusion agar	52 g
Water	1000 mL

Mix the ingredients, and sterilize at 121°C for 15 minutes. The pH must be 7.2–7.6 after sterilization. Dispense 9 mL potions into test tubes (16 mm in internal diameter) and prepare slant medium.

(iii) Assay Organism Suspension Incubate the test organism at 30°C for 48 hours on slant agar medium prepared in (ii) Section b above. Suspend the incubated organism in 7 mL of sterilized physiological saline. The inoculated slant medium can be stored for a maximum of 14 days at 4°C.

(iv) *Inoculated Assay Medium* To 100 mL of assay medium, held at 48–51°C, add 2 mL of a 1 in 10 dilute solution of the assay organism suspension in physiological saline, and mix well.

(v) *Perforated Agar Plate* Pour about 20-mL portions of the inoculated assay medium into Petri dishes (90 mm inter diameter, 20 mm height). Spread the medium evenly over the surface, and allow to solidify at room temperature. Use them as the inoculated agar plates. Prepare stainless-steel cylinders (7.9–8.1 mm external diameter, 5.9–6.1 mm internal diameter, 9.9–10.1 mm height) that do not interfere with the test. Place 4 cylinders in regular intervals on a circle with the radius of about 25- to 28-mm on each agar plate so that the inter-central distance between cylinders is 30 mm or more. Dispense 20 mL of the inoculated assay medium on the plate with the cylinders, solidify, and allow to stand at 4°C for 30–60 minutes. Remove the cylinders gently by means of appropriate sterilized equipment, such as tweezers. Prepare the perforated agar plate fresh before use.

(vi) *Nisin Standard Solutions* Suspend about 0.1 g of Nisin Reference Standard, exactly weighed, in 80 mL of diluted hydrochloric acid (1 in 600), and allow to stand for 2 hours at room temperature. Add diluted hydrochloric acid (1 in 600) to make 100 mL. Use the resulting solution as the standard stock solution. Before use, dilute the standard stock solution with diluted hydrochloric acid (1 in 600) to obtain 5 nisin standard solutions with concentrations of 1.25, 2.5, 5, 10, and 20 (IU/mL).

(vii) *Standard Curve* Use perforated agar plates in quintuplicate for each concentration. Transfer 0.2-mL portions from each standard solution into the holes. Cover the plates, and incubate them at 30°C for 18 hours. Measure the diameters of inhibition zones to the nearest 0.1 mm by means of a caliper or other appropriate device. Plot the values obtained on a graph, with the log of nisin concentration (x IU/mL) on the abscissa axis and the zone diameters (y mm) on the ordinate axis, to prepare the standard curve ($y = \alpha \log x + \beta$). Determine the constants α and β.

(viii) *Test Solution* Suspend 0.100 g of Nisin in 80 mL of diluted hydrochloric acid (1 in 600), and allow to stand at room temperature for 2 hours. Add diluted hydrochloric acid (1 in 600) to make exactly 100 mL. To exactly 1 mL of this solution, add diluted hydrochloric acid (1 in 600) to make exactly 200 mL. Use this solution as the test solution. The test solution should be prepared fresh before use.

(ix) *Determination of Activity* Measure the diameters of inhibition zones as directed for the preparation of the calibration curve. From the formulae, determine the activity (IU/mg).

$$I = (\text{Diameter (mm) of inhibition zone} - \beta)/\alpha$$

$$\text{Activity (IU/mL) of the test solution} = 10^{I}$$

$$\text{Activity (IU/mg) of Nisin} = \frac{\text{Activity (IU/mL) of the test solution} \times 20}{\text{Weight (g) of the sample}}$$

(2) Sodium Chloride

Dissolve 0.1 g, accurately weighed, in 100 mL of water, and make acidic with nitric acid. Titrate with 0.1 mol/L silver nitrate. To confirm the endpoint, use a potentiometer with a silver indicator electrode and a silver-silver chloride reference electrode. Separately, perform a blank test to make necessary corrections. Determine the sodium chloride content in the sample by the following formula:

$$\text{Content (\%) sodium chlorite (NaCl)} = \frac{(a - b) \times 5.85}{\text{Weight (g) of the sample} \times 10}$$

a = amount (mL) of 0.1 mol/L silver nitrate consumed in the test

b = amount (mL) of 0.1 mol/L silver nitrate consumed in the blank test

Nitrous Oxide

亜酸化窒素

N_2O Mol. Wt. 44.01

Nitrous oxide [10024-97-2]

Definition Nitrous Oxide is a gas consisting mainly of nitrous oxide (N_2O). It is filled in a hermetic, pressure-resistant metal container other than a cartridge-type container.

Content Nitrous Oxide contains not less than 97.0% (vol) of nitrous oxide (N_2O).

Description Nitrous Oxide is a colorless gas at room temperature under atmospheric pressure. It has no odor.

Identification

(1) A wood-chip ember bursts into flame when exposed to Nitrous Oxide.

(2) Analyze 1 mL each of Nitrous Oxide (the sample) and nitrous oxide (N_2O) by gas chromatography using the operating conditions specified for the Assay. The retention time of the main peak of the sample corresponds to that of nitrous oxide (NO_2).

Purity The amounts of Nitrous Oxide indicated in Purity are the volumes at 20°C at an atmospheric pressure of 101.3 kPa. Collect appropriate amounts of the sample, taking into account the temperature and pressure at the time of testing.

(1) Chloride To 2.5 mL of 0.1 mol/L silver nitrate TS, add water to make 50 mL. When 10 L of Nitrous Oxide is passed through this solution and allowed to stand for 5 minutes, a white turbidity appears. The turbidity is not higher than that of the solution prepared as follows: To 2.5 mL of 0.1 mol/L silver nitrate TS, add 1 mL of Chloride Ion

Standard Stock Solution, 0.15 mL of 10% nitric acid TS, and water to make 50 mL, and allow to stand for 5 minutes.

(2) Arsenic hydride and hydrogen phosphide Place 5 mL of silver diethyldithiocarbamate–quinoline TS into a Nessler tube. Into the Nessler tube, insert a gas introduction tube connected to a glass tube filled with absorbent cotton moistened with lead acetate TS. Keep the end of the gas introduction tube 2 mm above the bottom of the Nessler tube. Introduce 10 L of Nitrous Oxide into the Nessler tube over 10 minutes. The color of the silver diethyldithiocarbamate–quinoline TS does not change.

(3) Carbon monoxide Introduce 5 mL of Nitrous Oxide into a gas-measuring tube or syringe for gas chromatography, analyze it by gas chromatography using the operating conditions given below. No peak is observed at the retention time of carbon monoxide.

Operating Conditions

Detector: Thermal conductivity detector. When 5 mL of hydrogen or helium containing 0.1% (vol) carbon monoxide is charged, the peak height obtained is about 10 cm or more.

Column: A glass tube (about 3 mm internal diameter and about 3 m length).

Column packing material: 300- to 500-μm zeolite for gas chromatography.

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust so that the peak of carbon monoxide appears about 20 minutes after injection.

(4) Nitrogen monoxide and nitrogen dioxide Not more than 2 μL/L as total volume.

Use a detector tube type gas measuring instrument connected with a nitrogen oxide detector tube.

Assay The collection of Nitrous Oxide should follow the requirement given in Purity.

Measure 1.0 mL of Nitrous Oxide into a gas-measuring tube or syringe for gas chromatography, analyze by gas chromatography using the operating conditions given below, and measure the peak area (A_T) of air. Separately, introduce 3.0 mL of nitrogen into a gas mixer, and add the carrier gas to make exactly 100 mL. Mix them well to prepare a standard mixed gas. Using 1.0 mL of the mixed gas, measure the peak area (A_S) of nitrogen in the same manner as the sample.

$$\text{Content (\%(vol)) of nitrous oxide } (N_2O) = 100 - 3 \times \frac{A_T}{A_S}$$

Operating Conditions

Detector: Thermal-conductivity detector.

Column: A glass tube (about 3 mm internal diameter and about 3 m length).

Column packing material: 300- to 500-μm zeolite for gas chromatography.

Column temperature: A constant temperature of about 50°C.

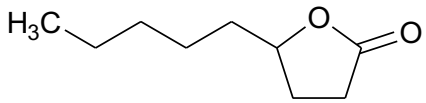
Carrier gas: Hydrogen or helium.

Flow rate: Adjust so that the peak of nitrogen appears about 2 minutes after injection.

γ-Nonalactone

Nonalactone
Nonano-1,4-lactone

γ-ノナラクトン



$C_9H_{16}O_2$ Mol. Wt. 156.22

5-Pentyldihydrofuran-2(3*H*)-one [104-61-0]

Content γ-Nonalactone contains not less than 98.0% of γ-nonalactone ($C_9H_{16}O_2$).

Description γ-Nonalactone is a colorless to light yellow, clear liquid having a sweet coconut-like odor.

Identification Determine the absorption spectrum of γ-Nonalactone as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.446–1.450.

Specific Gravity d_{25}^{25}: 0.958–0.966.

Purity Acid value Not more than 2.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Non-calcinated Coral Calcium

サンゴ未焼成カルシウム

Definition Non-calcinated Coral Calcium is obtained by sterilizing, drying, and triturating hermatypic corals of *Scleractinia*. It consists mainly of calcium carbonate.
Content Non-calcinated Coral Calcium, when dried, contains the equivalent of not less

than 85.0% of calcium carbonate ($CaCO_3$ = 100.09).

Description Non-calcinated Coral Calcium occurs as a white to yellowish-white powder.

Identification

To 1 g of Non-calcinated Coral Calcium, add 10 mL of water and 7 mL of diluted acetic acid (1 in 4). It effervesces and dissolves. When boiled to let the carbon dioxide out and neutralized with ammonia TS, this solution responds to all the tests for Calcium Salt in the Qualitative Tests .

Purity

(1) Hydrochloric acid-insoluble substances Not more than 3.0%.

To 5.0 g of Non-calcinated Coral Calcium, add 10 mL of water, then gradually add 12 mL of hydrochloric acid dropwise while stirring, and add water to make 200 mL. Filter through a filter paper for quantitative analysis (5C), and thoroughly wash the residue on the filter paper with boiling water until the washings are free of chloride. Incinerate the residue with the filter paper, and weigh.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Non-calcinated Coral Calcium, add 20 mL of diluted hydrochloric acid solution (1 in 4), cover with a watch glass, and boil gently for 15 minutes. After cooling, add 30 mL of water. Use the resulting solution as the sample solution. If the sample does not dissolve, evaporate to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes being covered with a watch glass, cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2), 1mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(3) Alkali metals and magnesium Not more than 12.0%.

To 1.0 g of Non-calcinated Coral Calcium, gradually add 30 mL of diluted hydrochloric acid (1 in 10) to dissolve it, and let the carbon dioxide out by boiling. After cooling, neutralize this solution with ammonia TS, add 60 mL of a solution of ammonium oxalate monohydrate (1 in 25), and heat on a water bath for 1 hour. After cooling, add water to make 100 mL, stir thoroughly, and filter. To 50 mL of the filtrate, add 0.5 mL of sulfuric acid, evaporate to dryness, ignite to constant weight, and weigh.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Moisten the specified amount of Non-calcinated Coral Calcium with 1 mL of water, add 5 mL of diluted hydrochloric acid (1 in 4), boil, and cool. If necessary, filter, wash the residue with water, and combine the washings with the filtrate.

Loss on Drying Not more than 2.0% (105°C, 3 hours).

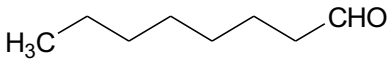
Assay Weigh accurately about 1 g of Non-calcinated Coral Calcium, previously dried, and dissolve it by gradually adding 10 mL of diluted hydrochloric acid (1 in 4). If necessary, filter it, wash the residue on the filter paper with water, and combine the washings with the filtrate. Add water to the resulting solution to make exactly 100 mL. Use this solution as the test solution. Proceed as directed in Method 1 under Calcium Salt Determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 5.004 mg of $CaCO_3$

Octanal

Caprylic Aldehyde
Octyl Aldehyde

オクタナール



$C_8H_{16}O$ Mol. Wt. 128.21

Octanal [124-13-0]

Content Octanal contains not less than 92.0% of octanal ($C_8H_{16}O$).

Description Octanal is a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Octanal as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.417–1.425.

Specific Gravity d_{25}^{25}: 0.810–0.830.

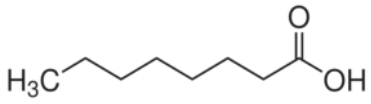
Purity Acid value Not more than 10.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (1).

Octanoic Acid

Caprylic Acid

オクタン酸



$C_8H_{16}O_2$ Mol. Wt. 144.21

Octanoic acid [124-07-2]

Content Octanoic Acid contains not less than 95.0% of octanoic acid ($C_8H_{16}O_2$).

Description Octanoic Acid is a colorless, oily liquid. It has a faint odor.

Identification Determine the absorption spectrum of Octanoic Acid as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Acid value 366–396.

Weigh accurately about 0.3 of Octanoic Acid, and proceed as directed in Acid Value under the Flavoring Substances Tests.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Decanoic acid Not more than 3.0%.

Test Solution Use Octanoic Acid as the sample.

Control Solution Measure 0.3 mL of decanoic acid, and add the sample to make 10 mL.

Procedure Analyze the test solution and the control solution by gas chromatography using the operating conditions specified in Assay. Confirm the peak of decanoic acid in the test solution using the control solution. Determine the sum (A_T) of the peak areas of all the peaks that appear within 40 minutes of the injection of the test solution and the peak area (A_S) of decanoic acid. Calculate the amount of decanoic acid by the formula:

$$\text{Amount (\%) of decanoic acid} = \frac{A_S}{A_T} \times 100$$

Water Content Not more than 0.4% (5 g, Volumetric Titration Direct Titration).

Residue on Ignition Not more than 0.1% (10 g, 800°C, 15 minutes).

Assay Proceed as directed under the Peak Area Percentage Method in the Gas

Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4). Use a fused silica capillary column (0.25–0.53 mm internal diameter and 30–60 m length) coated with a 0.25- to 1-μm thick layer of polyethylene glycol for gas chromatography. The column temperature should be raised from 150 to 230°C at a rate of 5°C/minute, and then maintained at 230°C for 24 minutes.

Onion Color

タマネギ色素

Definition Onion Color is obtained from the bulbs of onion, *Allium cepa* L., by extraction with water or hydrous ethanol or by extraction with an alkaline solution and neutralization. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Onion Color is not less than 50 and is in the range of 90–110% of the labeled value.

Description Onion Color occurs as a brown to dark brown powder, paste, or liquid. It has a slight, characteristic odor.

Identification

(1) Weigh an amount of Onion Color equivalent to 1 g of onion color with a Color Value 50, and dissolve it in 500 mL of citrate buffer (pH 7.0). The resulting solution is yellow-brown to red-brown.

(2) Weigh an amount of Onion Color equivalent to 1 g of onion color with a Color Value 50, and dissolve it in 500 mL of water. The resulting solution is yellow-brown to red-brown. To 10 mL of this solution, add 1 mL of iron(III) chloride hexahydrate solution (1 in 10). A brown to dark brown color is produced.

(3) Weigh an amount of Onion Color equivalent to 0.8 g of onion color with a Color Value 50, and dissolve it in 100 mL of sodium hydroxide solution (1 in 250). To 5 mL of this solution, add 10 mL of diluted hydrochloric acid (9 in 1000), then add 0.1mL of zinc chloride TS (pH 3.0), and shake. Stopper, warm at 50°C for 20 minutes, and centrifuge at 3000 rpm for 10 minutes if necessary. A brown to dark brown precipitate is produced.

Purity

(1) Lead Not more than 8 μg/g as Pb (0.50 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination. Prepare a test solution as directed given below. Weigh accurately a specified amount of Onion Color, dissolve it in 50 mL of sodium carbonate solution (1 in 1000), and then add

water to make exactly 100 mL. Refer to this as the sample solution. Dilute the sample solution exactly with citrate buffer (pH 7.0). Centrifuge if necessary. Use the supernatant as the test solution. Determine the color value using the following operation conditions.

Operating Conditions

Reference: Citrate buffer (pH 7.0).

Wavelength: A maximum absorption wavelength of 480–500 nm. If any absorption maximum is not exhibited, use a wavelength of 490 nm.

γ-Oryzanol

γ-オリザノール

Definition γ-Oryzanol is obtained from rice bran or rice germ oil and consists mainly of ferulic acid esters of sterols and triterpene alcohols.

Content γ-Oryzanol contains not less than 96.0% of ferulic acid esters when calculated on the dried basis.

Description γ-Oryzanol occurs as a white to yellowish-white powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Dissolve 10 mg of γ-Oryzanol in 10 mL of 3.5% (w/v) potassium hydroxide–ethanol TS by warming. A yellow color is produced.

(2) Dissolve 10 mg of γ-Oryzanol in 2 mL of ethyl acetate, add 0.2 mL of sulfuric acid, and shake. A yellow to orange color is produced. On the addition of 1 mL of acetic anhydride, the color of the solution gradually changes from red-purple through purple to gradually green.

(3) A solution (1 in 100,000) of γ-Oryzanol in heptane exhibits absorption maxima at wavelengths of 229–233 nm, 289–293 nm, and 313–317 nm.

(4) Prepare a test solution by dissolving 60 mg of γ-Oryzanol in ethyl acetate to make 10 mL. Prepare a control solution by dissolving 15 mg of cycloartenyl ferulate in ethyl acetate to make 50 mL. Analyze 5 μL each of the test solution and the control solution by thin-layer chromatography using a 70:30:1 mixture of hexane/ethyl acetate/acetic acid as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. Spray with a solution (1 in 10) of sulfuric acid in ethanol (95), and heat at 110°C for 10 minutes. The main spot from the test solution corresponds to the spot of cycloartenyl ferulate from the control solution.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead

Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Related Substances Perform thin-layer chromatography as directed in Identification (4) above. The main spot from the test solution is at the same position as the spot of cycloartenyl ferulate from the control solution, and any other single spot from the test solution is not more intense than that from the control solution.

Loss on Drying Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on Ignition Not more than 0.1% (1 g, 600°C, 3 hours).

Assay

Test Solution Place about 20 mg of γ-Oryzanol, accurately weighed, into a 200-mL Erlenmeyer flask, and add about 170 mL of heptane. Cover the mouth of the flask, warm in a water bath at 70–80°C for 30 minutes with occasional stirring. Then, treat ultrasonically for 20 minutes to dissolve the sample, cool to 20–30°C, and add heptane to make exactly 200 mL. Measure exactly 10 mL of this solution, and add heptane to make exactly 100 mL.

Procedure Measure the absorbance (A) of the test solution at the maximum around 315 nm against heptane as the reference, and calculate the content of ferulic acid esters by the following formula. Conduct the measurement within 15 minutes after the preparation of the test solution.

$$\text{Contenyt (\%) of ferulic acid esters} = \frac{A \times 20 \times 1000}{\text{Dry basis weight (mg) of the sample} \times 359} \times 100$$

Oxalic Acid

シュウ酸

$C_2H_2O_4 \cdot 2H_2O$ Mol. Wt. 126.07

Ethanedioic acid dihydrate [6153-56-6]

Content Oxalic Acid contains not less than 99.5% of oxalic acid ($C_2H_2O_4 \cdot 2H_2O$).

Description Oxalic Acid occurs as colorless crystals. It is odorless.

Identification

(1) Oxalic Acid sublimes upon heating.

(2) To 1 mL of a solution of Oxalic Acid (1 in 10), add 2 drops of sulfuric acid, add 1 mL of potassium permanganate solution (1 in 300), and heat. The pink color of the

solution disappears.

(3) Make a solution of Oxalic Acid (1 in 10) alkaline with ammonia TS, and add 1 mL of a solution of calcium chloride dihydrate (3 in 40). A white precipitate is formed.

Purity

(1) Clarity of solution　Colorless and almost clear.

Test Solution　Weigh 1.0 g of Oxalic Acid, add 20 mL of water, and dissolve it by boiling.

(2) Sulfate　Not more than 0.077% as SO_4.

Sample Solution　Weigh 1.0 g of Oxalic Acid, add 20 mL of water and 1 mL of sodium carbonate solution (1 in 8), evaporate to dryness on a water bath, heat gradually, and ignite to 600–700°C. To the residue, add 10 mL of water and 0.5 mL of nitric acid, boil, add 2 mL of hydrochloric acid, and evaporate to dryness on a water bath. Add water to the residue to make 100 mL, and filter. Use 25 mL of the filtrate as the sample solution.

Control Solution　To 0.40 mL of 0.005 mol/L sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(3) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition　Not more than 0.3% (1 g).

Assay　Weigh accurately about 1 g of Oxalic Acid, and dissolve it in water to make exactly 250 mL. Measure accurately 50 mL of this solution, add 3 mL of sulfuric acid, and warm to about 80°C. Titrate with 0.02 mol/L potassium permanganate while hot.

Each mL of 0.02 mol/L potassium permanganate = 6.303 mg of $C_2H_2O_4 \cdot 2H_2O$

Oxidized Starch

酸化デンプン

Definition　Oxidized Starch is obtained by treating starch with sodium hypochlorite.

Description　Oxidized Acetate occurs as a white to off-white powder, or as flakes or granules. It is odorless.

Identification

(1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

(3) Carboxyl groups　Proceed as directed in Identification (4) for Acetylated Oxidize Starch.

Purity

(1) Carboxyl groups　Not more than 1.1%.

Proceed as directed in Purity (2) for Acetylated Oxidize Starch.

(2) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Sulfur dioxide　Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying　Not more than 21.0% (not more than 13.3 kPa, 120°C, 4 hours).

Palm Oil Carotene

パーム油カロテン

Definition　Palm Oil Carotene is obtained from the fruits of the oil palm *Elaeis guineensis* Jacq. and consists mainly of carotene. It may contain edible fats or oils.

Content (Color Value)　Palm Oil Carotene contains the equivalent of not less than 30% of β-carotene ($C_{40}H_{56}$ = 536.87) or its Color Value ($E_{1cm}^{10\%}$) is not less than 7500. The actual value is in the range of 95–115% of the labeled value.

Description　Palm Oil Carotene is a red-brown to brown turbid oily substance having a slight, characteristic odor.

Identification

(1) Weigh an amount of Palm Oil Carotene equivalent to 15 mg of palm oil carotene with a Color Value 7500, and dissolve it in 5 mL of a 1:1 mixture of acetone/cyclohexane. An orange color develops.

(2) Proceed as directed in Identification (2) for Dunaliella Carotene.

(3) Proceed as directed in Identification (3) for Dunaliella Carotene.

Purity

(1) Lead　Not more than 5 μg/g as Pb (0.80 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic　Not more than 3 μg /g as As (0.50g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay (Color Value Determination)　Proceed as directed under Color Value Determination for Dunaliella Carotene.

Pancreatin

パンクレアチン

Definition Pancreatin is a mixture of enzymes that degrade protein, starch, and fats. It is derived from the pancreas of animals. It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Pancreatin occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Pancreatin complies with Methods 1 through 3 of the Pancreatin Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Pancreatin Activity Test

Method 1 Proceed as directed in Method 1 of the β-Amylase Activity Test in the monograph for β-Amylase. Use sodium chloride solution (29 in 5000) as the sample diluent and potato starch as the substrate, respectively.

Method 2 Proceed as directed in Method 1 of the Protease Activity Test in the monograph for Protease. Use casein TS (pH 8.0) as the substrate solution and trichloroacetate TS (for protease activity test) as a TS to make a precipitate.

Method 3 Proceed as directed in Method 1 of the Lipase Activity Test in the monograph for Lipase. Use polyvinyl alcohol I– polyvinyl alcohol II TS as the solution to emulsify olive oil.

Papain

パパイン

Definition Papain is a proteolytic enzyme derived from the fruit of the papaya *Carica papaya* L.. It may contain lactose, dextrin, or a food additive used for stabilizing the product.

Enzyme Activity Papain has an enzyme activity of not less than 300,000 units per gram.

Description Papain occurs as a white to light yellow-brown powder. It is odorless or has a characteristic odor.

Identification Papain shows activity when tested as directed under Enzyme Activity Determination.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If during the preparation of the test solution, the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100), use Method 3 in the Lead Limit Test

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed in the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escerichia coli* test and Method 2 for the *Salmonella* test.

Enzyme Activity Determination

Sample Solution Dissolve 8.75 g of L-cysteine hydrochloride monohydrate in about 800 mL of water, and add 2.23 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate to dissolve. Adjust the pH to 4.5 with sodium hydroxide TS (1 mol/L), and add water to make 1000 mL. Use this solution as the diluent.

Weigh accurately about 0.50 g of Papain, dissolve it in the diluent, and make exactly 100 mL. Measure exactly 1 mL of the solution, and add the diluent to make exactly 50 mL. Centrifuge this solution, if necessary. Dilute the supernatant liquid with the diluent to prepare a solution containing 20 to 100 units per mL.

Procedure Measure exactly 5 mL of casein TS (pH 8.0), transfer into a test tube, and warm for 5 minutes at 37 ± 0.5°C. Add 1 mL of the sample solution, shake immediately, and allow to react for 10 minutes at 37 ± 0.5°C. Add 5 mL of trichloroacetic acid TS, shake, allow to stand for 30 minutes at 37 ± 0.5°C, and filter through a filter paper for

quantitative analysis (5C). Discard the first 3 mL of the filtrate, and measure the absorbance (A_T) of the subsequent filtrate at 275 nm using water as the reference.

Separately, measure exactly 1 mL of the sample solution, add 5 mL of trichloroacetic acid TS, and shake well. Add 5 mL of casein TS (pH 8.0), shake well, allow to stand for 30 minutes at 37 ± 0.5°C. Measure the absorbance (A_b) of this solution in the same manner. Then measure the absorbance (A_S and A_{S0}) of Tyrosine Standard Solution and hydrochloric acid TS (0.1 mol/L) at 275 nm using water as the reference. Calculate the enzyme activity by the formula given below. One unit of the enzyme activity is the quantity of enzyme that increases the absorbance equivalent to 1 μg of tyrosine per minute when the test is performed as directed in the procedure.

$$\text{The Enzyme Activity (units/g) of Papain} = \frac{(A_T - A_b) \times 50}{A_S - A_{S0}} \times \frac{11}{10} \times \frac{1000}{M}$$

M = weight (mg) of Papain in 1 mL of the sample solution.

Paprika Color

Paprika Oleoresin

トウガラシ色素

Definition Paprika Color is obtained from the fruits of the pepper *Capsicum annuum* L. and consists mainly of capsanthins. It may contain edible fats or oils.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Paprika Colour is not less than 300 and is in the range of 95–115% of the labeled value.

Description Paprika Color is a dark red, viscous liquid having a characteristic odor.

Identification

(1) Weigh an amount of Paprika Color equivalent to 0.1 g of paprika color with a Color Value 300, and dissolve it in 100 mL of acetone. A yellow-orange color develops.

(2) Weigh 0.5 g of Paprika Color, dissolve it in 2 mL of toluene, and add 0.2 mL of sulfuric acid. A dark blue color develops.

(3) A solution of Paprika Color in acetone exhibits an absorption maximum at a wavelength of 450–460 nm or 465–475 nm or absorption maxima at both 450–460 nm and 465–475 nm.

(4) Weigh an amount of Paprika Color equivalent to 0.2 g of paprika color with a Color Value 300, dissolve it in 20 mL of acetone, and use this solution as the test solution. Analyze a 5-μL portion of the test solution by thin-layer chromatography using a 1:1 mixture of ethanol (95)/cyclohexane as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front

has ascended to a point about 10 cm above the starting line, and air-dry. Two yellow-red main spots are observed: one at an R_f of approximately 0.88–0.96 and the other at an R_f of approximately 0.75–0.90. When the spots are sprayed with sodium nitrite solution (1 in 20) followed by sulfuric acid TS (0.5 mol/L), the spots are decolorized immediately.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination, using the conditions below.

Operating Conditions

Solvent: Acetone.

Wavelength: Maximum absorption wavelength near 460nm.

Paraffin Wax

パラフィンワックス

Definition Paraffin Wax is a mixture of solid hydrocarbons obtained from petroleum atmospheric and vacuum distillates. It consists mainly of linear saturated hydrocarbons.

Description Paraffin Wax is a colorless or white, somewhat translucent solid at room temperature. It has a slight, characteristic odor.

Identification Determine the absorption spectrum of Paraffin Wax as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 43–75°C (Method 2).

Purity

(1) Lead Not more than 3 μg/g as Pb (3.0 g, Method 2, Control Solution: Lead Standard Solution 9.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Sulfur compound To 4.0 g of Paraffin Wax, add 2 mL of ethanol (99.5) and 2 drops of a transparent solution of sodium hydroxide (1 in 5) saturated with lead(II) oxide. Warm the mixture at 80°C for 10 minutes with occasional shaking, and allow to cool. A

dark brown color does not develop.

(4) Polycyclic aromatic hydrocarbons Before use, rinse all glass instruments used in the testing with 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement and examine them under ultraviolet light to confirm that no fluorescent contamination is present. Because some of the polycyclic aromatic hydrocarbons sought in this test are very susceptible to photo-oxidation, the entire procedure should be performed under subdued light.

Test Solution Weigh 150 g of Paraffin Wax into a 500-mL beaker, and melt by heating to make it homogenous. Place 25 g ± 0.2 g of the melted sample into a 500-mL separating funnel, add 100 mL of dimethyl sulfoxide TS, add 50 mL of 2,2,4-trimethylpentane TS while warming to keep the sample from solidifying, and shake vigorously for 2 minutes. Set up three 300-mL separating funnels with each containing 30 mL of 2,2,4-trimethylpentane TS. Allow to cool until the liquid phases in the 500-mL separating funnel separate and a waxy substance precipitates. Filter the lower layer (dimethyl sulfoxide TS phase) through glass wool fitted loosely in the separating funnel or filter paper, previously rinsed with 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement. Wash the filtrate in tandem with the 30-mL portions of 2,2,4-trimethylpentane TS contained in the 300-mL separating funnels already prepared. First, transfer the filtrate into a first separating funnel, and shake vigorously for 1 minute. Allow to stand, and use the lower layer for next washing. Repeat the same washing operation twice using the second and third separating funnels. Transfer the lower layer into a 2-L separating funnel. The individual upper layers (2,2,4-trimethylpentane TS phase) should be kept in each separating funnel since they will be used later.

Conduct extraction from the 2,2,4-trimethylpentane TS phase in the 500-mL separating funnel with an additional 100-mL portion of dimethyl sulfoxide TS, and filter the extractive through glass wool or filter paper in the same manner as previously done. Wash the extractive in tandem with the three 2,2,4-trimethylpentane TS phases kept in the three 300-mL separating funnels. Collect the dimethyl sulfoxide layer washed in the 2-L separating funnel. Conduct the extraction operation again from the 2,2,4-trimethylpentane TS phase in the 500-mL separating funnel with an additional 100-mL portion of dimethyl sulfoxide TS, and filter. Wash the extractive in tandem with the three 2,2,4-trimethylpentane TS phases in the same manner. Collect the dimethyl sulfoxide layer in the 2-L separating funnel. Discard the 2,2,4-trimethylpentaneTS layers in the 300-mL separating funnels.

Add 480 mL of water and 80 mL of isooctane2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement to the 2-L separating funnel containing 300 mL of the dimethyl sulfoxide TS phase, and extract by shaking vigorously for 2 minutes (first extraction). Allow to stand, transfer the lower layer into a second 2-L separating funnel,

add an additional 80 mL of 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement, and extract by shaking vigorously for 2 minutes (second extraction). Discard the lower layer. Wash the upper layer left in the former 2-L separating funnel with 100 mL of water by shaking vigorously for 1 minute, and discard the aqueous layer. Repeat this washing operation two more times. Refer to the extractive obtained as the first 2,2,4-trimethylpentane extractive. Wash the upper layer obtained in the second extraction with 100 mL of water by shaking vigorously for 1 minute. Discard the aqueous layer. Repeat this washing operation two more times. Refer to the extractive obtained as the second 2,2,4-trimethylpentane extractive.

Place the first 2,2,4-trimethylpentane extractive in a 300-mL Erlenmeyer flask through a 30-mL glass filter packed with 35 g of sodium sulfate pre-rinsed with 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement. Wash the first 2-L separating funnel with the second 2,2,4-trimethylpentane extractive, and place it in the 300-mL Erlenmeyer flask through the 30-mL glass filter (G3) packed with the sodium sulfate. Again, wash the second and first separating funnels successively with 20 mL of 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement, and place into the Erlenmeyer flask through the sodium sulfate. Transfer the 2,2,4-trimethylpentane extractives into a distilling flask, add 1 mL of hexadecane for ultraviolet absorption spectrum measurement, and evaporate the 2,2,4-trimethylpentane under nitrogen until 1 mL of residue remains. To the residue, add 10 mL of 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement, and evaporate again until 1 mL of residue remains. Repeat this operation.

Dissolve the residue in 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement, transfer into a 25-mL volumetric flask, and make exactly 25 mL adding 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement. Use this as the test solution.

Reference Solution Prepare a reference solution in the same manner as the preparation of the test solution without using the sample.

Procedure Measure the absorbance of the test solution in a 5-cm path length cell. The corrected absorbance does not exceed the limits given below.

Wavelength (nm)	Absorbance/cm path length
280–289	0.15
290–299	0.12
300–359	0.08
360–400	0.02

(5) Readily carbonizable substances Place 5.0 g of Paraffin Wax in a Nessler tube, melt by warming in a water bath at 80°C, and add 5 mL of sulfuric acid for readily

carbonizable substances test. Warm again in the water bath at 80°C for 1 minute, remove from the bath, and immediately shake vigorously for a few seconds. Repeat this operation three more times. Leave in the water bath at 80°C for 30 seconds. The separated sulfur layer is not darker in color than a solution prepared by mixing 3.0 mL of Iron(III) Chloride CSSS, 1.5 mL of Cobalt(II) Chloride CSSS, and 0.5 mL of Copper(II) Sulfate CSSS in a Nessler tube.

Residue on Ignition Not more than 0.1%.

Pectin

ペクチン

Definition Pectin is obtained from citrus fruits, apples, and other plants. It consists of water-soluble polysaccharides including partially methyl-esterified polygalacturonic acid. It may contain sucrose, glucose, lactose and dextrin.

Description Pectin occurs as a white to light brown powder or as granules. It is odorless or has a slight, characteristic odor.

Identification

Test Solution Weigh 50 mg of Pectin, and add 1 mL of 2-propanol. Next, add 50 mL of water while stirring magnetically. Adjust the pH to 12 with sodium hydroxide TS (0.5 mol/L), and allow to stand for 15 minutes. Adjust the pH to 7.0 with hydrochloric acid TS (0.5 mol/L), and add water to make exactly 100 mL. Use this solution as the sample solution. Place 0.5 mL of Tris buffer (pH 7.0) for pectin determination in a quartz cell, add 1.0 mL of the sample solution, 0.5 mL of water, and 0.5 mL of pectate lyase solution for pectin determination (enzyme solution), and then mix.

Enzyme Blank Place 0.5 mL Tris buffer (pH 7.0) for pectin determination in a quartz cell, add 1.0 mL of the sample solution and 1.0 mL of water, and mix.

Sample Blank Place 0.5 mL Tris buffer (pH 7.0) for pectin determination in a quartz cell, add 1.5 mL of water and 0.5 mL of the enzyme solution, and mix.

Procedure Measure the absorbance of the test solution, enzyme blank, and sample blank at 235 nm at 0 and at 10 minutes. Calculate the absorbance value (A_0) at 0 minutes and the absorbance value (A_{10}) at 10 minutes by the following equation given below. The change ($A_{10} - A_0$) in absorbance is not less than 0.023.

Absorbance value (A_0) at 0 minutes
= Absorbane value of the test solution at 0 minutes
– (Absorbane value of the enyme blank at 0 minutes
+ Absorbane value of the sample blank at 0 minutes)

Absorbance value (A_0) at 10 minutes
= Absorbane value of the test solution at 10 minutes
– (Absorbane value of the enyme blank at 10 minutes
+ Absorbane value of the sample blank at 10 minutes)

Purity

(1) Amide group Not more than 25% of total carboxyl groups.

Weigh accurately about 5 g of Pectin, place in a beaker, and add 5 mL of hydrochloric acid and 100 mL of 60% (vol) ethanol. Stir for 10 minutes, and filter with a glass filter (1G3). Wash the residue 6 times with 15 mL of a 20:1 mixture of 60% (vol) ethanol/hydrochloric acid each time. Next, wash the residue on the glass filter with 60% (vol) ethanol until the washings are free of chlorides, wash with 20 mL of ethanol (95), and dry at 105°C for 150 minutes. After cooling, weigh the residue. Weigh accurately an amount (M mg) of the residue equivalent to about one-tenth of the weight. Add 2 mL of ethanol (95) to moisten, add 100 mL of water previously boiled and cooled, and hydrate well with occasional stirring. Add 5 drops of Phenolphthalein TS, titrate with 0.1 mol/L sodium hydroxide, and express the number of mL as V_1. Next, add exactly 20 mL of 0.5 mol/L sodium hydroxide, shake well, and allow to stand for 15 minutes. Add exactly 20 mL of 0.5 mol/L hydrochloric acid, and shake until the pink color of the solution disappears. Titrate with 0.1 mol/L sodium hydroxide, and express the number of mL as V_2. The end of titration is when a light pink color develops.

Using the apparatus as directed under the Kjeldahl Method in Nitrogen Determination, distill the solution obtained by the titration. Transfer the solution in a 500-mL Kjeldahl flask. Attach a spray trap and a condenser to the flask. Place 20 mL of 0.1 mol/L hydrochloric acid and 150 mL of newly boiled and cooled water in an absorption flask, and immerse the lower end of the condenser into the solution in the absorption flask. Add 20 mL of sodium hydroxide solution (1 in 10) to the Kjeldahl flask, and heat, taking care to prevent bubble formation. Distill until 80–120 mL of distillate is obtained. Add a few drops of methyl red TS to the distillate, titrate with 0.1 mol/L sodium hydroxide, and express the number of mL as S. Perform a blank test in the same manner, and express the number of mL as B.

$$\text{Content (\%) of amide group of total carboxyl groups} = (B - S)/(V_1 + V_2 + (B - S)) \times 100$$

(2) Galacturonic acid Not less than 65%.

Calculate the content by the following formula from M, V_1, V_2, B, and S obtained in

Purity (1).

$$\text{Content (\%) of galacturonic acid} = \{[19.41 \times (V_1 + V_2 + (B - S))]/M\} \times 100$$

(3) Total nitrogen Not more than 2.5%.

Weigh about 2 g of Pectin, add 5 mL of hydrochloric acid and 100 mL of 60% (vol) ethanol, stir for 10 minutes, and filter through a glass filter (1G3). Wash the residue on the filter 6 times with 15 mL of a 20:1 mixture of 60% (vol) ethanol/hydrochloric acid each time. Then wash with 60% (vol) ethanol until the washings are free of chlorides and then with 20 mL of ethanol (95). Dry the glass filter containing the residue at 105°C for 150 minutes. Weigh accurately about 0.2 g of the dried residue, and determine the content of nitrogen according to the semi-micro Kjeldahl method.

(4) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (3) for Quillaja Extract.

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(7) Total insolubles Not more than 3.0%.

Weigh about 1 g of Pectin into a 250-mL beaker, and add 5 mL of 2-propanol to disperse the sample. While stirring magnetically, add 100 mL of disodium dihydrogen ethylenediaminetetraacetate–sodium hydroxide TS, previously filtered through a glass fiber filter paper. Stir for 30 minutes, and heat until boiling. If excessive bubbling occurs, reduce heat. While the solution is hot, filter it through a 70-mm glass fiber filter under vacuum. The 70-mm glass fiber filter to be used should be previous dried in an oven at 105°C for about 1 hour, cooled in a desiccator, and weighed. Wash the beaker 5 times with five 100-mL portions of warm water, previously filtered through a glass fiber filter, and filter the washings through the glass fiber filter paper. Dry the filter paper with the residue at 105°C for about 1 hour, and cool in a desiccator. Weigh the filter paper.

$$\text{Total insolubles (\%)} = \frac{\text{(Weight (g) of the residue)} - \text{(Weight (g) of the filter paper)}}{\text{Weight (g) of the sample}} \times 100$$

(8) Sum of methanol and 2-propanol Not more than 1.0%.

Test Solution Weigh accurately about 0.1 g of Pectin, add exactly 10 mL of diluted internal standard solution (1 in 25), and stopper tightly. Mix well until the sample disperses uniformly. Centrifuge it at 5000 rpm for 30 minutes using a centrifugal ultrafiltration unit. Use the filtrate as the test solution.

Internal Standard Solution Use a solution of 2-methyl-2-propanol (1 in 1000).

Standard Solution Weigh accurately about 0.1 g each of methanol and 2-propanol in a volumetric flask, and add water to make exactly 100 mL. Place exactly 10 mL of this solution and 4 mL of the internal standard solution into a volumetric flask, and add water to make exactly 100 mL.

Procedure Analyze 2.0 μL each of the test solution and standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratios of 2-propanol and methanol to 2-methyl-2-propanol for the test solution and the standard solution. Express them as Q_{T1} and Q_{T2} for the test solution, and as Q_{S1} and Q_{S2} for the standard solution. Obtain the contents of methanol and 2-propanol by the following formulae:

$$\text{Amount (\%) of 2-propanol} = \frac{\text{Weight (g) of 2-propanol}}{\text{Weight (g) of the sample}} \times \frac{Q_{T1}}{Q_{S1}}$$

$$\text{Amount (\%) of methanol} = \frac{\text{Weight (g) of methanol}}{\text{Weight (g) of the sample}} \times \frac{Q_{T2}}{Q_{S2}}$$

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube (3 mm internal diameter and 2 m length).

Column packing material: 180- to 250-μm styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Injection port temperature: A constant temperature of about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention times of methanol and 2-propanol to about 2 minutes and 10 minutes, respectively.

Loss on Drying Not more than 12.0% (105°C, 2 hours).

Acid-insoluble Ash Not more than 1.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per g.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 2 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture Prepare as directed in Method 2 for the *Escerichia coli* test. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 5 g of Pectin with 500 mL of lactose broth to disperse completely, and incubate it at 35 ± 1°C for 24 ± 2 hours.

Pectinase

ペクチナーゼ

Definition Pectinase includes enzymes that degrade pectin and pectic acid. It is derived from the culture of basidiomycetes (limited to species from the genus *Corticium*), filamentous fungi (limited to *Aspergillus aculeatus*, *Aspergillus alliaceus*, *Aspergillus awamori*, *Aspergillus carbonarius*, *Aspergillus japonicus*, *Aspergillus niger*, *Aspergillus pulverulentus*, *Aspergillus usamii*, *Rhizopus oryzae*, and species of the genus *Trichoderma*), yeasts (limited to *Geotrichum klebahnii* and species of the genus *Trichosporon*), actinomycetes (limited to *Streptomyces thermoviolaceu* and *Streptomyces violaceoruber*), or bacteria (limited to *Bacillus subtilis*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Pectinase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Pectinase complies with the Pectinase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Pectinase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement

of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of Pectinase, add citric acid–hydrochloric acid buffer (0.1 mol/L) at pH 4.0 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Weigh 0.6 g of pectin (citrus-derived) or pectic acid (citrus-derived), and dissolve it in 80 mL of citric acid–hydrochloric acid buffer (0.1 mol/L) at pH 4.0. Adjust the pH of this solution to 4.0 with trisodium citrate (1 mol/L) or hydrochloric acid TS (0.1 mol/L), and add citric acid–hydrochloric acid buffer (0.1 mol/L) at pH 4.0 to make 100 mL.

Test Solution Equilibrate 10 mL of substrate solution at 40°C for 5 minutes, add 1 mL of the sample solution, and mix immediately. Incubate the mixture at 40°C for 30 minutes, and add 3 mL of sodium carbonate TS (1 mol/L). To this solution, add 6 mL of 0.05 mol/L iodine, shake well, allow to stand at a dark place for 30 minutes, and then add 6 mL of sulfuric acid TS (2 mol/L).

Control Solution To 3 mL of sodium carbonate TS (1 mol/L), add 1 mL of the sample solution, and mix. Add 10 mL of substrate solution and 6 mL of 0.05 mol/L iodine, allow to stand at a dark place for 30 minutes, and then add 6 mL of sulfuric acid TS (2 mol/L).

Procedure Titrate the test solution and the control solution with sodium thiosulfate TS (0.02 mol/L) (indicator: 1 to 2 drops of soluble starch). The amount of sodium thiosulfate TS (0.02 mol/L) consumed by the test solution is less than that of sodium thiosulfate TS (0.02 mol/L) consumed by the control solution. The endpoint is when the blue color produced disappears.

Method 2

Sample Solution Weigh 1.0 g of Pectinase, add cold water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding cold water to the resulting solution.

Substrate Solution Weigh 0.95 g of pectin (citrus-derived) or pectin (apple-derived), dissolve it in about 70 mL of water, warmed to 70–90°C, and allow to cool. Adjust the pH of this solution to 3.5 with citric acid monohydrate solution (21 in 1000) or disodium hydrogen phosphate (71 in 2500), and add 10 mL of McIlvain buffer at pH 3.5 and water to make 100 mL.

Procedure Introduce 6 mL of the substrate solution and 6 mL of McIlvain buffer at pH 3.5 gently in tube A of the capillary tube viscometer as specified in Method 1 “Viscosity Measurement” under the General Tests. Place the viscometer vertically in a thermostatic water bath at 40°C, and allow to stand for 10–15 minutes. Add 2 mL of the sample solution, close tube C with a finger, and mix the content by blowing air into tube B. Measure the time (second) required for the mixture to drop according to the procedure for viscosity measurement while warming at 40°C. Repeat this step five times, and determine the average of the five measurements. Designate the obtained value as the

flow time of the test solution. Separately, proceed in the same manner using 2 mL of water instead of the sample solution, and measure the time. Designate the obtained value as the flow time of the control solution. The time for the test solution is less than that for the control solution.

Method 3

Sample Solution Weigh 0.83 g of Pectinase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 25-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 5.0 g of esterified pectin, and add it gradually to 800 mL of water, warmed to 40°C, to suspend it. Warm the suspension at a temperature below 60°C while stirring to dissolve it completely. After cooling, add 2.03 g of magnesium chloride hexahydrate, adjust the pH to 4.80 ± 0.04 with sodium hydroxide TS (1 mol/L), and add water to make 1000 mL.

Procedure Equilibrate 20 mL of the substrate solution at 30°C for 15 minutes, immerse a pH electrode into the solution, adjust the pH to 4.80 ± 0.04 with 0.05 mol/L sodium hydroxide, and add 1 mL of the sample solution. To this solution, add 0.05 mol/L sodium hydroxide dropwise continuously so that the pH is maintained at 4.80 ± 0.04 for 2 minutes after the addition of the sample solution. Record the amount of the sodium hydroxide added as the consumption by the test solution. Separately, using 1 mL of water instead of the sample solution, proceed as directed for test solution. Record the amount of the sodium hydroxide added as the consumption by the control solution. The consumption by the test solution is more than that by the control solution. The whole procedure given here should be carried out while stirring.

Method 4

Sample Solution Weigh 0.71 g of Pectinase, add acetate buffer (0.02 mol/L, pH 5.0, containing albumin) to dissolve it or disperse it uniformly, and make 250 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Add 0.5 g of polygalacturonic acid sodium salt gently to about 80 mL of water while stirring so that it is suspended in 5 minutes. Warm the suspension at 80–85°C for 2 minutes, and quickly cool to room temperature. To this solution, add 5 mL of acetate buffer (1 mol/L) at pH 5.0, and then add water to make 100 mL.

Test Solution To 0.5 mL of the sample solution, equilibrated at 40°C for 1 minute, add 0.5 mL of the substrate solution, equilibrated at 40°C, stir immediately, and allow to incubate at 40°C for 10 minute. To this solution, add 1 mL of 3,5-dinitro-salicylate TS (for pectinase activity test), and mix. Heat the mixture in a water bath for 5 minutes, allow to cool, and add 5 mL of water.

Control Solution Proceed as directed for the test solution using acetate buffer (0.02 mol/L, pH 5.0, containing albumin) instead of the sample solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 550 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 5

Sample Solution Weigh 1.0 g of Pectinase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 50-fold dilution by adding water to the resulting solution.

Substrate Solution Add 50 mL of water to 100 mL of citric acid–phosphate buffer (0.1 mol/L) at pH 5.5, and warm to 60°C. Add gradually 1 g of pectin (apple-derived), and stir for about 20 minutes to dissolve it completely. After cooling, add water to make 200 mL.

Test Solution To 0.5 mL of the sample solution, add 2.5 mL of the substrate solution, equilibrated at 45°C. Incubate the mixture at 45°C for 10 minutes. Add 1 mL of hydrochloric acid TS (0.5 mol/L), and mix.

Control Solution Proceed as directed for the test solution using water instead of the sample solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 235 nm. The absorbance value of the test solution is higher than that of the control solution. The measurement of absorbance should be done at 45°C. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 6

Sample Solution Weigh 1.0 g of Pectinase, add Tris buffer (0.1 mol/L, pH 7.8, containing calcium chloride) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution To 30 mL of a solution of 2-amino-2-hydroxymethyl-1,3-propan diol (969 in 20,000), add 6.6 mL of hydrochloric acid TS and 10 mL of water, and mix. To this solution, add 0.27 g of polygalacturonic acid sodium salt, and dissolve it by stirring at room temperature for at least 20 minutes. Adjust the pH to 7.8 with hydrochloric acid TS (1 mol/L), and add water to make 60 mL.

Test Solution To 0.9 mL of the substrate solution, add 0.9 mL of calcium chloride dihydrate solution (1 in 10,000), mix, and equilibrate the mixture at 37°C for about 5 minutes. To this solution, add 0.2 mL of the sample solution, and mix. Incubate the mixture at 37°C for 10 minutes, and then add 2 mL of hydrochloric acid TS (0.05 mol/L).

Control Solution To 0.9 mL of the substrate solution, add 0.9 mL of calcium chloride dihydrate solution (1 in 10,000), mix, and warm the mixture at 37°C for about 15 minutes. To this solution, add 2 mL of hydrochloric acid TS (0.05 mol/L) and 0.2 mL of the sample solution, and mix.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 235 nm within 30 minutes after their preparation. The absorbance value

of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Pectin Digests

ペクチン分解物

Definition Pectin Digests are obtained by enzymatically decomposing pectin [Pectin is defined as a substance that is produced from extracts of sugar beets (*Beta vulgaris* L. var. *rapa* Dum.) sunflower (*Helianthus annuus* L.), *amadaidai* (*Citrus sinensis* (L.) Osbeck), grape fruits (*Citrus* × *paradisi* Macfad.), limes (*Citrus aurantiifolia* (Christm.) Swingle), lemons (*Citrus limon* (L.) Burm. f.), or apples (*Malus pumila* Mill.) with water or an acidic solution or from the extracts further decomposed by an alkaline solution or enzyme and that consists mainly of the polysaccharides of methylated polygalacturonic acid]. They consist mainly of galacturonic acid.

Content Pectin Digests, when calculated on the dried basis, contain not less than 40% of galacturonic acid ($C_6H_{10}O_7$ = 194.14).

Description Pectin Digests occur as brown to blackish brown liquids.

Identification

(1) Add 1 g of the sample to 9 mL of water, and stir well. No gel is produced.

(2) Add 1 mL of a solution of the sample (1 in 1000) to 5 mL of sodium tetraborate–sulfuric acid TS cooled with ice, heat the mixture in a water bath for 10 minutes, and immediately cool with cold water. To the resulting solution, add 0.2 mL of carbazole-ethanol TS, and heat in a water bath for 15 minutes. A purple color is produced.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 70% (105°C, 3 hours).

Assay

Test Solution Weigh accurately about 1 g of the sample, and dissolve it in water to make exactly 100 mL. To exactly 1 mL of this solution, add water to make exactly 100 mL. Designate the resulting solution as the sample solution. Transfer 5 mL of sodium tetraborate–sulfuric acid TS into a test tube, cool with ice, and add exactly 1 mL of the sample solution. Heat the test tube, covered with a lid, in a water bath for 10 minutes, and immediately cool with ice for 5 minutes. To the resulting solution, add 0.2 mL of carbazole-ethanol TS, heat in a water bath for 15 minutes, and immediately cool with ice for 5 minutes.

Standard Solutions Dissolve appropriate amounts of galacturonic acid for assay in

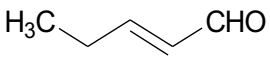
water to make solutions of 0.01 mg/mL, 0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL as anhydride, and proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the standard solutions at 530 nm. Prepare a calibration curve, determine the concentration of galacturonic acid in the test solution from the calibration curve, and calculate it on dried basis.

trans-2-Pentenal

(*E*)-2-Pentenal

***trans*-2-ペンテナール**



C_5H_8O Mol. Wt. 84.12

(2*E*)-Pent-2-enal [1576-87-0]

Content *trans*-2-Pentenal contains not less than 95.0% of *trans*-2-pentenal (C_5H_8O).

Description *trans*-2-Pentenal occurs as a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of *trans*-2-Pentenal as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{21}: 1.440–1.447.

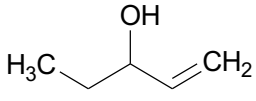
Specific Gravity d_{21}^{21}: 0.850–0.856.

Purity Acid value Not more than 6.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests using operating conditions (3). For the column, use a fused silica tube (0.25–0.53 mm in internal diameter and 50–60 m in length) coated with a 0.25–1 μm thick layer of polyethylene glycol for gas chromatography.

1-Penten-3-ol

1-ペンテン-3-オール



$C_5H_{10}O$ Mol. Wt. 86.13

Pent-1-en-3-ol [616-25-1]

Content 1-Penten-3-ol contains not less than 98.0% of 1-penten-3-ol ($C_5H_{10}O$).

Description 1-Penten-3-ol occurs as a colorless, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 1-Penten-3-ol, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.419–1.427.

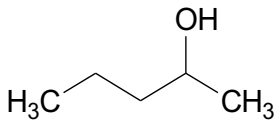
Specific Gravity d_{25}^{25}: 0.834–0.840.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2).

2-Pentanol

sec-Amyl Alcohol

2-ペンタノール



$C_5H_{12}O$ Mol. Wt. 88.15

Pentan-2-ol [6032-29-7]

Content 2-Pentanol contains not less than 98.0% of 2-pentanol ($C_5H_{12}O$).

Description 2-Pentanol occurs as a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of 2-Pentanol, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

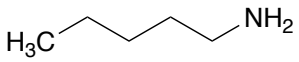
Refractive Index n_D^{20}: 1.403–1.409.

Specific Gravity d_{25}^{25}: 0.802–0.809.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2).

Pentylamine

ペンチルアミン



$C_5H_{13}N$ Mol. Wt. 87.16

Pentan-1-amine [110-58-7]

Content Pentylamine contains not less than 95.0% of pentylamine ($C_5H_{13}N$).

Description Pentylamine occurs as a colorless to yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Pentylamine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive index n_D^{20}: 1.408–1.424

Specific gravity d_{25}^{25}: 0.750–0.759

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operating conditions (2) except for the column. Use a fused silica tube (0.25–0.53 mm internal diameter and 30–60 m length) coated with a 0.25–1 μm thick layer of dimethylpolysiloxane for gas chromatography.

Pepsin

ペプシン

Definition Pepsin is a proteolytic enzyme obtained from animals or fish. It may contain lactose or dextrin.

Enzyme Activity Pepsin has an enzyme activity of not less than 110,000 units per gram.

Description Pepsin occurs as a slightly hygroscopic, white to light yellow-brown powder, or as a light yellow-brown to brown paste or liquid. It is odorless or has slight, characteristic odors.

Identification Pepsin has activity when tested as directed under Enzyme Activity Determination

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method). In the prepration of the test solution, if the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100), proceed as direted in Method 3 under the Lead Limit Test.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed in the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escerichia coli* test and Method 2 for the *Salmonella* test.

Enzyme Activity Determination

(i) Preparation of Test Solution and Standard Solution

Test Solution Weigh accurately an amount of Pepsin equivalent to about 1250 units enzyme activity, and add hydrochloric acid TS (0.01 mol/L), previously cooled with ice, to make exactly 50 mL.

Standard Solution Weigh accurately an amount of Saccharated Pepsin Reference Standard equivalent to about 1250 units, and add hydrochloric acid TS (0.01 mol/L), previously cooled with ice, to make exactly 50 mL.

(ii) Procedure Add exactly 1 mL each of the test solution and the standard solution, while cooling with ice, to separate 5-mL portions of casein TS (pH 2.0), exactly measured and warmed for 10 minutes at 37 ± 0.5°C. Shake immediately, and react for exactly 10 minutes at 37 ± 0.5°C. Add 5 mL of trichloroacetic acid solution (9 in 125) to each, shake, allow to stand for 30 minutes at 37 ± 0.5°C, and separately filter through a filter paper for quantitative analysis (5C). Discard the first 3 mL of each filtrate. To the subsequent 2 mL of each filtrate, exactly measured, add 5 mL of sodium carbonate TS (0.55 mol/L) and 1 mL of diluted Folin's TS (1 in 3). Allow to stand for 30 minutes at 37 ± 0.5°C. Measure the absorbance of them at 660 nm against water as the reference. The absorbance values are expressed as A_T and A_S, respectively.

Separately, measure exactly 1 mL each of the test solution and the standard solution, add 5 mL of trichloroacetic acid solution (9 in 125) to each, and shake. Then add 5 mL of casein TS (pH 2.0), warm for 30 minutes at 37 ± 0.5°C, and separately filter through a filter paper for quantitative analysis (5C). Discard the first 3 mL of each filtrate, and measure exactly 2 mL each of the subsequent filtrates. Measure absorbance (A_{TB} and A_{SB}) in the same manner. Calculate the enzyme activity by the formula:

$$\text{The Enxyme Activity (units/g) of Pepsin} = \frac{U_S \times (A_T - A_{TB})}{A_S - A_{SB}} \times \frac{1}{M}$$

U_S = units in 1 mL of the standard solution,

M = weight (g) of Pepsin in 1 mL of the test solution.

Peptidase

ペプチダーゼ

Definition Peptidase includes enzymes that degrade proteins and peptides. It is derived from the culture of filamentous fungi (limited to *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus sojae*, and *Rhizopus oryzae*), actinomycetes (limited to *Streptomyces avermitilis*, *Streptomyces cinnamoneus*, *Streptomyces griseus*, *Streptomyces thermoviolaceus*, and *Streptomyces violaceoruber*), or bacteria (limited to *Lactococcus lactis* and specices of the genus *Bacillus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Peptidase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Peptidase complies with the Peptidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Peptidase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Proceed as directed in Method 1 of the Aminopeptidase Activity Test in the monograph for Aminopeptidase.

Method 2

Proceed as directed in Method 2 of the Aminopeptidase Activity Test in the monograph for Aminopeptidase.

Method 3

Proceed as directed in Method 3 of the Aminopeptidase Activity Test in the monograph for Aminopeptidase.

Peracetic Acid Composition

過酢酸製剤

[79-21-0, Peracetic acid]

Definition Peracetic Acid Composition is an aqueous solution containing peracetic acid, "Glacial Acetic Acid," "Hydrogen Peroxide," and "1-Hydroxyethylidene-1,1-diphosphonic Acid" or an aqueous solution containing these four substances and "Octanoic Acid." Peroctanoic acid may be produced from "Octanoic Acid" contained in Peracetic Acid Composition.

Content Peracetic Acid Composition contains 12–15% of peracetic acid, 30–50% of acetic acid, 4–12% of hydrogen peroxide, and less than 1% of 1-hydroxyethylidene-1,1-diphosphonic acid. In addition, it can contain not more than 10% of octanoic acid.

Description Peracetic Acid Composition is a colorless, clear liquid having a characteristic, pungent odor.

Assay

(1) Peracetic acid and acetic acid

Sample Solution Weigh accurately about 1 g of Peracetic Acid Composition, and add water to make exactly 100 mL.

Procedure Pour 5 mL of methanol, then 10 mL of water into an octadecylsilanized silica gel minicolumn (500 mg), and discard the effluent. Pour exactly 10 mL of the sample solution into the column, and collect the effluent in a 100-mL beaker. Pour 10 mL of water into the column, add the effluent to the beaker, and add about 50 mL of water to the beaker. Titrate the resulting solution with 0.1 mol/L sodium hydroxide using a potentiometer. Use a glass electrode and a silver-silver chloride electrode as the indication and reference electrodes, respectively. Record the amounts (a mL and b mL) of the sodium hydroxide solution consumed at the first and second inflection points to determine each content by the following formulae.

$$\text{Content (\%) of peracetic acid } (C_2H_4O_3) = \frac{(b - a) \times 0.1 \times 76.05}{\text{Weight (g) of the sample}}$$

$$\text{Content (\%) of acetic acid } (C_2H_4O_2) = \frac{a \times 0.1 \times 60.05}{\text{Weight (g) of the sample}}$$

(2) Hydrogen peroxide

Test Solution Weigh accurately about 1 g of Peracetic Acid Composition, and add water to make exactly 100 mL. Transfer exactly 10 mL of this solution into a 250-mL Erlenmeyer flask, and add 75 mL of sulfuric acid TS (0.5 mol/L), cooled with ice.

Procedure Add 2 drops of ferroin TS to the test solution, and titrate with 0.1 mol/L cerium(IV) sulfate. The endpoint is when the color of the solution changes from orange to colorless through light red. Determine the content by the formula:

$$\text{Content (\%) of hydrogen peroxide } (H_2O_2) = \frac{\text{Consumption (mL) of 0.1 mol/L cerium (IV) sulfate} \times 0.1 \times 17.00}{\text{Weight (g) of the sample}}$$

(3) 1-Hydroxyethylidene-1,1-diphosphonic acid

Test Solution Weigh accurately about 0.2 g of Peracetic Acid Composition, and add water to make exactly 50 mL. Transfer exactly 3 mL of this solution into a 100-mL beaker, and add 50 mL of water. To this solution, add 1 drop of phenolphthalein TS. When the solution turns light red, add sulfuric acid TS (2.5 mol/L) until the light red color disappears. Add 2 mL of sulfuric acid TS (2.5 mol/L) further, and stir. Then add 0.4 g of ammonium peroxodisulfate, and stir. Heat the resulting solution with boiling chips on a hot plate for 90 minutes while replenishing the lost water, and then continue to heat until the volume of the solution is about 10 mL. After cooling, add 2 drops of phenolphthalein TS, then add sodium hydroxide solution (1 in 40) until the solution becomes faint red. Transfer this solution to a 50-mL volumetric flask, wash the boiling chips and the beaker a few times with a small amount of water, and add the washings to the flask. Make up with water to exactly 50 mL and refer to the resulting solution as the sample solution. Measure exactly 10 mL of the sample solution, add 2.0 mL of antimony tartrate–molybdic acid TS, shake well, and allow to stand for 20 minutes.

Reference Solution Using 10 mL of water instead of the sample solution, proceed as directed for the test solution, beginning with "add 2.0 mL of antimony tartrate–molybdic acid TS."

Standard Solutions Dissolve 0.2195 g of potassium dihydrogen phosphate in water to make exactly 1000 mL. To exactly 5 mL of this solution, add water to make exactly 1000 mL. Use the resulting solution as the standard stock solution. Measure exactly 0 mL, 3 mL, 5 mL, 10 mL, 15 mL, and 20 mL of the standard stock solution in separate volumetric flasks, and add water to each solution to make exactly 50 mL of each.

Measure exactly 10 mL each of the resulting solutions, and proceed as directed for the test solution, beginning with "add 2.0 mL of antimony tartrate–molybdic acid TS".

Procedure Measure the absorbance of the test solution and the standard solutions at 650 nm to prepare a calibrate curve. Determine the concentration of phosphorus in the test solution from the calibration curve and the absorbance of the test solution, and calculate the content of 1-hydroxyethylidene-1,1-diphosphonic acid ($C_2H_8O_7P_2$) by the formula:

$$\text{Content (\%) of } (C_2H_8O_7P_2) = \frac{\text{Concentration (μg/mL) of phosphorus} \times 206.0}{\text{Weight (g) of the sample} \times 61.94 \times 12}$$

(4) Octanoic acid

Test Solution Weigh accurately about 0.7 g of Peracetic Acid Composition, add a 1:1 mixture of water and acetonitrile to make 50 mL. To exactly 5 mL of this solution, add a 1:1 mixture of water and acetonitrile to make exactly 20 mL.

Standard Solutions Weigh accurately about 0.2 g of octanoic acid for assay, add a 1:1 mixture of water and acetonitrile to make 100 mL. Use this solution as the standard stock solution. Measure exactly 0.5 mL, 1 mL, 2.5 mL, 5 mL, and 10 mL of the standard stock solution in separate volumetric flasks, and add a 1:1 mixture of water and acetonitrile to each solution to make exactly 20 mL of each.

Procedure Analyze 20 μL each of the test solution and the standard solutions by liquid chromatography using the operating condition given below. Measure the peak areas of octanoic acid in the standard solutions to prepare a calibration curve. Determine the concentration (μg/mL) of octanoic acid in the test solution from the calibration curve and the peak area of octanoic acid in the test solution, and calculate the content of octanoic acid ($C_8H_{16}O_2$) by the formula:

$$\text{Content (\%) of octanoic acid } (C_8H_{16}O_2) = \frac{\text{Concentration (μg/mL) of octanoic acid in the test solution}}{\text{Weight (g) of the smple} \times 50}$$

Operating Conditions

Detector: Ultraviolet absorption spectrophotometer (determination wavelength 210 nm)

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

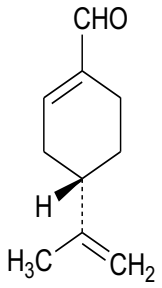
Column temperature: 30°C.

Mobile phase: A solution prepared by dissolving 0.12 g of acetic acid in 350 mL of water and adding 650 mL of acetonitrile.

Flow rate: 1.0 mL/minute.

l-Perillaldehyde

l-ペリルアルデヒド



$C_{10}H_{14}O$ Mol. Wt. 150.22

(4S)-4-(1-Methylethenyl)cyclohex -1-ene-1-carbaldehyde [18031-40-8]

Content *l*-Perillaldehyde contains not less than 90.0% of *l*-perillaldehyde ($C_{10}H_{14}O$).

Description *l*-Perillaldehyde is a colorless to light yellow, clear liquid having a strong perilla-like odor.

Identification Determine the absorption spectrum of *l*-Perillaldehyde as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.504–1.510.

Angular Rotation α_D^{20}: –110.0 to –150.0°.

Specific Gravity d_{25}^{25}: 0.962–0.970.

Purity Acid value Not more than 3.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Perlite

パーライト

Definition Perlite is obtained by calcining mineral silicon dioxide at 800–1200°C.

Description Perlite occurs as a white or light gray powder.

Identification Place 0.2 g of Perlite in a platinum crucible, dissolve it in 5 mL of hydrofluoric acid, and heat. It almost completely evaporates.

pH 5.0–9.0.

Test Solution Weigh 10.0 g of Perlite into an appropriate container, add 100 mL of water, and heat on a water bath for 2 hours with occasional shaking while replenishing the evaporated water. Cool, and filter with suction, using a filter holder equipped with a 47-mm membrane filter (0.45 μm pore size). If the filtrate is turbid, repeat the filtration with suction through the same filter. Wash the container and the residue on the filter with water, combine the washings with filtrate, and add water to make 100 mL.

Purity

(1) Water-soluble substances Not more than 0.20%.

Measure 50 mL of the test solution prepared for pH determination, evaporate to dryness, and dry the residue at 105°C for 2 hours. Weigh the residue.

(2) Hydrochloric acid-soluble substances Not more than 2.5%.

Weigh 2.0 g of Perlite, add 50 mL of diluted hydrochloric acid (1 in 4), and heat at 50°C for 15 minutes with occasional shaking. Cool, and filter. Wash the container and the residue on the filter paper with 3 mL of diluted hydrochloric acid (1 in 4), and combine the washings with the filtrate. Add 5 mL of diluted sulfuric acid (1 in 20), evaporate to dryness, ignite at 450–550°C to constant weight, and weigh the residue.

(3) Lead Not more than 10 μg/g as Pb (0.40 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Perlite, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernantant to remove the insoluble matter. Wash the residue on filter paper and the container used for centrifugation with 5 mL of hot water. Combine the washings with the filtrate, and allow to cool.

(4) Arsenic Not more than 3 μg/g as As (2.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Perlite, add 50 mL of diluted hydrochloric acid (1 in 4), and warm at 70°C for 15 minutes with a watch glass covering it while stirring occasionally. After cooling, filter the supernatant through a filter paper for quantitative analysis (5C). Wash the residue in the container three times with 10 mL each time, and add the washings to the filtrate through the filter paper. Wash the filter paper and the residue on the filter paper with 15 mL of water, combine the washings with the filtrate, and add water to make 100 mL. Use 25 mL of the resulting solution.

Loss on Ignition Not more than 3.0% (105°C 2 hours, then 1000°C 30 minutes).

Hydrofluoric Acid-insoluble Substances Not more than 37.5%.

Weigh accurately about 0.2 g of Perlite into a platinum crucible, previously ignited at 1000°C for 30 minutes, cooled in a desiccator, and weighed accurately. Weigh the crucible with the sample accurately. Add 5 mL of hydrofluoric acid and 2 drops of diluted sulfuric acid (1 in 2), evaporate to almost dryness on a water bath, and cool. Add 5 mL of hydrofluoric acid to the residue, and evaporate gently to dryness on a hot plate. Heat at 550°C for 1 hour, raise the temperature gradually, ignite at 1000°C for 30 minutes, allow

to cool in a desiccator, and weigh accurately.

Peroxidase

パーオキシダーゼ

Definition Peroxidase includes enzymes that reductively decompose hydrogen peroxide. It is derived from the cucumber *Cucumis sativus* L., horseradish (*Armoracia rusticana* P. *Gaertn*. or B. Mey. & Scherb.), the Japanese radish *Raphanus sativus* L., or the soybean *Glycine max* (L.) Merr., or the culture of basidiomycetes (limited to *Coprinus cinereus*), filamentous fungi (limited to *Aspergillus oryzae* and species of the genera *Alternari*a and *Oidiodendron*), actinomycetes (limited to *Streptomyces thermoviolaceus* and *Streptomyces violaceoruber*), or bacteria (limited to species of the genus *Bacillus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Peroxidase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Peroxidase complies with the Peroxidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Peroxidase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 0.10 g of Peroxidase, add water or potassium phosphate–

sodium hydroxide buffer (0.1 mol/L) at pH 7.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water or the same buffer to the resulting solution.

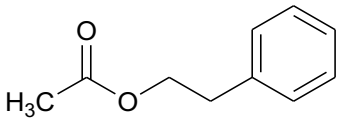
Substrate Solution Add water to 0.1 mL of hydrogen peroxide to make 100 mL.

Procedure Place 2 mL of potassium phosphate–sodium hydroxide buffer (0.1 mol/L, pH 7.0, containing phenol), 1 mL of the substrate solution, and 0.1 mL of 4-aminoantipyrine solution (1 in 250) into a quartz cell, and equilibrate at 37°C for 10 minutes. To this solution, add 0.1 mL of the sample solution, mix well, and incubate at 37°C. The absorbance of the resulting solution at a wavelength of 500 nm at 2 minutes after the addition of the sample solution is lower than that of the solution at the same wavelength at 5 minute after its addition.

Phenethyl Acetate

Phenylethyl Acetate

酢酸フェネチル



$C_{10}H_{12}O_2$ Mol. Wt. 164.20

2-Phenylethyl acetate [103-45-7]

Content Phenethyl Acetate contains not less than 98.0% of phenethyl acetate ($C_{10}H_{12}O_2$).

Description Phenethyl Acetate is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Phenethyl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.496–1.502.

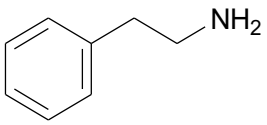
Specific Gravity d_{25}^{25}: 1.030–1.034.

Purity Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Phenethylamine

フェネチルアミン



$C_8H_{11}N$ Mol. Wt 121.18

2- Phenylethylamine [64-04-0]

Content Phenethylamine contains not less than 95.0% of phenethylamine ($C_8H_{11}N$).

Description Phenethylamine occurs as a colorless to light yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Phenethylamine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

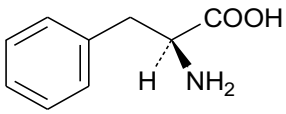
Refractive Index n_D^{25}: 1.526–1.532.

Specific Gravity d_{20}^{20}: 0.961–0.967.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (1).

L-Phenylalanine

L-フェニルアラニン



$C_9H_{11}NO_2$ Mol. Wt. 165.19

(2*S*)-2-Amino-3-phenylpropanoic acid [63-91-2]

Content L-Phenylalanine, when calculated on the dried basis, contains 98.5－102.0% of L-phenylalanine ($C_9H_{11}NO_2$).

Description L-Phenylalanine occurs as white crystals or crystalline powder having a slightly bitter taste.

Identification

(1) To 5 mL of a solution of L-Phenylalanine (1 in 1000), add 1 mL of ninhydrin

solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) To 10 mg of L-Phenylalanine, add 0.5 g of potassium nitrate and 2 mL of sulfuric acid, heat on a water bath for 20 minutes, and cool. Add 5 mL of hydroxylammonium chloride solution (1 in 10), allow to stand in ice water for 10 minutes, add 9 mL of sodium hydroxide solution (2 in 5), and allow to stand. A red-purple color develops.

(3) To 5 mL of a solution of L-Phenylalanine (1 in 100), add 1 mL of potassium permanganeta solution (1 in 100), and boil. A characteristic odor is evolved.

Specific Rotation $[\alpha]_D^{20}$: −33.0 to −35.2° (1 g, water, 50 mL, on the dried basis).

pH 5.4–6.0 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution Colorless and almost clear (0.50 g, hydrochloric acid TS (1 mol/L) 10 mL).

(2) Chloride Not more than 0.021% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of L-Phenylalanine in 5 mL of diluted hydrochloric acid (1 in 4).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

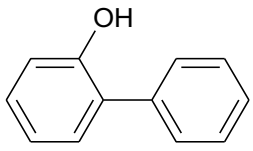
Residue on Ignition Not more than 0.1% .

Assay Weigh accurately about 0.3 g of L-Phenylalanine, and proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 16.52 mg of $C_9H_{11}NO_2$

o-Phenylphenol

オルトフェニルフェノール



$C_{12}H_{10}O$ Mol. Wt. 170.21

2-Phenylphenol [90-43-7]

Content *o*-Phenylphenol contains not less than 97.0% of *o*-phenylphenol ($C_{12}H_{10}O$).

Description *o*-Phenylphenol occurs as white, light yellow or light pink powder, or as flakes or lumps. It has a characteristic odor.

Identification

(1) To 1 mL of a solution (1 in 100) of *o*-Phenylphenol in ethanol (95), add 4 mL of a solution (1 in 500) of sodium tetraborate decahydrate and small crystals of 2,6-dichloroquinonechloroimide, and shake. The solution is blue to indigo-purple.

(2) On the surface of 1 mL of a solution (1 in 100) of *o*-Phenylphenol in ethanol (95), pour cautiously 1 mL of formaldehyde–sulfuric acid TS to form a layer. The boundary surface of the two layers turns to pink.

Melting Point 57–59 °C.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) *p*-Phenylphenol and other organic impurities Not more than 0.1% as *p*-Phenylphenol.

Test Solution Weigh 1.0 g of *o*-Phenylphenol, and dissolve it by adding 5 mL of ethanol (95) and 5 mL of a solution (1 in 1000) of caffeine monohydrate in ethanol (95).

Control Solution To 5 mL of a solution (1 in 5000) of *p*-phenylphenol in ethanol (95), add 5 mL of a solution (1 in 1000) of caffeine monohydrate in ethanol (95).

Analyze the test solution and the control solution by gas chromatography using the conditions given below. For the test solution, determine the ratio (A/A_s) of the sum (A) of the peak area of *p*-phenylphenol and the peak areas of all peaks appearing between the *o*-phenylphenol peak and the caffeine peak to the peak area (A_s) of caffeine, and for the control solution, determine the ratio (A'/A'_s) of the peak area (A′) of *p*-phenylphenol to the peak area (A'_s) of caffeine. The ratio (A/A_s) does not exceed the ratio (A'/A'_s).

Operating Conditions

Detector: Flame ionization detector.

Column: A stainless steel tube (3–4 mm internal diameter and 1 m length).

Column packing material

Liquid phase: 3% Diethylene glycol succinate polyester of the amount of support.

Support: 177- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature between 195–250°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the peak of caffeine appears about 12 minutes after injection.

Residue on Ignition Not more than 0.05% (5 g).

Assay

Test Solution Weigh accurately about 2 g of powdered *o*-Phenylphenol, add 25 mL of sodium hydroxide solution (1 in 25), dissolve it while warming if necessary, and cool. Add water to make exactly 500 mL.

Procedure Transfer exactly 25 mL of the test solution to an iodine flask, and add exactly 30 mL of potassium bromate solution (1 in 350), 5 mL of potassium bromide solution (2 in 25), and 50 mL of methanol, and shake well. Quickly add about 10 mL of diluted hydrochloric acid (1 in 2), stopper immediately, shake gently, and allow to react for 30 seconds. Place 15 mL of potassium iodide in the upper part of the iodine flask, allow to flow down by loosening the stopper, wash the stopper and mouth of the flask thoroughly with water, shake well, and allow to stand for 5 minutes.

Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 4 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner, and calculate the content by the formula:

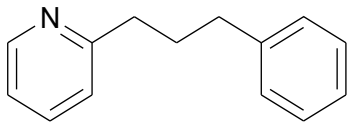
$$\text{Content (\%) of } o\text{-phenylphenol } (C_{12}H_{10}O) = \frac{4.255 \times (a - b)}{\text{Weight (g) of the sample} \times 50} \times 100$$

a = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the blank test,

b = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in this test.

2-(3-Phenylpropyl)pyridine

2-(3-フェニルプロピル)ピリジン



$C_{14}H_{15}N$ Mol. Wt. 197.28

2-(3-Phenylpropyl)pyridine [2110-18-1]

Content 2-(3-Phenylpropyl)pyridine contains not less than 97.0% of 2-(3-phenylpropyl)pyridine ($C_{14}H_{15}N$).

Description 2-(3-phenylpropyl)pyridine occurs as a colorless, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of 2-(3-phenylpropyl)pyridine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.558–1.563.

Specific Gravity d_{25}^{25}: 1.012–1.020.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (4), except for the column temperature. Adjust the column temperature by raising at a rate of 5°C/minute from 180°C to 230°C and maintaining at 230°C for 30 minutes.

Phosphated Distarch Phosphate

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Definition Phosphated Distarch Phoaphate is obtained through esterification of starch with ortho-phosphoric acid, its sodium or potassium salt, or sodium tripolyphosphate, combined with esterification with sodium trimetaphosphate or phosphorus oxychloride.

Description Phosphated Distarch Phoaphate occurs as a white to off-white powder, or as flakes or granules. It is odorless.

Identification

(1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

Purity

(1) Phosphorous Not more than 0.5% as P.

Proceed as directed in Purity (3) for Acetylated Distarch Phosphate.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (not more than 13.3 kPa, 120°C, 4 hours).

Phosphodiesterase

ホスホジエステラーゼ

Definition Phosphodiesterase includes enzymes that hydrolyze the phosphodiester linkages of nucleic acids and their analogs. It is derived from the culture of filamentous fungi (limited to *Aspergillus niger, Leptographium procerum,* and *Penicillium citrinum*)

or actinomycetes (limited to *Streptomyces aureus, Streptomyces avermitilis, Streptomyces cinnamoneus, Streptomyces griseus, Streptomyces thermoviolaceus,* and *Streptomyces violaceoruber*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Phosphodiesterase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Phosphodiesterase complies with the Phosphodiesterase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Phosphodiesterase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by either method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of Phosphodiesterase, add water to dissolve it or disperse it uniformly, and make 25 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 20 mg of adenosine 3′-monophosphate sodium salt, and dissolve it in 10 mL of sodium barbital–hydrochloric acid buffer (pH 5.0, containing sodium acetate–sodium chloride) or Tris buffer (1/7 mol/L) at pH 7.0, and filter the solution through a membrane filter (0.45 μm pore size). Prepare fresh before use.

Test Soliution Equilibrate 0.4 mL of substrate solution at 55°C for 5 minutes, add 0.1 mL of the sample solution, and shake immediately. Incubate the mixture at the same temperature for 15 minutes, add 4 mL of a 1 in 10 dilution of 60% perchloric acid, and shake. To this solution, add 0.4 mL of amidol TS, shake, then add 0.2 mL of

hexaammonium heptamolybdate tetrahydrate (83 in 1000), and shake. Cool this solution with running water for 15 minutes.

Control Solution To 0.4 mL of substrate solution, add 4 mL of a 1 in 10 dilution of 60% perchloric acid, and shake. Add 0.1 mL of the sample solution, and shake. To this solution, add 0.4 mL of amidol TS, shake, and proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 750 nm. The absorbance value of the test solution is higher than that of the control solution.

In the preparation of the test and control solutions, when the diluted perchloric acid (1 in 10) is added, the solution gets turbid, centrifuge it at 14,000 rpm for 3 minutes. To 2 mL of the supernatant, add 0.2 mL of amidol TS and 0.1 mL of hexaammonium heptamolybdate tetrahydrate (83 in 1000), shake the mixture, and cool with running water for 15 minutes.

Method 2

Sample Solution Weigh 0.25 g of Phosphodiesterase, add acetate buffer (pH5.6, containing zinc sulfate–albumin) to dissolve it or disperse it uniformly, and make 20 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Weigh 0.18 g of a mixture of guanosine 2′-and 3′-monophosphate disodium salts, and dissolve it in 40 mL of acetate buffer (pH5.6, containing zinc sulfate). Adjust the pH of this solution to 5.6 with acetic acid TS (0.1 mol/L) or sodium hydroxide TS (0.1 mol/L), and add acetate buffer (pH5.6, containing zinc sulfate) to make 50 mL. Prepare fresh before use.

Test Soliution Equilibrate 0.9 mL of the substrate solution at 65°C for 5 minutes, add 0.1 mL of the sample solution, and mix. Incubate the mixture at 65°C for 10 minutes, then add 1 mL of trichloroacetate–sodium dodecyl sulfate TS, and allow to cool. To this solution, add 2 mL of ammoniummolybdate–iron(II) sulfate TS, mix, and allow to stand at room temperature for 5 minutes.

Control Solution To 0.9 mL of the substrate solution, add 1 mL of trichloroacetate–sodium dodecylsulfate TS, and mix. Add 0.1 mL of the sample solution, and warm at 65°C for 5 minutes. After cooling, proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 750 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Phospholipase

ホスホリパーゼ

Definition Phospholipase includes enzymes that hydrolyze lecithin. It is derived from

the pancreas of animals, the cabbage *Brassica oleracea* L., or the soybean *Glycine max* (L.) Merr., or the culture of basidiomycetes (limited to species of the genus *Corticium*), filamentous fungi (limited to *Aspergillus oryzae* and *Aspergillus niger*), actinomycetes (limited to *Kitasatospora* sp., *Streptomyces avermitilis*, *Streptomyces cinnamoneus*, *Streptomyces griseus*, *Streptomyces lividans*, *Streptomyces polychromogenes*, *Streptomyces thermoviolaceus*, *Streptomyces violaceoruber*, and species of the genera *Actinomadura* and *Nocardiopsis*), or bacteria (limited to species of the genus *Bacillus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Phospholipase occurs as white to dark brown granules, powder, or paste or, as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Phospholipase complies with the Phospholipase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

The requirement of total plate count can be disregarded if unsterilized Phospholipase is used for captive consumption in the manufacture of food that will be sterilized or pasteurized before completion of the final product.

Phospholipase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Phospholipase, add water or acetate buffer (0.2 mol/L) at pH 4.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to

the resulting solution.

Substrate Solution Add gradually 1.0 g of L-α-lecithin (soybean-derived) to 50 mL of a solution of polyoxyethylene(10) octylphenylether (1 in 25) while stirring to dissolve it.

Test Solution To 0.5 mL of the substrate solution, add 0.25 mL of acetate buffer (0.2 mol/L) at pH 4.0 and 0.05 mL of calcium chloride dihydrate solution (147 in 10,000), and equilibrate at 37°C for 5 minutes. To this solution, add 0.1 mL of the sample solution, shake immediately. Incubate the mixture at 37°C for 10 minutes, then add 0.1 mL of diluted hydrochloric acid (9 in 100), and mix. To 0.028 mL of this solution, add 1.2 mL of TS A for free fatty acid determination, and mix. Warm the resulting solution at 37°C for 3 minutes in a dark place, add 0.6 mL of TS B for free fatty acid determination, and mix. Warm this solution at 37°C for 4.5 minutes in a dark place.

Control Solution To 0.5 mL of the substrate solution, add 0.25 mL of acetate buffer (0.2 mol/L) at pH 4.0 and 0.05 mL of calcium chloride dihydrate solution (147 in 10,000), and equilibrate at 37°C for 5 minutes. To this solution, add 0.1 mL of diluted hydrochloric acid (9 in 100) and 0.1 mL of the sample solution, and mix. To 0.028 mL of this solution, add 1.2 mL of the TS A, and mix. Warm the resulting solution at 37°C for 3 minutes in a dark place, add 0.6 mL of the TS B, and mix. Warm this solution at 37°C for 4.5 minutes in a dark place.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 550 nm. The absorbance value of the test solution is higher than that of control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 1.0 g of Phospholipase, add water or the buffer for the phospholipase activity test to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, 10,000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 0.5 g of L-α-lecithin (soybean-derived), dissolve it in 9.5 mL of water, and allow to stand for one night.

Test Solution To 0.1 mL of the substrate solution, add 0.1 mL of buffer for the phospholipase activity test, 0.05 mL of calcium chloride TS (0.1 mol/L), and 0.15 mL of a solution of 7.5% (w/v) polyoxyethylene(10) octylphenylether, shake, and equilibrate at 37°C for 5 minutes. To this solution, add 0.1 mL of the sample solution, and shake immediately. Incubate at 37°C for 10 minutes. Add 0.2 mL of Tris buffer (1 mol/L, pH 8.0, containing tetrasodium ethylenediaminetetraacetate), and mix. Immediately heat it in a water bath for 5 minutes. Cool this solution to 37°C, add 4 mL of TS for phospholipid determination, mix, and warm at 37°C for 20 minutes.

Control Solution Proceed as directed for the test solution using water or the buffer for the phospholipase activity test instead of the sample solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 500 nm. The absorbance value of the test solution is higher than that of control solution. If the test solution and the control solution are turbid, centrifuge them,

and use the supernatants.

Method 3

Sample Solution Weigh 1.0 g of Phospholipase, add water or hydrochloric acid TS (0.001 mol/L) to dissolve it, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold dilution by adding water or hydrochloric acid TS (0.001 mol/L) to the resulting solution.

Substrate Solution To 10.0 g of L-α-lecithin (soybean-derived), add 200 mL of water, 10 mL of calcium chloride TS (0.32 mol/L), and 100 mL of sodium deoxycholate TS (0.016 mol/L) to dissolve it, and then add water to make 500 mL. When egg yolk is used as the substrate, proceed as directed below: To one egg yolk, add 91 mL of water and 6 mL of calcium chloride TS (0.22 mol/L). Stir the mixture at 2500 rpm for 10 minutes using an emulsifying device while cooling with care not to let the mixture foam. To 25 mL of this solution, add 2.5 mL of sodium deoxycholate TS (3.3 mmol/L) and 2.5 mL of water. Store the solution in a cold place, and use it within one week.

Procedure Equilibrate 25 mL of the substrate solution at 40°C for 15 minutes (30 minutes when egg yolk is used as the substrate), and immerse a pH electrode into the solution. Adjust the pH to 8.00 ± 005 at 40°C with 0.01 mol/L sodium hydroxide, and add 2 mL of the sample solution immediately. To this solution, continuously add 0.01 mol/L sodium hydroxide dropwise so that the pH is kept at 8.00 ± 005 at 40°C for 5 minutes after the addition of the sample solution. Record the amount of sodium hydroxide added as the consumption by the test solution. Separately, using 2 mL of water or hydrochloric acid TS (0.001 mol/L) instead of the sample solution, proceed as directed for the test solution. Record the amount of sodium hydroxide added as the consumption by the control solution. The consumption by the test solution is more than that by the control solution. The whole procedure given here should be carried out while stirring.

Method 4

Sample Solution Weigh 1.0 g of Phospholipase, add water or a 100-fold dilution of Tris buffer (1 mol/L) at pH 8.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution To 3.0 mg of L-α-dipalmitoylphosphatidylcholine or L-α-phosphatidylinositol sodium salt, add 0.02 mL of Tris buffer (1 mol/L) at pH 8.0, 0.01 mL of magnesium chloride TS (0.1 mol/L), and 0.97 mL of water.

Test Solution To 1 mL of the substrate solution, add 0.1 mL of the sample solution, and incubate the mixture at 37°C for 60 minutes while stirring. After cooling, add 1 mL of a 2:1 mixture of chloroform/methanol, shake for 2 minutes, and allow to stand. Use the lower layer.

Standard Solution Dissolve 3 mg of diacylglycerol TS in 1 mL of 2:1 mixture of chloroform/methanol.

Procedure Analyze 10 μL each of the test solution and the standard solution by thin-layer chromatography, using a 30:20:1 mixture of heptane/diethyl ether/acetic acid as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer

chromatography as the solid support. When the solvent front has ascended to a point 10 cm above the starting line, stop developing. Air-dry the plate, spray with amido black TS, and examine. The spot from the test solution has the same Rf value as that from the standard solution.

Method 5

Sample Solution Weigh 1.0 g of Phospholipase, add water or acetate buffer (0.01 mol/L pH 5.5, containing magnesium chloride and calcium chloride) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 0.10 g of L-α-lyso-phosphatidylcholine, and dissolve it in 20 mL of acetate buffer (0.01 mol/L pH 5.5, containing magnesium chloride and calcium chloride), and adjust the pH to 5.5 with hydrochloric acid TS (2 mol/L) or sodium hydroxide TS (1 mol/L).

Test Solution To 1.0 mL of the substrate solution, equilibrated at 37°C for about 5 minutes, add 0.1 mL of the sample solution, and shake immediately. Incubate the mixture at 37°C for 5 minutes. To 0.05 mL of this solution, add 0.5 mL of TS A for free fatty acid determination, mix, and warm the mixture at 37°C for 5 minutes in a dark place. Then add 1.0 mL of TS B for free fatty acid determination, mix, and warm at 37°C for 5 minutes in a dark place.

Control Solution Proceed as directed for the test solution using acetate buffer (0.01 mol/L pH 5.5, containing magnesium chloride and calcium chloride) instead of the sample solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 550 nm. The absorbance value of the test solution is higher than that of control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Phosphoric Acid

リン酸

H_3PO_4 Mol. Wt. 98.00

Phosphoric acid [7664-38-2]

Content Phosphoric Acid contains not less than 75.0% of phosphoric acid (H_3PO_4).

Description Phosphoric acid is a colorless, clear syrupy liquid. It is odorless.

Identification To a solution of Phosphoric Acid (1 in 20), add 2–3 drops of phenolphthalein TS, and neutralize with sodium hydroxide (1 in 25). The solution responds to all the tests for Phosphate in the Qualitative Tests.

Specific Gravity d_{20}^{20}: Not less than 1.579.

Purity

(1) Clarity of solution　Colorless and almost clear (4.0 mL, ethanol (95) 16 mL).

(2) Sulfate　Not more than 0.14% as SO_4.

Test Solution　Weigh 0.20 g of Phosphoric Acid, and add water to make 50 mL.

Control Solution　To 0.60 mL of 0.005 mol/L sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(3) Lead　Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution　To the specified amount of Phosphoric Acid, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter.　Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay　Weigh accurately about 1.5 g of Phosphoric Acid, dissolve it in 25 mL of water, keep at about 15°C, and titrate with 1 mol/L sodium hydroxide (indicator: 5 drops of thymolphthalein TS) until the color of the solution changes to light blue.

Each mL of 1 mol/L sodium hydroxide = 49.00 mg of H_3PO_4

Phytase

フィターゼ

Definition　Phytase includes enzymes that degrade phytic acid. It is derived from the culture of filamentous fungi (limited to *Aspergillus niger*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description　Phytase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification　Phytase complies with the Phytase Activity Test.

Purity

(1) Lead　Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits　Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid　Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture　Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Phytase Activity Test　Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution　Weigh 0.40 g of Phytase, and add acetate buffer (0.005 mol/L) at pH 5.5 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution　Weigh 0.200 g of sodium phytate, and dissolve it in about 50 mL of acetate buffer (0.2 mol/L) at pH 5.5. Adjust its pH to 5.5 with diluted acetic acid (3 in 250), and add the same buffer to make 100 mL. Prepare fresh before use.

Test Solution　Equilibrate 0.5 mL of the sample solution at 37°C for 5 minutes, add 0.5 mL of the substrate solution, and shake immediately.　Incubate the mixture at 37°C for 10 minutes. To this solution, add 2 mL of ammonium molybdate–sulfuric acid TS (for phytase activity test) cooled in icy water, and shake.

Control Solution　To 0.5 mL of the sample solution, add 2 mL of ammonium molybdate–sulfuric acid TS (for phytase activity test), previously cooled in icy water, shake well, then add 0.5 mL of the substrate solution, and shake well.

Procedure　Add 0.1 mL each of a solution of citric acid monohydrate (21 in 100) to the test solution and the control solution, and shake. Measure the absorbance of them at a wavelength of 380 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Phytic Acid

フィチン酸

Definition　Phytic Acid is obtained from the seed bran of the rice plant *Oryza sativa* L. or the seeds of the corn plant *Zea mays* L. by extraction with water or acidic solution and purification. It consists mainly of inositol hexaphosphate. There are two types of products: Liquid Phytic Acid and Powder Phytic Acid. Powder Phytic Acid may contain

dextrin or reduced sugar syrup.

Liquid Phytic Acid

Content Liquid Phytic Acid contains 48.0–52.0% of phytic acid (inositol hexaphosphate) ($C_6H_{18}O_{24}P_6$ = 660.04).

Description Liquid Phytic Acid occurs as a colorless to light yellow-brown syrup-like liquid. It is odorless.

Identification

(1) A solution of Liquid Phytic Acid (1 in 10) is acidic.

(2) To a solution of Liquid Phytic Acid (1 in 10), add 3 drops of phenolphthalein TS, neutralize with sodium hydroxide solution (1 in 10), and add silver nitrate solution (1 in 100) dropwise. A white colloidal precipitate is produced.

(3) Decompose 1 mL of Liquid Phytic Acid with 3 mL of sulfuric acid in a Kjeldahl flask by heating for 3 hours. After cooling, add 8 mL of water and 3 drops of phenolphthalein TS, and neutralize with sodium hydroxide solution (1 in 10). The resulting solution responds to test (2) for Phosphate in the Qualitative Tests.

(4) Decompose 3 mL of Liquid Phytic Acid with 7 mL of 30% sulfuric acid in a well stoppered pressure-resistant test tube by heating at 130°C for 5 hours. Neutralize with sodium hydroxide solution (1 in 10), and add water to make 50 mL. To the resulting solution, add 0.5 g of active carbon, stir for 10 minutes, and filter. To 30 mL of the filtrate, add 0.5 mL of barium chloride dihydrate solution (1 in 10), and evaporate to dryness. The residue is a light red.

Purity

(1) Chloride Not more than 0.040% as Cl (0.40 g, Control Solution: 0.01 mol/L hydrochloric acid 0.45 mL).

(2) Sulfate Not more than 0.072% as SO_4 (0.40 g, Control Solution: 0.005 mol/L sulfuric acid 0.60 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Free inorganic phosphorus Not more than 1.0%.

Test Solution Weigh 0.5 g of Liquid Phytic Acid, and dissolve it in water to make exactly 200 mL. To exactly 3 mL of this solution, add 5 mL of L(+)-ascorbic acid solution (1 in 100), and add 5 mL of a solution prepared by dissolving 1 g of hexaammonium heptamolybdate tetrahydrate in 100 mL of sulfuric acid TS (0.025 mol/L). Then add acetic acid buffer (pH 4.0) to make exactly 50 mL, and allow to stand for 15 minutes.

Reference Solution To 5 mL of L(+)-ascorbic acid solution (1 in 100), add 5 mL of a

solution prepared by dissolving 1 g of hexaammonium heptamolybdate tetrahydrate in 100 mL of sulfuric acid TS (0.025 mol/L). Then add acetic acid buffer (pH 4.0) to make exactly 50 mL.

Procedure Measure the absorbance of the test solution at a wavelength of 750 nm against the reference solution. Prepare a calibration curve by measuring the absorbance of the following three solutions. Determine the concentration of free inorganic phosphorus from the calibration curve and the absorbance of the test solution, and then calculate the amount (%) of free inorganic phosphorus in the sample.

Solutions for Calibration Curve To exactly 5 mL of Phosphorus Standard Solution, add water to make exactly 1000 mL. Place exactly 5 mL, 10 mL and 20 mL of this solution into separate volumetric flasks, add 5 mL of L(+)-ascorbic acid solution (1 in 100) to each, and proceed as directed for the test solution.

Assay

Test Solution Transfer about 1.5 g of Liquid Phytic Acid, accurately weighed, into a 300-mL Kjeldahl flask, add 10 mL of sulfuric acid and 2.5 mL of nitric acid, and heat until the solution becomes transparent to decompose it. After cooling, transfer the resulting solution into a 500-mL volumetric flask, and add water to the volume. Transfer exactly 3 mL of the resulting solution into a 100-mL volumetric flask, neutralize with ammonia solution (1 in 4), and then add diluted nitric acid (1 in 10) to make slightly acidic. To this solution, add 20 mL of vanadic acid–molybdic acid TS and water to make exactly 100 mL. Agitate the solution and allow to stand for 30 minutes.

Procedure Measure the absorbance of the test solution at a wavelength of 420 nm. Prepare a calibration curve by measuring the absorbance of the following three solutions. Determine the total phosphorus concentration in the test solution from the calibration curve and the absorbance of the test solution, and then calculate the total phosphorus amount (%) in the sample. Determine the content of phytic acid from the total phosphorus amount (%) and the amount (%) of free inorganic phosphorus obtained in Purity (5).

$$\text{Content (\%) } \textit{of phytic acid } (\textit{inositol hexaphosphate}) \ (C_6H_{18}O_{24}P_6)$$
$$= \left(\textit{total phosphrus amount } (\%) - \textit{free inorganic phosphrus amount } (\%)\right) \times 3.552$$

Solutions for calibration curve To exactly 10 mL of Phosphorus Standard Solution, add water to make exactly 100 mL. Place exactly 5 mL, 10 mL and 20 mL of this solution into 100-mL separate volumetric flasks, proceed as directed for the test solution, and allow them to color.

Powder Phytic Acid

Content Powder Phytic Acid contains the equivalent of not less than 27.0% of phytic acid (inositol hexaphosphate) ($C_6H_{18}O_{24}P_6$ = 660.04) and the equivalent of 90–110% of the labeled content of phytic acid.

Description Powder Phytic Acid occurs as a light yellow to brown powder. It is odorless.

Identification

(1) A solution of Powder Phytic Acid (1 in 10) is acidic.

(2) To a solution of Powder Phytic Acid (1 in 10), add 3 drops of phenolphthalein TS, neutralize with sodium hydroxide (1 in 10), and add silver nitrate solution (1 in 100) dropwise. A white colloidal precipitate is produced.

(3) Decompose 1.5 g of Powder Phytic Acid with 3 mL of sulfuric acid in a 300-mL Kjeldahl flask by heating for 3 hours. After cooling, add 8 mL of water and 3 drops of phenolphthalein TS, and neutralize with sodium hydroxide (1 in 10). The resulting solution responds to test (2) for Phosphate in the Qualitative Tests.

(4) Weigh 3.5 g of Powder Phytic Acid, and dissolve it in 100 mL of water. Pour this solution into a column packed with 42 mL of weakly basic anion-exchange resin (free form), and run it through at a flow rate of 100–200 mL/hour. Wash the column with 200 mL of water by running it through at the same flow rate. Run 100 mL of sulfuric acid TS (0.5 mol/L) and then 100 mL of water in the same manner. Heat 200 mL of the eluate under reduced pressure to remove water and concentrate to 10 mL. Transfer it into a stoppered pressure-resistant test tube, and proceed as directed in Identification (4) for Liquid Phytic Acid.

Purity

(1) Chloride Not more than 0.040% as Cl (0.40 g, Control Solution: 0.01 mol/L hydrochloric acid 0.45 mL).

(2) Sulfate Not more than 0.072% as SO_4 (0.40 g, Control Solution: 0.005 mol/L sulfuric acid 0.60 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

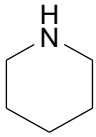
(5) Free inorganic phosphorus Not more than 1.0%.

Proceed as directed in Purity (5) for Liquid Phytic Acid.

Assay Proceed as directed in Assay for Liquid Phytic Acid.

Piperidine

ピペリジン



$C_5H_{11}N$ Mol. Wt. 85.15

Piperidine [110-89-4]

Content: Piperidine contains not less than 98.0% of piperidine ($C_5H_{11}N$).

Description: Piperidine occurs as a colorless to light yellow, clear liquid having a characteristic odor.

Identification: Determine the infrared absorption spectrum of Piperidine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.450–1.454.

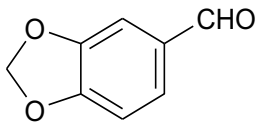
Specific Gravity d_{25}^{25}: 0.858–0.862.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2).

Piperonal

Heliotropin

ピペロナール



$C_8H_6O_3$ Mol. Wt. 150.13

Benzo[*d*][1,3]dioxole-5-carbaldehyde [120-57-0]

Content Piperonal contains not less than 98.0% of piperonal ($C_8H_6O_3$).

Description Piperonal occurs as white crystals or lumps having a heliotrope-like odor.

Identification If the sample is a solid, melt it by warming to prepare the sample. Determine the absorption spectrum of Piperonal as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

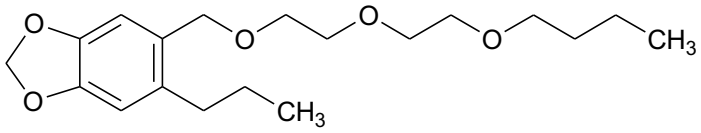
Melting Point 36–37.5°C.

Purity Acid value Not more than 3.0 (Flavoring Substances Tests).

Assay Using a solution (1 in 10) of Piperonal in acetone as the test solution, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Piperonyl Butoxide

ピペロニルブトキシド



$C_{19}H_{30}O_5$ Mol. Wt. 338.44

5-{[2-(2-Butoxyethoxy)ethoxy]methyl}-6-propylbenzo[*d*][1,3]dioxole [51-03-6]

Description Piperonyl Butoxide is a colorless to light brown, transparent oily liquid. It is odorless or has a slightly odor.

Identification

(1) To 0.5 mL of a solution (1 in 1000) of Piperonyl Butoxide in methanol, add 20 mL of tannic acid–acetic acid TS, and heat in a water bath with occasional shaking. A blue color develops.

(2) A solution (1 in 100,000) of Piperonyl Butoxide in 90% (vol) methanol exhibits absorption maxima at wavelengths of 236–240 nm and 288–292 nm, and the ratio of the absorbance at a wavelength 236–240 nm to that at 288–292 nm is 1.13–1.24.

Refractive Index n_D^{20}: 1.497–1.512.

Specific Gravity d_{20}^{20}: 1.05–1.07.

Purity

(1) Color The color of Piperonyl Butoxide is not deeper than that of the solution prepared by mixing 1.4 mL of Cobalt(II) Chloride CSSS, 4.3 mL of Iron(III) Chloride CSSS, and 0.3 mL of Copper(II) Sulfate CSSS.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Chlorinated compounds Not more than 0.035% as Cl.

Test Solution Weigh 0.50 g of Piperonyl Butoxide, transfer into a porcelain crucible, add 2 mL of sodium carbonate solution (1 in 8), heat on a water bath for 1 hour with occasional shaking, and evaporate almost completely to dryness. Add 1 g of calcium carbonate, carbonize almost completely by heating weakly, and incinerate almost completely by heating to about 600°C, and cool. Dissolve the residue, by adding 35 mL of diluted nitric acid (1 in 10) gradually, and filter. Wash the insoluble residue with 10 mL of water, combine the washings with filtrate, and add water to make 50 mL.

Control Solution Weigh 1 g of calcium carbonate, add 2 mL of sodium carbonate solution (1 in 8), dissolve it by gradually adding 35 mL of diluted nitric acid (1 in 10), and filter. Wash the insoluble residue with 10 mL of water, combine the washings with the filtrate, and add 0.5 mL of 0.01 mol/L hydrochloric acid and water to make 50 mL.

Procedure Add 0.5 mL of silver nitrate solution (1 in 50) to the test solution and the control solution, shake well, and allow to stand for 5 minutes. The test solution is not more turbid than the control solution.

(4) Distillation test

Residue after distillation at up to 194°C Not less than 85.0%.

Residue after distillation at up to 203°C Not more than 5.0%.

Weigh 25 g of Piperonyl Butoxide into a 100-mL eggplant shaped flask, previously accurately weighed, then weigh accurately the flask containing the sample, distill to 194°C under reduced pressure of 0.53 kPa, and weigh the residue in the flask. Distill up to 203°C under reduced pressure of 0.53 kPa, and weigh the residue in the flask.

Polybutene

Polybutylene

ポリブテン

Definition Polybutene is a polymer consisting mainly of isobutylene.

Description Polybutene is a colorless to pale yellow, viscous liquid. It is odorless or has a slight, characteristic odor. It is tasteless.

Identification Dissolve about 1 g of Polybutene in 5 mL of hexane, and proceed as directed in the Thin Film Method under Infrared Spectrophotometry. Polybutene exhibits absorption bands at about 1393 cm^{-1}, 1370 cm^{-1}, 1230 cm^{-1}, 950 cm^{-1}, and 920 cm^{-1}.

Purity

(1) Clarity of solution Clear (0.50 g, hexane 5.0 mL).

(2) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Chlorinated compounds Not more than 0.014% as Cl.

Proceed as directed in Purity (4) for Polyisobutylene, using 0.20 mL of 0.01 mol/L hydrochloric acid.

(5) Low molecular weight polymer Not more than 0.40%.

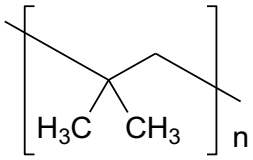
Weigh accurately about 10 g of Polybutene, and add 10 mL of methanol. Heat under a reflux condenser on a water bath for 1 hour with occasional shaking, and allow to stand in a cool place for 1 hour. Filter it into a flask, previously dried and accurately weighed, and evaporate the filtrate to dryness under reduced pressure at about 50°C. Dry in a vacuum desiccator for 20 hours, and weigh the residue accurately.

Residue on Ignition Not more than 0.05% (5 g).

Polyisobutylene

Butyl Rubber

ポリイソブチレン



$(C_4H_8)_n$

Poly(1,1-dimethylethylene) [9003-27-4]

Definition Polyisobutylene is a polymer of isobutylene. It may contain up to 2% of isoprene as a polymer component.

Description Polyisobutylene occurs as a colorless to light yellow, elastic rubbery semi-solid or viscous substance. It is odorless or has a slight, characteristic odor, and is tasteless.

Identification Dissolve about 1 g of Polyisobutylene in 5 mL of hexane, and proceed as directed in the Thin Film Method under Infrared Spectrophotometry. Polyisobutylene exhibits absorption bands at about 1393 cm^{-1}, 1370 cm^{-1}, 1230 cm^{-1}, 950 cm^{-1}, and 920 cm^{-1}.

Purity

(1) Clarity of solution Slightly turbid.

Test Solution Weigh 0.50 g of Polyisobutylene, add 50 mL of hexane, and dissolve it while heating in a water bath at about 80°C.

(2) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Chlorinated compounds Not more than 0.028% as Cl.

Test Solution Weigh 0.50 g of Polyisobutylene and 0.7 g of calcium carbonate, transfer them into a porcelain crucible, mix with a small amount of water, dry at 100°C, and heat at about 600°C for 10 minutes. After cooling, dissolve the residue by adding 20 mL of diluted nitric acid (1 in 10), filter, and wash the insoluble residue with about 15 mL of water. Combine the washings with filtrate, and add water to make 50 mL.

Control Solution Dissolve 0.7 g of calcium carbonate in 20 mL of diluted nitric acid

(1 in 10), filter if necessary, and add 0.40 mL of 0.01 mol/L hydrochloric acid and water to make 50 mL.

Procedure Add 0.5 mL of silver nitrate solution (1 in 50) to the test solution and the control solution, shake well, and allow to stand for 5 minutes. The test solution is not more turbid than the control solution.

(5) Total unsaturated substances Not more than 2.0%.

Weigh accurately about 0.5 g of Polyisobutylene, previously prepared by cutting up in thin strips, into a flask, and add 100 mL of cyclohexane. Stopper the flask, allow to stand over night to dissolve. If insoluble substances remain, shake for about 1 hour to dissolve them completely. Transfer in a 500-mL flask, wash the flask with a small amount of cyclohexane, and add the washings to the 500-mL flask. Add exactly 15 mL of Wijs TS, and mix well. If the solution is not clear, add cyclohexane until it becomes clear. Stopper the flask, and allow to stand for 30 minutes at 20–30°C, protected from light, with occasional shaking. Add 20 mL of potassium iodine (1 in 10) and 100 mL of water, and shake. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner, and make any necessary correction. Calculate the content of total unsaturated substances by formula:

$$\text{Content (\%) of total unsaturated substances} = [1.87 \times (a - b) \times 0.1]/\text{Weight (g) of the sample}$$

a = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the blank test,

b = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the test.

(6) Low molecular weight polymer Not more than 1.2%.

Weigh accurately about 10 g of Polyisobutylene in a flask, and add 40 mL of cyclohexane. Dissolve it by heating under a reflux condenser on a water bath for 1 hour with occasional shaking. After cooling, add 40 mL of methanol, shake well, and allow to stand in a cold place for 1 hour. Filter the liquid obtained into a flask, previously dried and accurately weighed, and evaporate the filtrate to dryness under reduced pressure at about 50°C. Dry the residue in a vacuum desiccator for 20 hours, and weigh it accurately.

Residue on Ignition Not more than 0.2%.

ε-Polylysine

ε-ポリリシン

Definition ε-Polylysine is obtained from the culture fluid of the actinomycete *Streptomyces albulus* by adsorption and isolation using ion-exchange resin. It consists mainly of ε-polylysine and may contain dextrin.

Content ε-Polylysine contains not less than 25% of ε-polylysine and contains 95–115% of the labeled content of ε-polylysine.

Description ε-Polylysine occurs as a light yellow liquid or highly hygroscopic powder. It has a slightly bitter-taste.

Identification

(1) To 1 mL of a solution of ε-Polylysine (1 in 1000), add 1 mL of Dragendorff reagent. A red-brown precipitate is produced.

(2) To 1 mL of a solution of 0.1 g of ε-Polylysine in 100 mL of phosphate buffer (pH 6.8), add 1 mL of methyl orange TS. A red-brown precipitate is produced.

(3) To 1 mL of a solution of ε-Polylysine (1 in 100), add 1 mL of hydrochloric acid, and heat at 110°C for 24 hours. After cooling, add sodium hydroxide solution (1 in 5) to adjust its pH to 6–8. Use this solution as the test solution. Separately, prepare a control solution by dissolving 10 mg of L-lysine monohydrochloride in 10 mL of water. Analyze 2 μL each of the test solution and the control solution by thin-layer chromatography using a 4:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry. Spray evenly with ninhydrin solution (1 in 50) in acetone, and heat at 90°C for 10 minutes to allow the color to develop. Examine the plate in daylight. The spot from the test solution corresponds in color tone and R_f value to the red-violet spot from the control solution.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (an amount equivalent to 0.5 g of ε-polylysine, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Residue on Ignition Not more than 1.0% (sample amount: equivalent of 0.5 g ε-polylysine).

Assay

Test Solution Weigh accurately an amount of the sample equivalent to about 0.25 g of ε-polylysine, and dissolve it in the mobile phase prepared as directed in Operating Conditions to make exactly 50 mL. To 1 mL of this solution, add 10 mL of the internal standard solution, prepared as directed below, and then add the mobile phase to make exactly 50 mL.

Internal Standard Dissolve 0.15 g of L-phenylalanine in the mobile phase to make exactly 100 mL, and dilute 5 mL of the resulting solution with the mobile phase to make exactly 100 mL.

Standard Solutions Weigh accurately about 0.3 g of ε-polylysine monohydrochloride for assay, previously dried at 105°C for 3 hours, and add the mobile phase to make exactly

100 mL. To exactly 25 mL of this solution, add the mobile phase to make exactly 100 mL (standard stock solution). Transfer 6 mL, 8 mL, and 10 mL of the standard stock solution into separate 50-mL volumetric flasks, add 10 mL of the internal standard solution to each, and dilute with the mobile phase to volume to prepare standard solutions. Calculate the concentration of ε-polylysine in each standard solution, assuming that the weight ratio of ε-polylysine to ε-polylysine monohydrochloride is 0.7785.

Procedure Analyze 100 μL each of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Prepare a calibration curve, using the concentrations of ε-polylysine in the standard solutions and the peak area ratios of ε-polylysine to L-phenylalanine for the standard solutions. Determine the peak area ratio of ε-polylysine to L-phenylalanine for the test solution, and calculate the ε-polylysine content using the calibration curve.

Operating Conditions

Detector: Ultraviolet absorption spectrophotometer (wavelength: 215 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of about 40°C.

Mobile phase: Use the solution prepared as follows: Dissolve 1.74 g of dipotassium hydrogen phosphate and 1.42 g of sodium sulfate decahydrate in about 800 mL of water, adjust the pH to 3.4 with phosphoric acid, and add water to make 1000 mL. To 920 mL of this solution, add 80 mL of acetonitrile.

Flow rate: Adjust the retention time of polylysine to about 4 minutes.

Polyphenol Oxidase

ポリフェノールオキシダーゼ

Definition Polyphenol Oxidase includes enzymes that oxidize the hydroxyl group of polyphenols. It is derived from the culture of basidiomycetes (limited to *Polyporus cinereus*, *Pycnoporus coccineus*, *Polyporus versicolor*, and species of the genera *Cyathus and Trametes*), filamentous fungi (limited to *Aspergillus niger*, *Myrothecium verrucaria,* and species of the genera *Alternaria and Coriolus*), actinomycetes (limited to *Streptomyces avermitilis*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Polyphenol Oxidase occurs as white to dark brown or white to greenish-

white granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Polyphenol Oxidase complies with the Polyphenol Oxidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Polyphenol Oxidase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 1.0 g of Polyphenol Oxidase, add borate buffer (0.02 mol/L) at pH 8.0 or water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Procedure Place 1 mL of phenol TS (0.25 mol/L) into a glass cell, add 1 mL of 4-aminoantipyrine TS (0.009 mol/L) and 0.5 mL of buffer for the polyphenol oxidase activity test, mix, and equilibrate the mixture at 30°C for 10 minutes. To this solution, add 0.5 mL of the sample solution, equilibrated at 30°C. Measure the absorbance of each solution at a wavelength of 505 nm at both 10 seconds and 40 seconds after the addition of the sample solution. The absorbance at 10 seconds is lower than that at 40 seconds.

Polysorbate 20

Polyoxyethylene (20) Sorbitan Monolaurate

ポリソルベート 20

[9005-64-5]

Definition Polysorbate 20 is a mixture of laurate partial esters of D-sorbitol and D-sorbitol anhydrides condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

Content Polysorbate 20 contains 70.0–74.0% of oxyethylene groups ($-OCH_2CH_2$ = 44.05).

Description Polysorbate 20 occurs as a colorless to orange-yellow oily liquid having a faint, characteristic odor.

Identification

(1) Determine the absorption spectrum of Polysorbate 20 as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Weigh 0.1 g of Polysorbate 20 in a flask, add 2 mL of a solution (1 in 50) of sodium hydroxide in methanol, and heat under a reflux condenser for 30 minutes in a water bath. Add 2 mL of boron trifluoride–methanol TS through the condenser, and heat for 30 minutes. Then add 4 mL of heptane through the condenser, and heat for 5 minutes. After cooling, add 10 mL of saturated sodium chloride solution, shake for about 15 seconds, and further add saturated sodium chloride solution to raise the liquid surface to the mouth of the flask. Collect 2 mL of the upper layer, wash three times with 2 mL of water each time, and dehydrate with sodium sulfate. Use the resulting solution as the test solution. Separately, prepare a control solution by dissolving 50 mg of methyl laurate, 50 mg of methyl palmitate, 80 mg of methyl stearate, and 0.10 g of methyl oleate in 50 mL of heptane. Analyze 1 μL each of the test solution and the control solution by gas chromatography using the conditions given below. The chromatogram of the test solution shows a peak at the retention time of methyl laurate.

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 30 m length) coated with a 0.5-μm thick layer of polyethylene glycol for gas chromatography.

Column temperature: Upon injection at 80°C, raise the temperature at a rate of 10°C/minute to 220°C, and maintain at 220°C for 40 minutes.

Inlet temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the peak of methyl laurate appears 10 minutes after injection and the peaks of methyl stearate and methyl oleate are separated.

Injection method: Split.

Split ratio: 1:50.

Saponification Value 40–55 (2.0 g, Flavorings Substances Tests).

Hydroxyl Value 96–108 (Fats and Related Substances Tests).

Purity

(1) Acid value Not more than 2.0 (Flavorings Substances Tests).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Ethylene oxide and dioxane

Not more than 1.0 μg/g for ethylene oxide.

Not more than 10 μg/g for 1,4-dioxane.

Test Solution Weigh accurately about 1 g of Polysorbate 20 into a specified head space vial, and add exactly 1 mL of water.

Standard Solution To 2.5 mL of ethylene oxide–tetrahydrofuran TS for polysorbate, measured exactly, add water to make exactly 100 mL. Measure exactly 1 mL of this solution, add water to make exactly 100 mL, and use the resulting solution as the ethylene oxide standard stock solution. To about 1 g of 1,4-dioxane, weighed accurately, add water to make exactly 100 mL. Measure 1 mL of this solution, add water to make exactly 200 mL, and use the resulting solution as the 1,4-dioxane standard stock solution. Prepare a standard solution by exactly measuring 5 mL of the ethylene oxide standard stock solution and 10 mL of the dioxane standard stock solution in a 50-mL volumetric flask and diluting to volume with water.

Control Solution Weigh accurately about 1 g of Polysorbate 20 in a specified head space vial, and add exactly 1 mL of the standard solution.

Procedure Stopper the vials, shake them well while warming until they are uniform. Analyze by head-space gas chromatography using the following operating conditions. Measure the peak areas, (A_{Te} and A_{Td}) and (A_{Re} and A_{Rd}), of ethylene oxide and 1,4-dioxane for each of the test solution and the control solution. Determine the each amount of ethylene oxide and 1,4-dioxane in the sample by the formula:

$$\text{Amount (μg/g) of ethylene oxide} = \frac{A_{Te} \times C_e}{(A_{Re} \times M_T) - (A_{Te} \times M_R)}$$

$$\text{Amount } (\mu g/g) \text{ of 1,4-dioxane} = \frac{A_{Td} \times C_d}{(A_{Rd} \times M_T) - (A_{Td} \times M_R)}$$

M_T = weight (g) of the sample in the test solution,

M_R = weight (g) of the sample in the control solution,

C_e = amount (μg/g) of ethylene oxide added to the control solution,

C_d = amount (μg/g) of 1,4-dioxane added to the control solution.

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 60 m length) coated with a 1.4-μm thick layer of 25% diphenyl/75% dimethylpolysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 40°C for 10 minutes, raise at 10°C/minute to 100°C, and maintain for 10 minutes. Then raise the temperature at 20°C/minute to 230°C.

Injection port temperature: A constant temperature of about 150°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Helium or nitrogen.

Flow rate: Adjust so that the peak of 1,4-dioxane appears 22 minutes after injection.

Injection method: Split.

Split ratio: 1:20.

Headspace sampler

Vial equilibration temperature: 70°C

Vial equilibration time: 45 minutes

Inlet-line temperature: 80°C

Injection amount: 1.0 mL

Column selection: Use a column capable of completely resolving peaks of acetaldehyde, ethylene oxide, and 1,4-dioxane in that order, when the solution prepared as directed below is chromatographed using the above conditions.

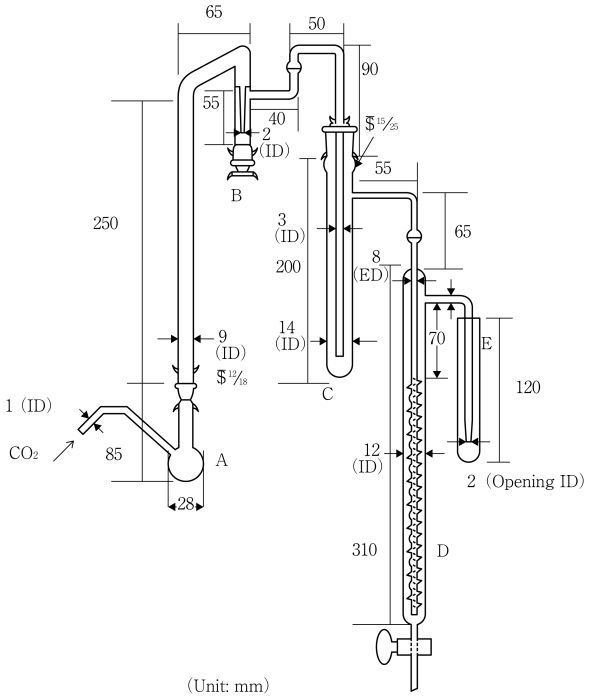
Solution: Place 1.0 mL of the standard solution into a specified headspace vial, add 0.10 mL of acetaldehyde solution (1 in 500,000), prepared fresh, tightly stopper, and shake well.

Water Content Not more than 3.0% (1 g, Volumetric Titration, Back Titration).

Residue on Ignition Not more than 0.25% (5 g, 800°C, 15 minutes).

Assay

(1) *Apparatus*　Use the apparatus as illustrated in the figure.



A: Side-arm reaction flask.

B: Condenser trap

C: Absorption tube

D: Absorption tube (the stopcock is lubricated with silicon grease)

E: Terminal absorption tube.

(2) *Procedure*　Fill trap B with a suspension of 60 mg of red phosphorus in 100 mL of water. Place exactly 10 mL of silver nitrate–ethanol TS in absorption tube C, exactly 15 mL bromine–potassium bromide TS for oxyethylene determination in absorption tube D, and exactly 10 mL of potassium iodide solution (1 in 10) in tube E. Place about 65 mg of the sample, weighed accurately, in reaction flask A, together with 10 mL of hydriodic acid and boiling chips. Connect reaction flask A to condenser trap B. Pass a slow stream of carbon dioxide through the apparatus at a rate allowing about one bubble to come out each second. Heat the flask gently in an oil bath to 140–150°C, and maintain at this temperature for at least 40 minutes to allow the mixture to react. Continue heating until the cloudy reflux in the condenser becomes clear and until the supernatant liquid in absorption tube C is almost completely clarified. Five minutes before the reaction is terminated, heat absorption tube C to 50–60°C in a hot water bath to expel the dissolved olefin completely. At the completion of decomposition, cautiously disconnect tubes D and C in the order named, then disconnect the carbon dioxide source, and remove flask A from the oil bath. Connect flask D, by its lower adapter, to a 500-mL iodine flask containing 150 mL of water and 10 mL of potassium iodide solution (1 in 10). Disconnect

tube E, rinse the side-arm of tube D with water into tube E. Allow the solution in tube D to run into the iodine flask through the stopcock, rinse the inner tube and spiral with water, and add the washings to the iodine flask. Add the contents in tube E to the iodine flask, rinse the inside of tube E with water, add the washings to the iodine flask, stopper, and allow to stand for 5 minutes. Add 5 mL of 10% sulfuric acid TS, and immediately titrate with 0.05 mol/L sodium thiosulfate (indicator: 2 mL of starch TS). Separately, perform a blank test to make necessary correction. Transfer the liquid in tube C into a flask, rinse the inside of tube C with water, and add the washings to the iodine flask to make 150 mL. Heat to boiling. After cooling, titrate with 0.05 mol/L ammonium thiocyanate (indicator: 3 mL of ammonium iron(III) sulfate TS for oxyethylene determination). Perform a blank test to make necessary correction. Calculate the oxyethylene content in the sample by the following formula:

$$\text{Content (\%) of oxyethylene} = \frac{(B - S) \times 0.05 \times 2.203}{M} + \frac{(B' - S') \times 0.05 \times 4.405}{M}$$

B = amount (mL) of 0.05 mol/L sodium thiosulfate consumed in the blank test,

S = amount (mL) of 0.05 mol/L sodium thiosulfate consumed in the test,

B′ = amount (mL) of 0.05 mol/L ammonium thiocyanate consumed in the blank test,

S′ = amount (mL) of 0.05 mol/L ammonium thiocyanate consumed in the test,

M = weight (g) of the sample.

Polysorbate 60

Polyoxyethylene (20) sorbitan monostearate

ポリソルベート 60

[9005-67-8]

Definition Polysorbate 60 is a mixture of stearate and palmitate partial esters of sorbitol and sorbitol anhydrides condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

Content Polysorbate 60 contains 65.0–69.5% of oxyethylene groups ($-OCH_2CH_2$ = 44.05).

Description Polysorbate 60 occurs as a colorless to orange oily liquid or semigel having a faint, characteristic odor.

Identification

(1) If necessary, warm the sample to dissolve. Determine the absorption spectrum of Polysorbate 60 as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Proceed as directed under Identification (2) for Polysorbate 20. The chromatogram of the test solution shows peaks at the retention times of methyl stearate and methyl palmitate.

Saponification Value 45–55 (2.0 g, Flavorings Substances Tests).

Hydroxyl Value 81–96 (Fats and Related Substances Tests).

Purity

(1) Acid value Not more than 2.0 (Flavorings Substances Tests).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Ethylene oxide and dioxane

Not more than 1.0 μg/g for ethylene oxide.

Not more than 10 μg/g for 1,4-dioxane.

Proceed as directed under Purity (4) for Polysorbate 20.

Water Content Not more than 3.0% (1 g, Volumetric Titration, Back Titration).

Residue on Ignition Not more than 0.25% (5 g, 800°C, 15 minutes).

Assay Using about 65 mg of the sample, weighed accurately, proceed as directed under Assay for Polysorbate 20.

Polysorbate 65

Polyoxyethylene (20) sorbitan tristearate

ポリソルベート 65

[9005-71-4]

Definition Polysorbate 65 is a mixture of stearate and palmitate partial esters of D-sorbitol and D-sorbitol anhydrides condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

Content Polysorbate 65 contains 46.0–50.0% of oxyethylene groups ($-OCH_2CH_2-$ = 44.05).

Description Polysorbate 65 occurs as a white to yellow-brown solid having a faint, characteristic odor.

Identification

(1) Dissolve the sample by heating. Determine the absorption spectrum of Polysorbate 65 as directed in the Thin Film Method under Infrared Spectrophotometry, and compare

with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Proceed as directed under Identification (2) for Polysorbate 20. The chromatogram of the test solution shows peaks at the retention times of methyl stearate and methyl palmitate.

Congealing Point 29–33°C.

Saponification Value 88–98 (2.0 g, Flavorings Substances Tests).

Hydroxyl Value 40–60 (Fats and Related Substances Tests).

Purity

(1) Acid value Not more than 2.0 (Flavorings Substances Tests).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Ethylene oxide and dioxane

Not more than 1.0 μg/g for ethylene oxide.

Not more than 10 μg/g for 1,4-dioxane.

Proceed as directed under Purity (4) for Polysorbate 20.

Water Not more than 3.0% (1 g, Volumetric Titration, Back Titration).

Residue on Ignition Not more than 0.25% (5 g, 800°C, 15 minutes).

Assay Using about 90 mg of the sample, weighed accurately, proceed as directed under Assay for Polysorbate 20.

Polysorbate 80

Polyoxyethylene (20) sorbitan monooleate

ポルソルベート 80

[9005-65-6]

Definition Polysorbate 80 is a mixture of oleate partial esters of sorbitol and sorbitol anhydrides condensed with approximately 20 moles of ethylene oxide per mole of D-sorbitol and its anhydrides.

Content Polysorbate 80 contains 65.0–69.5% of oxyethylene groups ($-OCH_2CH_2$ = 44.05).

Description Polysorbate 80 occurs as a colorless to orange-yellow oily liquid having a faint, characteristic odor.

Identification

(1) Determine the absorption spectrum of Polysorbate 80 as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Proceed as directed under Identification (2) for Polysorbate 20. The chromatogram from the test solution shows a peak at the retention time of methyl oleate.

Saponification Value 44–55 (2.0 g, Flavorings Substances Tests).

Hydroxyl Value 65–80 (Fats and Related Substances Tests).

Purity

(1) Acid value Not more than 2.0 (Flavorings Substances Tests).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Ethylene oxide and 1,4-dioxane

Not more than 1.0 μg/g for ethylene oxide.

Not more than 10 μg/g for 1,4-dioxane.

Proceed as directed under Purity (4) for Polysorbate 20.

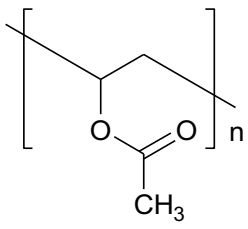
Water Content Not more than 3.0% (1 g, Volumetric Titration, Back Titration).

Residue on Ignition Not more than 0.25% (5 g, 800°C, 15 minutes).

Assay Using about 65 mg of the sample, weighed accurately, proceed as directed under Assay for Polysorbate 20.

Polyvinyl Acetate

酢酸ビニル樹脂



Poly(1-acetoxyethylene)

Definition Polyvinyl Acetate is a polymer of vinyl acetate.

Description Polyvinyl Acetate occurs as colorless to light yellow granules or glassy

lumps.

Identification Dissolve about 1 g of Polyvinyl Acetate in 5 mL of ethyl acetate, and proceed as directed in the Thin Film Method under Infrared Spectrophotometry. The solution exhibits absorption bands at about 1725 cm^{-1}, 1230 cm^{-1}, 1015 cm^{-1}, 937 cm^{-1}, and 785 cm^{-1}.

Purity

(1) Free acids Not more than 0.20% as CH_3COOH.

Weigh accurately about 2 g of Polyvinyl Acetate, add 50 mL of methanol, and dissolve it by shaking occasionally. Add 10 mL of water, and titrate with 0.1 mol/L sodium hydroxide (indicator: 4-5 drops of phenolphthalein TS). Perform a blank test, and make any necessary correction. Calculate the content of free acids as acetic acid (CH_3COOH) by the formula:

$$\text{Content (\%) of free acid} = \frac{\text{Volume (mL) of 0.1 mol/L sodium hydroxide consumed} \times 60}{\text{Weight (g) of the sample} \times 10 \times 100} \times 100$$

(2) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Residual monomer Not more than 5 μg/g.

Test Solution Wrap a portion of Polyvinyl Acetate in a powder paper and then a wrapping film, and smash into fine pieces with wooden hammer. Weigh 2.5 g, and dissolve it in toluene to make 25 mL.

Standard Solutions Measure 50 mg of vinyl acetate, add toluene to make exactly 50 mL, and refer to the resulting solution as Solution A. Transfer 1.0 mL, 0.3 mL, 0.1 mL, 0.03 mL, and 0.01 mL of Solution A into separate 100-mL volumetric flasks, and dilute each with toluene to volume.

Procedure Analyze 1 μL each of the test solution and the standard solutions by gas chromatography using the operating conditions given below. Measure the peak height or peak area for each standard solution and prepare a calibration curve. Measure the peak height or peak area for the test solution and determine the content using the calibration curve.

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.32 mm internal diameter and 30 cm length) coated with a 5-μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 100°C for 8 minutes, thereafter raise to 250°C at a rate of 20°C/minute, and maintain at 250°C for 5

minutes.

Injection port temperature: 150°C.

Carrier gas: Helium.

Flow rate: Adjust so that the peak of vinyl acetate appears around 7 minutes after injection.

Injection method: Split.

Split ratio: 1:8.

Loss on Drying Not more than 1.0% (not higher than 0.7 kPa, 80°C, 3 hours).

Residue on Ignition Not more than 0.05% (5 g).

Polyvinylpolypyrrolidone

ポリビニルポリピロリドン

Cross linked poly[(2-oxopyrrolidin-1-yl)ethylene] [25249-54-1]

Content Polyvinylpolypyrrolidone, when calculated on the anhydrous basis, contains 11.0–12.8% of nitrogen (N = 14.01).

Description Polyvinylpolypyrrolidone occurs a white to slightly yellowish-white powder. It is odorless.

Identification Determine the absorption spectrum of Polyvinylpolypyrrolidone as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH 5.0–8.0 (1.0g, water 100mL).

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 2, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Water-soluble substances Not more than 1.5%.

Weigh accurately about 25 g of Polyvinylpolypyrrolidone, transfer to a flat-bottom flask, and add 225 mL of water. Boil gently under a reflux condenser for 20 hours while stirring using a stirrer. After cooling, transfer the contents into a volumetric flask, add water to make exactly 250 mL, and allow to stand for 15 minutes. Take the supernatant into a centrifuge tube and centrifuge at 10,000×g for an hour. Filter the supernatant through a membrane filter with 0.45 μm pore size, measure exactly 50 mL of the filtrate into a glass evaporating dish, previously weighed accurately, evaporate to dryness, and dry at 90°C for 3 hours. Allow to stand in a desiccator, and weigh the residue accurately.

(4) Vinylpyrrolidone　Not more than 0.1%.

Weigh accurately about 4 g of Polyvinylpolypyrrolidone, add 30 mL of water, and shake for 15 minuets. Transfer it to a centrifuge tube, add 20 mL of water, centrifuge, and filter the supernatant a crucible-type glass filter (1G4). Wash both the residue in the centrifuge tube and residue on the filter with 50 mL each of water, combine the washings with filtrate, and add 0.50 g of sodium acetate trihydrate. Add 0.05 mol/L iodine solution until the color of iodine no longer disappear. Add another 3.0 mL of 0.05 mol/L iodine solution, allow to stand for 10 minutes, and titrate the excess iodine with 0.1 mol/L sodium thiosulfate solution. The consumption of 0.05 mol/L iodine solution is not more than 0.72 mL (Indicator Starch TS 3 mL). Separately, perform a blank test to make any necessary correction.

Water Content　Not more than 6.0% (1 g, Volumetric Titration, Direct Titraion).

Residue on Ignition　Not more than 0.4%.

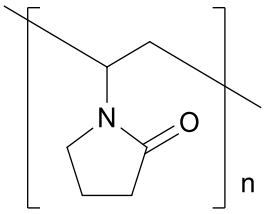
Assay　Weigh accurately about 0.2 g of Polyvinylpolypyrrolidone, determine nitrogen as directed in the Kjeldahl Method under Nitrogen Determination, and calculate on the anhydrous basis.

Each mL of 0.05 mol/L sulfuric acid = 1.401 mg of N

Polyvinylpyrrolidone

Povidone

ポリビニルピロリドン



$(C_6H_9NO)_n$

Poly[1-(2-oxopyrrolidin-1-yl)ethylene]　[9003-39-8]

Content　Polyvinylpyrrolidone, when calculated on the anhydrous basis, contains 11.5–12.8% of nitrogen (N = 14.01).

Description　Polyvinylpyrrolidone is a white to pale yellow powder.

Identification　Determine the infrared absorption spectrum of Polyvinylpyrrolidone, previously dried at 105°C for 6 hours, as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH　3.0–7.0 (1.0 g, water 20 mL).

Purity

(1) Viscosity

Test Solution Weigh accurately an amount of Polyvinylpyrrolidone equivalent to 1.00 g on the anhydrous basis, add water to dissolve, and make exactly 100 mL. Allow the solution to stand for 60 minutes.

Procedure Measure the kinematic viscosity of the test solution and water at 25°C as directed under Method 1 in Viscosity, and determine K value by the following formula. The K value is 90–108% of the labeled value.

$$K = \frac{1.5\log\nu_{rel} - 1}{0.15 + 0.003c} + \frac{\sqrt{300c\log\nu_{rel} + (c + 1.5c\log\nu_{rel})^2}}{0.15c + 0.003c^2}$$

c = the amount (g) of the sample, on the anhydrous basis, in 100 mL of the test solution

ν_{rel} = ratio of the kinematic viscosity of the test solution to the kinematic viscosity of water

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Aldehyde Not more than 500 μg/g as acetaldehyde.

Test Solution Weigh accurately about 1 g of Polyvinylpyrrolidone , and dissolve it in potassium pyrophosphate–hydrochloric acid buffer (0.05 mol/L, pH9.0) to make exactly 100 mL. Stopper tightly, heat at 60°C for 60 minutes, and cool to room temperature.

Standard Solution Weigh 0.100 g of freshly distilled acetaldehyde, and dissolve it in 4°C water to make exactly 100 mL. Allow this solution to stand at 4°C for about 20 hours. Take exactly 1 mL of this solution, add potassium pyrophosphate–hydrochloric acid buffer (0.05 mol/L, pH 9.0) to make exactly 100 mL.

Procedure Place 0.5 mL each of the test solution, standard solution, and water in separate cells, and add exactly 2.5 mL of potassium pyrophosphate–hydrochloric acid buffer (0.05 mol/L, pH9.0) and 0.2 mL of β-nicotinamide adenine dinucleotide TS in each cell. Stir them well, stopper tightly, and allow to stand at 22 ± 2°C for 2–3 minutes. Measure the absorbance (A_{T1}, A_{S1}, and A_{B1}) of these solutions at 340 nm against water as reference. To each solution, add 0.05 mL of aldehyde dehydrogenase TS, stir, stopper, and allow to stand at 22 ± 2°C for 5 minutes. Measure the absorbance (A_{T2}, A_{S2}, and A_{B2}) of these solutions in the same manner. Determine the amount of aldehyde by the formula:

$$\text{Amount of aldehyde (μg/g)} = \frac{1000}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{(A_{T2} - A_{T1}) - (A_{B2} - A_{B1})}{(A_{S2} - A_{S1}) - (A_{B2} - A_{B1})}$$

(4) 1-Vinyl-2-pyrrolidone Not more than 10 μg/g as 1-vinyl-2-pyrrolidone.

Test Solution Weigh accurately about 0.25 g of Polyvinylpyrrolidone, and dissolve it in methanol (1 in 5) to make exactly 10 mL.

Standard Solution Weigh exactly 50 mg of 1-vinyl-2-pyrrolidone, add methanol to dissolve, and make exactly 100 mL. Measure exactly 1 mL of this solution, add methanol to make exactly 100 mL. Measure exactly 5 mL of the second solution, and add methanol (1 in 5) to make exactly 100 mL.

Procedure Analyze 50 μL potions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of 1-vinyl-2-pyrrolidone for the test solution and the standard solution, and determine its amount by the formula:

$$\text{Amount (μg/g) of 1-vinyl-2-pyrrolidone} = \frac{2.5}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T}{A_S}$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 254 nm).

Column: A stainless steel tube (about 4 mm internal diameter and about 25 cm length).

Column packing material: 5-μm octylsilanized silica gel for liquid chromatography.

Guard column: A column with the same internal diameter as the main column, packed with the same material as the main column.

Column temperature: A constant temperature of around 40°C.

Mobile phase: A 4:1 mixture of water/methanol.

Flow rate: Adjust the retention time of 1-vinyl-2-pyrrolidone to about 10 minutes.

Column selection: Use a column that is capable of eluting 1-vinyl-2-pyrrolidone and vinyl acetate in that order and whose separation rate is not less than 2.0 when 50 μL of the solution prepared as directed below is chromatographed according to the above operating conditions. When the test is repeated 6 times according to the above operating conditions, the relative standard deviation of the peak areas of 1-vinyl-2-pyrrolidone is not more than 2%.

Solution: Dissolve 10 mg of Polyvinylpyrrolidone and 0.5 g of vinyl acetate in 100 mL of methanol. To 1 mL of this solution, add methanol (1 in 5) to make 100 mL.

Rinse of Guard Column

After the test solution is analyzed, run the mobile phase through the guard column

in reverse direction to testing operation at the above flow rate for 30 minute.

(5) Hydrazine Not more than 1 μg/g as hydrazine.

Test Solution Transfer about 2.5 g of Polyvinylpyrrolidone, weighed accurately, into a 50-mL centrifuge tube, add 25 mL of water, and dissolve it by stirring. Add 500 μL of a solution (1 in 20) of salicylaldehyde in methanol, shake, and heat in a water bath at 60°C for 15 minutes. After cooling, add 2.0 mL of toluene, stopper, and shake vigorously for 2 minutes. Centrifuge, and use the upper layer as the test solution.

Standard Solution Weigh 90 mg of salicylaldazine, and dissolve it in toluene to make exactly 100 mL. To exactly 1 mL of this solution, add toluene to make exactly 100 mL.

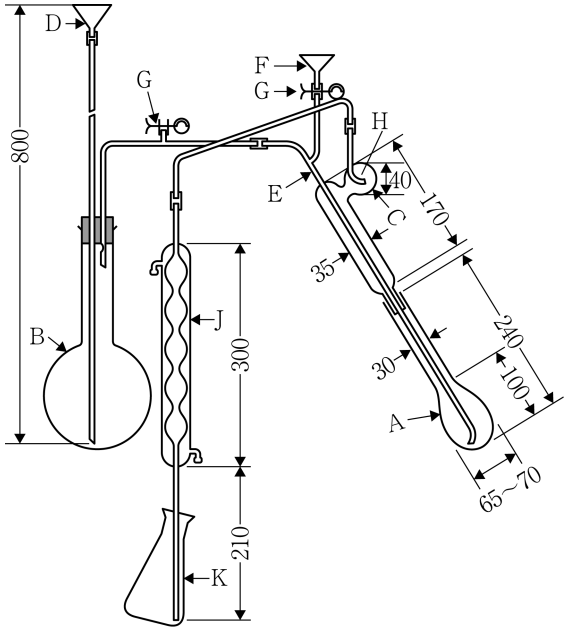
Procedure Analyze 10 μL each of the test solution and the standard solution by thin-layer chromatography, using methanol solution (2 in 3) as the developing solvent. Use a thin-layer plate coated with fluorescent dimethylsilanized silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 15 cm above the starting line, and air-dry the plate. Examine under ultraviolet light (around 365 nm). A spot from the test solution appears at the position corresponding to the spot from the standard solution. The fluorescence of the spot from the test solution is not more intense than that produced by the spot from the standard solution.

Water Content Not more than 5.0% (0.5g, Volumetric Titration, Direct Titration).

Residue on Ignition Not more than 0.1% (1 g, 600 ± 50°C)

Assay

(1) Apparatus Use hard-glass apparatus, as illustrated in the figure below. Ground-glass may be used for joint parts. Before use, all rubber parts used for the apparatus should be boiled in sodium hydroxide solution (1 in 25) for 10–30 minutes and then in water for 30–60 minutes, and finally rinsed thoroughly with water.



(Unit: mm)

A: Kjeldahl flask.

B: Steam generator (filled with water containing 2 to 3 drops of sulfuric acid, and boiling chips to prevent bumping).

C: Spray trap.

D: Water supply funnel.

E: Steam tube.

F: Injection funnel for alkali solution.

G: Rubber tube with pinch cock.

H: Small hole (the diameter is almost the same as the internal diameter of the tube).

J: Condenser (with the lower end cut diagonally).

K: Absorption flask.

(2) Procedure

Transfer about 0.1 g of Polyvinylpyrrolidone, accurately weighed, in Kjeldahl flask A, and add 5 g of a powdered mixture of 33 g of potassium sulfate, 1 g of cupper(II) sulfate pentahydrate, and 1 g of titanium(IV) oxide. Wash down the sample adhering to the neck of A with a small amount of water, and then pour 7 mL of sulfuric acid down the inside wall of A. Heat A gradually until the solution in the flask exhibits a clear yellow-green color and the inside walls of flask A are free from carbonized material, and heat for another 45 minute. After cooling, add 20 mL of water carefully, and cool. Connect A to the distillation apparatus, washed by passing steam through it. To absorption flask K, add 30 mL of boric acid solution (1 in 25) and 3 drops of bromocresol green–methyl red

mixture TS, and add an adequate amount of water to immerse the lower end of condenser J in the solution. Add 30 mL of sodium hydroxide solution (2 in 5) through funnel F, carefully wash down with 10 mL of water, and immediately close the pinch cock attached to rubber tube G. Distill with steam until 80–100 mL of distillate is obtained. Lower absorption flask K so that the lower end of J comes above the solution, and rinse the end of J with a small amount of water. Titrate with 0.025 mol/L sulfuric acid. The endpoint is when the color of the solution changes from green through slightly grayish blue to slightly grayish red-violet. Separately, perform a blank test to make necessary correction.

Each mL of 0.025 mol/L sulfuric acid = 0.7003 mg of N

Potassium Alginate

アルギン酸カリウム

Potassium alginate [9005-36-1]

Content Potassium Alginate, when calculated on the dried basis, contains 89.2–105.5% of potassium alginate.

Description Potassium Alginate occurs in white to yellowish-white filamentous, granular, or powdered form.

Identification

(1) Proceed as directed in Identification (1) for Ammonium Alginate.

(2) Ignite 1 g of Potassium Alginate at 550–600°C for 3 hours, and add 10 mL of water to the residue. The solution obtained responds to all the tests for Potassium Salt described in the Qualitative Tests.

Purity

(1) Water-insoluble substances Not more than 2.0% (on the dried basis).

Proceed as directed in Purity (1) for Ammonium Alginate in Monographs.

(2) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 15.0% (105°C, 4 hours).

Microbial Limit Proceed as directed in the Microbial Limit Test (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Coliforms: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 2 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture Prepare as directed in Method 2 for the coliform test. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 5 g of Potassium Alginate with 500 mL of lactose broth to disperse completely, and incubate it at 35 ± 1°C for 24 ± 2 hours.

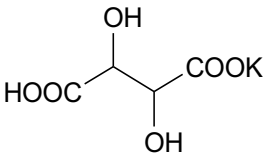
Assay Proceed as directed in the Assay for Alginic Acid.

Each mL of 0.25 mol/L sodium hydroxide = 29.75 mg of potassium alginate

Potassium DL-Bitartrate

Potassium Hydrogen *dl*-Tartrate
Potassium Hydrogen DL-Tartrate

DL-酒石酸水素カリウム



$C_4H_5KO_6$ Mol. Wt. 188.18

Monopotassium monohydrogen 2,3-dihydroxybutanedioate

Content Potassium DL-Bitartrate, when dried, contains not less than 99.0% of potassium DL-bitartrate ($C_4H_5KO_6$).

Description Potassium DL-Bitartrate occurs as colorless crystals or as a white crystalline powder. It has a cool, acidic taste.

Identification

(1) Dissolve 1 g of Potassium DL-Bitartrate in 10 mL of ammonia TS. The solution has no optical activity.

(2) Heat gradually 0.5 g of Potassium DL-Bitartrate. It is carbonized, emitting an odor like burning sucrose. To the residue, add 5 mL of water, and stir well. The mixture is alkaline. When neutralized with diluted hydrochloric acid (1 in 4) and filtered, the solution responds to all tests for Potassium Salt in the Qualitative Tests.

(3) Potassium DL-Bitartrate responds to all the tests for Tartrate in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and almost clear (0.50 g, ammonium TS 3.0 mL).

(2) Sulfate Not more than 0.019% as SO_4.

Test Solution Weigh 0.50 g of Potassium DL-Bitartrate, add 2 mL of diluted hydrochloric acid (1 in 4) and 30 mL of water, dissolve it while heating, and add water to make 50 mL.

Control Solution To 0.20 mL of 0.005 mol/L sulfuric acid, add 2 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(3) Ammonium salt Weigh 0.50 g of Potassium DL-Bitartrate, add 5 mL of sodium hydroxide solution (1 in 25), and heat. No odor of ammonia is evolved.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B)

Test Solution Weigh the specified amount of Potassium DL-Bitartrate, and add 10 mL of water, dissolve by heating, and cool.

(6) Readily oxidizable substances Weigh 2.0 g of Potassium DL-Bitartrate, add 20 mL of water and 30 mL of diluted sulfuric acid (1 in 20) to dissolve, keep the solution at 20°C, and add 4.0 mL of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 3 minutes.

Loss on Drying Not more than 0.5% (105°C, 3 hours).

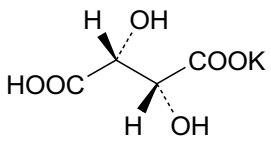
Assay Weigh accurately about 0.4 g of Potassium DL-Bitartrate, previously dried, and dissolve it in 20 mL of boiling water. Titrate the solution with 0.1 mol/L sodium hydroxide while it is hot (indicator: 2–3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide = 18.82 mg of $C_4H_5KO_6$

Potassium L-Bitartrate

Potassium Acid Tartrate
Potassium Hydrogen *d*-Tartrate
Potassium Hydrogen L-Tartrate

L-酒石酸水素カリウム



$C_4H_5KO_6$ Mol. Wt. 188.18

Monopotassium monohydrogen (2*R*,3*R*)-2,3-dihydroxybutanedioate [868-14-4]

Content Potassium L-Bitartrate, when dried, contains not less than 99.0% of potassium L-bitartrate ($C_4H_5KO_6$).

Description Potassium L-Bitartrate occurs as colorless crystals or as a white crystalline powder. It has a cool, acidic taste.

Identification

(1) Dissolve 1 g of Potassium L-Bitartrate in 10 mL of ammonia TS. The solution is dextrorotary.

(2) Proceed as directed in Identification (2) and (3) for Potassium DL-Bitartrate.

Specific Rotation $[\alpha]_D^{20}$: +32.5 to +35.5°.

Weigh accurately about 5 g of the Potassium L-Bitartrate, previously dried, add 10 mL of ammonia TS and water to make exactly 50 mL, and measure the angular rotation.

Purity

(1) Clarity of solution Colorless and almost clear (0.50 g, ammonia TS 3.0 mL).

(2) Sulfate Not more than 0.019% as SO_4.

Proceed as directed in Purity (2) for Potassium DL-Bitartrate.

(3) Ammonium salt Proceed as directed in Purity (3) for Potassium DL-Bitartrate.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Proceed as directed in Purity (5) for Potassium DL-Bitartrate.

Loss on Drying Not more than 0.5% (105°C, 3 hours).

Assay Proceed as directed in the Assay for Potassium DL-Bitartrate.

Each mL of 0.1 mol/L sodium hydroxide = 18.82 mg of $C_4H_5KO_6$

Potassium Bromate

臭素酸カリウム

$KBrO_3$ Mol. Wt. 167.00

Potassium bromate [7758-01-2]

Content Potassium Bromate, when dried, contains not less than 99.0% of potassium bromate ($KBrO_3$).

Description Potassium Bromate occurs as white crystals or crystalline powder.

Identification Potassium Bromate responds to all the tests for Potassium Salt and for Bromate in the Qualitative Tests.

Purity

(1) Free acid and free alkali Weigh 5.0 g of Potassium Bromate, dissolve it in 60 mL of freshly boiled and cooled water while warming, cool, and add 3 drops of phenolphthalein TS. Perform the following tests on this solution:

(i) If the solution is colorless, add 1.2 mL of 0.01 mol/L sodium hydroxide. A pink color develops.

(ii) If the solution is pink, add 0.40 mL of 0.01 mol/L hydrochloric acid. The color disappears.

(2) Bromide Weigh 2.0 g of Potassium Bromate, dissolve it in 40 mL of water, add 0.25 mL of diluted sulfuric acid (3 in 100), and add 1 drop of methyl orange TS. A pink-red color develops. Shake the solution. The pink-red color does not immediately disappear.

(3) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Potassium Bromate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of Potassium Bromate, dissolve it in 5 mL of water while warming, add 5 mL of hydrochloric acid, and evaporate to dryness on a water bath. Dissolve the residue in 5 mL of water.

Loss on Drying Not more than 0.5% (105°C, 2 hours).

Assay Weigh accurately about 0.1 g of Potassium Bromate, previously dried, transfer into a 200-mL ground-glass stoppered flask, add 50 mL of water, 1.5 g of potassium iodide, and 10 mL of diluted sulfuric acid (1 in 5), and immediately stopper tightly. Allow to stand in a dark place for 5 minutes, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 2.783 mg of $KBrO_3$

Potassium Carbonate, Anhydrous

Potassium Carbonate

炭酸カリウム(無水)

K_2CO_3 Mol. Wt. 138.21

Potassium carbonate [584-08-7]

Content Potassium Carbonate, when dried, contains not less than 99.0% of potassium carbonate (K_2CO_3).

Description Potassium Carbonate occurs as a white powder or as granules.

Identification A solution of Potassium Carbonate (1 in 10) responds to all the tests for Potassium Salt and for Carbonate in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.053% as Cl.

Sample Solution Weigh 0.20 g of Potassium Carbonate, add 3 mL of diluted nitric acid (1 in 10), boil, and cool.

Control Solution Use 0.30 mL of 0.01 mol/L hydrochloric acid.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Potassium Carbonate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(4) Arsenic Not more than 3 μg/g as As (2.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of Potassium Carbonate, dissolve it in 10 mL of water, add gradually 2 mL of hydrochloric acid, and add water to make 20 mL. Use 5 mL of this solution as the test solution.

Loss on Drying Not more than 5.0% (180°C, 4 hours).

Assay Weigh accurately about 1 g of Potassium Carbonate, previously dried, dissolve it in 25 mL of water, and titrate with 0.25 mol/L sulfuric acid (indicator: 3 drops of bromophenol blue TS). Boil near the endpoint to let the carbon dioxide out, cool, and continue the titration.

Each mL of 0.25 mol/L sulfuric acid = 34.55 mg of K_2CO_3

Potassium Chloride

塩化カリウム

KCl Mol. Wt. 74.55

Potassium chloride [7447-40-7]

Content Potassium Chloride, when dried, contains not less than 99.0% of potassium chloride (KCl).

Description Potassium Chloride occurs as colorless crystals or as a white powder. It is odorless and has a salty taste.

Identification Potassium Chloride responds to all the tests for Potassium Salt and for Chloride in the Qualitative Tests.

Purity

(1) Free acid and free alkali Weigh 5.0 g of Potassium Chloride, dissolve it in 50 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS. The color of the solution does not change to pink. Add 0.30 mL of 0.02 mol/L sodium hydroxide. The color of the solution changes to pink.

(2) Bromide Not more than 0.13%.

Test Solution Weigh 0.75 g of Potassium Chloride, add water to dissolve it, and make exactly 500 mL. Measure 5 mL of this solution, add 2 mL of phenol red TS (pH 4.7) and 1 mL of a solution of sodium *p*-toluenesulfonchloramide trihydrate (1 in 10,000), mix immediately, and allow to stand for 2 minutes. Add 0.15 mL of 0.1 mol/L sodium thiosulfate, stir, and dilute with water to 10 mL.

Control Solution Weigh 2.979 g of potassium bromide, previously dried at 110°C for 4 hours, add water to dissolve, and make exactly 1000 mL. To exactly 1 mL of this solution, add water to make exactly 1000 mL. Then measure exactly 5 mL of the second solution, add 2 mL of phenol red TS (pH 4.7) and 1 mL of a solution of sodium *p*-toluenesulfonchloramide trihydrate (1 in 10,000), and proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at 590 nm. The absorbance of the test solution is not greater than that of the control solution.

(3) Iodide Moisten 5 g of Potassium Chloride by the dropwise addition of a freshly prepared mixture of 0.15 mL of sodium nitrite solution (1 in 10), 1 mL of 10% sulfuric acid TS, 25 mL of starch TS, and 25 mL of water. After 5 minutes, examine in daylight. No blue color is produced.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Potassium Chloride, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool.

(5) Calcium or magnesium　Weigh 0.20 g of Potassium Chloride, and dissolve it in 20 mL of water. Add 2 mL of ammonia TS, 2 mL of a solution of ammonium oxalate monohydrate (1 in 30), and 2 mL of a solution of disodium hydrogenphosphate dodecahydrate (1 in 8), and allow to stand for 5 minutes. The solution does not become turbid.

(6) Sodium　Weigh 0.20 g of Potassium Chloride, dissolve it in 100 mL of water, and proceed as directed in the Flame Coloration Test. No yellow color persists.

(7) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more than 1.0% (105°C, 2 hours).

Assay　Weigh accurately about 0.25 g of Potassium Chloride, previously dried, into a ground-glass stoppered flask, dissolve it in 50 mL of water, add exactly 50 mL of 0.1 mol/L silver nitrate while shaking. Then add 3 mL of nitric acid and 5 mL of nitrobenzene while shaking, and shake vigorously. Add 2 mL of ammonium iron(III) sulfate–sulfuric acid TS, and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate. Perform a blank test in the same manner.

Each mL of 0.1 mol/L silver nitrate = 7.455 mg of KCl

Potassium Dihydrogen Phosphate

Monopotassium Phosphate
Potassium Phosphate, Monobasic
Primary Potassium Phosphate

リン酸二水素カリウム

KH_2PO_4　Mol. Wt. 136.09

Potassium dihydrogenphosphate　[7778-77-0]

Content　Potassium Dihydrogen Phosphate, when dried, contains not less than 98.0% of potassium dihydrogen phosphate (KH_2PO_4).

Description　Potassium Dihydrogen Phosphate occurs as colorless crystals or as a white crystalline powder.

Identification　A solution of Potassium Dihydrogen Phosphate (1 in 20) responds to all the tests for Potassium Salt and for Phosphate in the Qualitative Tests.

pH　4.4–4.9 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution　Colorless and slightly turbid (1.0 g, water 20 mL).

(2) Chloride　Not more than 0.011% as Cl (1.0 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Sulfate　Not more than 0.019% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(4) Lead　Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution　To the specified amount of Potassium Dihydrogen Phosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter.　Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

(5) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more than 0.5% (105°C, 4 hours).

Assay　Weigh accurately about 3 g of Potassium Dihydrogen Phosphate, previously dried, dissolve it in 30 mL of water, add 5 g of sodium chloride, and dissolve by shaking well. While keeping at about 15°C, titrate with 1 mol/L sodium hydroxide (indicator: 3–4 drops of thymol blue TS).

Each mL of 1 mol/L sodium hydroxide = 136.1 mg of KH_2PO_4

Potassium Ferrocyanide

Potassium Hexacyanoferrate(II)

フェロシアン化カリウム

$K_4[Fe(CN)_6] \cdot 3H_2O$　　Mol. Wt.422.39

Potassium hexacyanoferrate(II) trihydrate　[13943-58-3]

Content　Potassium Ferrocyanide contains not less than 99.0% of potassium ferrocyanide ($K_4[Fe(CN)_6] \cdot 3H_2O$).

Description　Potassium Ferrocyanide occurs as yellow crystals or crystalline powder.

Identification

(1) To 10 mL of a solution of Potassium Ferrocyanide (1 in 100), add 1 mL of iron(III) chloride TS. A dark blue precipitate is formed.

(2) Potassium Ferrocyanide responds to all the tests for Potassium Salt in the Qualitative Tests.

Purity

(1) Cyanide　To 10 mg of copper(II) sulfate pentahydrate, add 8 mL of water and 2 mL of ammonia TS to dissolve the sample. Immerse a strip of filter paper in this solution, and expose to hydrogen sulfite. A brown color develops. When 1 drop of a solution of

Potassium Ferrocyanide (1 in 100) is dropped on the browned strip, no white circle is produced.

(2) Ferricyanide

Test Solution Dissolve 10 mg of Potassium Ferrocyanide in 10 mL of water to make exactly 100 mL.

Control Solution Add water to 10 mg of potassium hexacyanoferrate(III) to make exactly 100 mL.

Procedure Analyze 10 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. The peak area of hexacyanoferrate(III) ion from the test solution does not exceed that of hexacyanoferrate(III) ion from the control solution.

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 205 nm).

Column: A stainless steel (4.6 mm internal diameter and 15 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: Use a solution prepared by adding 325 mL of phosphate buffer (0.05 mol/L) with pH 7, 20 mL of tetra-n-butylammonium dihydrogen phosphate TS (0.5 mol/L), and 350 mL of acetonitrile to 200 mL of water and making up to 1000 mL with water.

Flow rate: 1 mL/minute.

(3) Lead Not more than 5 μg/g as Pb (0.80g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

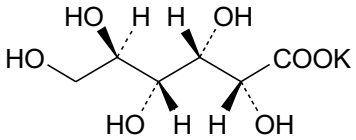
Sample Solution To the specified amount of Potassium Ferrocyanide, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

Assay Weigh accurately about 1 g of Potassium Ferrocyanide, and dissolve it in 200 mL of water. To this solution, add 10 mL of sulfuric acid, and titrate with 0.02 mol/L potassium permanganate. The endpoint is when the red color of the solution persists for 30 seconds.

Each mL of 0.02 mol/L potassium permanganate = 42.24 mg of $K_4[Fe(CN)_6] \cdot 3H_2O$

Potassium Gluconate

グルコン酸カリウム



$C_6H_{11}KO_7$ Mol. Wt. 234.25

Monopotassium D-gluconate [299-27-4]

Content Potassium Gluconate, when dried, contains 97.0–103.0% of potassium gluconate ($C_6H_{11}KO_7$).

Description Potassium Gluconate occurs as a white to yellowish-white crystalline powder or as granules. It is odorless.

Identification

(1) Potassium Gluconate responds to all the tests for Potassium Salt in the Qualitative Tests.

(2) Measure 5 mL of a solution of Potassium Gluconate (1 in 10), and proceed as directed in Identification (2) for Glucono-δ-Lactone.

pH 7.3–8.5 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Colorless and almost clear.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Reducing sugars Not more than 0.50% as D-glucose.

Weigh 1.0 g of Potassium Gluconate and proceed as directed in Purity (3) for Zinc Gluconate. Titrate excess iodine with 0.1 mol/L sodium thiosulfate. The volume of the sodium thiosulfate solution consumed is not less than 8.15 mL.

Loss on Drying Not more than 3.0% (105°C, 4 hours).

Assay Weigh accurately about 0.15 g of Potassium Gluconate, previously dried, and dissolve it in 75 mL of acetic acid, and titrate with 0.1 mol/L perchloric acid until the red color of the solution disappears (indicator: 10 drops of quinaldine red TS). Perform a blank test in the same manner.

Each mL of 0.1 mol/L perchloric acid = 23.43 mg of $C_6H_{11}KO_7$

Potassium Hydrogen Sulfite Solution

亜硫酸水素カリウム液

Content Potassium Hydrogen Sulfite Solution contains not less than 25.0% of potassium hydrogen sulfite ($KHSO_3$ = 120.17).

Description Potassium Hydrogen Sulfite Solution is a light yellow liquid having an odor of sulfur dioxide.

Identification Diluted Potassium Hydrogen Sulfite Solution (1 in 5) responds to all the tests for Potassium Salt and for Sulfite in the Qualitative Tests.

Purity

(1) Clarity of solution Slightly turbid (3.0 g, water 20 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Potassium Hydrogen Sulfite Solution, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow it to cool, and use as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Gently boil for 5 minutes, and allow to cool.

(3) Arsenic Not more than 1.5 μg/g as As (10 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Add water to the specified amount of Potassium Hydrogen Sulfite Solution to make 25 mL. Measure 5 mL of this solution, add 2 mL of sulfuric acid, and heat on a water bath until sulfur dioxide is no longer evolved. Evaporate to about 2 mL, and add water to make 10 mL. Use 5 mL of this solution as the test solution.

Assay Weigh accurately about 0.5 g of Potassium Hydrogen Sulfite Solution, and proceed as directed under Sulfite Determination.

Each mL of 0.05 mol/L iodine = 6.009 mg of $KHSO_3$

Potassium Hydroxide

Caustic Potash

水酸化カリウム

KOH Mol. Wt. 56.11

Potassium hydroxide [1310-58-3]

Content Potassium Hydroxide contains not less than 85.0% of potassium hydroxide (KOH).

Description Potassium Hydroxide occurs as white lumps having various shapes including pellets, flakes, and rods, or as a white powder.

Identification

(1) A solution of Potassium Hydroxide (1 in 50) is strongly alkaline.

(2) Potassium Hydroxide responds to all the tests for Potassium Salt in the Qualitative Test.

Purity

(1) Clarity of solution Colorless and almost clear.

Test Solution Weigh 50 g of Potassium Hydroxide, dissolve it in newly boiled and cooled water to make 250 mL, and use this solution as the sample solution. To 5 mL of the sample solution, add 20 mL of water, and mix.

(2) Potassium Carbonate The content of potassium carbonate (K_2CO_3) determined in the Assay is not more than 2.0%.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Potassium Hydroxide, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool.

(4) Mercury Not more than 0.10 μg/g as Hg.

Test Solution To 10 mL of the sample solution prepared in Purity (1) above, measured exactly, add 1 mL of potassium permanganate solution (3 in 50) and about 30 mL of water, and shake well. Neutralize by gradually adding hydrochloric acid (purified), add 5 mL of diluted sulfuric acid (1 in 2), and cool. Use this solution as the sample solution. To the sample solution, add hydroxylammonium chloride solution (1 in 5) until the purple color of the potassium permanganate disappears and the precipitate of manganese dioxide dissolves, and then add water to make 100 mL.

Control Solution To 2.0 mL of Mercury Standard Solution, add 1 mL of potassium permanganate solution (3 in 50), 30 mL of water, hydrochloric acid (purified) (the same amount as used for preparing the test solution), and 5 mL of diluted sulfuric acid(1 in 2), and proceed in the same manner as the preparation of the test solution.

Procedure Analyze the test solution and the control solution as directed under Cold-Vapor Atomic Absorption Spectrophotometry. Transfer appropriate portions of the test solution and the control solution into separate testing vials, add 10 mL of tin(II) chloride–sulfuric acid TS, and immediately fit them into the atomic absorption spectrophotometer, and set off the pump to circulate the air in a closed state. Measure the absorbance using conditions given below. The absorbance of the test solution is not greater than that of the control solution.

Operating Conditions

Light source: Mercury hollow cathode lamp.

Analytical line wavelength: 253.7 nm.

Carrier gas: Air.

(5) Arsenic Not more than 3 μg/g as As (Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Measure exactly 2.5 mL of the sample solution prepared in Purity (1) above, add 5 mL of water, and neutralize by gradually adding hydrochloric acid.

Assay Weigh accurately about 50 g of Potassium Hydroxide, and dissolve it in freshly boiled and cooled water to make exactly 1000 mL. Use this solution as the sample solution.

Measure exactly 25 mL of the sample solution, add 10 mL of freshly boiled and cooled water, and titrate with 1 mol/L hydrochloric acid (indicator: 1 mL of bromophenol blue TS). When the solution reaches neutral, add exactly 1 mL of 1 mol/L hydrochloric acid, and boil for about 5 minutes. After cooling, titrate the excess acid with 0.1 mol/L sodium hydroxide, and determine the volume (a mL) of 1 mol/L hydrochloric acid consumed.

Separately, measure exactly 25 mL of the sample solution, transfer into a ground-glass stoppered flask, and add 25 mL of freshly boiled and cooled water. To the solution, add 10 mL of a solution of barium chloride dihydrate (3 in 25), stopper, shake gently, and titrate with 1 mol/L hydrochloric acid (indicator: 1 mL of phenolphthalein TS). Record the volume consumed as b (mL).

$$\text{Content (\%) of potassium hydroxide (KOH)} = \frac{0.05611 \times b \times 40}{\text{Weight (g) of the sample}} \times 100$$

$$\text{Content (\%) of potassium carbonate } (K_2CO_3) = \frac{0.06910 \times (a - b) \times 40}{\text{Weight (g) of the sample}} \times 100$$

Potassium Hydroxide Solution

水酸化カリウム液

Content Potassium Hydroxide Solution contains 95–120% of the labeled content of potassium hydroxide (KOH = 56.11).

Description Potassium Hydroxide Solution is a colorless or slightly colored liquid.

Identification

(1) Diluted Potassium Hydroxide Solution (1 in 50) is strongly alkaline.

(2) Potassium Hydroxide Solution responds to all the tests for Potassium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution　Colorless and almost clear.

Sample Solution　To Potassium Hydroxide Solution, add freshly boiled and cooled water to prepare a solution equivalent to 20% (w/v) solution of KOH, calculated from the labeled content.

Test Solution　Mix 5 mL of the sample solution with 20 mL of water.

(2) Potassium Carbonate　Not more than 2.0% as K_2CO_3 per KOH.

(3) Lead　Not more than 2 μg/g of KOH as Pb (an amount equivalent to 2.0 g of KOH, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution　To the specified amount of Potassium Hydroxide Solution, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool.

(4) Mercury　Not more than 0.10 μg/g of KOH as Hg.

Proceed as directed in Purity (4) for Potassium Hydroxide.

(5) Arsenic　Not more than 3 μg/g of KOH as As (Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution　To 2.5 mL of the sample solution prepared in Purity (1), measured exactly, add 5 mL of water, and neutralize by gradually adding hydrochloric acid.

Assay　Weigh accurately an amount of Potassium Hydroxide Solution equivalent to about 5 g of potassium hydroxide (KOH). Add freshly boiled and cooled water to make 100 mL, and use this solution as the sample solution. Measure exactly 25 mL of the sample solution, and proceed as directed in the Assay for Potassium Hydroxide.

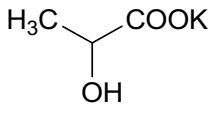
$$\text{Content (\%) of potassium hydroxide (KOH)} = \frac{0.05611 \times b \times 4}{\text{Weight (g) of the sample}} \times 100$$

$$\text{Content (\%) of potassium carbonate } (K_2CO_3) \text{ per potassium hydroxide (KOH)}$$

$$= \frac{0.06910 \times (a - b) \times 4}{\text{Weight (g) of the sample}} \times \frac{100}{\text{Content (\%) of the potassium hydroxide}} \times 100$$

Potassium Lactate

乳酸カリウム
乳酸カリウム液



$C_3H_5KO_3$ Mol. Wt.128.17

Monopotassium 2-hydroxypropanoate [996-31-6]

Content Potassium Lactate contains not less than 50.0% of potassium lactate ($C_3H_5KO_3$) and contains 95−110% of the labeled content of potassium lactate.

Description Potassium Lactate is a colorless, clear, slightly viscose liquid. It is odorless or has a slight, characteristic odor.

Identification Potassium Lactate responds to all the tests for Potassium Salt and Lactate in the Qualitative Tests.

Purity

(1) Free acid Weigh exactly an amount of Potassium Lactate equivalent to 0.60 g of potassium lactate, and add 20 mL of freshly boiled and cooled water and 3 drops of phenolphthalein TS. Titrate with 0.1 mol/L sodium hydroxide. Its consumption is not more than 0.2 mL.

(2) Lead Not more than 2.0 μg/g as Pb for 60% potassium lactate (an amount equivalent to 1.2 g of potassium lactate, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As for 60% potassium lactate (an amount equivalent to 0.60 g of potassium lactate, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Potassium Lactate in water to make 10 mL. Use 5 mL of this solution.

(4) Reducing substances Add 5 drops of Potassium Lactate to 10 mL of Fehling's TS, and boil for 5 minutes. No red precipitate is produced.

Assay Weigh accurately an amount of Potassium Lactate equivalent to about 0.3 g of potassium lactate, and evaporate to dryness on a water bath. To the residue, add 60 mL of a 5:1 mixture of acetic acid/acetic anhydride, dissolve completely, and titrate with 0.1 mol/L perchloric acid. Usually, a potentiometer is used to confirm the endpoint. When crystal violet-acetic acid TS (1 mL) is used as the indicator, the endpoint is when the color of the solution changes from violet through blue to green. Separately perform a blank test to make necessary correction.

Each mL of 0.1 mol/L perchloric acid = 12.82 mg of $C_3H_5KO_3$

Potassium Metabisulfite

Potassium Pyrosulfite

ピロ亜硫酸カリウム

$K_2S_2O_5$ Mol. Wt. 222.33

Potassium disulfite [16731-55-8]

Content Potassium Metabisulfite contains not less than 93.0% of potassium metabisulfite ($K_2S_2O_5$).

Description Potassium Metabisulfite occurs as white crystals or crystalline powder having an odor of sulfur dioxide.

Identification Potassium Metabisulfite responds to all the tests for Potassium Salt and for Sulfite in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear (1.0 g, water 10 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Potassium Metabisulfite, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(3) Arsenic Not more than 3 μg/g as As (5.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Potassium Metabisulfite in water to make 25 mL. Measure 5 mL of this solution, add 1 mL of sulfuric acid, evaporate to about 2 mL, and add water to make 10 mL. Use 5 mL of this solution.

Assay Weigh accurately about 0.2 g of Potassium Metabisulfite, and proceed as directed under Sulfite Determination.

Each mL of 0.05 mol/L iodine = 5.558 mg of $K_2S_2O_5$

Potassium Metaphosphate

メタリン酸カリウム

Content Potassium Metaphosphate, when dried, contains the equivalent of 53.0–80.0% of phosphorus(V) oxide (P_2O_5 = 141.94).

Description Potassium Metaphosphate occurs as white fibrous crystals or powder, or as colorless to white glassy flakes or lumps.

Identification

(1) Dissolve 0.1 g of Potassium Metaphosphate by adding 0.4 g of sodium acetate trihydrate and 10 mL of water, make the solution slightly acidic with diluted acetic acid (1 in 20) or sodium hydroxide solution (1 in 20), and add 5 mL of egg white TS. A white precipitate is formed.

(2) Potassium Metaphosphate responds to all the tests for Potassium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution　Colorless and slight turbid.

Test Solution　Weigh 1.0 g of powdered Potassium Metaphosphate, add 50 mL of water, heat in a water bath, and dissolve by vigorously stirring. Add gradually 50 mL of sodium hydroxide solution (1 in 25), heat in a water bath for 10 minutes with occasional stirring, and cool to 35–45°C.

(2) Chloride　Not more than 0.11% as Cl (0.10 g in powder form, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Orthophosphate　Weigh 1.0 g of powdered Potassium Metaphosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(4) Sulfate　Not more than 0.096% as SO_4.

Test Solution　Weigh 0.20 g of powdered Potassium Metaphosphate, add 30 mL of water and 2 mL of diluted hydrochloric acid (1 in 4), and dissolve it by boiling for 1 minute. Cool, and add water to make 50 mL.

Control Solution　To 0.40 mL of 0.005 mol/L sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(5) Lead　Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution　To the specified amount of Potassium Metaphosphate, add 5 mL of nitric acid and 25 mL of water, boil gently for 15 minutes with a watch glass covering it, and cool.

(6) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more than 5.0% (110°C, 4 hours).

Assay　Proceed as directed in the Assay for Potassium Polyphosphate.

Potassium Nitrate

硝酸カリウム

KNO_3　　Mol. Wt. 101.10

Potassium nitrate　[7757-79-1]

Content　Potassium Nitrate, when dried, contains not less than 99.0% of potassium nitrate (KNO_3).

Description　Potassium Nitrate occurs as colorless prismatic crystals or as a white crystalline powder. It is odorless and has a salty and cool taste.

Identification Potassium Nitrate responds to all the tests for Potassium Salt and for Nitrate in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.021% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Potassium Nitrate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of Potassium Nitrate, dissolve it in 3 mL of water, add 2 mL of sulfuric acid, and heat until white fumes are evolved. Then add a small amount of water to dissolve, heat until white fumes are evolved, cool, and add 5 mL of water to dissolve.

Loss on Drying Not more than 1.0% (105°C, 4 hours).

Assay Weigh accurately about 0.4 g of Potassium Nitrate, previously dried, into a 500-mL round-bottom flask, and dissolve it in about 300 mL of water. Add 3 g of powdered Devarda's alloy and 15 mL of sodium hydroxide solution (2 in 5). Connect the flask immediately to the distillation apparatus that is fit to a spray trap and a receiver with a condenser, containing exactly 50 mL of 0.05 mol/L sulfuric acid. Allow to stand for 2 hours, and distill until about 250 mL of the distillate is obtained. Titrate the excess sulfuric acid with 0.1 mol/L sodium hydroxide (indicator: 3 drops of methyl red–methylene blue mixture TS). Perform a blank test in the same manner.

Each mL of 0.05 mol/L sulfuric acid = 10.11 mg of KNO_3

Potassium Polyphosphate

Potassium Tripolyphosphate
Pentapotassium Triphosphate

ポリリン酸カリウム

Content Potassium Polyphosphate, when dried, contains the equivalent of 43.0–76.0% of phosphorus(V) oxide (P_2O_5 = 141.94).

Description Potassium Polyphosphate occurs as white fibrous crystals or powder, or as colorless to white glassy flakes or lumps.

Identification

(1) To 0.1 g of Potassium Polyphosphate, add 0.4 g of sodium acetate trihydrate and 10 mL of water to dissolve the sample, add diluted acetic acid (1 in 20) to make the solution slightly acidic, and add 3 mL of silver nitrate solution (1 in 50). A white precipitate is formed.

(2) Potassium Polyphosphate responds to all the tests for Potassium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and slightly turbid (1.0 g, sodium acetate trihydrate 4.0 g and water 100 mL).

(2) Chloride Not more than 0.11% as Cl (0.10 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Orthophosphate Weigh 1.0 g of Potassium Polyphosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(4) Sulfate Not more than 0.096% as SO_4.

Test Solution Weigh 0.20 g of Potassium Polyphosphate, and add 30 mL of water and 2 mL of diluted hydrochloric acid (1 in 4), and dissolve it by boiling for 1 minute. Cool, and add water to make 50 mL.

Control Solution To 0.40 mL of 0.005 mol/L sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(5) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Potassium Polyphosphate, add 5 mL of nitric acid and 25 mL of water, boil gently for 15 minutes with a watch glass covering it, and cool.

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 5.0% (110°C, 4 hours).

Assay

Test Solution Weigh accurately about 0.2 g of Potassium Polyphosphate, previously dried, dissolve it by adding 5 mL of nitric acid and 25 mL of water, boil for 30 minutes while replenishing the lost water, and cool. Add water to make exactly 500 mL, and filter through a dry filter paper if necessary.

Procedure Measure exactly 5 mL of the test solution, add 20 mL of vanadic acid–molybdic acid TS and water to make exactly 100 mL, shake well, and allow to stand for 30 minutes. Measure the absorbance of this solution at a wavelength of 400 nm against a reference solution prepared in the same manner as for the test solution, using 5 mL of water.

Separately, measure exactly 10 mL of Monopotassium Phosphate Standard Solution,

add 20 mL of diluted nitric acid (1 in 25), and add water to make exactly 250 mL. Measure exactly 10 mL, 15 mL, and 20 mL, respectively, of this solution, and proceed in the same manner as for the test solution. Measure the absorbance of each solution, and prepare a calibration curve.

Determine the weight (g) of phosphorus (P) in 5 mL of the test solution from the calibration curve and the absorbance of the test solution, and calculate the content of phosphorus(V) oxide (P_2O_5) by the formula:

Content (%) of phosphorus(V) oxide (P_2O_5)
= {[(Weight (g) of phosphorus(V) in 5 mL of the test solution) × 2.291 × 100]
/[Weight (g) of the sample]} × 100

Potassium Pyrophosphate

Tetrapotassium Pyrophosphate
Tetrapotassium Diphosphate

ピロリン酸四カリウム

$K_4P_2O_7$ Mol. Wt. 330.34

Potassium diphosphate [7320-34-5]

Content Potassium Pyrophosphate, when dried, contains not less than 98.0% of potassium pyrophosphate ($K_4P_2O_7$).

Description Potassium Pyrophosphate occurs as colorless to white lumps or crystalline powder, or as a white powder.

Identification

(1) Dissolve 0.1 g of Potassium Pyrophosphate by adding 10 mL of water and 2–3 drops of nitric acid, and add 1 mL of silver nitrate solution (1 in 50). A white precipitate is formed.

(2) Potassium Pyrophosphate responds to all the tests for Potassium Salt in the Qualitative Tests.

pH 10.0–10.7 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution Colorless and slightly turbid (0.50 g, water 20 mL).

(2) Chloride Not more than 0.011% as Cl (1.0 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Orthophosphate Weigh 1.0 g of Potassium Pyrophosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(4) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(5) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Potassium Pyrophosphate, add 5 mL of nitric acid and 25 mL of water, boil gently for 15 minutes with a watch glass covering it, and cool.

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

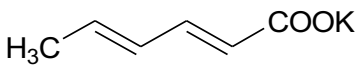
Loss on Drying Not more than 7.0% (110°C, 4 hours).

Assay Weigh accurately about 3 g of Potassium Pyrophosphate, previously dried, dissolve it in 75 mL of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 3–4 drops of methyl orange–xylene cyanol FF TS).

Each mL of 1 mol/L hydrochloric acid = 165.2 mg of $K_4P_2O_7$

Potassium Sorbate

ソルビン酸カリウム



$C_6H_7KO_2$ Mol. Wt. 150.22

Monopotassium (2*E*,4*E*)-hexa-2,4-dienoate [24634-61-5]

Content Potassium Sorbate, when dried, contains 98.0–102.0% of potassium sorbate ($C_6H_7KO_2$).

Description Potassium Sorbate occurs as white to light yellow-brown, flaky crystals, crystalline powder, or granules. It is odorless or has a slight odor.

Identification

(1) To a solution of Potassium Sorbate (1 in 100), add 1 mL of acetone. Add diluted hydrochloric acid (1 in 4) dropwise to make the solution slightly acidic, add 2 drops of bromine TS, and shake. The color of the solution disappears immediately.

(2) Potassium Sorbate responds to all the tests for Potassium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Weigh 0.20 g of Potassium Sorbate, and dissolve it in 5.0 mL of water. The solution is not darker in color than Matching Fluid F.

(2) Free alkali Weigh 1.0 g of Potassium Sorbate, and dissolve it in 20 mL of freshly boiled and cooled water. When 2 drops of phenolphthalein TS are added, a pink color

develops. The color disappears on the addition of 0.40 mL of 0.05 mol/L sulfuric acid.

(3) Chloride　Not more than 0.018% as Cl.

Test Solution　Weigh 1.0 g of Potassium Sorbate, dissolve it in about 30 mL of water, and add 11 mL of diluted nitric acid (1 in 10) while shaking well. Filter, wash with water, combine the washings with filtrate, and add water to make 50 mL.

Control Solution　To 0.50 mL of 0.01 mol/L hydrochloric acid, add 6 mL of diluted nitric acid (1 in 10) and water to make 50 mL.

(4) Sulfate　Not more than 0.038% as SO_4.

Test Solution　Weigh 0.50 g of Potassium Sorbate, dissolve it in about 30 mL of water, and add 3 mL of diluted hydrochloric acid (1 in 4) while shaking well. Filter, wash with water, combine the washings with the filtrate, and add water to make 50 mL.

Control Solution　To 0.40 mL of 0.005 mol/L sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(5) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(6) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more than 1.0% (105°C, 3 hours).

Assay　Weigh accurately about 0.3 g of Potassium Sorbate, previously dried, add 50 mL of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid (indicator: 10 drops of *p*-naphtholbenzein TS) until the brown color of the solution changes to green.

Each mL of 0.1 mol/L perchloric acid = 15.02 mg of $C_6H_7KO_2$

Potassium Sulfate

硫酸カリウム

K_2SO_4　　Mol. Wt.174.26

Potassium sulfate　[7778-80-5]

Content　Potassium Sulfate contains not less than 99.0% of potassium sulfate (K_2SO_4).

Description　Potassium Sulfate occurs as colorless or white crystals or crystalline powder.

Identification　Potassium Sulfate responds to all the tests for Potassium Salt and Sulfate in the Qualitative Tests.

pH　5.5–8.5 (1.0 g, water 20 mL).

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Potassium Sulfate, add 20 mL of diluted hydrochloric acid (1 in 4), boil gently for 5 minutes with a watch glass covering it, and allow to cool.

(2) Selenium Not more than 30 μg/g.

Test solution and control solution Weigh 0.20 g of Potassium Sulfate in a beaker, add 25 mL of hydrochloric acid TS (4 mol/L), shake, and add 25 mL of water. Use this solution as the sample solution. Measure exactly 3 mL of Selenium Standard Stock Solution, and add water to make exactly 1000 mL. Measure exactly 2 mL of this solution in a beaker, and add 50 mL of hydrochloric acid TS (2 mol/L). Use this solution as the control stock solution.

Carefully add 5 mL of ammonia solution to each of the sample solution and control stock solution. Do this step in a draft chamber. After cooling, adjust the pH of each solution to 1.8–2.2 with diluted ammonia solution (1 in 2), and add water to make 60 mL each. Transfer them to separate separating funnels, wash each beaker with 10 mL of water, and add the washings to each separating funnel. Add 0.2 g of hydroxylammonium chloride to each, and dissolve them while gently shaking. Add 5 mL of 2,3-diamononaphthalene TS, shake, and allow to stand for 100 minutes. To each, add 5.0 mL of cyclohexane, shake well for 2 minutes. Collect the cyclohexane layers, and separately centrifuge at 3000 rmp for10 minutes. Use the resulting upper layers as the test solution and control solution, respectively.

Procedure Measure the absorbance of them at the maximum at around 378 nm against a reference that is prepared using 50 mL of hydrochloric acid TS (2 mol/L) in the same manner as for the sample solution. The absorbance value of the test solution is not greater than that of the control solution.

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay Weigh accurately about 0.5 g of Potassium Sulfate, dissolve it in 200 mL of water, add 1 mL of hydrochloric acid, and boil. Add 8 mL of a solution of barium chloride dihydrate (3 in 25) little by little while stirring, and heat on a water bath for 1 hour. After cooling, filter the solution through a filter paper for quantitative analysis (5C), and wash the residue on the filter paper with water until the washing no longer shows chloride reaction. Place the filter paper with residue in a crucible, already ignited and weighed, dry it, and ignite at 500–600°C to constant weight. Weigh accurately the residue, and calculate the content by the formula:

$$\text{Content (\%) of potassium sulfate } (K_2SO_4) = \frac{\text{Weight (g) of the residue} \times 0.7466}{\text{Weight (g) of the sample}} \times 100$$

Powdered Cellulose

粉末セルロース

Definition Powdered Cellulose is obtained by decomposing pulp and consists mainly of cellulose.

Description Powdered Cellulose occurs as a white powder. It is odorless.

Identification

(1) To 10 g of Powdered Cellulose, add 290 mL of water, and mix in a high-speed (12,000 rpm or more) power blender for 5 minutes. Transfer 100 mL of the mixture to a 100-mL measuring cylinder, and allow to stand for 1 hour. The suspension separates into a clear or white supernatant liquid and a precipitate.

(2) Determine the absorption spectrum of Powdered Cellulose, previously dried, as directed in the Disk Method under the Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

pH 5.0–7.5.

To 10.0 g of Powdered Cellulose, add 90 mL of water, and allow to stand for 1 hour with occasional stirring. Centrifuge the mixture, and use the supernatant for pH measurement.

Purity

(1) Water-soluble substances Not more than 1.5%.

Weigh accurately about 6 g of Powdered Cellulose, previously dried, add 90 mL of water freshly boiled and cooled. Allow to stand for 10 minutes with occasional stirring, filter through a glass filter (1G4), and discard the initial 10 mL of filtrate. If necessary, filter again, using the same filter to obtain a clear filtrate. Place 15 mL of the filtrate in an evaporation dish, previously dried and weighed, heat on a water bath, taking care not to scorch it, and evaporate to dryness. Dry the residue at 105°C for 1 hour, and accurately weigh the evaporation dish containing the residue. Separately, perform blank test for correction.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

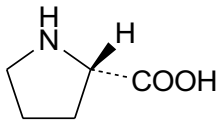
(4) Starch To 20 mL of the liquid obtained in Identification (1), add a few drops of iodine TS, and mix. No bluish-purple or blue color develops.

Loss on drying Not more than 10.0% (105°C, 3 hours).

Ash Not more than 0.3% (about 800°C, 2 hours).

L-Proline

L-プロリン



$C_5H_9NO_2$ Mol. Wt. 115.13

(2*S*)-Pyrrolidine-2-carboxylic acid [147-85-3]

Content L-Proline, when calculated on the dried basis, contains 98.0–102.0% of L-proline ($C_5H_9NO_2$).

Description L-Proline occurs as white crystals or crystalline powder. It is odorless, or has a very slight characteristic odor, and has a very slight sweet taste.

Identification

(1) To 5 mL of a solution of L-Proline (1 in 1000), add 1 mL of ninhydrin solution (1 in 50), and heat for 1 minute in a water bath. A yellow color develops.

(2) To 1 mL of a solution of L-Proline (1 in 500), add 1 mL of a solution of sodium carbonate decahydrate (1 in 50), 1 mL of a solution of sodium pentacyanonitrosylferrate(III) dihydrate (1 in 100), and 1 mL of acetaldehyde solution (1 in 10). A blue color develops.

Specific Rotation $[\alpha]_D^{20}$: – 84.0 to – 86.0° (4 g, water, 100 mL, on the dried basis).

pH 5.9–6.9 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.1% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.25 g of L-Proline and proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 11.51 mg of $C_5H_9NO_2$

L-Proline Solution

L-プロリン液

Content L-Proline Solution contains not more than 50% of L-proline ($C_5H_9NO_2$ = 115.13) and contains 95.0–110.0% of the labeled content of L-proline.

Description L-Proline Solution is a colorless liquid. It is odorless or has a very slight characteristic odor, and has a very slight sweet taste.

Identification

(1) To 5 mL of diluted L-Proline Solution (1 in 200), add 1 mL of ninhydrin solution (1 in 50), and heat for 1 minute in a water bath. A yellow color develops.

(2) To 4 g of L-Proline Solution, add 100 mL of water, and mix. It shows levorotatory.

Purity

(1) Lead Not more than 2 μg/g of $C_5H_9NO_2$ as Pb (an amount equivalent to 2.0 g of L-proline ($C_5H_9NO_2$), Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g of $C_5H_9NO_2$ as As (an amount equivalent to 0.50 g of L-proline ($C_5H_9NO_2$), Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of L-Proline Solution in 5 mL of water, heating if necessary.

Residue on Ignition Not more than 0.1% per L-proline ($C_5H_9NO_2$).

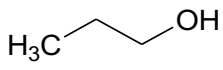
Assay Weigh accurately an amount of L-Proline Solution equivalent to about 0.25 g of L-proline ($C_5H_9NO_2$), and proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 11.51 mg of $C_5H_9NO_2$

Propanol

Propyl Alcohol

プロパノール



C_3H_8O Mol. Wt. 60.10

Propan-1-ol [71-23-8]

Content Propanol contains not lass than 99.0% of propanol (C_3H_8O).

Description Propanol is a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Propanol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

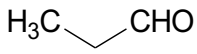
Refractive Index n_D^{20}: 1.383–1.388.

Specific Gravity d_{25}^{25}: 0.800–0.805.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay in Flavoring Agents in the Flavoring Substances Tests, using operating conditions (2).

Propionaldehyde

プロピオンアルデヒド



C_3H_6O Mol. Wt. 58.08

Propanal [123-38-6]

Content Propionaldehyde contains not less than 97.0% of propionaldehyde (C_3H_6O).

Description Propionaldehyde occurs as a colorless, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Propionaldehyde, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.360–1.380.

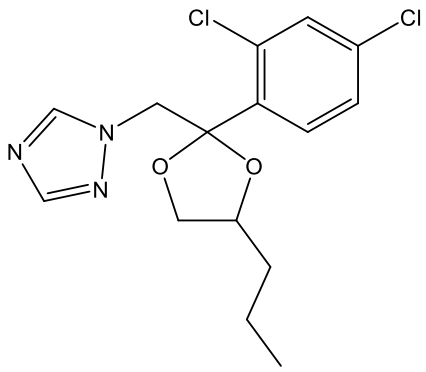
Specific Gravity d_{25}^{25}: 0.796–0.814.

Purity Acid value Not more than 5.0 (Flavoring Substance Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (3).

Propiconazole

プロピコナゾール



$C_{15}H_{17}Cl_2N_3O_2$ Mol. Wt. 342.22

(2*RS*, 4*RS*; 2*RS*, 4*SR*)-1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]- 1*H*-1,2,4-triazole［60207-90-1］

Content Propiconazole contains not less than 95.0% of propiconazole ($C_{15}H_{17}Cl_2N_3O_2$).

Description Propiconazole is a colorless to dark yellow-red viscous liquid. It is odorless.

Identification Determine the infrared absorption spectrum of Propiconazole, as directed in the Liquid Film Paste Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers. Use optical plates made from sodium chloride.

Specific Gravity d_{20}^{20}: 1.288–1.290.

Purity

Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method). In the preparation of the test solution, ignite the charred sample at 450°C.

Assay

Test Solution and Standard Solution Weigh accurately about 50 mg each of Propiconazole and propiconazole for assay, add 20 mL of the internal standard solution to each, add acetone to dissolve the mixture, and then make each of the solutions up to exactly 100 mL with acetone.

Internal Standard Solution Dissolve 75 mg of fludioxonil for assay in acetone to make exactly 50 mL.

Procedure Analyze 1 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratios of propiconazole to fludioxonil for the test solution and the standard solution, and express as Q_T and Q_S, respectively. Calculate the amount of propiconazole by the

formula:

$$\text{Content (\%) of propiconazole } (C_{15}H_{17}Cl_2N_3O_2) = \frac{\text{Weight (mg) of propiconazole for assay}}{\text{Weight (mg) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of dimethylpolysiloxan for gas chromatography.

Column temperature: Inject at 200°C, then raise the temperature at a rate of 5°C/minute to 280°C.

Injection port temperature: A constant temperature of about 250°C.

Detector temperature: A constant temperature of about 300°C.

Carrier gas: Helium.

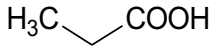
Flow rate: Adjust the retention time of propiconazole to 10–15 minutes.

Injection method: Split.

Split ratio: 1 : 10.

Propionic Acid

プロピオン酸



$C_3H_6O_2$ Mol. Wt. 74.08

Propanoic acid [79-09-4]

Content Propionic Acid contains not less than 99.5% of propionic acid ($C_3H_6O_2$).

Description Propionic Acid is an oily, clear liquid having a characteristic odor.

Identification To 1 mL of Propionic Acid, add 3 drops of sulfuric acid and 1 mL of ethanol (95), and heat. An aroma is evolved.

Specific Gravity d_{20}^{20}: 0.993–0.997.

Purity

(1) Distillation test Not less than 95% (vol) is distilled at 138.5–142.5°C (Method 2).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead

Standard Solution 4.0 mL, Flame Method).

(3) Arsenic　Not more than　3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Aldehyde　Not more than 0.2% as propionic aldehyde.

Measure 10 mL of Propionic Acid into a 250-mL ground-glass stoppered Erlenmeyer flask containing 50 mL of water and 10 mL of sodium hydrogen sulfite solution (1 in 80), stopper, shake vigorously, allow to stand for 30 minutes, and titrate with 0.05 mol/L iodine until the color of the solution changes to yellow-brown. The volume of the iodine consumed is not more than 7 mL. Perform a blank test in the same manner, and make any necessary correction.

(5) Residue on evaporation　Not more than 0.01%.

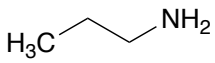
Weigh 20 g of Propionic Acid, evaporate at 140°C to constant weight, and weigh the residue.

Assay　Weigh accurately about 3 g of Propionic Acid, dissolve it in 40 mL of freshly boiled and cooled water, and titrate with 1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide = 74.08 mg of $C_3H_6O_2$

Propylamine

プロピルアミン



C_3H_9N　　Mol. Wt. 59.11

Propan-1-amine　[107-10-8]

Content　Propylamine contains not less than 95.0% of propylamine (C_3H_9N).

Description　Propylamine occurs as a colorless to yellow, clear liquid having a characteristic odor.

Identification　Determine the infrared absorption spectrum of Propylamine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

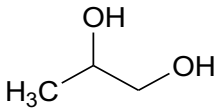
Refractive index　n_D^{20}: 1.384–1.392

Specific gravity　d_{25}^{25}: 0.710–0.720

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operating conditions (2) except for the column. Use a fused silica tube (0.25–0.53 mm internal diameter and 30–60 m length) coated with a 0.25–1 μm thick layer of dimethylpolysiloxane for gas chromatography.

Propylene Glycol

プロピレングリコール



$C_3H_8O_2$ Mol. Wt. 76.09

Propane-1,2-diol [57-55-6]

Content Propylene Glycol contains not less than 98.0% of propylene glycol ($C_3H_8O_2$).

Description Propylene Glycol is a colorless, clear, viscous liquid. It is odorless and has a slightly bitter-sweet taste.

Identification

(1) To 1 mL of Propylene Glycol, add 0.5 g of potassium hydrogen sulfate, and heat. A fruity odor is evolved.

(2) With 2–3 drops of Propylene Glycol, mix 0.7 g triphenylchloromethane, add 1 mL of pyridine, heat under a reflux condenser on a water bath for an hour, and cool. Add 20 mL of acetone, and dissolve by heating. Add 20 mg of active carbon, stir, and filter. Concentrate the filtrate to about 10 mL, and cool. Collect the deposited crystals by filtration, and dry for 4 hours in a desiccator. The melting point of the crystals obtained is 174–178°C.

Specific Gravity d_{20}^{20}: 1.036–1.040.

Purity

(1) Distillation test Not less than 95% (vol) is distilled at 185–189°C (Method 2).

(2) Free acid To 50 mL of water, add 1 mL of phenolphthalein TS, then add sodium hydroxide solution (1 in 2500) until the pink color of the solution persists for 30 seconds. To the prepared water, add 10 mL of Propylene Glycol, mix, and add 0.20 mL of 0.1 mol/L sodium hydroxide. A pink color persists for not less than 30 seconds.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Water Content Not more than 0.2% (10 g, Volumetric Titration, Direct Titration).

Residue on Ignition Not more than 0.05% (10 g).

Assay Weigh accurately about 1 g of Propylene Glycol, and add water to make exactly 250 mL. Measure exactly 10 mL of this solution, transfer into a ground-glass stoppered flask, add exactly 10 mL of sodium periodate TS and 4 mL of diluted sulfuric acid (1 in 2), shake well, and allow to stand for 40 minutes. To this solution, add 5 g of potassium iodide, immediately stopper tightly, shake well, and allow to stand in a dark place for 5 minutes. Titrate with 0.1 mol/L sodium thiosulfate (indicator: 1 mL of starch TS). Perform a blank test in the same manner, and calculate the content by the formula:

$$\text{Content (\%) of propylene glycol } (C_3H_8O_2) = \frac{(a - b) \times 3.805 \times 25}{\text{Weight (g) of the sample} \times 1000} \times 100$$

a = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the blank test,

b = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the test.

Propylene Glycol Alginate

Propan-1,2-diol Alginate

アルギン酸プロピレングリコールエステル

Description Propylene Glycol Alginate occurs as a white to yellowish-white coarse or fine powder. It is almost odorless.

Identification Prepare a test solution as follows: To 1 g of Propylene Glycol Alginate, add 100 mL of water to produce a pasty solution.

(1) To 5 mL of the test solution, add 5 mL of lead(II) acetate TS. It immediately becomes gelled.

(2) To 10 mL of the test solution, add 1 mL of sodium hydroxide solution (1 in 25), heat the mixture in a water bath for 5–6 minutes, cool it, and add 1 mL of diluted sulfuric acid (1 in 20). It immediately becomes gelled.

(3) To 1 mL of the test solution, add 4 mL of water, and shake vigorously. Effervescence persists.

Purity

(1) Degree of esterification Not less than 40.0%.

Calculate the esterification value of Propylene Glycol Alginate by the formula:

$$\text{Degree of esterification (\%)} = 100 - (a + b + c)$$

a = content (%) of free alginic acid,

b = content (%) of sodium alginate,

c = content (%) of insoluble ash.

Determine a, b, and c as directed in (1)(i), (1)(ii), and (2), respectively.

(i) *Free alginic acid* Weigh accurately about 0.5 g of Propylene Glycol Alginate, previously dried, dissolve it in 200 mL of freshly boiled and cooled water, add 2 drops of phenolphthalein TS, and titrate with 0.02 mol/L sodium hydroxide to the first pink color that persists for about 20 seconds. Calculate the content by the formula below. Perform a blank test in the same manner, and make any necessary correction.

$$\text{Content (\%) of free alginic acid} = \frac{\text{Volume (mL) of 0.02 mol/L sodium hydroxide consumed} \times 0.00352}{\text{Weight (g) of the sample}} \times 100$$

(ii) *Sodium alginate* Weigh accurately about 1 g of Propylene Glycol Alginate, previously dried, transfer into a porcelain or platinum crucible (diameter: 20–30 mm), heat very gently at first, then gradually raise the temperature, and heat at 300–400°C for about 2 hours until completely carbonized. After cooling, crush the carbonized substance with a glass rod, transfer together with the crucible into a beaker, add about 50 mL of water, and then add 20 mL of 0.05 mol/L sulfuric acid. Cover the beaker with a watch glass, heat on a water bath for 1 hour, and filter. If the filtrate is colored, take a new sample, carbonize thoroughly, and repeat the procedure in the same manner. Wash thoroughly with hot water the beaker, the crucible, and the residue on the filter paper until the washings do not turn the litmus paper (blue) red, then combine the washings with the filtrate. Titrate the excess sulfuric acid with 0.1 mol/L sodium hydroxide (indicator: 3 drops of methyl red TS) and calculate the content by the formula:

$$\text{Content (\%) of sodium alginate} = \frac{\text{Volume (mL) of 0.05 mol/L sulfuric acid consumed} \times 0.0198}{\text{Weight (g) of the sample}} \times 100$$

(2) Insoluble ash Not more than 1.5%.

Dry the residue on the filter paper obtained under (1)(ii), ignite to constant weight, cool, and weigh accurately.

(3) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 20.0% (105°C, 4 hours).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Coliforms: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 2 for total plate count and the enumeration of yeasts and molds. Previously add sodium hydroxide solution to the diluent to be used.

Pre-enrichment Culture Prepare as directed in Method 2 for the coliform test. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 5 g of Propylene Glycol Alginate with 500 mL of lactose broth to disperse completely, and incubate it at 35 ± 1°C for 24 ± 2 hours.

Propylene Glycol Esters of Fatty Acids

Propylene Glycol Mono- and Diesters
Propane-1,2-diol Esters of Fatty Acids

プロピレングリコール脂肪酸エステル

Definition Propylene Glycol Esters of Fatty Acids are esters of fatty acids and propylene glycol, or transesterified substances of fats and oils and propylene glycol.

Description Propylene Glycol Esters of Fatty Acids occur as white to light yellow-brown powders, flakes, granules, or waxy lumps, or as colorless to light yellow-brown, viscous liquids. They are odorless or have a slight, characteristic odor.

Identification

(1) To 0.1 g of the sample, add 2 mL of ethanol (95), dissolve it by warming, add 5 mL of diluted sulfuric acid (1 in 20), heat in a water bath for 30 minutes, and cool. Oil drops or white to yellow-white solids are formed. To the separated oil drops or solids, add 3 mL of diethyl ether, and shake. They dissolve.

(2) *Test Solution* To about 5 g of the sample, add 50 mL of 3.5% (w/v) potassium hydroxide–ethanol TS, and heat under a reflux condenser in a water bath for 1 hour. Dilute the mixture with methanol (1 in 5), and use the resulting solution as the test solution.

Control Solutions Use both a 9:1 mixture of methanol/propylene glycol and a 9:1 mixture of methanol/glycerol.

Procedure Analyze 5 μL each of the test solution and the control solutions by thin-layer chromatography using a 9:1 mixture of acetone/water as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point 15 cm above the starting line. Air-dry the plate, and heat at 110°C for 10 minutes to remove the solvent. After cooling, spray with thymol–sulfuric

acid TS, and heat at 110°C for 20 minutes to develop a color. A yellow spot is observed at the position corresponding to propylene glycol in the control solution. A yellow-brown spot may be observed at the position corresponding to glycerol in the control solution.

Purity

(1) Acid value　Not more than 8.0 (Fats and Related Substances Tests).

(2) Lead　Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10.0 mL, Flame Method).

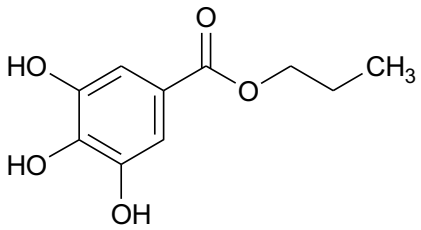
(3) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Polyoxyethylene　Proceed as directed in Purity (4) for Sorbitan Esters of Fatty Acids.

Residue on Ignition　Not more than 1.5%.

Propyl Gallate

没食子酸プロピル



$C_{10}H_{12}O_5$　　Mol. Wt. 212.20

Propyl 3,4,5-trihydroxybenzoate　[121-79-9]

Content　Propyl Gallate, when dried, contains 98.0–102.0% of propyl gallate ($C_{10}H_{12}O_5$).

Description　Propyl Gallate occurs as a white to light brown-yellow crystalline powder. It is odorless and has a slightly bitter taste.

Identification

(1) Dissolve 0.5 g of Propyl Gallate in 10 mL of sodium hydroxide solution (1 in 25), distill, and collect the initial about 4 mL of distillate. The solution is clear, and it emits an odor of propanol when heated.

(2) To 5 mL of a solution (1 in 50) of Propyl Gallate in ethanol (95), add 1 drop of a solution of iron(III) chloride hexahydrate (1 in 500). A purple color develops.

Melting Point　146–150°C (dried sample).

Purity

(1) Clarity of solution　Weigh 0.50 g of Propyl Gallate, and dissolve it in 10 mL of

ethanol (95). The color of the solution is not darker than that of Matching Fluid C.

(2) Chloride　Not more than 0.028% as Cl.

Sample Solution　Weigh 1.50 g of Propyl Gallate, add 75 mL of water, warm for 5 minutes at about 70°C, cool to about 20°C, and filter. Use 25 mL of the filtrate as the sample solution.

Control Solution　Use 0.40 mL of 0.01 mol/L hydrochloric acid for the preparation.

(3) Sulfate　Not more than 0.048% as SO_4.

Sample Solution　Use 25 mL of the filtrate obtained in Purity (2) as the sample solution.

Control Solution　Use 0.50 mL of 0.005 mol/L sulfuric acid for the preparation.

(4) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more than 1.5% (105°C, 2 hours).

Residue on Ignition　Not more than 0.1% .

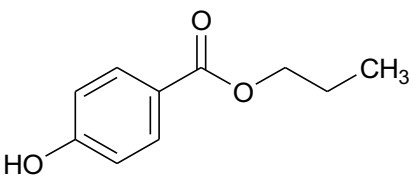
Assay　Dry a glass filter (1G4) at 110°C for 30 minutes, allow it to cool in a desiccator, and weigh accurately. Weigh accurately about 0.2 g of Propyl Gallate, previously dried, add 150 mL of water, and boil. To the mixture, add 50 mL of bismuth nitrate TS while stirring vigorously, stir for an additional several minutes, and filter the precipitate through the glass filter, previously prepared. Wash the precipitate twice with two 5-mL portions of diluted nitric acid (1 in 300) cooled in ice, and wash it with icy water until a litmus paper (blue) does not turn red. Dry the glass filter with the residue at 110° for 3 hours, allow to cool in a desiccator, weigh accurately, and calculate the content by the formula:

$$\text{Content (\%) of propyl gallate } (C_{10}H_{12}O_5) = \frac{\text{Weight (g) the precipitate} \times 0.4865}{\text{Weight (g) of the sample}} \times 100$$

Propyl *p*-Hydroxybenzoate

Propylparaben

パラオキシ安息香酸プロピル



$C_{10}H_{12}O_3$ Mol. Wt. 180.20

Propyl 4-hydroxybenzoate [94-13-3]

Content Propyl *p*-Hydroxybenzoate, when dried, contains not less than 99.0% of propyl *p*-hydroxybenzoate ($C_{10}H_{12}O_3$).

Description Propyl *p*-Hydroxybenzoate occurs as colorless crystals or as a white crystalline powder. It is odorless.

Identification

(1) Proceed as directed in Identification (1) for Isobutyl *p*-Hydroxybenzoate.

(2) To 50 mg of Propyl *p*-Hydroxybenzoate, add 2 drops of acetic acid and 5 drops of sulfuric acid, and warm for 5 minutes. An odor of propyl acetate is evolved.

Melting Point 95–98°C.

Purity

(1) Free acid Not more than 0.55% as *p*-hydroxybenzoic acid.

Proceed as directed in Purity (1) for Isobutyl *p*-Hydroxybenzoate.

(2) Sulfate Not more than 0.024% as SO_4.

Proceed as directed in Purity (2) for Isobutyl *p*-Hydroxybenzoate.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.5% (5 hours).

Residue on Ignition Not more than 0.05% (5 g).

Assay Proceed as directed in the Assay for Isobutyl *p*-Hydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide = 180.2 mg of $C_{10}H_{12}O_3$

Protease

プロテアーゼ

Definition Protease includes enzymes that degrade protein. It is derived from the muscle or internal organs of terrestrial animals, fish, or crustaceans, or the culture of basidiomycetes (limited to *Pycnoporus coccineus*), filamentous fungi (limited to *Aspergillus melleus*, *Aspergillus niger, Aspergillus oryzae*, *Aspergillus phoenicis*, *Aspergillus saitoi*, *Aspergillus sojae*, *Monascus pilosus*, *Monascus purpureus*, *Mucor circinelloides, Mucor javanicus, Mucor miehei*, *Mucor rouxii*, *Penicillium citrinum*, *Penicillium duponti*, *Rhizomucor miehei*, *Rhizopus chinensis*, *Rhizopus delemar*, *Rhizopus niveus*, and *Rhizopus oryzae*), yeasts (limited to species of the genuse *Saccharomyces*), actinomycetes (limited to

species from the genus *Streptomyces*), or bacteria (limited to*Bacillus amyloliquefaciens*, *Bacillus clausii*, *Bacillus coagulans* J4, *Bacillus halodurans*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus polymixa*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bacillus thermoproteolyticus*, *Geobacillus caldoproteolyticus*, *Geobacillus stearothermophilus*, *Lysobacter enzymogenes*, and *Pseudomonas paucimobilis*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Protease occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Protease complies with the Protease Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Protease Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of Protease, add water, cooled water, or protease sample diluent to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water, cooled water, or the same diluent to the resulting solution.

Test Soliution Equilibrate 5 mL of an appropriate substrate solution for protease at 37°C for 10 minutes, add 1 mL of the sample solution, and shake immediately. Incubate the mixture at the same temperature for 10 minutes, add 5 mL of trichloroacetic acid solution (9 in 125) or trichloroacetic acid TS (for protease activity test), and shake. Warm it 37°C for 30 minutes, and filter. Discard 3 mL of the first filtrate, and collect 2 mL of

the filtrate, add 5 mL of sodium carbonate TS (0.55 mol/L) and 1 mL of folin TS (1 in 3), mix, and warm the mixture at 37°C for 30 minutes.

Control Solution To 1 mL of the sample solution, add 5 mL of trichloroacetic acid solution (9 in 125) or trichloroacetic acid TS (for protease activity test), and shake. Add 5 mL of the substrate solution for protease, mix immediately, warm this solution at 37°C for 30 minutes, and filter. Proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 660 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 0.50 g of Protease, add water or acetate buffer (0.1 mol/L) at pH 4.7 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 4.0 g of hemoglobin (bovine-derived), and dissolve it in 100 mL of water while stirring for 10 minutes. Adjust the pH of the solution to 1.7 with hydrochloric acid TS (0.3 mol/L), and stir for 10 minutes. Again adjust the pH of this solution to 4.7 with sodium acetate TS (0.5 mol/L), and add water to make 200 mL.

Test Solution Transfer 10 mL of the substrate solution into a stoppered test tube, equilibrate it at 40°C for about 5 minutes, add 2 mL of the sample solution, and stir gently for 30 seconds with the tube stoppered. Incubate the mixture at 40°C for 30 minutes. To this solution, add 10 mL of trichloroacetic acid solution (7 in 50), shake well for about 40 seconds, allow to stand at room temperature for 60 minutes while occasional shaking at about 10 minute intervals. Shake this solution vigorously to disperse the contents, and filter the dispersion through a filter paper. Filter again the first half volume of the filtrate through the filter paper, and use the whole filtrate as the test solution.

Control Solution Transfer 10 mL of the substrate solution into a stoppered test tube, and equilibrate it at 40°C for about 30 minutes. Add 10 mL of trichloroacetic acid solution (7 in 50), and shake well for about 40 seconds. To this solution, add 2 mL of the sample solution, equilibrated at 40°C for about 30 minutes, shake well, and allow to stand at room temperature for 60 minutes while occasional shaking at about 10 minute intervals. Proceed as directed for the test solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 275 nm against the reference solution prepared as directed below. The absorbance value of the test solution is higher than that of the control solution.

Reference Solution Transfer 10 mL of the substrate solution into a stoppered test tube, warm at 40°C for about 5 minutes, and add 2 mL of water or acetate buffer (0.1 mol/L) at pH 4.7 instead of the sample solution. Proceed as directed for the test solution.

Method 3

Sample Solution Weigh 1.0 g of Protease, add water to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 0.5 g of azocasein or azocollagen, and add Tris buffer (0.05 mol/L, pH 7.5, containing calcium chloride–polyethylene glycol) to dissolve or suspend it. Adjust the pH to 7.5 with hydrochloric acid TS (0.5 mol/L) or sodium hydroxide TS (0.5 mol/L), and add the same buffer to make 100 mL.

Test solution Equilibrate 0.2 mL of the sample solution at 30°C for 2 minutes, add 1 mL of the substrate solution, equilibrated at 30°C, and shake immediately. Incubate the mixture at 30°C for 5 minutes, add 0.2 mL of trichloroacetic acid solution (1 in 10), shake, and allow to stand at room temperature for 5 minutes. Centrifuge it at 14,000 rpm for 5 minutes, and add 0.25 mL of sodium hydroxide TS (0.5 mol/L) to 1 mL of the supernatant.

Control Solution Proceed as directed for the test solution using Tris buffer (0.05 mol/L, pH 7.5, containing calcium chloride–polyethylene glycol) instead of the sample solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 420 nm. The absorbance value of the test solution is higher than that of the control solution.

Method 4

Sample Solution Weigh 1.5 g of Protease, add sodium borate–hydrochloric acid buffer (0.01 mol/L, pH 8.5, containing polysorbate) to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding the buffer to the resulting solution.

Substrate Solution Weigh 30 mg of succinyl trialanine *p*-nitroanilide, and dissolve it in 1 mL of dimethyl sulfoxide, and add 15 mL of sodium borate-hydrochloric acid buffer (0.01 mol/L, pH 8.5, containing polysorbate).

Test Solution Equilibrate 0.1 mL of the sample solution at 25°C for 3 minutes, add 1 mL of the substrate solution, and shake immediately. Incubate the mixture at 25°C for 10 minutes, add 0.25 mL of diluted acetic acid (1 in 5), and shake.

Control Solution Proceed as directed for the test solution using sodium borate-hydrochloric acid buffer (0.01 mol/L, pH 8.5, containing polysorbate) instead of the sample solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 405 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Psicose Epimerase

プシコースエピメラーゼ

[1618683-38-7]

Definition Psicose Epimerase is an enzyme that mutually isomerizes fructose and psicose to each other. It is derived from the culture of *Escherichia coli* (limited to *E. coli* K12 W3110 strain) in which the psicose epimerase gene, intrinsically occurring in the bacterium (limited to *Arthrobacter globiformis*), is introduced. It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Enzyme Activity Psicose Epimerase has an enzyme activity of not less than 230 units per gram.

Description Psicose Epimerase occurs as a light to dark brown liquid or as a gray powder.

Identification When tested by the enzyme activity determination specified below, Psicose Epimerase shows activity.

Purity

(1) Lead Not more than 5 µg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution specified in Method 1, proceed as directed in Method 3.

(2) Arsenic Not more than 3 µg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Enzyme Activity Determination

(i) Substrate Solution Weigh 0.18 g of D(+)-psicose, dissolve it in water, and add water to make exactly 5 mL. Prepare fresh before use.

(ii) Sample Solution Weigh accurately about 1.0 g of Psicose Epimerase, and dissolve it in the diluent prepared as directed below to make a constant volume so that the resulting solution has 4–10 units per mL. The diluent: Mix phosphate buffer (0.05

mol/L) at pH 8.0 and magnesium chloride TS (1 mol/L) at a rate of 199 : 1.

(iii) D(−)-Fructose Standard Solutions Weigh accurately about 0.27 g of D(−)-fructose for enzyme activity determination, dissolve it in water to make exactly 100 mL. Use this solution as the standard stock solution. Prepare four standard solutions with different concentrations—10 μmol, 5 μmol, 3 μmol, and 1 μmol of D(−)-fructose ($C_6H_{12}O_6$ = 180.16) per mL—by exactly diluting the standard stock solution with water to 1.5, 3, 5, and 15 times, respectively.

(iv) Procedure

Test Solution Place 0.100 mL of the sample solution into a test tube, mix it with 0.400 mL of the diluent prepared in (ii), lid the test tube, and equilibrate at 50°C ± 0.5°C for 5 minutes. Add 0.500 mL of the substrate solution to the test tube, mix, incubate at 50°C ± 0.5°C for exactly 10 minutes, and then heat in a water bath for 2 minutes. After cooling, add about 100 mg of strongly acidic cation-exchange resin and about 100 mg of weakly basic anion-exchange resin (free-form), both whose surface water was previously removed with a filter paper, shake for 15 minutes, and filter through a membrane filter (pore size: 0.2 μm).

For strongly acidic cation-exchange resin, wash with water before use as directed in the Strongly Acidic Cation-exchange Resin section under C. REAGENTS, SOLUTIONS, AND OTHER REFERENCE MATERIALS, beginning with "Weigh about 50 g of strongly acidic cation-exchange resin," and confirm that the pH of the effluent is 5.0–6.5.

Reference Solution Place 0.100 mL of the diluent into a test tube instead of the sample solution, and proceed as directed for the test solution.

Determination Analyze 10 μL each of the test solution, the reference solution, and the four D(−)-fructose standard solutions by liquid chromatography using the operating conditions given below. Prepare a calibration curve from the peak area and the concentration (μmol/mL) of each D(−)-fructose standard solution. Measure the peak areas of D(−)-fructose in the test solution and the reference solution, and then determine the concentration (μmol/mL) of D(−)-fructose in each solution from the calibration curve. Calculate the enzyme activity by the following formula. One unit of the enzyme activity is equivalent to the amount of the enzyme required to liberate 1 μmol of D(−)-fructose per minute when determined as directed in the Procedure.

$$\text{Enzyme activity (unit/g)} = \frac{(C_T - C_B) \times V_T}{M}$$

C_T = concentration (μmol/mL) of D(−)-fructose in the test solution,
C_B = concentration (μmol/mL) of D(−)-fructose in the reference solution,
V_T = whole volume (mL) of the sample solution prepared,
M = weight (g) of the sample.

Operating conditions

Detector: Differential refractometer.

Column: A stainless steel tube (8 mm internal diameter and 30 cm length).

Column packing material: About 9-μm cation-exchange resin for liquid chromatography (Ca-form).

Column temperature: 80°C.

Mobile phase: Water.

Flow rate: 0.4 mL/min.

Psyllium Seed Gum

サイリウムシードガム

Definition Psyllium Seed Gum is obtained from the seed coats of the blonde psyllium *Plantago ovata* Forssk. and consists mainly of polysaccharides. It may contain sucrose, glucose, lactose, dextrin, or maltose.

Description Psyllium Seed Gum occurs as an off-white or light yellow-brown powder or as granules. It is odorless or has a slight characteristic odor.

Identification Place 2 g of Psyllium Seed Gum in a 400-mL beaker, add 200 mL of water, and dissolve it while stirring at 80°C for 10 minutes. When cooled to room temperature, the solution becomes a characteristic, flowable substance in a partial gel or partial sol state.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Protein Not more than 2.0%.

Weigh accurately about 1 g of Psyllium Seed Gum, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

Each mL of 0.005 mol/L sulfuric acid = 0.8754 mg of protein

Loss on Drying Not more than 12.0% (105°C, 5 hours).

Ash Not more than 5.0% (on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 2 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture For the *Escherichia coli* test, prepare as follows: Mix 1 g of Psyllium Seed Gum with 200 mL of lauryl sulfate broth to disperse uniformly and incubate at 35 ± 1°C for 48 ± 2 hours. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 1 g of Psyllium Seed Gum with 200 mL of lactose broth to disperse completely, and incubate it at 35 ± 1°C for 24 ± 2 hours.

Pullulan

プルラン

Definition Pullulan is obtained by isolation from the culture fluid of the filamentous fungus *Aureobasidium pullulans*. It consists of the polysaccharide pullulan.

Description Pullulan occurs as a white to light yellowish-white powder. It is odorless or has a slight characteristic odor.

Identification

(1) Dissolve 10 g of Pullulan in 100 mL of water by adding in small amounts while stirring. A viscous solution is produced.

(2) To 10 mL of the solution prepared in Identification (1), add 0.1 mL of pullulanase TS, mix, and allow to stand. It is not viscous.

(3) To 10 mL of a solution of Pullulan (1 in 50), add 2 mL of polyethylene glycol 600. A white precipitate forms immediately.

Kinematic Viscosity 15–180 mm^2/s.

Weigh 10.0 g of Pullulan, previously dried, dissolve it in water, and make exactly 100 g of solution. Measure the viscosity at 30 ± 0.1°C.

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Total nitrogen Not more than 0.05%.

Weigh accurately about 3 g of Pullulan, and determine the total nitrogen by the semi-micro Kjeldahl method. Use 12 mL of sulfuric acid to decompose the sample. The amount of sodium hydroxide solution (2 in 5) added in the distillation procedure is 40 mL.

(4) Mono- and oligo-saccharides Not more than 12.0%.

Test Solution Dissolve 0.800 g of Pullulan, dried previously, in 100 mL of water, and use this solution as the sample stock solution. Place 1 mL of the stock solution in a centrifuge tube, add 0.1 mL of saturated potassium chloride solution and 3 mL of methanol, mix vigorously, and centrifuge. Use the supernatant as the sample solution. Gently add exactly 0.2 mL of the sample solution to 5 mL of a solution (1 in 500) of anthrone in diluted sulfuric acid (3 in 4), previously cooled in icy water, and mix immediately. Warm the mixture at 90ºC for 10 minutes, and then cool immediately.

Standard Solution and Blank Test Solution To exactly 1 mL of the sample stock solution, add water to make exactly 50 mL. With exactly 0.2 mL each of the resulting solution and water in place of 0.2 mL of the sample solution, proceed in the same manner as the preparation of the test solution, and use the solutions obtained as a standard solution and a blank test solution, respectively.

Procedure Measure the absorbance (A_T, A_S, and A_0) of the test solution, standard solution, and blank test solution at a wavelength of 620 nm. Use water as the reference.

Calculate the content by the formula:

$$\text{Content (\%) of mono-and oligo-saccharides} = \frac{A_T - A_0}{A_S - A_0} \times 8.2$$

Loss on Drying Not more than 8.0% (90°C, reduced pressure, 6 hours).

Residue on Ignition Not more than 5.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 100 per gram.

Coliforms: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 1 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture Prepare as directed in Method 1, respectively, for the *coliform* test and the *Salmonella* test.

Pullulanase

プルラナーゼ

Definition Pullulanase includes enzymes that degrade pullulan. It is derived from the culture of bacteria (limited to *Pullulanibacillus naganoensis*, *Sulfolobus solfataricus*, and species of the genera *Bacillus and Klebsiella*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Pullulanase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Pullulanase complies with the Pullulanase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Pullulanase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Pullulanase, add water or citric acid–sodium hydroxide buffer (0.02 mol/L) at pH 5.0 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution Weigh 0.40 g of pullulan, and dissolve it in citric acid–sodium hydroxide buffer (0.02 mol/L) at pH 5.0 to make 100 mL. Prepare fresh before use.

Test Solution Transfer 1 mL of the substrate solution into a test tube, equilibrate at

40°C, add 1 mL of the sample solution, equilibrated at 40°C, and shake immediately. Incubate the mixture at 40°C for 30 minutes, add 2 mL of Somogyi TS (I), and mix. Cover the mouth of the test tube with a glass bead, heat it in a water bath for 20 minutes, and allow to cool to room temperature. Add 2 mL of Nelson TS to dissolve the red precipitate, add 4 mL of water, and allow to stand for 30 minutes.

Control Solution Transfer 1 mL of the sample solution into a test tube, add 2 mL of Somogyi TS (I), mix, add 1 mL of the substrate solution, and mix. Cover the mouth of the test tube with a glass bead, heat it in a water bath for 20 minutes, and allow to cool to room temperature. To this solution, add 2 mL of Nelson TS to dissolve the red precipitate, then add 4 mL of water, and allow to stand for 30 minutes.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 520 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Method 2

Sample Solution Weigh 1.0 g of Pullulanase, add water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water to the resulting solution.

Substrate Solution Weigh 1.0 g of pullulan (red), and dissolve it in 50 mL of acetate buffer (0.2 mol/L) at pH 5.0.

Test Solution Add 1 mL of the substrate solution to 1 mL of the sample solution, and shake immediately. Incubate the mixture at 40°C for 20 minutes. Add 4.0 mL of ethanol (99.5), mix, and allow to stand at room temperature for 5 minutes. Centrifuge, and use the supernatant.

Control Solution Proceed as directed for the test solution using acetate buffer (0.2 mol/L) at pH 5.0 instead of the sample solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 510 nm. The absorbance value of the test solution is higher than that of the control solution.

Method 3

Sample Solution Weigh 1.0 g of Pullulanase, add citric acid–sodium hydroxide buffer (0.05 mol/L, pH 5.0, containing cysteine) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 5-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Weigh 0.3 g of pullulan (reduced), and dissolve it in citric acid–sodium hydroxide buffer (0.05 mol/L, pH 5.0, containing cysteine) to make 50 mL.

Test Solution Equilibrate 3.3 mL of the substrate solution at 50°C for 8 minutes, add 0.6 mL of the sample solution, and incubate the mixture at 50°C for 20 minutes. To this solution, add 1.8 mL of *p*-hydroxybenzoic acid hydrazide TS, shake immediately, and allow to stand at room temperature for 20 minutes.

Control Solution Proceed as directed for the test solution using citric acid–sodium hydroxide buffer (0.05 mol/L, pH 5.0, containing cysteine) instead of the sample solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 405 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Purified Carrageenan

Refined Carrageenan

精製カラギナン

Definition Purified Carrageenan is one of carrageenans. "Carrageenan"* is defined as a substance that is obtained from the whole algae of the genus *Hypnea*, *Eucheuma*, *Iridaea*, *Gigartina,* or *Chondrus* and that consists mainly of ι-carrageenan, κ-carrageenan, and λ-carrageenan. It may contain sucrose, glucose, maltose, lactose, or dextrin.

Description Purified Carrageenan occurs as a white to light-brown powder or as granules. It has no or slight odor.

Identification

(1) Proceed as directed in Identification (1) for Semirefined Carrageenan.

(2) To 20 mL of water, add 0.1 g of Purified Carrageenan, then add 3 mL of a solution of barium chloride dihydrate (3 in 25) and 5 mL of diluted hydrochloric acid (1 in 5), stir well, and remove the precipitate if necessary. Boil the solution for 5 minutes, and a white crystalline precipitate is formed.

Viscosity Not less than 5.0 mPa・s. Proceed as directed the viscosity test for Semirefined Carrageenan.

Purity

(1) Sulfate group 15–40 %.

Weigh accurately about 8 g Purified Carrageenan, and disperse in 400 mL of 60% (vol) 2-propanol. Stir gently for 4 hours, and filter through a filter paper (5C) for quantitative analysis. Wash the residue on the filter paper four times with two 10-mL portions of 60% (vol) 2-propanol and then with two 10-mL portions of 2-propanol. Dry the residue at 105°C to constant weight, and use as the test sample. Weigh accurately about 1 g of the test sample into a 100-mL Kjeldahl flask, and add 50 mL of diluted hydrochloric acid (1 in 10). Boil under a reflux condenser for 1 hour. Add 25 mL of 10% (vol) hydrogen peroxide, and boil for an additional 5 hours. Filter to remove the precipitate if necessary,

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* "Carrageenan" is defined in the List of Existing Food Additives.

transfer the filtrate to a 500-mL beaker, and gently add 10 mL of a solution of barium chloride dihydrate (3 in 25) while boiling. Heat for 2 hours in a water bath, cool, and filter through a filter paper (5C) for quantitative analysis. Wash the residue on the filter paper with warm water until the filtrate is free from chloride. Dry the residue with the filter paper, place in a ceramic crucible, and incinerate. Weigh the crucible containing ash, determine the amount of ash (as barium sulfate), and calculate the percentage of sulfate group (SO_4) by formula:

$$\text{Amount (\%) of sulfate group } (SO_4) = \frac{\text{Weight (g) of barium sulfate} \times 0.4116}{\text{Weight (g) of the sample}} \times 100$$

(2) Acid-insoluble substances Not more than 2.0%.

Weigh accurately about 2 g of the test sample prepared in Purity (1), and proceed as directed in Purity (4) for Semirefined Carrageenan.

(3) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) 2-Propanol and methanol Not more than 0.10% as the sum of 2-propanol and methanol.

Proceed as directed in Purity (7) for Semirefined Carrageenan.

Loss on Drying Not more than 12.0% (105°C, 4 hours).

Ash 15.0–40.0 % (2.0 g of the sample prepared in Purity (1)).

Acid-insoluble Ash Not more than 1.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid For total plate count and the enumeration of yeasts and molds, prepare as follows: Mix 1 g of Purified Carrageenan with 200 mL of phosphate buffer, 0.1% peptone solution, or sodium chloride-peptone buffer to disperse uniformly.

Pre-enrichment Culture For the *Escherichia coli* test, prepare as follows: Mix 1 g of Purified Carrageenan with 200 mL of lauryl sulfate broth to disperse uniformly, and incubate at 35 ± 1°C for 48 ± 2 hours. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 1 g of Purified Carrageenan with 200 mL of lactose broth to disperse uniformly, and incubate at 35 ± 1°C for 24 ± 2 hours.

Purple Corn Color

ムラサキトウモロコシ色素

Definition Purple Corn Color is obtained from the seeds or ears of the corn plant *Zea mays* L. and consists mainly of cyanidin 3-glucoside. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Purple Corn Color is not less than 30 and is in the range of 90–120% of the labeled value.

Description Purple Corn Color occurs as a dark red powder, paste, or liquid. It has a slight characteristic odor.

Identification

(1) Weigh an amount of Purple Corn Color equivalent to 1 g of purple corn color with a Color Value 30, and dissolve it in 100 mL of citrate buffer (pH 3.0). A red to dark red-orange color develops.

(2) To the solution prepared in Identification (1), add sodium hydroxide solution (1 in 25) to make it alkaline. The solution turns dark green.

(3) A solution of Purple Corn Color in citrate buffer (pH 3.0) exhibits an absorption maximum at a wavelength of 505–525 nm.

(4) Prepare a test solution by diluting 10 mL of the solution prepared in Identification (1) to 100 mL with citrate buffer (pH 3.0). Separately, prepare a standard solution by dissolving 1 mg of cyanidin 3-glucoside chloride in citrate buffer (pH 3.0) to make 5 mL. Analyze 10 μL each of the test solution and the standard solution by high-pressure liquid chromatography using the operating conditions given below. The main peak of the test solution corresponds to the retention time of cyanidin 3-glucoside chloride.

Operating Conditions

Detector: Visible absorption spectrophotometer (wavelength: 515 nm).

Column: A stainless steel tube (4–5 mm internal diameter and 15–30 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 73:27 mixture of 4 % phosphoric acid solution/methanol.

Flow rate: Adjust the flow rate the retention time of cyanidin 3-glucoside chloride to about 10 minutes.

Purity

(1) Lead Not more than 8 μg/g as Pb (0.50 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Fumonisin B_1 Not more than 0.3 μg/g (on the basis of a Color Value 30).

Test Solution Set up a glass or polypropylene column (15 mm internal diameter). Pack it with about 2 g of trimethylaminopropyl-bonded silica gel and rinse with methanol and then with a 3:1 mixture of methanol/water. Weigh accurately an amount of Purple Corn Color equivalent to about 5 g of purple corn color with a Color Value 30, add 80 mL of a 3:1 mixture of methanol/water, and shake. Adjust the pH to 8–9 with sodium hydroxide solution (1 in 10), and add a 3:1 mixture of methanol/water to make exactly 100 mL (sample solution). Pour 10 mL of the sample solution into the column, and discard the effluent. Rinse the column with 20 mL of a 3:1 mixture of methanol/water and then with 10 mL of methanol. Pour 20 mL of a 99:1 mixture of methanol/acetic acid onto the column, and collect the effluent. Evaporate it to dryness under reduced pressure at less than 40°C, and dissolve the residue by adding 0.2 mL of a 1:1 mixture of water/acetonitrile.

Standard Solution Weigh accurately about 10 mg of fumonisin B_1, and add a 1:1 mixture of water/acetonitrile to make exactly 100 mL. Measure exactly 10 mL, 5 mL, and 1 mL of this solution into separate 200-mL volumetric flasks, and add a 1:1 mixture of water/acetonitrile to volume.

Procedure To 0.1 mL of each of the test solution and the standard solutions, add 0.1 mL of phthalaldehyde TS, and shake. Measure exactly 20 μL of each solution, inject each into the liquid chromatograph within one minute after the addition of phthalaldehyde TS, and analyze by liquid chromatography according to the operating conditions given below. Measure the fumonisin B_1 peak areas for the test solution and the standard solutions, and calculate the content using the calibration curve prepared.

Operating Conditions

Detector: Fluorescence spectrophotometer (excitation wavelength: 335 nm, fluorescent wavelength: 440 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 15 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 25°C.

Mobile phase: A 7:3 mixture of methanol/phosphate buffer (pH 3.3).

Flow rate: Adjust the retention time of fumonisin B_1 to about 17 minutes.

Color Value Determination Proceed as directed under Color Value Determination, using the following operating conditions.

Operating Conditions

Solvent: Citrate buffer (pH 3.0).

Wavelength: Maximum absorption wavelength of 505–525 nm.

Purple Sweet Potato Color

ムラサキイモ色素

Definition Purple Sweet Potato Color is obtained from the tuberous roots of the sweet potato plant *Ipomoea batatas* (L.) Poir. and consists mainly of cyanidin acylglucosides and peonidin acylglucosides. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Purple Sweet Potato Color is not less than 50 and is in the range of 90–110% of the labeled value.

Description Purple Sweet Potato Color occurs as a dark red powder, paste, or liquid. It has a slight characteristic odor.

Identification

(1) Weigh an amount of Purple Sweet Potato Color equivalent to 1.0 g of purple sweet potato color with a Color Value 50, and dissolve it in 100 mL of citrate buffer (pH 3.0). A red to dark purple-red color develops.

(2) To the solution prepared in Identification (1), add sodium hydroxide solution (1 in 25) to make it alkaline. The solution turns dark green.

(3) A solution of Purple Sweet Potato Color in citrate buffer (pH 3.0) exhibits an absorption maximum at a wavelength of 515–535 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination, using the following operating conditions.

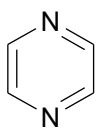
Operating Conditions

Solvent: Citrate buffer (pH 3.0).

Wavelength: Maximum absorption wavelength of 515–535 nm.

Pyrazine

ピラジン



$C_4H_4N_2$ Mol. Wt. 80.09

Pyrazine [290-37-9]

Content Pyrazine contains not less than 98.0% of pyrazine ($C_4H_4N_2$).

Description Pyrazine occurs as a white to light yellow solid having a characteristic odor.

Identification Hold a powdered sample of Pyrazine between two optical plates, warm to melt, and cool. Determine the infrared absorption spectrum of Pyrazine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

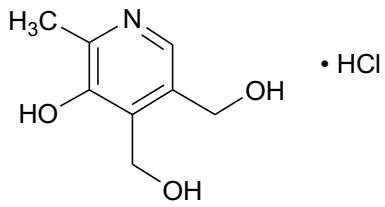
Melting Point 51–55°C.

Assay Using a solution (1 in 10) of Pyrazine in ethanol (95) as the test solution, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2).

Pyridoxine Hydrochloride

Vitamin B_6

ピリドキシン塩酸塩



$C_8H_{11}NO_3 \cdot HCl$ Mol. Wt. 205.64

(5-Hydroxy-6-methylpyridine-3,4-diyl)dimethanol monohydrochloride [58-56-0]

Content Pyridoxine Hydrochloride, when calculated on the dried basis, contains not less than 98.0% of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$).

Description Pyridoxine Hydrochloride occurs as white to light yellow crystals or crystalline powder. It is odorless.

Identification

(1) To 1 mL of a solution of Pyridoxine Hydrochloride (1 in 10,000), add 2 mL of a solution (1 in 4000) of 2,6-dibromo-*N*-chloro-*p*-benzoquinone monoimine in ethanol (95) and 1 drop of ammonia TS. A blue color develops. When the same test is performed using Pyridoxine Hydrochloride to which 1 mL of boric acid saturated solution is previously added, no blue color develops.

(2) Pyridoxine Hydrochloride responds to all the tests for Chloride in the Qualitative Tests.

Melting Point 203–209°C (decomposition).

pH 2.5–3.5 (0.50 g, water 25 mL).

Purity Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Loss on Drying Not more than 0.5% (4 hours).

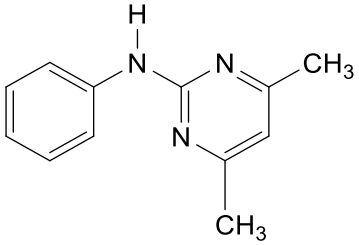
Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.4 g of Pyridoxine Hydrochloride, add 5 mL of acetic acid and 5 mL of acetic anhydride, and dissolve it by boiling gently. After cooling, add 30 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid (indicator: 1 mL of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction. Calculate on the dried basis.

Each mL of 0.1 mol/L perchloric acid = 20.56 mg of $C_8H_{11}NO_3 \cdot HCl$

Pyrimethanil

ピリメタニル



$C_{12}H_{13}N_3$ Mol. Wt. 199.25

N-(4,6-dimethylpyrimidin-2-yl)aniline [53112-28-0]

Content Pyrimethanil contains not less than 96.0% of pyrimethanil ($C_{12}H_{13}N_3$).

Description Pyrimethanil occurs as a white to yellowish-white, odorless powder.

Identification Determine the infrared absorption spectrum of Pyrimethanil as directed in the Disk Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 96–98°C.

Purity Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Water Content Not more than 1.0% (2.0 g, Volumetric Titration, Direct Titration).

Assay Weigh accurately about 50 mg each of Pyrimethanil and pyrimethanil for assay.

Dissolve them separately in methanol to make exactly 50 mL of each. Take 1 mL each, and add a 3:1 mixture of acetonitrile/water to make exactly 20 mL each. Use them as test solution and standard solution, respectively. Analyze 10 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of pyrimethanil for the test solution and the standard solution. Calculate the pyrimethanil content by the formula:

$$\text{Content (\%) of pyrimethanil } (C_{12}H_{13}N_3) = \frac{\text{Weight (g) of pyrimethanil for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 268 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μm octadecyl silanized silica gel for liquidchromatography.

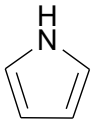
Column temperature: A constant temperature of 24–40°C.

Mobile phase: To 750 mL of acetonitrile, add 250 mL of water and 2 g of ammonium acetate, and dissolve.

Flow rate: Adjust the retention time of pyrimethanil to about 5–6 minutes.

Pyrrole

ピロール



C_4H_5N Mol. Wt. 67.09

Pyrrole [109-97-7]

Content Pyrrole contains not less than 98.0% of pyrrole (C_4H_5N).

Description Pyrrole occurs as a colorless to yellow, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Pyrrole, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

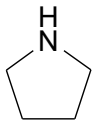
Refractive Index n_D^{20}: 1.507–1.511.

Specific Gravity d_{25}^{25}: 0.955–0.975.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2).

Pyrrolidine

ピロリジン



C_4H_9N Mol. Wt. 71.12

Pyrrolidine [123-75-1]

Content: Pyrrolidine contains not less than 95.0% of pyrrolidine (C_4H_9N).

Description: Pyrrolidine occurs as a colorless, clear liquid having a characteristic odor.

Identification: Determine the infrared absorption spectrum of Pyrrolidine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare it with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.440–1.446.

Specific Gravity d_{25}^{25}: 0.853–0.863.

Assay: Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavoring Substances Tests. Use operating conditions (2), except for the column. Use a fused silica tube (0.25–0.53 mm internal diameter and 30–60 m length) coated with a 0.25–1 μm thick layer of dimethyl polysiloxane.

Quicklime

生石灰

Definition Quicklime is obtained by calcinating limestones. It consists mainly of calcium oxide.

Content Quicklime, when ignited, contains not less than 93.0% of calcium oxide (CaO = 56.08).

Description Quicklime occurs as white to grayish-white lumps, granules, or powder.

Identification

(1) Moisten 1 g of Quicklime with water. It generates heat. Then add 5 mL of water. The resulting suspension is alkali.

(2) To 1 g of Quicklime, add 20 mL of water and 6 mL of acetic acid (1 in 3), and filter. The filtrate responds to all the tests for Calcium Salt as described in the Quantitative Tests.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 1.0%.

Weigh accurately a crucible-type glass filter (1G4), previously dried at 105°C for 30 minutes and cooled in a desiccator. Weigh 5.0 g of Quicklime, add 100 mL of water, then add hydrochloric acid dropwise while shaking until the sample no longer dissolves, and boil. After cooling, add hydrochloric acid to acidify if necessary. Filter through with the previously weighed glass filter. Wash the residue well on the filter with water until the washings are free of chloride. Dry the residue with the filter at 105°C for 1 hour. Allow to cool in a desiccator, and weigh accurately.

(2) Carbonate Weigh 2.0 g of Quicklime, add 50 mL of water, shake thoroughly, and add 25 mL of diluted hydrochloric acid (1 in 4). It does not effervesce vigorously.

(3) Lead Not more than 5 µg/g as Pb (0.80 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution Weigh the specified amount of Quicklime into a crucible. Heat it gradually to carbonize, and ignite at 500°C with a lid. Add 20 mL of diluted hydrochloric acid (1 in 4) to the residue, cover with a watch glass, and boil gently for 15 minutes. After cooling, add 30 mL of water. If the sample does not dissolve, evaporate to dryness, add 20 mL of diluted hydrochloric acid (1 in 4) to the residue, and boil gently for 5 minutes. After cooling, add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1 mL of bromothymol blue TS as the indicator instead of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(4) Alkali metals and magnesium Not more than 6.0%.

Weigh accurately about 0.5 g of Quicklime, add 30 mL of water and 15 mL of diluted hydrochloric acid (1 in 4) to dissolve it. Heat the solution, and boil for 1 minute. Immediately add 40 mL of a solution of oxalic acid dihydrate (3 in 50), agitate, and add 2 drops of methyl red TS, then add ammonia solution dropwise until the solution exhibits a yellow color to precipitate the calcium. Heat this solution on a water bath for 1 hour, cool, and add water to make 100 mL. Mix well, and filter. Transfer 50 mL of the filtrate into a platinum crucible, previously ignited at 800°C for 30 minutes, cooled in a desiccator, and weighed accurately. Add 0.5 mL of sulfuric acid, evaporate to dryness, ignite at 800°C to constant weight, and weigh the crucible to determine the weight of the residue.

(5) Barium Not more than 300 µg /g as Ba.

Test Solution Weigh 1.50 g of Quicklime, and add water to make it muddy. Add 20 mL of diluted hydrochloric acid (1 in 4) to dissolve it, and add water to make 30 mL. Filter the solution, and use 20 mL of the filtrate as the test solution.

Procedure To the test solution, add 2 g of sodium acetate, 1 mL of diluted acetic acid (1 in 20), and 0.5 mL of potassium chromate solution (1 in 20), and allow to stand for 15 minutes. The solution is not more turbid than a control solution prepared as follows: To 0.30 mL of Barium Standard Solution, add water to make 20 mL, and then treated in the same manner as for the test solution.

(6) Arsenic Not more than 3 µg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Quicklime in 8 mL of diluted hydrochloric acid (1 in 4).

Loss on Ignition Not more than 10.0% (800°C, constant weight).

Assay Weigh accurately about 1.5 g of Quicklime, previously ignited, add 30 mL of diluted hydrochloric acid (1 in 4) to dissolve, and add water to make exactly 250 mL. Use the resulting solution as the test solution. Proceed as directed under Method 1 in Calcium Salt Determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.804 mg of CaO.

Quillaia Extract

Quillaja Extract

キラヤ抽出物

Definition Quillaia Extract is obtained from the bark of the soapbark tree *Quillaja saponaria* Molina and consists mainly of saponins.

Content Quillaia Extract, when dried, contains not less than 30.0% of partially hydrolyzed saponins.

Description Quillaia Extract occurs as a reddish light-brown powder or brown liquid having a characteristic pungent taste.

Identification

(1) To 1.0 g of the powder-form sample, add the same quantity of water, and mix at room temperature. It dissolves, making a slightly suspended solution.

(2) Weigh 0.50 g of the powder-form sample or previously dried liquid-form sample,

dissolve it in 20 mL of water, and use the resulting solution as the test solution. Analyze a 2-μL portion of the test solution by thin-layer chromatography using a 30:16:8:1 mixture of ethyl acetate/ethanol (95)/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate, coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 15 cm above the initial spot. Air-dry the plate, spray with 4-methoxybenzaldehyde–sulfuric acid TS uniformly, heat at 110°C for 10 minutes, and examine. Four successive purple-brown spots are observed at R_f values of about 0.1–0.5.

pH 4.5–5.5 (4.0 g of the powder-form sample or previously dried liquid-form sample, water 100 mL).

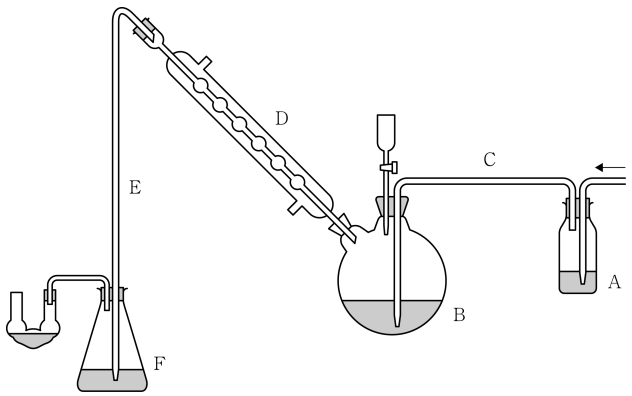
Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g of the powder-form sample or previously dried liquid-form sample, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 2 μg/g as As (0.75 g of the powder-form sample or previously dried liquid-form sample, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Sulfur dioxide Not more than 30 μg/g.

(i) Apparatus Use the apparatus as illustrated in the figure.



A: Gas washing bottle
B: Round-bottom flask
C: Gas introducing tube
D: Reflux condenser
E: Glass joint tube
F: Absorption flask

(ii) Procedure Weigh accurately about 100 g of Quillaia Extract, place in 1000-mL round-bottom flask B, and add 500 mL of methanol to produce a suspension. Equip gas-introducing tube C, so that the tube can reach the flask bottom, and join reflux condenser D to flask B. Place in absorption flask F 10 mL of hydrogen peroxide TS, previously confirmed to be neutral with methyl red TS. Equip glass joint tube E between D and F. Allow carbon dioxide or nitrogen gas to flow through introducing tube C, and remove air

in the apparatus. After air is removed, immediately add 30 mL of diluted hydrochloric acid (1 in 3) to round-bottom flask B, connect reflux condenser D to joint glass tube E. Heat round-bottom flask B slowly until methanol begins to be refluxed, and keep heating mildly for 2 hours. Detach absorption flask F from the apparatus and cool. Titrate the solution in flask F with 0.01 mol/L sodium hydroxide solution (indicator, 3 drops of methyl red TS).

Each mL of 0.01 mol/L sodium hydroxide solution = 0.3203 mg of SO_2

Water Content Powder-form sample Not more than 6.0% (1 g, Volumetric Titration, Direct Titration).

Loss on Drying Liquid-form sample 50.1–70.0% (1.0 g, 105°C, 5 hours).

Residue on Ignition Not more than 10.0% (1.0 g of the powder-form sample or previously dried liquid-form sample).

Assay

Test Solution Weigh accurately about 2 g of the powder-form sample or previously dried liquid-form sample, dissolve it in water to make exactly 100 mL. Measure exactly 10 mL of this solution, add 10 mL of potassium hydroxide solution (1 in 50), attach a reflux condenser, and heat in a water bath for 2 hours. After cooling, dissolve it in 25 mL of ethanol (95), add 0.5 mL of phosphoric acid, and add water to make exactly 50 mL.

Standard Solution Weigh accurately about 20 mg of partially hydrolyzed saponin for quantitative analysis, previously dried at 105°C for 3 hours, dissolve it in 50% (vol) ethanol to make exactly 50 mL.

Procedure Analyze 20 μL each of the test solution and the standard solution by high performance liquid chromatography using the operating conditions given below. Measure the peak area (A_{T1}) of partially hydrolyzed saponin and the peak area (A_{T2}) of analogous saponin (the relative retention time of the analogous saponin to partially hydrolyzed saponin is about 0.95) for the test solution, and the peak area (A_S) of partially hydrolyzed saponin for the standard solution.

$$\text{Content (\%) of partially hydrolyzed saponin} = \frac{\text{Weight (g) of partially hydrolyzed saponin}}{\text{Weight (g) of the sample}} \times \frac{(A_{T1} + A_{T2}) \times 10}{A_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 210 nm).

Column: Stainless steel tube (4–6 mm internal diameter and 15–30 cm length).

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 13:7 mixture of 0.1% phosphoric acid/acetonitrile.

Flow rate: Adjust the retention time of partially hydrolyzed saponin to about 10 minutes.

Red Cabbage Color

アカキャベツ色素

Definition Red Cabbage Color is obtained by extraction from the leaves of the cabbage plant *Brassica oleracea* var. capitata L. It consists mainly of cyanidin acylglucosides. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Red Cabbage Color is not less than 50 and is in the range of 90–110% of the labeled value.

Description Red Cabbage Color occurs as a dark red powder, paste, or liquid. It has a slight characteristic odor.

Identification

(1) Weigh an amount of Red Cabbage Color equivalent to 0.1 g of red cabbage color with a Color Value 50, and dissolve it in 100 mL of citrate buffer (pH 3.0). A red to dark purple-red color develops.

(2) To the solution prepared in Identification (1), add sodium hydroxide solution (1 in 25) to make it alkaline. The solution turns dark green to light yellow-green.

(3) A solution of Red Cabbage Color in citrate buffer (pH 3.0) exhibits an absorption maximum at a wavelength of 520–540 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standards Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination, using the following operating conditions.

Operating Conditions

Solvent: Citrate buffer (pH 3.0).

Wavelength: Maximum absorption wavelength of 520–540 nm.

Rennet

レンネット

Definition Rennet includes enzymes that coagulate milk. It is derived from the abomasum of ruminants or the culture of basidiomycetes (limited to *Irpex lacteus*), filamentous fungi (limited to *Cryphonectria parasitica*, *Mucor miehei*, *Mucor pusillus* Lindt, *Mucor* spp., *Rhizomucor miehei*, and *Rhizomucor pusillus*), yeasts (limited to *Kluyveromyces lactis*), or bacteria (limited to *Bacillus cereus* and *Escherichia coli*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Rennet occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Rennet complies with the Rennet Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Rennet Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 5.0 g of Rennet, add acetate buffer (pH 5.5) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold dilution by adding the same buffer to the resulting solution.

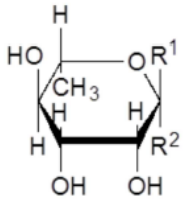
Substrate Solution To 110.0 g of nonfat dry milk, add 100 mL of calcium chloride dehydrate solution (1 in 2000) to uniformly mix. To this solution, add 900 mL of calcium

chloride dehydrate solution (1 in 2000), stir gently for 30 minute so that the solution does not effervesce, and allow to stand in a dark place for 30 minutes. Prepare fresh before use.

Procedure Transfer 25 mL of the substrate solution into a transparent glass container, equilibrate at 32°C for 15 minutes, add 0.5 mL of the sample solution, and stir gently so that the solution does not effervesce. Incubate this solution at 32°C. Fine granular scales of coagulated milk are observed on the skin of the substrate solution formed on the wall surface of the container.

L-Rhamnose

L-ラムノース



・H_2O

α-L-Rhamnopyranose: R^1 = OH, R^2 = H

β-L-Rhamnopyranose: R^1 = H, R^2 = OH

$C_6H_{12}O_5 \cdot H_2O$ Mol. Wt. 182.17

L-Rhamnopyranose monohydrate [10030-85-0]

Definition L-Rhamnose is produced by hydrolysis and separation from glycosides contained in rutin (extract)—"Rutin (extract)" is defined in the List of Existing Food Additives as a substance that is obtained from the entire part of the *azuki* plant *Vigna angularis* (Willd.) Ohwi & H. Ohashi, the buds or flowers of the plant *Styphnolobium japonicum* (L.) Schott (*Sophora japonica* L.) or the entire part of the buckwheat plant *Fagopyrum esculentum* Moench and that consists mainly of rutin—or in the fruit skins, barks, or flowers of *Citrus sinensis* (L.) Osbeck or *Citrus unshiu* (Swingle) S. Malcov, or from rhamnolipids obtained from soybean oil, rapeseed oil, or cone oil by fermentation, concentration, and separation. Its principal constituent is L-rhamnose.

Content L-Rhamnose, when dried, contains 98.0–101.5% of L-rhamnose ($C_6H_{12}O_5 \cdot H_2O$).

Description L-Rhamnose occurs as white crystals or crystalline powder. It is odorless or has a slight characteristic odor. It has a sweet taste.

Identification Analyze the test and the standard solutions prepared in Assay by liquid chromatography using operating conditions given in Assay. The retention time of the main peak from the test solution corresponds to that of the peak of L-rhamnose from the standard solution.

Specific rotation $[\alpha]_D^{20}$: +7.7 to +8.6° (after dried, 2 g, water, 50 mL).

Determination should be conducted 1 hour after the test solution is prepared.

Purity

(1) Clarity of solution Colorless and clear (1 g, water 10 mL).

(2) Sulfate Not more than 0.048% as SO_4 (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(3) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (24 hours).

Residue on Ignition Not more than 0.1% (500–550°C, 3 hours).

Assay

Test Solution and Standard Solution Weigh accurately about 0.5 g each of L-Rhamnose and L-rhamnose for assay, previously dried, and dissolve them separately in a 4:1 mixture of acetonitrile/water to make exactly 50 mL.

Procedure Analyze 10 μL each of the test solution and the standard solution by liquid chromatography using the following operating conditions. Measure the peak areas (A_T *and* A_S) of L-rhamnose for the test solution and the standard solution, and calculate the content by the formula:

$$\text{Content (\%) of L-rhamnose } (C_6H_{12}O_5 \cdot H_2O) = \frac{\text{Weight (g) of L-rhamnose for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (4–6 mm internal diameter and 15–30 cm length).

Column packing material: About 5 μm amino-bonded silica gel for liquid chromatography.

Column temperature: A constant temperature of about 35°C.

Mobile phase: A 4:1 mixture of acetonitrile/water.

Flow rate: Adjust the retention time of L-rhamnose to about 8 minutes.

Column selection: Use a column capable of producing well-resolved peaks of L-rhamnose and sucrose, in that order listed, when 20 μL of a solution prepared by dissolving 0.8 g of L-rhamnose for assay and 80 mg of sucrose in 50 mL of a 4:1 mixture of acetonitrile/water is chromatographed using the above conditions.

Rhamsan Gum

ラムザンガム

Definition Rhamsan Gum is obtained from the culture fluid of *Sphingomonas* sp. and consists mainly of polysaccharides. It may include sucrose, glucose, lactose, dextrin, or maltose.

Description Rhamsan Gum occurs as a whitish or brownish powder having a slight odor.

Identification

(1) Add 0.3 g of Rhamsan Gum gradually to 100 mL of water with vigorous stirring. A viscous solution is produced. When the solution is heated to 80°C, its viscosity remains almost unchanged.

(2) Heat the solution obtained in Identification (1) to 80°C, add 0.3 g of carob bean gum gradually with vigorous stirring, and continue to stir for an additional 10 minutes. When the solution is cooled to about 10°C, it is not gelatinized.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Total nitrogen Not more than 5.0% (on the dried basis).

Proceed as directed in the Kjeldahl method in Nitrogen Determination, using about 1 g of Rhamsan Gum, weighed accurately.

(4) 2-Propanol Not more than 0.10%.

Proceed as directed in Purity (7) for Semirefined Carrageenan in the Monographs. Methanol determination is not conducted.

Loss on Drying Not more than 15.0% (105°C, 2.5 hours).

Ash Not more than 16.0% (on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid For total plate count and the enumeration of yeasts and molds, prepare as follows: Mix 1 g of Rhamsan Gum with 500 mL of phosphate buffer, 0.1% peptone solution, or sodium chloride-peptone buffer to disperse uniformly. For the

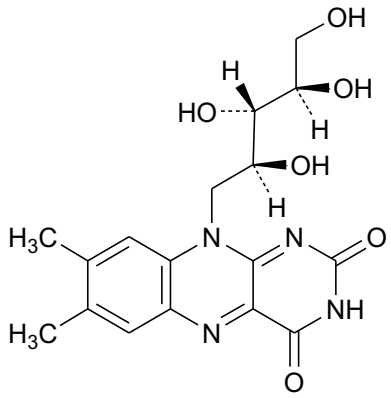
enumeration of yeasts and molds, use 2 mL of the sample fluid.

Pre-enrichment Culture　For the *Escherichia coli* test, prepare as follows: Mix 1 g of Rhamsan Gum with 500 mL of lauryl sulfate broth to disperse uniformly, and incubate at 35 ± 1°C for 48 ± 2 hours. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture:　Mix 1 g of the sample with 500 mL of lactose broth to disperse uniformly, and incubate at 35 ± 1°C for 24 ± 2 hours.

Riboflavin

Vitamin B_2

リボフラビン



$C_{17}H_{20}N_4O_6$　　Mol. Wt. 376.36

7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[*g*]pteridine-2,4(*3H*,*10H*)-dione　[83-88-5]

Content　Riboflavin, when dried, contains 98.0–102.0% of riboflavin ($C_{17}H_{20}N_4O_6$).

Description　Riboflavin occurs as yellow to orange-yellow crystals or crystalline powder having a slight odor and a bitter taste.

Identification　A solution of Riboflavin (1 in 100,000) is light yellow-green and emits a strong yellowish-green fluorescence, which disappears on the addition of diluted hydrochloric acid (1 in 4) or sodium hydroxide solution (1 in 25).

Specific Rotation　$[\alpha]_D^{20}$: –128.0 to –142.0°.

Weigh accurately about 0.1 g of Riboflavin, previously dried, dissolve it in 4 mL of potassium hydroxide solution (1 in 150), add 10 mL of freshly boiled and cooled water, and add 4 mL of ethanol (95) with sufficient shaking. Add freshly boiled and cooled water to make exactly 20 mL, and measure the angular rotation of the resulting solution within 30 minutes.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Lumiflavin Weigh 25 mg of Riboflavin, add 10 mL of chloroform (ethanol-free), shake for 5 minutes, and filter. The color of the filtrate is not darker than that of the solution prepared by adding water to 3.0 mL of 1/60 mol/L potassium dichromate to make 1000 mL.

Loss on Drying Not more than 1.5% (105°C, 2 hours).

Residue on Ignition Not more than 0.3%.

Assay Throughout this assay, all procedures should be protected from direct light and the apparatus used should be light-resistant.

Test Solution Weigh accurately about 15 mg of Riboflavin, previously dried, add 800 mL of diluted acetic acid (1 in 400), dissolve it by warming, cool, and add water to make exactly 1000 mL.

Standard Solution Weigh accurately about 15 mg of Riboflavin Reference Standard, previously dried at 105° for 2 hours, and proceed as directed for the test solution.

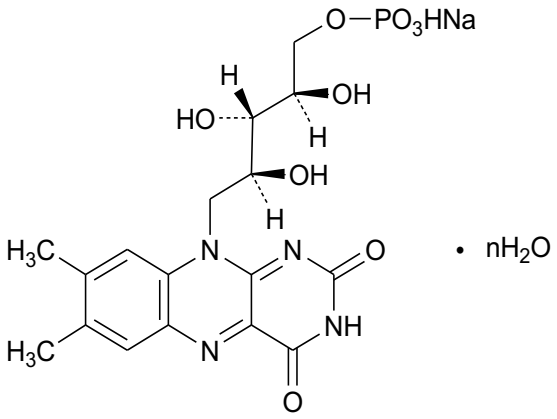
Procedure Using water as the reference solution, measure the absorbance (A_T and A_S) of the test solution and standard solution, respectively, at a wavelength of 445 nm. Add 20 mg of sodium dithionite to 5 mL of each solution, and shake well to discolor, and immediately measure the absorbance (A_T' and A_S'). Calculate the content by the formula:

$$\text{Content (\%) of riboflavin } (C_{17}H_{20}N_4O_6) = \frac{\text{Weight (mg) of Riboflavin Reference Standard}}{\text{Weight (mg) of the sample}} \times \frac{A_T - A_T'}{A_S - A_S'} \times 100$$

Riboflavin 5′-Phosphate Sodium

Sodium Riboflavin Phosphate
Sodium Vitamin B_2 Phosphate

リボフラビン 5′-リン酸エステルナトリウム



n = 2 or 0

$C_{17}H_{20}N_4NaO_9P \cdot nH_2O$ (n = 2 or 0) Mol. Wt. dihydrate 514.36

anhydrous 478.33

Monosodium (2*R*,3*S*,4*S*)-5-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10 (2*H*)-yl)-2,3,4-trihydroxypenthyl monohydrogenphosphate dihydrate

Monosodium (2*R*,3*S*,4*S*)-5-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)-2,3,4-trihydroxypenthyl monohydrogenphosphate [130-40-5]

Content Riboflavin 5′-Phosphate Sodium, when calculated on the anhydrous basis, contains not less than 95.0% of riboflavin 5′-phosphate sodium ($C_{17}H_{20}N_4NaO_9P$).

Description Riboflavin 5′-Phosphate Sodium occurs as yellow to orange crystals or crystalline powder. It is almost odorless and has a bitter taste.

Identification

(1) Proceed as directed under Identification for Riboflavin.

(2) To 50 mg of Riboflavin 5′-Phosphate Sodium, add 10 mL of nitric acid, evaporate to dryness on a water bath, and ignite. To the residue, add 10 mL of diluted nitric acid (1 in 50), and boil for 5 minutes. Cool, neutralize with ammonia TS, and filter if necessary. The solution responds to all the tests for Sodium Salt and for Phosphate in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +38.0 to +43.0° (0.3 g, diluted hydrochloric acid (9 in 20), 20 mL, on the anhydrous basis).

Purity

(1) Clarity of solution Clear (0.20 g, water 10 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Lumiflavin Proceed as directed in Purity (2) for Riboflavin, using 35 mg of Riboflavin 5′-Phosphate Sodium.

Water Content Not more than 10.0% (0.1 g, Volumetric Titration, Back Titration).

Instead of 20 mL of methanol for water determination, use 25 mL of a 1:1 mixture of methanol for water determination/ethylene glycol for water determination.

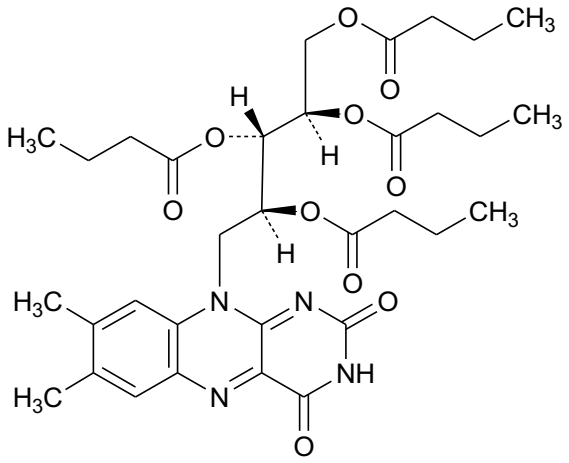
Assay Weigh accurately about 20 mg of Riboflavin 5′-Phosphate Sodium, and proceed as directed in the Assay for Riboflavin. Calculate the content by the formula:

$$\text{Content (\%) of riboflavin 5′-phosphate sodium } (C_{17}H_{20}N_4NaO_9P) = \frac{\text{Weight (g) of Riboflavin Reference Standard}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T - A_T'}{A_S - A_S'} \times 1.271 \times 100$$

Riboflavin Tetrabutyrate

Vitamin B_2 Tetrabutyrate

リボフラビン酪酸エステル



$C_{33}H_{44}N_4O_{10}$ Mol. Wt. 656.72

(2*R*,3*S*,4*S*)-5-(7,8-Dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)pentane-1,2,3,4-tetrayl tetrabutanoate [752-56-7]

Content Riboflavin Tetrabutyrate, when dried, contains 97.0–102.0% of riboflavin tetrabutyrate ($C_{33}H_{44}N_4O_{10}$).

Description Riboflavin Tetrabutyrate occurs as yellow-orange crystals or crystalline powder. It is almost tasteless and has a slight, characteristic odor.

Identification

(1) To 5 mL of a solution (1 in 500) of Riboflavin Tetrabutyrate in ethanol (95), add 2 mL of a 1:1 mixture of hydroxylammonium chloride solution (3 in 20)/sodium hydroxide solution (3 in 20), shake well, and add 0.8 mL of hydrochloric acid, 0.5 mL of a solution of iron(III) chloride hexahydrate (1 in 10), and 8 mL of ethanol (95). A deep red-brown

color develops.

(2) A solution (1 in 100,000) of Riboflavin Tetrabutyrate in ethanol (95) is light yellow-green and emits a strong yellowish-green fluorescence, which disappears on the addition of diluted hydrochloric acid (1 in 4) or sodium hydroxide solution (1 in 25).

Purity

(1) Clarity of solution Clear (0.10 g, chloroform 10 mL).

(2) Absorbance ratio Weigh 0.10 g of Riboflavin Tetrabutyrate, and dissolve it in ethanol (95) to make 200 mL. Measure 10 mL of this solution, and add ethanol (95) to make 200 mL. The solution obtained exhibits absorption maxima at wavelengths of 270 nm, 350 nm, and 445 nm. When the absorbance values at the respective maximum wavelengths are expressed as A_1, A_2, and A_3, A_1/A_3 is 2.47–2.77, A_1/A_2 is 3.50–3.90, and A_2/A_3 is 0.65–0.75.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Loss on Drying Not more than 1.0% (reduced pressure, 4 hours).

Residue on Ignition Not more than 0.5%.

Assay Throughout this assay, all procedures should be protected from direct light and the apparatus used should be light-resistant.

Test Solution Weigh accurately about 40 mg of Riboflavin Tetrabutyrate, previously dried, and dissolve it in ethanol (95) to make exactly 500 mL. Measure exactly 10 mL of this solution, and add ethanol (95) to make exactly 50 mL.

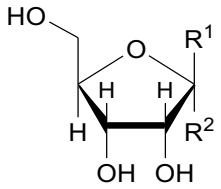
Standard Solution Weigh accurately about 50 mg of Riboflavin Reference Standard, previously dried at 105°C for 2 hours, add 160 mL of diluted acetic acid (1 in 40), dissolve it by heating, cool, and add water to make exactly 500 mL. Measure exactly 5 mL of this solution, and add ethanol (95) to make exactly 50 mL.

Procedure Measure the absorbance (A_T and A_S) of the test solution and the standard solution at a wavelength of 445 nm against ethanol (95) as the reference solution. Calculate the content by the formula:

$$\text{Content (\%) of riboflavin tetrabutyrate } (C_{33}H_{44}N_4O_{10}) = \frac{\text{Weight (g) of Riboflavin Reference Standard}}{\text{Weight (g) of the sample} \times 2} \times \frac{A_T \times 1.745}{A_S} \times 100$$

D-Ribose

D-リボース



α-D-Ribose: R^1 = H, R^2 = OH

β-D-Ribose: R^1 = OH, R^2 = H

$C_5H_{10}O_5$ Mol. Wt. 150.13

D-Ribofuranose [50-69-1]

Definition D-Ribose is obtained by isolation from the fermentation culture fluid of D-glucose by the bacterium *Bacillus pumilus* or *Bacillus subtilis*. It consists mainly of D-ribose.

Content D-Ribose, when calculated on the anhydrous basis, contains 90.0–102.0% of D-ribose ($C_5H_{10}O_5$).

Description D-Ribose occurs as white or light brown crystals or powder. It is odorless or has a slight characteristic odor.

Identification

(1) Add 2–3 drops of a solution of D-Ribose (1 in 20) to 5 mL of boiling Feling's TS. A red precipitation is produced.

(2) A solution of D-Ribose (1 in 50) is levorotatory.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Other Sugars Analyze the test solution and the standard solution by liquid chromatography as directed in the Assay. The sum of the peak areas of all solutes, other than D-ribose, in the test solution that appear within two times the retention time of D-ribose is not greater than 10.0% of the total area of all the peaks.

Water Content Not more than 5.0% (1 g, Volumetric Titration, Direct Titration).

Residue on Ignition Not more than 1.0%.

Assay

Test Solution and Standard Solution Weigh accurately about 1 g each of D-Ribose and D-ribose for assay, separately dissolve them in water, and make 2 solutions of exactly

50 mL each. Use them as the test solution and the standard solution, respectively.

Procedure Analyze 10 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of D-ribose for the test solution and the standard solution. Calculate the content by the following formula:

$$\text{Content (\%) of D-ribose } (C_5H_{10}O_5) = \frac{\text{Anhydrous basis weight (g) of D-ribose for assay}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (8 mm internal diameter and 25–35 cm length).

Column packing material: About 6-μm gel strongly acidic anion exchange resin for liquid chromatography.

Column temperature: 80°C.

Mobile phase: Water.

Flow rate: Adjust the retention time of D-ribose to about 14 minutes.

Rice Bran Oil Extract

コメヌカ油抽出物

Definition Rice Bran Oil Extract is obtained from rice bran oil by extraction and consist mainly of ferulic acid and its esters.

Content Rice Bran Oil Extract, when calculated on the dried basis, contains the equivalent of not less than 60% of ferulic acid ($C_{10}H_{10}O_4$ = 194.18).

Description Rice Bran Oil Extract occurs as a white to yellowish-white powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Dissolve 10 mg of Rice Bran Oil Extract in 10 mL of 3.5% (w/v) potassium hydroxide–ethanol TS by warming. A light yellow to yellow color is produced.

(2) Dissolve 10 mg of Rice Bran Oil Extract in 2 mL of acetone, and add 0.1 mL of a solution (1 in 50) of iron(III) chloride hexahydrate in ethanol (95). A brown to red-brown color is produced.

(3) A solution (1 in 100,000) of Rice Bran Oil Extract in methanol exhibits absorption maxima at wavelengths of 231–235 nm and 319–323 nm.

(4) Prepare a test solution by dissolving 60 mg of Rice Bran Oil Extract in ethyl

acetate to make 10 mL. Prepare two control solutions by separately dissolving 15 mg each of Rice Bran Oil Extract for assay and cycloartenyl ferulate in ethyl acetate to make 50 mL each. Analyze 5 μL each of the test solution and the control solutions by thin-layer chromatography as directed in Identification (4) for γ-Oryzanol. Two spots from the test solution correspond to the respective spots of ferulic acid and cycloartenyl ferulate in the control solutions.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Related substances Perform thin-layer chromatography as directed in Identification (4) above. The two main spots from the test solution are at the same position as the respective spots of ferulic acid and cycloartenyl ferulate in the control solution and any other single spot from the test solution is not more intense than the spot of ferulic acid in the control solution.

Loss on Drying Not more than 2.0% (105°C, 3 hours).

Residue on Ignition Not more than 0.5% (1 g).

Assay

Test Solution Weigh accurately about 30 mg of Rice Bran Oil Extract, dissolve it in 70 mL of ethanol (95) by warming. After cooling, add ethanol (95) to make exactly 100 mL. To exactly 2 mL of this solution, add ethanol (95) to make exactly 100 mL.

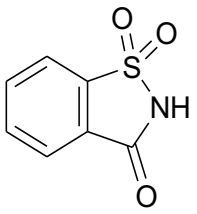
Standard Solutions Weigh accurately about 20 mg of ferulic acid for assay, previously dried at 105°C for 3 hours, and dissolve it in ethanol (95) to make exactly 100 mL. Measure exactly 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL of this solution into separate 100-mL volumetric flasks, and add ethanol (95) to make exactly 100 mL each.

Procedure Measure the absorbance of the standard solutions at the maximum at near 322 nm to prepare a calibration curve. Measure the absorbance of the test solution at the same wavelength, determine the concentration of ferulic acid in the test solution from the calibration curve, and calculate its content in the sample by the formula:

$$\text{Content (\%) of ferulic acid} = \frac{\text{Content (mg/mL) of ferulic acid in the test solution} \times 50 \times 100}{\text{Dry basis weight (mg) of the sample}} \times 100$$

Saccharin

サッカリン



$C_7H_5NO_3S$ Mol. Wt. 183.18

1,2-Benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide [81-07-2]

Content Saccharin, when dried, contains not less than 99.0% of saccharin ($C_7H_5NO_3S$).

Description Saccharin occurs as colorless to white crystals or as a white crystalline powder. It is odorless or has a slight aroma, and has an extremely sweet taste.

Identification

(1) Mix 20 mg of Saccharin with 40 mg of resorcinol, add 10 drops of sulfuric acid, and heat gently until the color of the mixture changes to dark green. Cool, and dissolve it adding 10 mL of water and 10 mL of sodium hydroxide solution (1 in 25). The solution emits a green fluorescence.

(2) Dissolve 0.1 g of Saccharin in 5 mL of sodium hydroxide solution (1 in 25), evaporate to dryness while gently heating, and fuse the residue, being careful not to carbonize it. Continue heating until the odor of ammonia is no longer evolved, and cool. Dissolve the residue by adding about 20 mL of water, neutralize with diluted hydrochloric acid (1 in 10), filter, and then add 1 drop of a solution of iron(III) chloride hexahydrate (1 in 10) to the filtrate. A purple to red-purple color develops.

Melting Point 226–230°C.

Purity

(1) Clarity of solution

Colorless and clear (1.0 g, hot water 30 mL).

Colorless and clear (1.0 g, ethanol (95) 35 mL).

(2) Lead Not more than 1 μg/g as Pb (10 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (5.0 g, Standard Color: Arsenic Standard Solution 15 mL, Apparatus B).

Test Solution Weigh the specified amount of Saccharin, transfer into a Kjeldahl flask, add 10 mL of nitric acid and 5 mL of sulfuric acid, and heat. If the solution is still brown, cool the solution, add 1 mL of nitric acid, and heat. Repeat this procedure until the solution becomes colorless or light yellow, and heat until white fumes are evolved.

After cooling, add 10 mL of water and 15 mL of ammonium oxalate saturated solution, and heat until white fumes are evolved again. After cooling, add water to make 50 mL. Use 5 mL of this solution as the test solution.

Standard Color Measure 15 mL of Arsenic Standard Solution into a Kjeldahl flask, add 10 mL of nitric acid and 5 mL of sulfuric acid, heat until white fumes are evolved, and allow to cool. Add 10 mL of water and 15 mL of a saturated solution of ammonium oxalate, heat until white fumes are evolved again, and allow to cool. Add water to make 50 mL. Measure 10 mL of this solution, and proceed as directed for the test solution in Method using Apparatus B in Procedure under the Arsenic Limit Test in the General Tests, starting with "Transfer the test solution into …."

(4) Benzoic acid and Salicylic acid Weigh 0.5 g of Saccharin, dissolve it in 15 mL of hot water, and add 3 drops of a solution of iron(III) chloride hexahydrate (1 in 10). No precipitate is formed, and no purple to red-purple color develops.

(5) *o*-Toluenesulfonamide Not more than 25 μg/g as *o*-toluenesulfonamide.

Test Solution Weigh 10 g of Saccharin, dissolve it in 70 mL of sodium hydroxide solution (1 in 25), and extract three times with 30 mL of ethyl acetate each time. Combine all the ethyl acetate layers, and wash with 30 mL of sodium chloride solution (1 in 4), and add about 10 g of sodium sulfate, and shake. Transfer the ethyl acetate layer quantitatively to an eggplant-shaped flask, evaporate the ethyl acetate, and dissolve the residue in 1.0 mL of a solution (1 in 4000) of caffeine monohydrate in ethyl acetate.

Control Solution Measure 1.0 mL of a solution (1 in 4000) of *o*-toluenesulfonamide in ethyl acetate, remove the ethyl acetate while heating on a water bath, and dissolve the residue in 1.0 mL of a solution (1 in 4000) of caffeine monohydrate in ethyl acetate.

Procedure Analyze the test solution and the control solution by gas chromatography using the conditions given below. The peak height ratio H/Hs of *o*-toluenesulfonamide (H) to caffeine (Hs) for the test solution does not exceed the peak height ratio H′/Hs′ of *o*-toluenesulfonamide (H′) to caffeine (Hs′) for the control solution.

Operating Conditions

Detector: Flame ionization detector.

Column: A glass or stainless steel tube (3–4 mm internal diameter and 1 m length).

Column packing material

Liquid phase: 3% Diethylene glycol succinate polyester of the amount of support.

Support: 177- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of 195–205°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the caffeine peak appears after about 6 minutes.

Loss on Drying Not more than 1.0% (105°C, 2 hours).

Assay Weigh accurately about 0.3 g of Saccharin, previously dried, dissolve it in 75 mL of boiling water, cool, and titrate with 0.1 mol/L sodium hydroxide (indicator: 3 drops of

phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide = 18.32 mg of $C_7H_5NO_3S$

Semirefined Carrageenan

Semirefined Carrageenan
Processed Red Algae
加工ユーケマ藻類

Definition Semirefined Carrageenan is one of carrageenans. "Carrageenan"* is defined as a substance that is obtained from the whole algae of the genus *Hypnea*, *Eucheuma*, *Iridaea*, *Gigartina,* or *Chondrus* and that consists mainly of ι-carrageenan, κ-carrageenan, and λ-carrageenan.

Description Semirefined Carrageenan occurs as a white to light brown powder or as granules. It has no or slight odor.

Identification

(1) To 4 g of Semirefined Carrageenan, add 200 mL of water. Keep at 80°C in a water bath while stirring to make a homogeneous viscous liquid. Replenish the lost water, and cool to room temperature. A viscous solution or gel is formed.

(2) To 20 mL of water, add 0.1 g of Semirefined Carrageenan and 5 mL of diluted hydrochloric acid (1 in 5), boil for 5 minutes, and remove the precipitate if necessary. When 3 mL of a solution of barium chloride dihydrate (3 in 25) is added to the resulting liquid, a white turbidity or white crystalline precipitate is formed.

Viscosity Not less than 5.0 mPa·s.

Weigh an amount of Semirefined Carrageenan equivalent to 7.5 g on the dried basis, add 450 mL of water, agitate for 10–20 minutes to disperse the sample, and add water to make 500 g of a dispersion. Heat it to 80°C in a water bath while stirring constantly. Replenish the water lost by evaporation, and measure the viscosity at 75°C as directed in Method 2 of Viscosity in the General Tests. In measurement, attach rotor No.1 and an adapter, heated to about 75°C, to the viscometer, and immerse the rotor into the dispersed sample to the specified position. Start the measurement at 30 rounds per minute, and take the readings after 6 rounds (12 seconds). If the viscosity is too low, use an adapter for low viscosity, and if it is too high, use rotor No. 2.

Purity

(1) Calcium Not more than 1.5 % as Ca.

Test Solution Weigh accurately about 10 g of Semirefined Carrageenan, previously

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* "Carrageenan" is defined in the List of Existing Food Additives.

dried, and transfer into a crucible. Heat gently to carbonize, and incinerate at 400–500°C for about 5 hours. Add 10 mL of water and 5 mL of nitric acid TS (1 mol/L) to the incinerated ash, and boil for 3 minutes. Filter it, and add water to make exactly 50 mL. Measure exactly 1 mL of the solution, add 1 mL of nitric acid TS (1 mol/L), and add water to make exactly 100 mL.

Standard Solution Weigh 2.497 g of calcium carbonate, previously dried at 180°C for 1 hour, add 20 mL of diluted hydrochloric acid (1 in 4) to dissolve, and add water to make 1000 mL exactly. Measure exactly a suitable volume of this solution, add 1 mL of nitric acid TS (1 mol/L), and exactly dilute this solution with water to make a solution containing 1–3 μg of calcium (Ca = 40.08) per mL.

Procedure Perform the tests on the test solution and standard solution as directed under Flame Atomic Absorption Spectrophotometry using the operating conditions below. Determine the calcium amount in test solution from the calibration curve prepared from the standard solution.

Operating Conditions

Light Source: Calcium hollow cathode lamp.

Wavelength: 422.7 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(2) Sodium Not more than 1.0 %.

Test Solution Weigh accurately about 1 g of Semirefined Carrageenan, previously dried, and transfer into a crucible. Heat gently to carbonize, and incinerate at 400–500°C for about 5 hours. To the ash obtained, add 5 mL of hydrochloric acid TS (3 mol/L) to disperse, and boil for 3 minutes. Using a little amount of hydrochloric acid TS (3 mol/L), wash out the contents in the crucible completely into a chromatography column (70 mm in height and 12 mm in inner diameter), stuffed with glass wool and attached to a 50-mL volumetric flask as a receiver. Elute with hydrochloric acid TS (3 mol/L) to gain about 45 mL of eluate, and add water to make exactly 50 mL. Measure exactly 2 mL of this solution, and add hydrochloric acid TS (0.02 mol/L) to make exactly 500 mL.

Standard Solution Weigh exactly 0.2542 g of sodium chloride, previously dried at 130°C for 2 hour, and dissolve it in hydrochloric acid TS (0.02 mol/L)to make exactly 1000 mL. Measure exactly a suitable amount of this solution, and dilute it with hydrochloric acid TS (0.02 mol/L) to make a solution containing exactly 1–3 μg of sodium (Na = 22.99) per mL.

Procedure Perform the tests on the test solution and the standard solution as directed under Flame Atomic Absorption Spectrophotometry using the operating conditions below. Determine the sodium amount in the test solution from the calibration curve prepared from the standard solution.

Operating Conditions

Light Source: Sodium hollow cathode lamp.

Wavelength: 589.0 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(3) Sulfate 15–40 % (on the dried basis).

Weigh accurately about 1 g of Semirefined Carrageenan, and transfer into a 100-mL Kjeldahl flask. Add 50 mL of diluted hydrochloric acid (1 in 10), attach a reflux condenser, and boil for 1 hour. Add 25 mL of 10% (vol) hydrogen peroxide solution, and boil for 5 hours. Filter the separate solution if necessary, and transfer the filtrate into a 500-mL beaker. Add gradually 10 mL of a solution of barium chloride dihydrate (3 in 25) while boiling. Heat for 2 hours in a water bath, and cool. Filter using filter paper for the quantitative analysis (5C), and wash the residue on the filter paper with warm water until the washings are free of chlorides. Dry the residue with the filter paper, and place into a porcelain crucible. Incinerate the content to white ash, weigh as barium sulfate. Calculate the content of Sulfate (SO_4) by the formula, and determine on the dried basis.

$$\text{Content (\%) sulfate } (SO_4) = \frac{\text{Weight (g) of basium sulfate} \times 0.4116}{\text{Weight (g) of the sample}} \times 100$$

(4) Acid-insoluble substances 8–18%.

Weigh accurately about 2 g of Semirefined Carrageenan, and transfer into a 300-mL beaker containing 150 mL of water and 1.5 mL of sulfuric acid. Cover the beaker with a watch glass, and heat for 6 hours in a water bath. Occasionally, rub down the adhered matter on the wall of the beaker with a glass rod, and wash down with water to replenish the water lost by evaporation. Weigh accurately about 0.5 g of diatomaceous earth for chromatography, dried for 3 hours at 105°C, add to the sample solution, and mix well. Weigh a glass filter (1G3), dried for 3 hours at 105°C. Filter, with suction, the mixture of diatomaceous earth and the sample solution, using the glass filter, and wash down the residue into the glass filter with warm water. Dry the glass filter with the residue for 3 hours at 105°C. Allow to cool in the desiccator, and measure the total weight. Calculate the amount of the acid-insoluble substances by the formula:

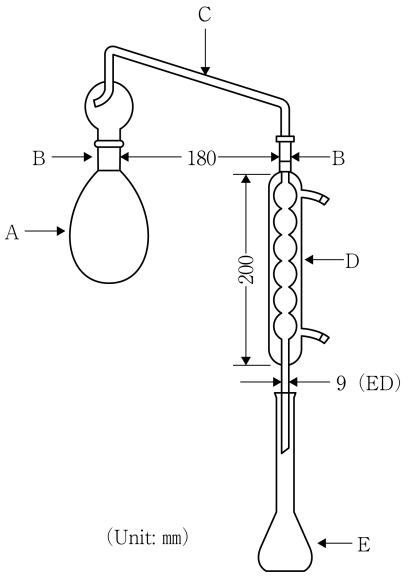
$$\text{Content (\%) of acid-insoluble substances} = \frac{\text{Total weight (g)} - \left[\left(\begin{matrix}\text{Weight (g) of}\\ \text{diatomaceous earth}\end{matrix}\right) + \left(\begin{matrix}\text{Weight (g) of}\\ \text{the glass filter}\end{matrix}\right)\right]}{\text{Weight (g) of the sample}}$$

(5) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(7) Total amount of 2-propanol and methanol Not more than 0.10%.

(i) Apparatus　Use the apparatus as illustrated in the figure.



A: Eggplant-shaped flask (300 mL)

B: Ground-glass joint

C: Delivery tube with a spray trap

D: Condenser

E: Volumetric flask (100 mL)

(ii) Method

Test Solution　Weigh accurately about 2 g of Semirefined Carrageenan in eggplant-shaped flask A, add 200 mL of water, a few boiling chips, and 1 mL of silicon resin, and stir well. Place exactly 4 mL of the internal standard solution in volumetric flask E, and set up the apparatus. Moisten the joint parts with water. Distill it at a rate of 2 to 3 mL/minute, being careful not to allow bubbles to come in delivery tube C, and collect about 90 mL of distillate. To the distillate, add water to make exactly 100 mL. Use 2-methyl-2-propanol solution (1 in 1000) as the internal standard solution.

Standard Solution　Weigh accurately about 0.5 g each of 2-propanol and methanol, and add water to make exactly 50 mL. Measure exactly 5 mL of this solution, and add water to make exactly 50 mL. Then measure exactly 2 mL of the second solution and 4 mL of the internal standard solution in a 100-mL volumetric flask, and add water to volume.

Procedure　Analyze 2.0 μL each of the test solution and the standard solution by gas chromatography using the operating conditions below. Determine the peak area ratios of each of 2-propanol and methanol to 2-methyl-2-propanol for each solution, and express as Q_{T1} and Q_{T2} for the test solution and as Q_{S1} and Q_{S2} for the standards solution. Calculate each content by the following formulae, and obtain the sum of both substances.

$$\text{Amount (\%) of 2-propanol} = \frac{\text{Weight (g) of 2-propanol}}{\text{Weight (g) of the sample}} \times \frac{Q_{T1}}{Q_{S1}} \times 0.4$$

$$\text{Amount (\%) of methanol} = \frac{\text{Weight (g) of methanol}}{\text{Weight (g) of the sample}} \times \frac{Q_{T2}}{Q_{S2}} \times 0.4$$

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube (3 mm internal diameter and 2 m length).

Column packing material: 180- to 250-μm styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Injection port temperature: A constant temperature of about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention times of methanol and for 2-propanol to about 2 minutes and about 10 minutes, respectively.

Loss on Drying Not more than 12.0% (105°C, 4 hours).

Ash 15.0–35.0% (on the dried basis).

Acid-insoluble Ash Not more than 2.0% (on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

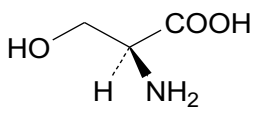
Salmonella: Negative per test.

Sample Fluid For total plate count and the enumeration of yeasts and molds, prepare as follows: Mix 10 g of Semirefined Carrageenan with 190 mL of phosphate buffer, 0.1% peptone solution, or sodium chloride-peptone buffer to disperse uniformly.

Pre-enrichment Culture For the *Escherichia coli* test, prepare as follows: Mix 10 g of Semirefined Carrageenan with 190 mL of phosphate buffer, 0.1% peptone solution, or sodium chloride-peptone buffer to disperse uniformly, mix 20 mL of the resulting solution with 200 mL of lauryl sulfate broth, and incubate at 35 ± 1°C for 48 ± 2 hours. For the *Salmonella* test, prepare as follows: Mix 25 g of Semirefined Carrageenan with 475 mL of lactose broth to disperse uniformly, and incubate at 35 ± 1°C for 24 ± 2 hours.

L-Serine

L-セリン



$C_3H_7NO_3$ Mol. Wt. 105.09

(2*S*)-2-Amino-3-hydroxypropanoic acid [56-45-1]

Content L-Serine, when calculated on the dried basis, contains 98.0–102.0% of L-serine ($C_3H_7NO_3$).

Description L-Serine occurs as white crystals or crystalline powder. It is odorless, and has a very slight sweet taste.

Identification

(1) To 5 mL of a solution of L-Serine (1 in 1000), add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A bluish-purple color develops.

(2) To 10 mL of a solution of L-Serine (1 in 20), add 0.2 g of *o*-periodic acid, and heat. The odor of formalin is evolved.

Specific Rotation $[\alpha]_D^{20}$: +13.5 to +16.0° (10 g, hydrochloric acid TS (2 mol/L), 100 mL, on the dried basis).

pH 5.2–6.2 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.1% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.2 g of L-Serine, and proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 10.51 mg of $C_3H_7NO_3$

Shellac

シェラック

Definition Shellac is obtained from the secretion of lac scale insects, *Laccifer* spp., and consists mainly of esters of aleuritic acid and shellolic acid or esters of aleuritic acid and jaralic acid. There are two types of products: White Shellac and Purified Shellac. These products are also divided into two types: wax-containing shellac, from which wax is not removed, and wax-free shellac, from which wax is removed.

White Shellac

白シェラック

Description White Shellac occurs as white to light yellow granules or small granular flakes. It is odorless or has a slight, characteristic odor.

Identification

(1) To 12 g of White Shellac, add 60 mL of ethanol (95), and shake. It dissolves within 3 hours at ordinary temperature. To 12 g of White Shellac, add 60 mL of toluene, and proceed in the same manner. It does not dissolve. A wax-containing product makes a solution containing dispersed fine particles of wax.

(2) Heat and melt 50 mg of White Shellac on the hot plate at 170°C, and continue heating. Gummy materials are formed by thermal polymerization. After cooling, add 1 mL of ethanol (95) and shake. It does not dissolve.

Purity

(1) Acid value 73–89.

Test Solution Weigh accurately about 1 g of White Shellac, dissolve it in 50 mL of ethanol (neutralized).

Procedure Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests. In titration, confirm the endpoint, using a potentiometer, or, visually by checking that a pink color persists for 30 seconds.

(2) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(3) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Wax

Wax-containing shellac : Not more than 5.5%.

Wax-free shellac : Not more than 0.2%.

To 10.0 g of White Shellac, add 150 mL of a solution of sodium carbonate decahydrate (1 in 60). Dissolve it by shaking on a water bath. Then heat on a water bath for an additional 3 hours with a watch glass covering the beaker. Cool with water more than 1 hour. Filter the floating wax, wash the wax and the filter paper with water. Transfer the wax and the filter into a beaker, and dry until almost all the water evaporate under 65°C. Transfer the wax and the filter paper into an extraction thimble in a Soxhlet extractor. Pour a suitable amount of hexane into the beaker, warm and dissolve the wax, and transfer into the extraction thimble. Extract with hexane for 2 hours. Evaporate hexane to dryness, and dry the residue for 3 hours at 105°C and weigh.

(5) Rosin Dissolve 2.0 g of White Shellac in 10 mL of ethanol (99.5). Add gradually 50 mL of hexane while shaking. Transfer the solution into a 200 mL separating funnel, and wash twice with 50 mL of water each time. Transfer the upper layer solution, and

filter. Evaporate the filtrate to dryness on a water bath. To the residue, add 5 mL of acetic anhydride, and dissolve the residue while heating on a water bath if necessary. Transfer the resulting solution to a test tube, and add 1 drop of sulfuric acid. The solution does not show a color change from purple-red through purple to khaki.

Loss on Drying Not more than 6.0% (dry for 4 hours at 40°C, then desiccate 15 hours in the desiccator).

Ash Not more than 1.0%.

Purified Shellac

精製シェラック

Description Purified Shellac occurs as yellow to dark brown small flakes. It is odorless or has slight characteristic odor.

Identification Proceed as directed in Identification (1) and (2) for White Shellac.

Purity

(1) Acid value 60–80.

Proceed as directed in Purity (1) for White Shellac. Use a potentiometer to confirm the endpoint.

(2) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(3) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Wax

Wax-containing shellac: Not more than 5.5%.

Wax-free shellac: Not more than 0.2%.

Proceed as directed in Purity (4) for White Shellac.

(5) Rosin Proceed as directed in Purity (5) for White Shellac.

Loss on Drying Not more than 2.0% (dry for 4 hours at 40°C, then desiccate 15 hours in the desiccator).

Ash Not more than 1.0%.

Silicon Dioxide

Silica Gel

二酸化ケイ素

SiO_2 Mol. Wt. 60.08

Silicon dioxide

Content Silicon Dioxide, when ignited, contains not less than 94.0% of silicon dioxide (SiO_2).

Description Silicon Dioxide occurs as white granules, powder, or colloidal liquid. It is odorless.

Identification Place 0.2 g of Silicon Dioxide in a platinum crucible, add 5 mL of hydrofluoric acid to dissolve, and heat. It almost completely evaporates.

Purity

(1) Water-soluble substances Not more than 5.0% of the dried substance.

Weigh 5.0 g of Silicon Dioxide, previously dried at 105°C for 2 hours, in an appropriate container, add 150 mL of water, and stir thoroughly for 15 minutes with a magnetic stirrer. Filter with suction, using a filter holder equipped with a 47-mm diameter membrane filter (0.45 μm pore size). If the filtrate is turbid, repeat the filtration with suction through the same filter. Wash the flask and the residue on the filter with water, combine the washings with the filtrate, and add water to make 250 mL. Measure 50 mL of this solution, evaporate to dryness, dry the residue at 105°C for 2 hours, and weigh the residue.

(2) Lead Not more than 5 μg/g as Pb (0.80 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Silicon Dioxide, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and allow to cool.

(3) Arsenic Not more than 3 μg/g of the dried substance as As (Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh 5.0 g of Silicon Dioxide, previously dried 105°C for 2 hours, add 50 mL of diluted hydrochloric acid (1 in 4), heat on a water bath for 1 hour with occasional shaking while replenishing the water lost, and allow to cool. Filter the mixture, wash the residue on the filter and in the beaker with water, combine the washings with the filtrate, and add water to make 100 mL. Use 10 mL of this solution as the test solution.

Loss on Ignition Not more than 70.0% (83.0% in the case of colloidal liquid) (105°C, 2 hours, then 1000°C, 30 minutes).

Assay Weigh accurately about 1 g of Silicon Dioxide, previously ignited, and transfer into a platinum crucible, previously ignited at 1000°C for 30 minutes and cooled in a desiccator. Accurately weigh the crucible containing the sample, M (g). Add 4 drops of ethanol (95) and 2 drops of sulfuric acid, then add a sufficient amount of hydrofluoric acid, and evaporate to dryness on a water bath. After cooling, add 5 mL of hydrofluoric acid to the residue, and evaporate to dryness. Heat at 550°C for 1 hour, gradually raise the temperature, ignite at 1000°C for 30 minutes, and allow to cool in a desiccator. Weigh accurately the crucible with the residue, m (g), and calculate the content by the formula:

$$\text{Content (\%) of silicon dioxide } (SiO_2) = \frac{\text{M (g)} - \text{m (g)}}{\text{Weight (g) of the sample}} \times 100$$

Silicon Dioxide (fine)

微粒二酸化ケイ素

SiO_2 Mol. Wt. 60.08

Silicon dioxide

Content Silicon Dioxide (fine), when ignited, contains not less than 99.0% of silicon dioxide (SiO_2).

Description Silicon Dioxide (fine) occurs as a white fine powder of less than 15 μm in average particle diameter having a smooth touch, and is odorless and tasteless.

Identification Place 0.2 g of Silicon Dioxide (fine) into a platinum crucible, dissolve it in 5 mL of hydrofluoric acid, and heat. It almost evaporates.

Purity

(1) Water-soluble substances Not more than 5.0% of the dried substance.

Weigh 2.0 g of Silicon Dioxide (fine) into an appropriate container, dried at 105°C for 2 hours, add 60 mL of water, mix thoroughly for 15 minutes with a magnetic stirrer, and filter with suction, using a filter holder equipped with a membrane filter (0.45 μm pore size). If the filtrate is turbid, repeat the filtration with suction through the same filter. Wash the container and the residue on the filter with water, combine the washings with the filtrate, and add water to make 100 mL. Measure 50 mL of this solution, evaporate to dryness, dry the residue at 105°C for 2 hours, and accurately weigh.

(2) Lead Not more than 5 μg/g as Pb (0.80 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Silicon Dioxide (fine), add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

(3) Arsenic Not more than 1.5 μg/g as As (5.0 g (previously dried at 105°C for 2 hours), Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Silicon Dioxide (fine), previously dried, add 50 mL of diluted hydrochloric acid (1 in 4), and heat on a water bath for 1 hour with occasional shaking while replenishing the lost water. After cooling, filter. Wash the container and the residue on the filter paper with water, and add the washings to the filtrate. To the resulting solution, add water to make 100 mL. Refer to this solution as Solution A. Use 20 mL of Solution A.

(4) Sodium Not more than 0.20% as Na_2O.

Test Solution To 5 mL of Solution A prepared in Purity (3), add water to make 100 mL.

Control Solution Weigh 1.886 g of sodium chloride, dried at 130°C for 2 hours, dissolve it in water to make exactly 1000 mL. Measure exactly 5.0 mL of this solution, and add water to make exactly 1000 mL.

Procedure Determine the atomic absorbance under the following operating conditions. The atomic absorbance of the test solution is not more than that of the control solution.

Operating Conditions

Light source: Sodium hollow cathode lamp.

Wavelength of analysis line: 589.0 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(5) Aluminum Not more than 0.20% as Al_2O_3.

Test Solution To 20 mL of Solution A prepared in Purity (3), add water to make 100 mL.

Control Solution Weigh 2.33 g of aluminum potassium sulfate dodecahydrate, and dissolve it by adding 5 mL of hydrochloric acid and water to make exactly 100 mL. Measure 2.0 mL of this solution, and add water to make exactly 250 mL.

Procedure Determine the atomic absorbance using the operating conditions below. The atomic absorbance of the test solution is not more than that of the control solution.

Operating Conditions

Light source: Aluminum hollow cathode lamp.

Wavelength of analysis line: 309.3 nm.

Supporting gas: Dinitrogen monoxide.

Combustible gas: Acetylene.

(6) Iron Not more than 0.50 mg/g as Fe_2O_3.

Test Solution To 20 mL of Solution A prepared in Purity (3), add water to make 100

mL.

Control Solution Weigh 6.04 g of ammonium iron(III) sulfate dodecahydrate, and dissolve it by adding 20 mL of hydrochloric acid and water to make exactly 1000 mL. Measure 5.0 mL of this solution, and add 10 mL of hydrochloric acid and water to make exactly 1000 mL.

Procedure Determine the atomic absorbance using the operating conditions below. The atomic absorbance of the test solution is not more than that of the control solution.

Operating Conditions

Light source: Iron hollow cathode lamp.

Wavelength of analysis line: 248.3 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

Loss on Drying Not more than 7.0% (105°C, 2 hours).

Loss on Ignition Not more than 8.5% (dried sample, 1000°C, 30 minutes).

Assay Weigh accurately about 1 g of Silicon Dioxide (fine), previously ignited, place it into a platinum crucible, ignited at 1000°C for 30 minutes and allowed to cool in a desiccator previously. Weigh accurately the crucible, M (g), containing the sample. Add 4 drops of ethanol (95) and 2 drops of sulfuric acid, then add a sufficient amount of hydrofluoric acid, and evaporate to dryness on a water bath. After cooling, add 5 mL of hydrofluoric acid to the residue, and evaporate to dryness. Heat at 550°C for 1 hour, gradually raise the temperature, ignite at 1000°C for 30 minutes, and allow to cool in a desiccator. Weigh accurately the crucible, m (g), containing the residue, and calculate the content by the formula:

$$\text{Content (\%) of silicon dioxide } (SiO_2) = \frac{\text{M (g)} - \text{m (g)}}{\text{Weight (g) of the sample}} \times 100$$

Silicone Resin

Dimethylpolysiloxane
Polydimethylsiloxane

シリコーン樹脂

Description Silicone Resin occurs as a colorless to light gray, transparent or translucent, viscous liquid or pasty substance. It is almost odorless.

Identification Determine the absorption spectrum of Silicone Resin as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index (Refractive index of extracted silicone oil)　n_D^{25}: 1.400–1.410.

Test Solution　Weigh 20 g of Silicone Resin, add 100 mL of hexane, shake reciprocally for 3 hours at about 200 strokes/minute, centrifuge for 30 minutes at 10,000 rpm, and collect the supernatant. To the residue, add 50 mL of hexane, shake thoroughly to disperse well, and centrifuge. Combine both supernatants, evaporate the hexane by warming in a water bath of 50–60°C under reduced pressure, and dry at 105°C for 1 hour.

Specific Gravity　d_{20}^{20}: 0.96–1.02.

Kinematic Viscosity (Kinematic viscosity of extracted silicone oil)　100–1100 mm^2/s.

Measure the kinematic viscosity of the test solution for the specific gravity test at 25°C.

Purity

(1) Lead　Not more than 1 μg/g as Pb (4.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Silicon dioxide　Not more than 15.0%.

Weigh accurately about 2 g of Silicone Resin into a fluororesin centrifuge tube, weighed previously, and add 10 mL of a solution of 10% (w/v) n-dodecylbenzenesulfonic acid in hexane. Shake reciprocally for 5 hours at about 200 strokes/minute, centrifuge for 20 minutes at 10,000 rpm, and remove the supernatant. To the residue, add 10 mL of hexane, and shake thoroughly to disperse well. Repeat three times the procedure of centrifuging and removing the supernatant. Dry the centrifuge tube with the residue at105°C for 1 hour. Weigh the residue.

Sodium Acetate

酢酸ナトリウム

$$H_3C-COONa \cdot nH_2O$$

n = 3 or 0

$C_2H_3NaO_2 \cdot nH_2O$ (n = 3 or 0)　　Mol. Wt. trihydrate 136.08

anhydrous 82.03

Monosodium acetate trihydrate　[6131-90-4]

Monosodium acetate　[127-09-3]

Definition　Sodium Acetate occurs in two forms: the crystalline form (trihydrate) called Sodium Acetate (crystal) and the anhydrous form called Sodium Acetate (anhydrous).

Content　Sodium Acetate, when dried, contains not less than 98.5% of sodium acetate ($C_2H_3NaO_2$).

Description　Sodium Acetate (crystal) occurs as colorless, transparent crystals or as a

white crystalline powder. Sodium Acetate (anhydrous) occurs as a white crystalline powder or as lumps. They are odorless.

Identification

(1) Heat the Sodium Acetate gradually. It fuses, and then decomposes, emitting an odor of acetone. The aqueous solution of the residue is alkaline.

(2) Sodium Acetate responds to all the tests for Sodium Salt and for Acetate in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 20 mL).

(2) Free acid and free alkali Weigh 2.0 g of Sodium Acetate (crystal) or 1.2 g of Sodium Acetate (anhydrous), and dissolve it in 20 mL of freshly boiled and cooled water. Add 2 drops of phenolphthalein TS, and while keeping the solution at 10°C, perform the following test:

(i) If the solution is colorless, add 0.10 mL of 0.1 mol/L sodium hydroxide. A pink color develops.

(ii) If the solution is pink, add 0.10 mL of 0.1 mol/L hydrochloric acid. The color disappears.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying

Crystal: 36.0–42.0% (120°C, 4 hours).

Anhydrous: Not more than 2.0% (120°C, 4 hours).

Assay Weigh accurately about 0.2 g of Sodium Acetate, previously dried, dissolve it in 40 mL of acetic acid, and titrate with 0.1 mol/L perchloric acid. The endpoint is usually confirmed using a potentiometer. When crystal violet–acetic acid TS (1 mL) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 8.203 mg of $C_2H_3NaO_2$

Sodium Alginate

アルギン酸ナトリウム

Sodium alginate [9005-38-3]

Content Sodium Alginate, when calculated on the dried basis, contains 90.8–106.0% of

sodium alginate.

Description Sodium Alginate occurs as a white to yellowish-white powder. It is almost odorless.

Identification

(1) Prepare a test solution as follows: To 0.5 g of Sodium Alginate, add 50 mL of water in small portions while stirring, warm the mixture at 60–70°C for 20 minutes with occasional shaking to make it homogenous, and cool.

(i) To 5 mL of the test solution, add 1 mL of a solution of calcium chloride dihydrate (3 in 40). A gelatinous precipitate is formed immediately.

(ii) To 10 mL of the test solution, add 1 mL of diluted sulfuric acid (1 in 20). A gelatinous precipitate is formed immediately.

(iii) To 1 mL of the test solution, add 1 mL of ammonium sulfate saturated solution. No precipitate is formed.

(2) The residue on ignition of Sodium Alginate responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 6.0–8.0. Add 0.50 g of Sodium Alginate to 50 mL of water gradually while stirring, and warm the mixture at 60–70°C for 20 minutes with occasional stirring to make it homogenous. Cool, and measure the pH.

Purity

(1) Sulfate Not more than 0.96% as SO_4.

To 0.10 g of Sodium Alginate, add 20 mL of water to make it pasty, then add 1 mL of hydrochloric acid, shake vigorously, heat in a water bath for several minutes, and proceed as directed in Purity (1) for Alginic Acid.

(2) Phosphate Add 0.10 g of Sodium Alginate to 20 mL of water gradually while stirring, and warm the mixture at 60–70°C for 20 minutes with occasional stirring to make it homogenous. Then proceed as directed in Purity (2) for Alginic Acid.

(3) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 15.0% (105°C, 4 hours).

Residue on Ignition 33.0–37.0% (calculated on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Coliforms: Negative per test.

Salmonella: Negative per test.

Sample Fluid　Prepare as directed in Method 2 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture　Prepare as directed in Method 2 for the coliform test. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each culture: Mix 5 g of the Sodium Alginate with 500 mL of lactose broth to disperse completely, and incubate at 35 ± 1°C for 24 ± 2 hours.

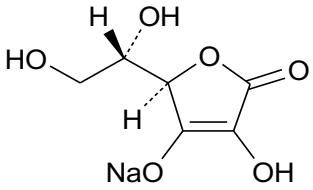
Assay　Proceed as directed in the Assay for Alginic acid.

Each mL of 0.25 mol/L sodium hydroxide solution = 27.75 mg of sodium alginate

Sodium L-Ascorbate

Sodium Ascorbate
Vitamin C Sodium

L-アスコルビン酸ナトリウム



$C_6H_7NaO_6$　　Mol. Wt. 198.11

Monosodium (2*R*)-2[(1*S*)-1,2-dihydroxyethyl]-4-hydroxy-5-oxo-2,5-dihydrofuran-3-olate [134-03-2]

Content　Sodium L-Ascorbate, when dried, contains not less than 99.0% of sodium L-ascorbate ($C_6H_7NaO_6$).

Description　Sodium L-Ascorbate occurs as a white to yellowish-white crystalline powder, or as granules or fine granules. It is odorless and has a slightly salty taste.

Identification

(1) Proceed as directed in Identification (1) and (2) for L-Ascorbic Acid.

(2) Sodium L-Ascorbate responds to all the tests for Sodium Salt in the Qualitative Tests.

Specific Rotation　$[\alpha]_D^{20}$: +103.0 to +108.0° (1 g, freshly boiled and cooled water, 10 mL, on the dried basis).

pH　6.5–8.0 (2.0 g, water 20 mL).

Purity

(1) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead

Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

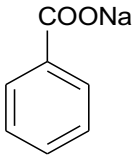
Loss on Drying Not more than 0.5% (reduced pressure, 24 hours).

Assay Weigh accurately about 0.2 g of Sodium L-Ascorbate, previously dried, dissolve it in 50 mL of metaphosphoric acid solution (1 in 50), and titrate with 0.05 mol/L iodine (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine = 9.905 mg of $C_6H_7NaO_6$

Sodium Benzoate

安息香酸ナトリウム



$C_7H_5NaO_2$ Mol. Wt. 144.10

Monosodium benzenecarboxylate [532-32-1]

Content Sodium Benzoate, when dried, contains not less than 99.0% of sodium benzoate ($C_7H_5NaO_2$).

Description Sodium Benzoate occurs as a white crystalline powder or as granules. It is odorless.

Identification Sodium Benzoate responds to all the tests for Sodium Salt and for Benzoate in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 5.0 mL).

(2) Free acid and free alkali Weigh 2.0 g of Sodium Benzoate, dissolve it in 20 mL of boiling water, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.05 mol/L sulfuric acid. The solution is colorless. To this solution, add 0.40 mL of 0.1 mol/L sodium hydroxide. The color of the solution changes to red.

(3) Sulfate Not more than 0.30% as SO_4.

Test Solution Weigh 0.20 g of Sodium Benzoate, and dissolve it in water to make 100 mL. To 40 mL of this solution, add 2.5 mL of diluted hydrochloric acid (1 in 4) dropwise while shaking well. Filter, wash with water, combine the washings with the filtrate, and add water to make 50 mL.

Control Solution To 0.50 mL of 0.005 mol/L sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Mix well the specified amount of Sodium Benzoate with 0.20 g of calcium hydroxide, and ignite the mixture. Dissolve the residue in 10 mL of diluted hydrochloric acid (1 in 4).

(6) Readily oxidizable substances Proceed as directed in Purity (3) for Benzoic Acid.

(7) Chlorinated compounds Not more than 0.014% as Cl.

Test Solution Weigh 0.50 g of Sodium Benzoate, transfer into a porcelain crucible, add 2.5 mL of diluted nitric acid (1 in 10), and mix thoroughly. Dry at 100°C, add 0.8 g of calcium carbonate and a small amount of water, mix, and dry at 100°C. Heat at about 600°C for 10 minutes and cool. Dissolve the residue in 20 mL of diluted nitric acid (1 in 10), filter, wash the insoluble substances with about 15 mL of water, combine the washings with the filtrate, and add water to make 50 mL.

Control Solution Weigh 0.8 g of calcium carbonate, dissolve it in 22.5 mL of diluted nitric acid (1 in 10), filter if necessary, and add 0.20 mL of 0.01 mol/L hydrochloric acid and water to make 50 mL.

Procedure Add 0.5 mL each of silver nitrate solution (1 in 50) to each solution, shake well, and allow to stand for 5 minutes. The test solution is not more turbid than the control solution.

(8) Phthalate Not more than 50 μg/g as phthalate.

Test Solution Weigh 1.0 g of Sodium Benzoate, and dissolve it in a 7:3 mixture of diluted acetic acid (1 in 100)/methanol to make exactly 50 mL.

Control Solution Prepared the control solution as directed in Purity (5) for Benzoic Acid, using a 7:3 mixture of diluted acetic acid (1 in 100)/methanol.

Procedure Proceed as directed in Purity (5) for Benzoic Acid.

Loss on Drying Not more than 1.5% (105°C, 4 hours).

Assay Weigh accurately about 1.5 g of Sodium Benzoate, previously dried and transfer into a 300-mL ground-glass stoppered flask. Dissolve it in 25 mL of water, add 75 mL of diethyl ether, and titrate with 0.5 mol/L hydrochloric acid (indicator: 10 drops of bromophenol blue TS). Perform the titration while mixing the water and diethyl ether layers well by shaking. Titrate until the aqueous layer produces a persistent light green color.

Each mL of 0.5 mol/L hydrochloric acid = 72.05 mg of $C_7H_5NaO_2$

Sodium Bicarbonate

Sodium Hydrogen Carbonate

Bicarbonate of Soda

炭酸水素ナトリウム

$NaHCO_3$ Mol. Wt. 84.01

Sodium hydrogencarbonate [144-55-8]

Content Sodium Bicarbonate, when dried, contains not less than 99.0% of sodium bicarbonate ($NaHCO_3$).

Description Sodium Bicarbonate occurs as a white crystalline powder or as crystalline lumps.

Identification Sodium Bicarbonate responds to all the tests for Sodium Salt and for Bicarbonate in the Qualitative Tests.

Purity

(1) Clarity of solution Clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.021% as Cl.

Sample Solution Weigh 0.50 g of Sodium Bicarbonate, add 5 mL of diluted nitric acid (1 in 10), boil, and cool.

Control Solution Use 0.30 mL of 0.01 mol/L hydrochloric acid.

(3) Carbonate Weigh 1.0 g of Sodium Bicarbonate, add carefully 20 mL of freshly boiled and cooled water, and dissolve it at 15°C or lower while shaking horizontally. Add 2.0 mL of 0.1 mol/L hydrochloric acid, and add 2 drops of phenolphthalein TS. No pink color develops immediately.

(4) Ammonium salt Weigh 1.0 g of Sodium Bicarbonate, and heat. No odor of ammonia is evolved.

(5) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Bicarbonate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of Sodium Bicarbonate, and dissolve it by adding 3 mL of water and 2 mL of hydrochloric acid.

Loss on Drying Not more than 0.25% (4 hours).

Assay Weigh accurately about 2 g of Sodium Bicarbonate, previously dried, dissolve it in 25 mL of water, and titrate with 0.5 mol/L sulfuric acid (indicator: 3 drops of

bromophenol blue TS). Just before the endpoint, boil to let the carbon dioxide out, cool, and continue the titration.

Each mL of 0.5 mol/L sulfuric acid = 84.01 mg of $NaHCO_3$

Sodium Carbonate

Crystal: Soda Carbonate
Anhydrous: Soda Ash

炭酸ナトリウム

$Na_2CO_3 \cdot nH_2O$ (n = 1 or 0) Mol. Wt. monohydrate 124.00

anhydrous 105.99

Sodium carbonate monohydrate [5968-11-6]

Sodium carbonate [497-19-8]

Definition Sodium Carbonate occurs as two forms: the crystalline form (monohydrate) called Sodium Carbonate (crystal) and the anhydrous form called Sodium Carbonate (anhydrous).

Content Sodium Carbonate, when dried, contains not less than 99.0% of sodium carbonate (Na_2CO_3).

Description Sodium Carbonate (crystal) occurs as a white crystalline powder or as colorless to white crystalline lumps. Sodium Carbonate (anhydrous) occurs as a white powder or as granules.

Identification Sodium Carbonate responds to all the tests for Sodium Salt and to tests (1) and (3) for Carbonate in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and very slightly turbid (1.0 g, water 20 mL).

(2) Chloride Not more than 0.35% as Cl.

Sample Solution Weigh 0.50 g of Sodium Carbonate, add 6 mL of diluted nitric acid (1 in 10), boil, and cool. Add water to make 100 mL and perform the test, using 10 mL of the solution as the sample solution.

Control Solution 0.50 mL of 0.01 mol/L hydrochloric acid.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Carbonate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric

acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 17.0% (105°C, 4 hours).

Assay Weigh accurately about 0.6 g of Sodium Carbonate, previously dried, dissolve it in 50 mL of water, and titrate with 0.5 mol/L hydrochloric acid (indicator: 3 drops of bromophenol blue TS). Soon before the titration reaches the endpoint, boil to expel carbon dioxide, cool, and continue the titration.

Each mL of 0.5 mol/L hydrochloric acid = 26.50 mg of Na_2CO_3

Sodium Carboxymethylcellulose

Sodium Cellulose Glycolate
Cellulose Gum

カルボキシメチルセルロースナトリウム

[9004-32-4]

Description Sodium Carboxymethylcellulose occurs as a white to light yellow powder, as granules, or as a fibrous substance. It is odorless.

Identification

(1) Determine the absorption spectrum of Sodium Carboxymethylcellulose, previously dried, as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Ignite 1 g of Sodium Carboxymethylcellulose at 550–600°C for 3 hours. The resulting residue responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 6.0–8.5.

Weigh 0.50 g of Sodium Carboxymethylcellulose, and add in small portions to 50 mL of water while stirring. Warm at 60–70°C for 20 minutes while stirring occasionally to make the solution homogeneous, and allow to cool.

Purity

(1) Chloride Not more than 0.64% as Cl.

Sample Solution Weigh 0.10 g of Sodium Carboxymethylcellulose, add 20 mL of water and 0.5 mL of hydrogen peroxide, and heat in a water bath for 30 minutes. After cooling, add water to make 100 mL, and filter through a dry filter paper. Use 25 mL of the filtrate as the sample solution.

Control Solution Use 0.45 mL of 0.01 mol/L hydrochloric acid.

(2) Sulfate　Not more than 0.96% as SO_4.

Sample Solution　20 mL of the filtrate obtained in Purity (1).

Control Solution　0.40 mL of 0.005 mol/L sulfuric acid.

(3) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more the 12.0% (105°C, 4 hours).

Sodium Carboxymethylstarch

デンプングリコール酸ナトリウム

Description　Sodium Carboxymethylstarch occurs as a white powder. It is odorless.

Identification

(1) To 5 mL of a solution of Sodium Carboxymethylstarch (1 in 1000), add 5 drops of diluted hydrochloric acid (1 in 4) and 1 drop of iodine TS, and shake. A blue to red-purple color develops.

(2) To 1 mL of a solution of Sodium Carboxymethylstarch (1 in 500), add 5 mL of chromotropic acid TS, and heat in a water bath for 10 minutes. A purple to red-purple color develops.

(3) To 5 mL of a solution of Sodium Carboxymethylstarch (1 in 500), add 5 mL of a solution of copper(II) sulfate pentahydrate (1 in 20), and shake. A light blue precipitate is formed.

(4) Ignite 1 g of Sodium Carboxymethylstarch at 450–550°C for 3 hours. The resulting residue responds to all the tests for Sodium Salt in the Qualitative Tests.

pH　6.0–8.5 (1.0 g, water 50 mL).

Purity

(1) Chloride　Not more than 0.43% as Cl.

Sample Solution　Weigh 0.10 g of Sodium Carboxymethylstarch, add 10 mL of water and 1 mL of nitric acid, heat in a water bath for 10 minutes, cool, and filter if necessary. Wash the residue with a small amount of water, combine the washings with the filtrate, and add water to make 100 mL. Use 25 mL of this solution as the sample solution.

Control Solution　0.30 mL of 0.01 mol/L hydrochloric acid.

(2) Sulfate　Not more than 0.96% as SO_4.

Sample Solution　Weigh 0.10 g of Sodium Carboxymethylstarch, add 10 mL of water and 1 mL of hydrochloric acid, heat in a water bath for 10 minutes, cool, and filter if necessary. Wash the residue with a small amount of water, combine the washings with

the filtrate, and add water to make 50 mL. Use 10 mL of this solution as the sample solution.

Control Solution 0.40 mL of 0.005 mol/L sulfuric acid.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 10.0% (105°C, 4 hours).

Sodium Caseinate

カゼインナトリウム

[9005-46-3]

Content Sodium Caseinate, when dried, contains 14.5–15.8% of nitrogen (N = 14.01).

Description Sodium Caseinate occurs as a white to light yellow powder, or as granules or flakes. It is odorless and tasteless or has a slight, characteristic odor and taste.

Identification

(1) Proceed as directed in Identification (1), (2), and (3) for Casein.

(2) The residue on ignition of Sodium Caseinate responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 6.0–7.5 (1.0 g, water 50 mL).

Purity

(1) Clarity of solution Colorless and slightly turbid.

Proceed as directed in Purity (1) for Casein.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Fat Not more than 2.0%.

Proceed as directed in Purity (4) for Casein.

Loss on Drying Not more than 15.0% (100°C, 3 hours).

Residue on Ignition Not more than 6.0% (dried sample).

Assay Weigh accurately about 0.15g of Sodium Caseinate, previously dried, and proceed as directed in the Kjeldahl Method in Nitrogen Determination.

Each mL of 0.05 mol/L sulfuric acid = 1.401 mg of N

Sodium Chlorite

亜塩素酸ナトリウム

$NaClO_2$ Mol. Wt. 90.44

Sodium chlorite [7758-19-2]

Content Sodium Chlorite contains not less than 70.0% of sodium chlorite ($NaClO_2$).

Description Sodium Chlorite occurs as a white powder. It is odorless or has a slight odor.

Identification

(1) Sodium Chlorite responds to all the tests for Sodium Salt and for Chlorite in the Qualitative Tests.

(2) To 2 mL of a solution of Sodium Chlorite (1 in 100), add 100 mL of phosphate buffer (pH8), and measure the absorbance. The solution exhibits its absorption maximum at a wavelength of 258–262 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Chlorite, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow it to cool, and use as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Gently boil for 5 minutes, allow to cool.

(2) Arsenic Not more than 0.8 μg/g as As (2.5 g, Standard Color: Arsenic Standard Solution 4.0 mL, Apparatus B).

Test Solution Add 20 mL of water to the specified amount of Sodium Chlorite, and then add 1 mL of nitric acid and 20 mL of hydrochloric acid. Evaporate the mixture on a water bath to dryness. Add water to the residue to make 25 mL.

Assay Weigh accurately about 1 g of Sodium Chlorite, and dissolve it in water to make exactly 250 mL. Measure exactly 20 mL of this solution, transfer into an iodine flask, and add 12 mL of diluted sulfuric acid (3 in 100), 20 mL of water, and 4 g of potassium iodide. Immediately stopper tightly, allow to stand in a dark place for 15 minutes, and titrate with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 2.261 mg of $NaClO_2$

Sodium Chlorite Solution

亜塩素酸ナトリウム液

Content Sodium Chlorite Solution contains 4.0–25.0 % of sodium chlorite ($NaClO_2$ = 90.44) and 95–100% of the labeled content of sodium chlorite.

Description Sodium Chlorite Solution is a clear, colorless to light yellow liquid. It is odorless or has a slight odor.

Identification

(1) Sodium Chlorite Solution responds to all the tests for Sodium Salt and for Chlorite in the Qualitative Tests.

(2) Sodium Chlorite Solution is alkaline.

(3) Measure an appropriate quantity of diluted Sodium Chlorite Solution (1 in 100) so that the absorbance of the resulting solution is between 0.2 and 0.7, and add a phosphate buffer solution (pH 8). The solution exhibits its absorption maximum at a wavelength of 258–262 nm.

Purity

(1) Lead Not more than 2 μg/g of $NaClO_2$ as Pb (an amount equivalent to 2.0 g of $NaClO_2$, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Chlorite Solution, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow it to cool, and use as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Gently boil for 5 minutes, and allow to cool.

(2) Arsenic Not more than 0.8 μg/g of $NaClO_2$ as As (an amount equivalent to 2.5 g of $NaClO_2$, Standard Color: Arsenic Standard Solution 4.0 mL, Apparatus B).

Test Solution To the specified amount of Sodium Chlorite Solution, add 2 mL of nitric acid and 20 mL of hydrochloric acid. Evaporate the mixture on a water bath to dryness. Add water to the residue to 25 mL.

Assay Measure exactly a quantity of this solution equivalent to about 60 mg of $NaClO_2$, transfer into an iodine flask, add 12 mL of diluted sulfuric acid (3 in 100), and add water to make about 55 mL. Add 4 g of potassium iodide, immediately stopper tightly, allow to stand in a dark place for 15 minutes, and titrate with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner as the sample solution, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 2.261 mg of $NaClO_2$

Sodium Chondroitin Sulfate

コンドロイチン硫酸ナトリウム

Content Sodium Chondroitin Sulfate, when dried, contains 2.5–3.8% of nitrogen (N = 14.01) and 5.5－7.0% of sulfur (S = 32.07).

Description Sodium Chondroitin Sulfate occurs as a white to whitish powder.

Identification

(1) To 5 mL of a solution of Sodium Chondroitin Sulfate (1 in 100), add 1 mL of acriflavine hydrochloride solution (1 in 200). A yellow-brown precipitate is formed.

(2) To 5 mL of a solution of Sodium Chondroitin Sulfate (1 in 100), add 1 mL of hydrochloric acid, heat in a water bath for 10 minutes, and cool. Add 1 mL of a solution of barium chloride dihydrate (3 in 25). A white precipitate is formed.

(3) The residue on ignition of Sodium Chondroitin Sulfate responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 5.5–7.5 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution Almost clear.

Test Solution Weigh 0.10 g of Sodium Chondroitin Sulfate, add 20 mL of water, and dissolve it while shaking well.

(2) Chloride Not more than 0.14% as Cl.

Test Solution Weigh 50 mg of Sodium Chondroitin Sulfate, dissolve it in 10 mL of water, add 15 mL of ethanol (95) and 6 mL of diluted nitric acid (1 in 10), shake, and filter. Wash the residue with 50% (vol) ethanol, combine the washings with the filtrate, and add 50% (vol) ethanol to make 50 mL.

Control Solution To 0.20 mL of 0.01 mol/L hydrochloric acid, add 6 mL of diluted nitric acid (1 in 10) and 50% (vol) ethanol to make 50 mL.

(3) Inorganic sulfate Not more than 0.24% as SO_4.

Sample Solution Weigh 0.10 g of Sodium Chondroitin Sulfate, dissolve it in 15 mL of water, add 1 mL of hydrochloric acid, and shake well. Add 2 mL of a solution of aluminum(III) chloride hexahydrate (1 in 5), shake well again, and add 5 mL of ammonia TS little by little while shaking. Centrifuge, and collect the supernatant. Add 5 mL of water to the residue, shake, centrifuge, and combine the washings with the supernatant. Repeat this procedure using 5 mL of water, combine the washings with the supernatant, and neutralize the obtained solution with diluted hydrochloric acid (1 in 4).

Control Solution Use 0.50 mL of 0.005 mol/L sulfuric acid.

Procedure Proceed as directed in the Sulfur Limit Test.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more than 10.0% (105°C, 4 hours).

Residue on Ignition　23.0–31.0% (dried sample).

Assay

(1) Nitrogen　Weigh accurately about 1 g of Sodium Chondroitin Sulfate, previously dried, and proceed as directed in the Kjeldahl Method under Nitrogen Determination.

Each mL of 0.05 mol/L sulfuric acid = 1.401 mg of N

(2) Sulfur　Weigh accurately about 0.5 g of Sodium Chondroitin Sulfate, previously dried, into a Kjeldahl flask, dissolve it in 30 mL of water, and add 5 g of potassium chlorate. Then add 30 mL of nitric acid in small portions, heat until the solution becomes about 5 mL, and cool. Transfer quantitatively to a beaker, using 25 mL of hydrochloric acid, and concentrate on a water bath to about 5 mL. Add 100 mL of water to the solution, neutralize with ammonia TS, add 5 mL of diluted hydrochloric acid (1 in 10), and add 5 mL of a solution of barium chloride dihydrate (3 in 25) while boiling. Cover the beaker with a watch glass, and heat on a water bath for 2 hours while replenishing the water. Cool, filter through a filter paper for quantitative analysis (5C), and wash the beaker and the residue on the filter paper with warm water until the washings do not respond to the tests for Chloride. Dry the residue together with the filter paper, ignite at 450–550°C to constant weight, and weigh accurately. Calculate the content of sulfur by the formula:

$$\text{Content (\%) of sulfur (S)} = \frac{\text{Weight (g) of the residue} \times 0.1374}{\text{Weight (g) of the sample}} \times 100$$

Sodium Copper Chlorophyllin

銅クロロフィリンナトリウム

Description　Sodium Copper Chlorophyllin occurs as a bluish-black to greenish-black powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Place 1 g of Sodium Copper Chlorophyllin into a porcelain crucible, moisten with a small amount of sulfuric acid, and heat gradually. After it is almost completely incinerated at the lowest possible temperature, allow to cool. Add 1 mL of sulfuric acid, heat gradually until fumes of sulfuric acid have practically ceased to be evolved, and allow to cool. Add 10 mL of diluted hydrochloric acid (1 in 4) to the residue, dissolve it by heating on a water bath, filter if necessary, and add water to make 10 mL. Perform the tests, given below, using this solution as the test solution.

(i) Perform the Flame Coloration Test on the test solution. A green color is imparted to the flame, and then changes to yellow color.

(ii) To 5 mL of the test solution, add 0.5 mL of a solution of sodium *N,N*-diethyldithiocarbamate trihydrate (1 in 1000). A brown precipitate is formed.

(2) To 1 mL of a solution of Sodium Copper Chlorophyllin (1 in 1000), add phosphate buffer (pH 7.5) to make 100 mL, and measure the absorbance. The solution exhibits absorption maxima at wavelengths of 403–407 nm and 627–633 nm. When the absorbance values at these absorption maxima are expressed as A_1 and A_2, respectively, A_1/A_2 is not more than 4.0.

Specific Absorbance $E_{1cm}^{10\%}$ (maximum absorption wavelength near 405 nm): Not less than 508 (on the dried basis).

This test should be protected from direct light, and the apparatus used in the test should be light-resistant.

Weigh accurately about 0.1 g of Sodium Copper Chlorophyllin, and dissolve it in water to make exactly 100 mL. Measure exactly 1 mL of this solution, add phosphate buffer (pH 7.5) to make exactly 100 mL, and promptly measure the absorbance.

pH 9.5–11.0 (1.0 g, water 100 mL).

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Inorganic copper salt Not more than 0.03% as Cu.

Test Solution Weigh 1.0 g of Sodium Copper Chlorophyllin, and dissolve it in 60 mL of water.

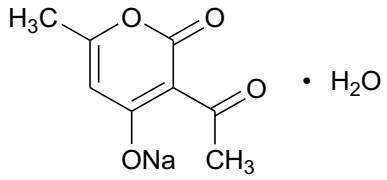
Procedure Analyze a 2-μL portion of the test solution by thin-layer chromatography using a 4:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm, and air-dry the plate. Spray with a solution of *N,N*-sodium diethyldithiocarbamate trihydrate (1 in 1000). No light brown spot is observed.

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 5.0% (105°C, 2 hours).

Sodium Dehydroacetate

デヒドロ酢酸ナトリウム



$C_8H_7NaO_4 \cdot H_2O$ Mol. Wt. 208.14

Monosodium 3-acetyl-4-oxido-6-methyl-2*H*-pyran-2-one monohydrate [64039-28-7]

Content Sodium Dehydroacetate, calculated on the anhydrous basis, contains 98.0–102.0% of sodium dehydroacetate ($C_8H_7NaO_4$ = 190.13).

Description Sodium Dehydroacetate occurs as a white crystalline powder. It is odorless or has a slight odor.

Identification

(1) To 0.1 g of Sodium Dehydroacetate, add 1 mL of water, 3 to 5 drops of a solution (1 in 5) of salicylaldehyde in ethanol (95), and 0.5 mL of sodium hydroxide solution (1 in 3), and heat in a water bath. A red color develops.

(2) To 2 mL of a solution of Sodium Dehydroacetate (1 in 100), add 3 drops of a solution of (+)–potassium sodium tartrate tetrahydrate (7 in 50) and 2 drops of copper(II) acetate TS, and shake. A whitish-purple precipitate is formed.

(3) Sodium Dehydroacetate responds to all the tests for Sodium Salt in the Qualitative Tests.

(4) Dissolve 0.5 g of Sodium Dehydroacetate in 10 mL of water, add 1 mL of diluted hydrochloric acid (1 in 4), filter the precipitate produced, and wash it thoroughly with water. The melting point of the residue is 109–112°C.

Purity

(1) Color of solution Colorless (0.50 g, water 10 mL).

(2) Free alkali Weigh 1.0 g of Sodium Dehydroacetate, and dissolve it in 20 mL of freshly boiled and cooled water. When 2 drops of phenolphthalein TS is added a pink color develops, but it disappears on the addition of 0.30 mL of 0.05 mol/L sulfuric acid.

(3) Chloride Not more than 0.011% as Cl.

Test Solution Weigh 1.0 g of Sodium Dehydroacetate, dissolve it in 30 mL of water, and add 9.5 mL of diluted nitric acid (1 in 10) dropwise while shaking well. Filter, wash with water, and combine the washings with the filtrate. Add water to make 50 mL.

Control Solution To 0.30 mL of 0.01 mol/L hydrochloric acid, add 6 mL of diluted nitric acid (1 in 10) and water to make 50 mL.

(4) Sulfate Not more than 0.014% as SO_4.

Test Solution Weigh 1.0 g of Sodium Dehydroacetate, dissolve it in 30 mL of water, and add 3 mL of diluted hydrochloric acid (1 in 4) dropwise while shaking well. Filter, wash with water, and combine the washings with the filtrate. Add water to make 50 mL.

Control Solution To 0.30 mL of 0.005 mol/L sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(5) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(7) Readily carbonizable substances Perform the test using 0.30 g of Sodium Dehydroacetate, as the test sample, and Matching Fluid C.

Water Content 8.3–10.0% (0.3 g, Volumetric Titration, Back Titration).

Assay Weigh accurately about 0.4 g of Sodium Dehydroacetate, add 50 mL of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid (indicator: 10 drops of *p*-naphtholbenzein TS) until the brown color of the solution changes to green. Calculate on the anhydrous basis.

Each mL of 0.1 mol/L perchloric acid = 19.01 mg of $C_8H_7NaO_4$

Sodium Dihydrogen Phosphate

Monosodium Phosphate
Sodium Phosphate, Monobasic

リン酸二水素ナトリウム

$NaH_2PO_4 \cdot nH_2O$ (n = 2 or 0) Mol. Wt. dihydrate 156.01
anhydrous 119.98

Sodium dihydrogenphosphate dihydrate [13472-35-0]

Sodium dihydrogenphosphate [7558-80-7]

Definition Sodium Dihydrogen Phosphate occurs in two forms: the crystalline form (dihydrate) called Sodium Dihydrogen Phosphate (crystal) and the anhydrous form called Sodium Dihydrogen Phosphate (anhydrous).

Content Sodium Dihydrogen Phosphate, when dried, contains 98.0–103.0% of sodium dihydrogen phosphate (NaH_2PO_4).

Description Sodium Dihydrogen Phosphate (crystal) occurs as colorless to white crystals or as a white crystalline powder. Sodium Dihydrogen Phosphate (anhydrous) occurs as a white powder or as granules.

Identification A solution of Sodium Dihydrogen Phosphate (1 in 20) responds to all the tests for Sodium Salt and for Phosphate in the Qualitative Tests.

pH 4.3–4.9 (1.0 g, water 100 mL).

Purity For Sodium Dihydrogen Phosphate (crystal), dry the sample before performing the tests.

(1) Clarity of solution Colorless and very slightly turbid (2.0 g, water 20 mL).

(2) Chloride Not more than 0.11% as Cl (0.20 g, Control Solution: 0.01 mol/L hydrochloric acid 0.60 mL).

(3) Sulfate Not more than 0.048% as SO_4 (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(4) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Dihydrogen Phosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying

Crystal: 22.0–24.0% (40°C, 16 hours, then 120°C, 4 hours).

Anhydrous: Not more than 2.0% (120°C, 4 hours).

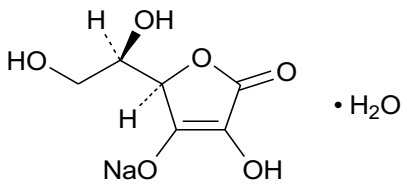
Assay Weigh accurately about 3 g of Sodium Dihydrogen Phosphate, previously dried, dissolve it in 30 mL of water. To this solution, add 5 g of sodium chloride, and dissolve it by shaking well. While keeping at about 15°C, titrate with 1 mol/L sodium hydroxide (indicator: 3–4 drops of thymol blue TS).

Each mL of 1 mol/L sodium hydroxide = 120.0 mg of NaH_2PO_4

Sodium Erythorbate

Sodium Isoascorbate

エリソルビン酸ナトリウム



$C_6H_7NaO_6 \cdot H_2O$ Mol. Wt. 216.12

Monosodium (2*R*)-2[(1*R*)-1,2-dihydroxyethyl]-4-hydroxy-5-oxo-2,5-dihydrofuran-3-olate monohydrate [63524-04-9]

Content Sodium Erythorbate, when dried, contains not less than 98.0% of sodium erythorbate ($C_6H_7NaO_6 \cdot H_2O$).

Description Sodium Erythorbate occurs as a white to yellowish-white crystalline powder, or as granules or fine granules. It is odorless and has a slightly salty taste.

Identification

(1) Proceed as directed in Identification (1) and (2) for Erythorbic Acid.

(2) Sodium Erythorbate responds to all the tests for Sodium Salt in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +95.5 to +98.0° (previously dried, 1 g, water 10 mL).

pH 6.0–8.0 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Weigh 1.0 g of Sodium Erythorbate, and dissolve it in 10 mL of water. The solution is clear, and its color is not darker than that of Matching Fluid J.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL Apparatus B).

Loss on Drying Not more than 0.25% (reduced pressure, 24 hours).

Assay Weigh accurately about 1 g of Sodium Erythorbate, previously dried, and dissolve it in metaphosphoric acid solution (1 in 50) to make exactly 250 mL. Measure exactly 50 mL of this solution, and titrate with 0.05 mol/L iodine (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine = 10.81 mg of $C_6H_7NaO_6 \cdot H_2O$

Sodium Ferrocyanide

Sodium Hexacyanoferrate(II)

フェロシアン化ナトリウム

$Na_4[Fe(CN)_6] \cdot 10H_2O$ Mol. Wt. 484.06

Sodium hexacyanoferrate(II) decahydrate [13601-19-9]

Content Sodium Ferrocyanide includes not less than 99.0% of sodium ferrocyanide ($Na_4[Fe(CN)_6] \cdot 10H_2O$).

Description Sodium Ferrocyanide occurs yellow crystals or crystalline powder.

Identification

(1) Proceed as directed in Identification (1) for Potassium Ferrocyanide.

(2) Sodium Ferrocyanide responds to all the tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Cyanide Proceed as directed in Purity (1) for Potassium Ferrocyanide.

(2) Ferricyanide Proceed as directed in Purity (2) for Potassium Ferrocyanide.

(3) Lead Not more than 5 μg/g as Pb (0.80 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method). Proceed as directed in Purity (3) for Potassium Ferrocyanide

Assay Weigh accurately about 1 g of Sodium Ferrocyanide, and dissolve it in 200 mL of water. To this solution, add 10 mL of sulfuric acid, and titrate with 0.02 mol/L potassium permanganate. The endpoint is when the pink color of the solution persists for 30 seconds.

Each mL of 0.02 mol/L potassium permanganate = 48.41 mg of $Na_4[Fe(CN)_6]\cdot 10H_2O$

Sodium Ferrous Citrate

Sodium Iron Citrate

クエン酸第一鉄ナトリウム

Iron(II) sodium salt of 2-hydroxypropane-1,2,3-tricarboxylic acid

Content Sodium Ferrous Citrate contains 10.0–11.0% of Fe (= 55.85).

Description Sodium Ferrous Citrate occurs as a greenish-white to greenish-yellow powder. It is odorless.

Identification

(1) To 5 mL of a solution of Sodium Ferrous Citrate (1 in 100), add 1 mL of diluted hydrochloric acid (1 in 4) and 0.5 mL of freshly prepared potassium hexacyanoferrate(III) solution (1 in 10). A blue color develops.

(2) To 5 mL of a solution of Sodium Ferrous Citrate (1 in 100), add 2 mL of ammonia solution. A red-brown color develops, but no precipitate is formed.

(3) Ignite 3 g of Sodium Ferrous Citrate at 500–600°C for 3 hours. The resulting residue responds to all the tests for Sodium Salt in the Qualitative Tests.

(4) To 0.5 g of Sodium Ferrous Citrate, add 5 mL of water and 10 mL of potassium hydroxide solution (1 in 25), heat in a water bath for 10 minutes while stirring well, cool, and filter. Take a portion of the filtrate, neutralize with diluted acetic acid (1 in 2), add an excessive amount of a solution of calcium chloride dihydrate (3 in 40), and boil. A

white, crystalline precipitate is formed. The precipitate does not dissolve in sodium hydroxide solution (1 in 25), but dissolves in diluted hydrochloric acid (1 in 4).

Purity

(1) Sulfate Not more than 0.48% as SO_4.

Test Solution Weigh 0.40 g of Sodium Ferrous Citrate, dissolve it in 50 mL of water, and add water to make 100 mL. Measure 10 mL of this solution, add 1 mL of diluted hydrochloric acid (1 in 4) and 0.1 g of hydroxylammonium chloride, and boil for 1 minute. Cool, and add water to make 50 mL.

Control Solution To 0.40 mL of 0.005 mol/L of sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(2) Iron(III) salt Weigh 2.0 g of Sodium Ferrous Citrate into a ground-glass stoppered flask, dissolve it in 5 mL of hydrochloric acid and 30 mL of water, add 4 g of potassium iodide, stopper, and allow to stand in a dark place for 15 minutes. Add 2 mL of starch TS, and shake well. A color develops, but it disappears on the addition of 1.0 mL of 0.1 mol/L sodium thiosulfate to the solution.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (1.0 g, Standard Color: Arsenic Standard Solution 6.0 mL, Apparatus B).

Test Solution To the specified amount of Sodium Ferrous Citrate, add 10 mL of water, 1 mL of sulfuric acid, and 10 mL of sulfurous acid solution, evaporate to about 2 mL, and add water to make 10 mL. Use 5 mL of this solution as the test solution.

Standard Color To the specified amount of Arsenic Standard Solution, add 10 mL of water, 1 mL of sulfuric acid, and 10 mL of sulfurous acid solution. Evaporate to about 2 mL, and add water to make10 mL. Measure 5 mL of the resulting solution, and proceed in the same manner as the preparation of the test solution.

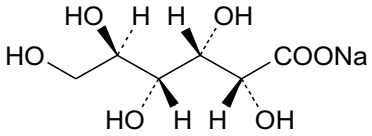
(5) Tartrate Weigh 1.0 g of Sodium Ferrous Citrate, add 5 mL of water and 10 mL of potassium hydroxide solution (1 in 15), heat in a water bath for 10 minutes while stirring well, cool, and filter. Measure 5 mL of the filtrate, add diluted acetic acid (1 in 4) to make it weakly acidic, then add 2 mL of acetic acid, and allow to stand for 24 hours. No white, crystalline precipitate is formed.

Assay Weigh accurately about 1 g of Sodium Ferrous Citrate, transfer into a ground-glass stoppered flask, add 25 mL of diluted sulfuric acid (1 in 20) and 2 mL of nitric acid, and boil for 10 minutes. After cooling, add 20 mL of water and 4 g of potassium iodide, immediately stopper tightly, allow to stand in a dark place for 15 minutes, and add 100 mL of water. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner.

Each mL of 0.1 mol/L sodium thiosulfate = 5.585 mg of Fe

Sodium Gluconate

グルコン酸ナトリウム



$C_6H_{11}NaO_7$ Mol. Wt. 218.14

Monosodium D-gluconate [527-07-1]

Content Sodium Gluconate, when dried, contains 98.0–102.0% of sodium gluconate ($C_6H_{11}NaO_7$).

Description Sodium Gluconate occurs as a white to yellowish-white crystalline powder or as granules. It has a slight, characteristic odor.

Identification

(1) Sodium Gluconate responds to all the tests for Sodium Salt in the Qualitative Tests.

(2) Measure 5 mL of a solution of Sodium Gluconate (1 in 10), and proceed as directed in Identification (2) for Glucono-δ-Lactone.

pH 6.2–7.8 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 10 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Reducing sugars Not more than 0.50% as D-glucose.

Using 1.0 g of Sodium Gluconate instead of Zinc Gluconate, proceed as directed in Purity (3) for Zinc Gluconate. Titrate excess iodine with 0.1 mol/L sodium thiosulfate. The volume of the sodium thiosulfate solution consumed is not less than 8.15 mL.

Loss on Drying Not more than 0.3% (105°C, 2 hours).

Assay Weigh accurately about 0.15 g of Sodium Gluconate, previously dried, and dissolve it in 75 mL of acetic acid, and titrate with 0.1 mol/L perchloric acid until the red color of the solution disappears (indicator: 10 drops of quinaldine red TS). Perform a blank test in the same manner.

Each mL of 0.1 mol/L perchloric acid = 21.81 mg of $C_6H_{11}NaO_7$

Sodium Hydrogen Sulfite Solution

亜硫酸水素ナトリウム液

Content Sodium Hydrogen Sulfite Solution contains not less than 34.0% of sodium hydrogen sulfite ($NaHSO_3$ = 104.06).

Description Sodium Hydrogen Sulfite Solution is a light yellow liquid having an odor of sulfur dioxide.

Identification Diluted Sodium Hydrogen Sulfite Solution (1 in 5) responds to all the tests for Sodium Salt and for Sulfite in the Qualitative Tests.

Purity

(1) Clarity of solution Very slightly turbid (3.0 g, water 20 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Hydrogen Sulfite Solution, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Leave it to cool, and use as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add diluted hydrochloric acid (1 in 4) to the residue. Gently boil for 5 minutes, leave to cool, and use it as the sample solution

(3) Arsenic Not more than 1.5 μg/g as As (10 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Add water to the specified amount of Sodium Hydrogen Sulfite Solution to make 25 mL. Measure 5 mL of this solution, add 2 mL of sulfuric acid, and heat on a water bath until sulfur dioxide no longer evolves. Evaporate to about 2 mL, and add water to make 10 mL. Use 5 mL of this solution as the test solution.

Assay Weigh accurately about 0.5 g of Sodium Hydrogen Sulfite Solution, and proceed as directed under Sulfite Determination.

Each mL of 0.05 mol/L iodine = 5.203 mg of $NaHSO_3$

Sodium Hydrosulfite

Hydrosulfite

次亜硫酸ナトリウム

$Na_2S_2O_4$ Mol. Wt. 174.11

Sodium dithionite [7775-14-6]

Content Sodium Hydrosulfite contains not less than 85.0% of sodium hydrosulfite ($Na_2S_2O_4$).

Description Sodium Hydrosulfite occurs as a white to bright grayish white crystalline powder. It is odorless or has a slight odor of sulfur dioxide.

Identification

(1) To 10 mL of a solution of Sodium Hydrosulfite (1 in 100), add 2 mL of a solution of copper(II) sulfate pentahydrate (1 in 20). A gray-black color develops.

(2) To 10 mL of a solution of Sodium Hydrosulfite (1 in 100), add 1 mL of potassium permanganate solution (1 in 300). The color of the solution disappears immediately.

(3) Sodium Hydrosulfite responds to all the tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Slightly turbid.

Test Solution To 10 mL of formaldehyde solution, add 10 mL of water, and neutralize with sodium hydroxide solution (1 in 25). Dissolve 0.50 g of Sodium Hydrosulfite in 10 mL of the solution obtained, and allow to stand for 5 minutes.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Hydrosulfite, add 40 mL of diluted hydrochloric acid (1 in 4), and evaporate it to dryness. To the residue, add 20 mL of diluted hydrochloric acid (1 in 4), boil gently for 15 minutes with a watch glass covering it, and allow to cool.

(3) Zinc Not more than 80 μg/g as Zn.

Procedure Measure 5 mL of the sample solution prepared in Purity (2) above, add 0.1 mL of ammonia TS, filter, and transfer the filtrate into a Nessler tube. Add water to make 20 mL, add 5 mL of diluted hydrochloric acid (1 in 4) and 0.1 mL of a freshly prepared solution of potassium hexacyanoferrate(II) trihydrate (1 in 10), and allow to stand for 15 minutes. The solution is not more turbid than a control solution prepared as follows: Place 8.0 mL of Zinc Standard Solution into a Nessler tube, add water to make 20 mL, add 5 mL of diluted hydrochloric acid (1 in 4) and 0.1 mL of a freshly prepared solution of potassium hexacyanoferrate(II) trihydrate (1 in 10), and allow to stand for 15 minutes.

(4) Arsenic Not more than 3 μg/g as As (5.0 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Sodium Hydrosulfite, in water to make 25 mL. Measure 5 mL of this solution, add 1 mL of sulfuric acid, evaporate to about 2 mL, and add water to make 10 mL. Use 5 mL of this solution as the test solution.

(5) Disodium dihydrogen ethylenediaminetetraacetate dihydrate Weigh 0.5 g of Sodium Hydrosulfite, dissolve it in 5 mL of water, add 2 mL of potassium chromate solution (1 in 200) and 2 mL of arsenic trioxide TS, and heat in a water bath for 2 minutes. No purple color develops.

(6) Formate Not more than 0.050% as HCHO.

Test Solution　Weigh 1.0 g of Sodium Hydrosulfite, and dissolve it in water to make 1000 mL. Measure 10 mL of this solution, add 5 mL of diluted hydrochloric acid (1 in 2), and add about 0.3 g of magnesium powder in small portions. When effervescence is almost no longer evolved, allow to stand for 2 hours with a watch glass covering it.

Control Solution　To 1.0 mL of Formaldehyde Standard Solution (2 μg/mL), add 5 mL of diluted hydrochloric acid (1 in 2).

Procedure　To 1 mL of test solution, add 2 mL of sulfuric acid and 0.5 mL of chromotropic acid TS, and heat in a water bath for 10 minutes. The color of the solution is not darker than that of the solution obtained by treating the control solution in the same manner as for the test solution.

Assay　Add 10 mL of water to 10 mL of formaldehyde solution, and neutralize with sodium hydroxide solution (1 in 25). To the resulting solution, add about 2 g of Sodium Hydrosulfite, accurately weighed, add water to dissolve it, and make exactly 500 mL. Measure exactly 25 mL of this solution, adjust the pH to 1.1–1.5 with diluted hydrochloric acid (1 in 10), and titrate with 0.05 mol/L iodine for sodium hydrosulfite (indicator: 1–3 mL of starch TS).

Each mL of 0.05 mol/L iodine = 4.353 mg of $Na_2S_2O_4$

Sodium Hydroxide

Caustic Soda

水酸化ナトリウム

$NaOH \cdot nH_2O$ (n = 1 or 0)　　Mol. Wt. monohydrate 58.01

anhydrous 40.00

Sodium hydroxide monohydrate　[12200-64-5]

Sodium hydroxide　[1310-73-2]

Definition　Sodium Hydroxide occurs in two forms: the crystalline form called Sodium Hydroxide (crystal) and the anhydrous form called Sodium Hydroxide. Sodium Hydroxide (crystal) is a mixture of sodium hydroxide (NaOH) and sodium hydroxide monohydrate ($NaOH \cdot H_2O$).

Content　Sodium Hydroxide (crystal) contains 70.0–75.0% of sodium hydroxide (NaOH). Sodium Hydroxide　contains not less than 95.0% of sodium hydroxide (NaOH).

Description　Sodium Hydroxide (crystal) occurs as a white crystalline powder or as granules. Sodium Hydroxide　occurs as white lumps having various shapes, including pellets, flakes, and rods, or as a white powder.

Identification

(1) A solution of Sodium Hydroxide (1 in 50) is strongly alkaline.

(2) Sodium Hydroxide responds to all the tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and almost clear.

Test Solution Weigh 50 g of Sodium Hydroxide, and dissolve it in freshly boiled and cooled water to make 250 mL. Use this solution as the sample solution. Measure 5.0 mL of the sample solution, and mix with 20 mL of water.

(2) Sodium carbonate The content of sodium carbonate (Na_2CO_3) obtained by the assay is not more than 2.0%.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Hydroxide, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool.

(4) Mercury Not more than 0.10 μg/g as Hg.

Test Solution Measure exactly 10 mL of the sample solution prepared in Purity (1) above, add 1 mL of potassium permanganate solution (3 in 50) and about 30 mL of water, and shake. Neutralize by gradually adding hydrochloric acid (purified), add 5 mL of diluted sulfuric acid (1 in 2), and cool. Use this solution as the sample solution. To the sample solution, add hydroxylamine chloride solution (1 in 5) until the purple color of the potassium permanganate disappears and the precipitate of manganese dioxide dissolves, and add water to make 100 mL.

Control Solution To 2.0 mL of Mercury Standard Solution, add 1 mL of potassium permanganate solution (3 in 50), 30 mL of water, and hydrochloric acid (purified) (the same amount as used for preparing the test solution), and 5 mL of diluted sulfuric acid(1 in 2), and proceed in the same manner as the preparation of the test solution.

Procedure Analyze the test solution and the control solution by cold-vapor atomic absorption spectrophotometry. Transfer appropriate portions of the test solution and the control solution into separate testing vials, add 10 mL of tin(II) chloride–sulfuric acid TS, and immediately fit them into the atomic absorption spectrophotometer, and set off the pump to circulate the air in a closed state. Measure the absorbance using conditions given below. The absorbance value of the test solution is not higher than that of the control solution.

Operating Conditions

Light source: Mercury hollow cathode lamp.

Analytical line wavelength: 253.7 nm.

Carrier gas: Air.

(5) Arsenic Not more than 3 μg/g as As (Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution　Measure 2.5 mL of the sample solution prepared in Purity (1), add 5 mL of water, and neutralize by gradually adding hydrochloric acid.

Assay　Weigh accurately about 50 g of Sodium Hydroxide, and add freshly boiled and cooled water to make exactly 1000 mL. Use this solution as the sample solution. Measure exactly 25 mL of the sample solution, add 10 mL of freshly boiled and cooled water, and titrate with 1 mol/L hydrochloric acid (indicator: 1 mL of bromophenol blue TS). When the solution reaches neutral, add exactly 1 mL of 1 mol/L hydrochloric acid, boil for about 5 minutes, and cool. Titrate the excess acid with 0.1 mol/L sodium hydroxide, and determine the volume (a mL) of 1 mol/L hydrochloric acid consumed.

Separately, measure exactly 25 mL of the sample solution, transfer into a ground-glass stoppered flask, and add 25 mL of freshly boiled and cooled water. To the solution, add 10 mL of a solution of barium chloride dihydrate (3 in 25), stopper, shake gently, and titrate with 1 mol/L hydrochloric acid (indicator: 1 mL of phenolphthalein TS). Record the volume of 1 mol/L hydrochloric acid consumed as b (mL).

$$\text{Content (\%) of sodium hydroxide (NaOH)} = \frac{0.04000 \times b \times 40}{\text{Weight (g) of the sample}} \times 100$$

$$\text{Content (\%) of sodium carbonate } (Na_2CO_3) = \frac{0.05299 \times (a - b) \times 40}{\text{Weight (g) of the sample}} \times 100$$

Sodium Hydroxide Solution

水酸化ナトリウム液

Content　Sodium Hydroxide Solution contains 95–120% of the labeled content of sodium hydroxide (NaOH = 40.00).

Description　Sodium Hydroxide Solution is a colorless or slightly colored liquid.

Identification

(1) Diluted Sodium Hydroxide Solution (1 in 50) is strongly alkaline.

(2) Sodium Hydroxide Solution responds to all the tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution　Colorless and almost clear.

Sample Solution　To Sodium Hydroxide Solution, add freshly boiled and cooled water to prepare a solution equivalent to 20% (w/v) solution of NaOH, calculated from the labeled content.

Test Solution　Mix 5.0 mL of the sample solution with 20 mL of water.

(2) Sodium Carbonate　Not more than 2.0% of Na_2CO_3 per sodium hydroxide (NaOH)

obtained by the assay.

(3) Lead　Not more than 2 μg/g of NaOH as Pb (an amount equivalent to 2.0 g of KOH, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution　To the specified amount of Sodium Hydroxide Solution, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool.

(4) Mercury　Not more than 0.10 μg/g of NaOH as Hg.

Proceed as directed in Purity (4) for Sodium Hydroxide.

(5) Arsenic　Not more than 3 μg/g of NaOH as As (Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Proceed as directed in Purity (5) for Sodium Hydroxide.

Assay　Weigh accurately an amount of Sodium Hydroxide Solution equivalent to about 5 g of sodium hydroxide (NaOH). Add freshly boiled and cooled water to make exactly 100 mL, and use this solution as the sample solution. Measure exactly 25 mL of the sample solution, and proceed as directed in the Assay for Sodium Hydroxide.

$$\text{Content (\%) of sodium hydroxide (NaOH)} = \frac{0.04000 \times b \times 4}{\text{Weight (g) of the sample}} \times 100$$

$$\text{Content (\%) of sodium carbonate } (Na_2CO_3) \text{ per sodium hydroxide (NaOH)}$$

$$= \frac{0.05299 \times (a - b) \times 4}{\text{Weight (g) of the sample}} \times \frac{100}{\text{Content (\%) of the sodium hydroxide}} \times 100$$

Sodium Hypochlorite

Hypochlorite of Soda

次亜塩素酸ナトリウム

NaClO　　Mol. Wt. 74.44

Sodium hypochlorite

Content　Sodium Hypochlorite contains not less than 4.0% of available chlorine.

Description　Sodium Hypochlorite is a colorless to light green-yellow liquid having an odor of chlorine.

Identification

(1) Sodium Hypochlorite responds to all the tests for Sodium Salt and for Chlorite in the Qualitative Tests.

(2) To 4 mL of a solution of Sodium Hypochlorite (1 in 25), add 100 mL of phosphate buffer (pH 8), and measure the absorbance. The solution exhibits an absorption maximum at a wavelength of 291–294 nm.

(3) Dip a litmus paper (red) in Sodium Hypochlorite. The color changes to blue, and then fades.

Assay Weigh accurately about 3 g of Sodium Hypochlorite, and add 50 mL of water. Add 2 g of potassium iodine and 10 mL of diluted acetic acid (1 in 4), immediately stopper tightly, and allow to stand in a dark place for 15 minutes. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 3.545 mg of Cl

Sodium Iron Chlorophyllin

鉄クロロフィリンナトリウム

Description Sodium Iron Chlorophyllin occurs as a green-black powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Place 1 g of Sodium Iron Chlorophyllin in a ceramic crucible, and add a small amount of sulfuric acid to moisten. Heat the crucible gradually to almost incinerate the sample at as low temperature as possible, and cool. Again add 1 mL of sulfuric acid, gradually heat until sulfuric acid vapor no longer develops, and cool. To the residue, add 10 mL of diluted hydrochloric acid (1 in 4), and dissolve it by heating on a water bath. Filter if necessary, and add water to make 10 mL. Make the resulting solution weakly alkaline with ammonia TS, add 10 mL of hydrogen sulfide TS, allow to stand for 30 minutes, and filter. Perform the following tests for the filtrate and the residue on the filter paper.

(i) To the filtrate, add 1 mL of diluted hydrochloric acid (1 in 4), and perform the Flame Coloration Test. A yellow color is imparted to the flame.

(ii) Dissolve the residue on the filter paper by adding 2 mL of diluted nitric acid (1 in 10), and add water to make 5 mL. To the resulting solution, add 2–3 drops of ammonium thiocyanate solution (2 in 25). A red color develops.

(2) To 1 mL of a solution of Sodium Iron Chlorophyllin (1 in 1000), add phosphate buffer (pH 7.5) to make 100 mL, and measure the absorbance. The solution exhibits absorption maxima at wavelengths of 396–400 nm and 652–658 nm. When the absorbance values at the absorption maxima are expressed as A_1 and A_2, respectively, A_1/A_2 is not more than 9.5.

Specific Absorbance $E_{1cm}^{1\%}$ (maximum absorbance wavelength near 398 nm): Not less

than 400 (on the dried basis).

This test should be protected from direct light, and the apparatus used in the test should be light-resistant.

Weigh accurately about 0.1 g of Sodium Iron Chlorophyllin, and dissolve it in water to make exactly 100 mL. Measure exactly 1 mL of this solution, add phosphate buffer (pH 7.5) to make exactly 100 mL, and promptly measure the absorbance.

pH 9.5–11.0 (1.0 g, water 100 mL).

Purity

(1) Inorganic iron salt Not more than 0.09% as Fe.

Test Solution Weigh 1.0 g of Sodium Iron Chlorophyllin, and dissolve it in 60 mL of water.

Procedure Analyze a 2-μL portion of the test solution by thin-layer chromatography using a 4:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm, and air-dry the plate. Spray with a solution of sodium hexacyanoferrate(II) decahydrate (1 in 1000). No blue spot is observed.

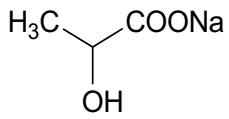
(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 5.0% (105°C, 2 hours).

Sodium Lactate

Sodium Lactate Solution

乳酸ナトリウム



$C_3H_5NaO_3$ Mol. Wt. 112.06

Monosodium 2-hydroxypropanoate [72-17-3]

Content Sodium Lactate contains not less than 40.0% of sodium lactate ($C_3H_5NaO_3$) and contains 95–110% of the labeled content of sodium lactate.

Description Sodium Lactate is a colorless, clear, syrupy liquid. It is odorless or has a slight, characteristic odor.

Identification Sodium Lactate responds to all the tests for Sodium Salt and for Lactate

in the Qualitative Tests.

pH 6.5–7.5.

Measure the pH of a solution prepared as follows: To 1.0 mL of Sodium Lactate, add 5 mL of water, and shake.

Purity

(1) Sulfate Not more than 0.012% as SO_4 for 60% sodium lactate (an amount equivalent to 0.60 g of sodium lactate, Control Solution: 0.005 mol/L sulfuric acid 0.25 mL).

(2) Lead Not more than 2 μg/g as Pb for 60% sodium lactate (an amount equivalent to 1.2 g of sodium lactate, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Iron Not more than 10 μg/g as Fe for 60% sodium lactate (an amount equivalent to 0.60 g of sodium lactate, Method 1, Control Solution: Iron Standard Solution 1.0 mL).

(4) Arsenic Not more than 3 μg/g as As for 60% sodium lactate (an amount equivalent to 0.60 g of sodium lactate, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of Sodium Lactate, add water to make 10 mL. Use 5 mL of this solution.

(5) Volatile fatty acids Weigh 5 g of Sodium Lactate, add 2 mL of diluted sulfuric acid (1 in 20), and heat on a water bath. No butyric acid-like odor is evolved.

(6) Methanol Not more than 0.20% v/w as CH_3OH for 60% sodium lactate.

Weigh an amount of Sodium Lactate equivalent to 3.0 g of sodium lactate, add 8 mL of water, distill the solution, collect 5 mL of the initial distillate, and add water to make 100 mL. Proceed as directed in Purity (9) for Lactic Acid, using 1.0 mL of this solution.

Assay Weigh accurately an amount of Sodium Lactate equivalent to about 0.3 g of sodium lactate, and evaporate to dryness on a water bath. Completely dissolve the residue in 60 mL of a 4:1 mixture of acetic acid/acetic anhydride, and titrate with 0.1 mol/L perchloric acid (indicator: 1 mL of crystal violet–acetic acid TS) until the color of the solution changes to blue. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 11.21 mg of $C_3H_5NaO_3$

Sodium DL-Malate

Sodium *dl*-Malate

Sodium Malate

DL-リンゴ酸ナトリウム

n = 3 or 1/2

$C_4H_4Na_2O_5 \cdot nH_2O$ (n = 3 or 1/2) Mol. Wt. trihydrate 232.10

hemihydrate 187.06

Disodium (2*RS*)-2-hydroxybutanedioate trihydrate

Disodium (2*RS*)-2-hydroxybutanedioate hemihydrate

[anhydrous 676-46-0]

Definition Sodium DL-Malate occurs as trihydrate and hemihydrate.

Content Sodium DL-Malate, when dried, contains 98.0–102.0% of sodium DL-malate ($C_4H_4Na_2O_5$ = 178.05).

Description Sodium DL-Malate occurs as a white crystalline powder or as lumps. It is odorless and has a salty taste.

Identification

(1) Place 1 mL of a solution of Sodium DL-Malate (1 in 20) into a porcelain dish, add 10 mg of sulfanilic acid, and proceed as directed in Identification (1) in DL-Malic Acid.

(2) Proceed as directed in Identification (2) for DL-Malic Acid.

(3) Sodium DL-Malate responds to all the tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Free alkali Not more than 0.2% as Na_2CO_3.

Weigh 1.0 g of Sodium DL-Malate, dissolve it in 20 mL of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS. A pink color develops, and it disappears on the addition of 0.40 mL of 0.05 mol/L sulfuric acid.

(3) Chloride Not more than 0.011% as Cl (1.0 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(6) Readily oxidizable substances Weigh 0.10 g of Sodium DL-Malate, dissolve it by adding 25 mL of water and 25 mL of diluted sulfuric acid (1 in 20), keep at 20°C, and add 1.0 mL of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 3 minutes.

Loss on Drying

Trihydrate: 20.5–23.5% (120°C, 1 hour, then 160°C, 2 hours).

Hemihydrate: Not more than 7.0% (120°C, 1 hour, then 160°C, 2 hours).

Assay Weigh accurately about 0.15 g of Sodium DL-Malate, previously dried, dissolve it in 30 mL of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid. The endpoint is usually confirmed by using a potentiometer. When crystal violet–acetic acid TS (1 mL) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 8.903 mg of $C_4H_4Na_2O_5$

Sodium Metabisulfite

Sodium Pyrosulfite

ピロ亜硫酸ナトリウム

$Na_2S_2O_5$ Mol. Wt. 190.11

Sodium disulfite [7681-57-4]

Content Sodium Metabisulfite contains not less than 93.0% of sodium pyrosulfite ($Na_2S_2O_5$).

Description Sodium Metabisulfite occurs as a white powder having an odor of sulfur dioxide.

Identification Sodium Metabisulfite responds to all the tests for Sodium Salt and for Sulfite in the Qualitative Tests .

Purity

(1) Clarity of solution Very slightly turbid (0.50 g, water 10 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Metabisulfite, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow to cool, and use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes, and allow to cool.

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard

Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Sodium Metabisulfite in 10 mL of water. Add 1 mL of sulfuric acid, heat on a hot plate until white fumes are evolved, and add water to make 5 mL.

Assay Weigh accurately about 0.2 g of Sodium Metabisulfite, and proceed as directed under Sulfite Determination.

Each mL of 0.05 mol/L iodine = 4.753 mg of $Na_2S_2O_5$

Sodium Metaphosphate

メタリン酸ナトリウム

Content Sodium Metaphosphate, when dried, contains the equivalent of 60.0–83.0% phosphorus(V) oxide (P_2O_5 = 141.94).

Description Sodium Metaphosphate occurs as white fibrous crystals or powder, or as colorless to white glassy flakes or lumps.

Identification

(1) To a solution of Sodium Metaphosphate (1 in 40), add diluted acetic acid (1 in 20) or sodium hydroxide solution (1 in 20) to make it weakly acidic, and add 5 mL of egg white TS. A white precipitate is formed.

(2) Sodium Metaphosphate responds to all the tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and very slightly turbid (powder 1.0 g, water 20 mL).

(2) Chloride Not more than 0.21% as Cl (powder 0.10 g, Control Solution: 0.01 mol/L hydrochloric acid 0.60 mL).

(3) Orthophosphate Weigh 1.0 g of powdered Sodium Metaphosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(4) Sulfate Not more than 0.048% as SO_4.

Test Solution Weigh 0.40 g of powdered Sodium Metaphosphate, add 30 mL of water and 2 mL of diluted hydrochloric acid (1 in 4), dissolve it by boiling for 1 minute, cool, and add water to make 50 mL.

Control Solution To 0.40 mL of 0.005 mol/L sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(5) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Metaphosphate, add 5mL of nitric acid and 25 mL of water, boil gently for 15 minutes with a watch glass covering it,

and cool.

(6) Arsenic Not more than 3 μg/g as As (0.50 g of powdered sample, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

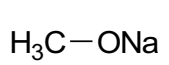
Loss on Drying Not more than 5.0% (110°C, 4 hours).

Assay Proceed as directed in the Assay for Potassium Polyphosphate.

Sodium Methoxide

Sodium Methylate

ナトリウムメトキシド



CH_3ONa Mol. Wt. 54.02

Sodium methoxide [124-41-4]

Content Sodium Methoxide contains not less than 95.0% of sodium methoxide (CH_3ONa).

Description Sodium Methoxide occurs as a white, hygroscopic, fine powder.

Identification

(1) A solution of Sodium Methoxide (1 in 100) is alkaline.

(2) To 1 drop of a solution of Sodium Methoxide (1 in 100), add 0.1 mL of diluted sulfuric acid (1 in 20) and 0.2 mL of potassium permanganate solution (1 in 300), and allow to stand for 5 minutes. Add 0.2 mL of sodium sulfite solution (1 in 5) and 3 mL of sulfuric acid, and then add 0.2 mL of chromotropic acid TS. A red-purple to purple color develops.

(3) Sodium Methoxide responds to all the tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Very slightly turbid.

Test Solution Weigh 5.0 g of Sodium Methoxide, and dissolve it in freshly boiled and cooled water to make 100 mL. Use this solution as the sample solution. Measure 20 mL of the sample solution, add 30 mL of freshly boiled and cooled water.

(2) Sodium carbonate Not more than 0.5% as Na_2CO_3.

Proceed as directed in Assay (iii).

(3) Sodium hydroxide Not more than 2.0% as NaOH.

Proceed as directed in Assay (iv).

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Measure 10 mL of the sample solution prepared in Purity (1) above, neutralize by gradually adding diluted hydrochloric acid (1 in 4), and evaporate to dryness on a water bath. Dissolve the residue by adding 5 mL of water.

Assay

(i) Promptly and accurately weigh about 0.5 g of Sodium Methoxide, using a titration flask for the Karl Fischer method, immediately add 10 mL of salicylic acid–methanol TS, stopper tightly, dissolve, and cool. Proceed as directed under Direct Titration in Volumetric Titration in Water Determination (Karl Fischer Method). Perform a blank test on 10 mL of salicylic acid–methanol TS in the same manner, and calculate the sum (A) of the contents of sodium hydroxide and sodium carbonate as sodium hydroxide by the formula:

$$\text{A (\%)} = \frac{(a - b) \times f \times 2.222}{\text{Weight (g) of the sample} \times 1000} \times 100$$

a = volume (mL) of water determination TS consumed in the test,

b = volume (mL) of water TS consumed in the blank test,

f = number of mg of water equivalent to 1 mL of water determination TS.

(ii) Quickly and accurately weigh about 2 g of Sodium Methoxide into a ground-glass stoppered Erlenmeyer flask, and immediately dissolve it by gently adding about 50 mL of freshly boiled and cooled water. Add 10 mL of a solution of barium chloride dihydrate (3 in 25), stopper, allow to stand for 5 minutes, and titrate with 1 mol/L hydrochloric acid (indicator: 2 drops of phenolphthalein TS). Calculate the sum (B) of the contents of sodium methoxide and sodium hydroxide as sodium methoxide (CH_3ONa) by the formula:

$$\text{B (\%)} = \frac{\text{Volume (mL) of 1 mol/L hydrochloric acid consumed} \times 0.054}{\text{Weight (g) of the sample}} \times 100$$

(iii) Add 1 mL of 1 mol/L hydrochloric acid to the solution left after titration in (ii) above, boil gently for about 5 minutes, cool, and titrate the excess acid with 0.1 mol/L sodium hydroxide. Calculate the content (C) of sodium carbonate (Na_2CO_3) by the formula:

$$\text{C (\%)} = \frac{[1 - (\text{Volume (mL) of 0.1 mol/L sodium hydroxide consumed} \times 0.1)] \times 0.053}{\text{Weight (g) of the sample taken in (ii)}} \times 100$$

(iv) Calculate the content (D) of sodium hydroxide (NaOH) by the formula:

$$D\ (\%) = A - (C \times 0.377)$$

(v) Calculate the content (E) of sodium methoxide (CH_3ONa) by the formula:

$$E\ (\%) = B - (D \times 1.350)$$

Storage Standards Store in a hermetic container.

Sodium Nitrate

硝酸ナトリウム

$NaNO_3$ Mol. Wt. 84.99

Sodium nitrate [7631-99-4]

Content Sodium Nitrate, when dried, contains not less than 99.0% of sodium nitrate ($NaNO_3$).

Description Sodium Nitrate occurs as colorless crystals or as a white crystalline powder. It is odorless and has a slightly salty taste.

Identification Sodium Nitrate responds to all the tests for Sodium Salt and for Nitrate in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 10 mL).

(2) Chloride Not more than 0.21% as Cl (0.10 g, Control Solution: 0.01 mol/L hydrochloric acid 0.60 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Proceed as directed in Purity (3) for Potassium Nitrate

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of Sodium Nitrate, dissolve it in 3 mL of water, add 2 mL of sulfuric acid, and heat until white fumes are evolved. Then add a small amount of water to dissolve, heat until white fumes are evolved, cool, and add 5 mL of water to dissolve.

Loss on Drying Not more than 1.0% (105°C, 4 hours).

Assay Proceed as directed in the Assay for Potassium Nitrate.

Each mL of 0.05 mol/L sulfuric acid = 8.499 mg of $NaNO_3$

Sodium Nitrite

亜硝酸ナトリウム

$NaNO_2$ Mol. Wt. 69.00

Sodium nitrite [7632-00-0]

Content Sodium Nitrite, when dried, contains not less than 97.0% of sodium nitrite ($NaNO_2$).

Description Sodium Nitrite occurs as a white to light yellow crystalline powder, or as granules or rod-shaped lumps.

Identification Sodium Nitrite responds to all the tests for Sodium Salt and for Nitrite in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.71% as Cl.

Test Solution Weigh 1.0 g of Sodium Nitrite, and dissolve it in water to make 500 mL. Measure 10 mL of this solution, add 3 mL of diluted acetic acid (1 in 4), and warm gradually. After the gas is no longer evolved, add 6 mL of diluted nitric acid (1 in 10), and add water to make 50 mL.

Control Solution To 0.40 mL of 0.01 mol/L hydrochloric acid, add 3 mL of diluted acetic acid (1 in 4), 6 mL of diluted nitric acid (1 in 10), and water to make 50 mL.

(3) Sulfate Not more than 0.24% as SO_4.

Test Solution Weigh 1.0 g of Sodium Nitrite and dissolve it in water to make 100 mL. Measure 10 mL of this solution, add 1 mL of hydrochloric acid, evaporate to dryness in a water bath, dissolve the residue in 1 mL of diluted hydrochloric acid (1 in 4) and 20 mL of water, and add water to make 50 mL.

Control Solution Measure 0.50 mL of 0.005 mol/L sulfuric acid, add 1 mL of hydrochloric acid, evaporate to dryness in a water bath, and then proceed as directed for the test solution.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Nitrite, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow it to cool, and use as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Gently boil for 5 minutes, and allow to cool.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Sodium Nitrite in 5 mL of water,

add 2 mL of hydrochloric acid, and evaporate to dryness in a water bath. Dissolve the residue by adding 5 mL of water.

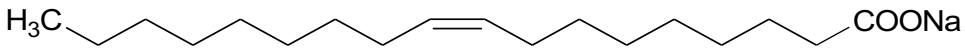
Loss on Drying Not more than 3.0% (100°C, 5 hours).

Assay Weigh accurately about 1 g of Sodium Nitrite, previously dried, dissolve it in water to make exactly 100 mL, and refer to this solution as Solution A. Weigh exactly 40 mL of 0.02 mol/L potassium permanganate, transfer into an Erlenmeyer flask, and add 100 mL of water and 5 mL of sulfuric acid. To this solution, add exactly 10 mL of Solution A while keeping the tip of the pipet below the surface of the liquid. Allow to stand for 5 minutes, add exactly 25 mL of 0.05 mol/L oxalic acid, warm to about 80°C, and titrate the excess oxalic acid with 0.02 mol/L potassium permanganate while hot.

Each mL of 0.02 mol/L potassium permanganate = 3.450 mg of $NaNO_2$

Sodium Oleate

オレイン酸ナトリウム



$C_{18}H_{33}NaO_2$ Mol. Wt. 304.44

Monosodium (9*Z*)-octadec-9-enoate [143-19-1]

Description Sodium Oleate occurs as a white to yellowish powder, or as light brown-yellow, granules or lumps. It has a characteristic odor and taste.

Identification

(1) To 50 mL of a solution of Sodium Oleate (2 in 25), add 5 mL of diluted sulfuric acid (1 in 20) while stirring, filter through a filter paper moistened previously with water, and wash the residue with water until the washings no longer shows acidity to methyl orange TS. Filter the oily residue through a dry filter paper, transfer 2–3 drops of the oily solution into a small test tube, and superimpose about a 1-mL layer of sulfuric acid. A brown-red band develops at the junction. Take another 1–3 drops of the oily solution, dissolve it in 3–4 mL of diluted acetic acid (1 in 4), add 1 drop of a solution (1 in 10) of chromium(VI) oxide in acetic acid, and add 10－30 drops of sulfuric acid while shaking. A dark purple color develops.

(2) The residue on ignition of Sodium Oleate responds to all the tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear (0.50 g, water 20 mL).

(2) Free Alkali Not more than 0.5%.

Weigh accurately about 5 g of powdered Sodium Oleate, add 100 mL of ethanol (neutralized), and dissolve it while heating. Filter the insoluble residue while the liquid

is hot, wash with ethanol (neutralized) at about 40°C until the washings become colorless, and combine the washings with the filtrate. Cool, titrate with 0.05 mol/L sulfuric acid, and determine the volume consumed (a mL). Wash the above residue 5 times with 10 mL of boiling water each time, combine all the washings, cool, add 3 drops of bromophenol blue TS, titrate with 0.05 mol/L sulfuric acid, and determine the volume consumed (b mL). Calculate the content of free alkali by the formula:

$$\text{Content (\%) of free alkali} = \frac{(0.0040 \times a) + (0.0053 \times b)}{\text{Weight (g) of the sample}} \times 100$$

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (5.0 g, Standard Color: Arsenic Standard Solution 15 mL, Apparatus B).

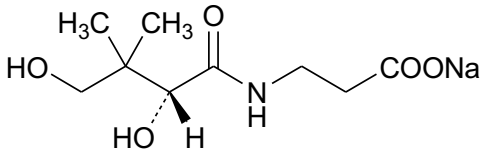
Test Solution Add 30 mL of boiling water to the specified amount of Sodium Oleate, and dissolve it while stirring well. Add 6 mL of diluted sulfuric acid (1 in 20) dropwise, remove the deposited fatty acid by extracting with diethyl ether, and add water to make 50 mL. Use 5 mL of this solution for the test.

Standard Color To the specified amount of Arsenic Standard Solution, add 30 mL of water and 6 mL of diluted sulfuric acid (1 in 20), and add water to make 50 mL. Measure10.0 mL of this solution, and proceed in the same manner as the test solution.

Residue on Ignition 22.0–25.0%.

Sodium Pantothenate

パントテン酸ナトリウム



$C_9H_{16}NNaO_5$ Mol. Wt. 241.22

Monosodium 3-[(2*R*)-2,4-dihydroxy-3,3-dimethylbutanoylamino]propanoate

[75033-16-8]

Content Sodium Pantothenate, when calculated on the dried basis, contains 5.6–6.0% of nitrogen (N = 14.01) and 9.3–9.7% of sodium (Na = 22.99).

Description Sodium Pantothenate occurs as a white powder. It is odorless and has a slightly acid taste.

Identification

(1) Proceed as directed in Identification (1) and (2) for Calcium Pantothenate.

(2) A solution of Sodium Pantothenate (1 in 20) responds to all the tests for Sodium Salt in the Qualitative Tests.

Specific Rotation $[\alpha]_D^{20}$: +25.0 to +28.5° (previously dried, 1.25 g, water, 25 mL).

pH 8.5–10.0 (2.0 g, water 10 mL).

Purity

(1) Calcium Weigh 1.0 g of Sodium Pantothenate, dissolve it in 10 mL of water, and add 0.5 mL of diluted acetic acid (1 in 20) and 0.5 mL of a solution of ammonium oxalate monohydrate (1 in 25). No precipitate is formed.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Alkaloid Proceed as directed in Purity (3) for Calcium Pantothenate.

Loss on Drying Not more than 5.0% (reduced pressure, 24 hours).

Assay

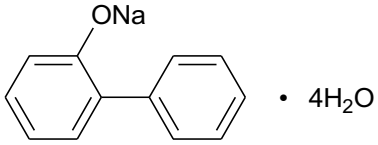
(1) Nitrogen Weigh accurately about 50 mg of Sodium Pantothenate, proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination, and calculate on the dried basis.

(2) Sodium Weigh accurately about 0.6 g of Sodium Pantothenate, dissolve it in 50 mL of acetic acid, and titrate with 0.1 mol/L perchloric acid (indicator: 1 mL of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, make any necessary correction, and calculate on the dried basis.

Each mL of 0.1 mol/L perchloric acid = 2.299 mg of Na

Sodium *o*-Phenylphenate

オルトフェニルフェノールナトリウム



$C_{12}H_9NaO \cdot 4H_2O$ Mol. Wt. 264.25

Monosodium 2-phenylphenolate tetrahydrate [anhydrous 132-27-4]

Content Sodium *o*-Phenylphenate, when calculated on the anhydrous basis, contains

not less than 95.0% of sodium *o*-phenylphenate ($C_{12}H_9NaO$ = 192.19).

Description Sodium *o*-Phenylphenate occurs as a white or light pink to pink powder, or as flakes or lumps. It has a characteristic odor.

Identification

(1) Proceed as directed in Identification (1) and (2) for *o*-Phenylphenol.

(2) Sodium *o*-Phenylphenate responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 11.1–12.2 (1.0 g, water 50 mL).

Purity

(1) *o*-Phenylphenol Weigh 1.0 g of Sodium o-Phenylphenate, dissolve it in 50 mL of water, add diluted hydrochloric acid (1 in 4) until the solution is weakly acidic, and allow to stand for 1 hour. Filter the formed precipitate, wash with a small amount of water, and dry in a desiccator (sulfuric acid) for 24 hours. The melting point is 55–58°C.

(2) Sodium hydroxide Not more than 1.0%.

Weigh accurately about 5 g of powdered Sodium *o*-Phenylphenate, dissolve it in 50 mL of 50% (vol) ethanol, and titrate with 1 mol/L hydrochloric acid (indicator: 1 mL of bromophenol blue TS). Calculate the content by the formula:

$$\text{Content (\%) of sodium hydroxide (NaOH)} = \text{Volume (mL) of 1 mol/L hydrochloric acid consumed} - \frac{\text{Weight (g) of the sample}}{0.264} \times \frac{0.04}{\text{Weight (g) of the sample}} \times 100$$

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (2.5 g, Standard Color: Arsenic Standard Solution 15 mL, Apparatus B).

Test Solution Weigh 2.5 g of powdered Sodium *o*-Phenylphenate, transfer into a Kjeldahl flask, add 20 mL of nitric acid, and heat weakly until the contents become fluid. After cooling, add 5 mL of sulfuric acid, and heat until white fumes are evolved. If the solution is still brown in color, cool, add 5 mL of nitric acid, and heat. Repeat this procedure until the color of the solution becomes colorless to light yellow. After cooling, add 15 mL of a solution of ammonium oxalate monohydrate (1 in 25), and heat until white fumes are evolved again. After cooling, add water to make 25 mL, and use 5 mL of this solution as the test solution.

Standard Color Place the specified amount of Arsenic Standard Solution into a Kjeldahl flask, add 20 mL of nitric acid and 5 mL of sulfuric acid, and heat until white fumes are evolved again. After cooling, add water to make 25 mL. Using 5 mL of this solution, proceed in the same manner as the preparation of the test solution.

(5) *p*-Phenylphenol and other organic impurities Not more than 0.1%, as *p*-phenylphenol, of *o*-phenylphenol.

Test Solution Weigh 2.0 g of Sodium *o*-Phenylphenate, dissolve it in 100 mL of water, add diluted hydrochloric acid (1 in 4) until the solution is weakly acidic, and allow to stand for 1 hour. Filter the formed precipitate, wash with a small amount of water, and dry in a desiccator (sulfuric acid) for 24 hours. Weigh 1.0 g of the prepared sample, and add 5 mL of ethanol (95) and 5 mL of a solution (1 in 1000) of caffeine monohydrate in ethanol (95) to dissolve.

Procedure Proceed as directed in (3) for *o*-Phenylphenol.

Water Content 25.0–28.0% (0.1 g, Volumetric Titration, Direct Titration).

Use 20 mL of methanol for water determination and 10 mL of acetic acid instead of 25 mL of methanol for water determination.

Assay Weigh accurately about 3 g of powdered Sodium *o*-Phenylphenate, and dissolve it in several drops of sodium hydroxide solution (1 in 25) and water to make exactly 500 mL. Proceed as directed in the Assay for *o*-Phenylphenol, using this solution as the test solution.

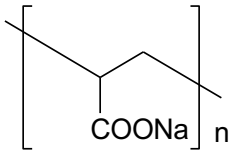
$$\text{Content (\%) of sodium } o\text{-phenylphenate } (C_{12}H_9NaO) = \frac{4.805 \times (a - b)}{\text{Anhydrous basis weight (g) of the sample} \times 50} \times 100$$

a = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the blank test,

b = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in this test.

Sodium Polyacrylate

ポリアクリル酸ナトリウム



$(C_3H_3NaO_2)_n$

Poly(sodium 1-carboxylatoethylene)

Description Sodium Polyacrylate occurs as a white powder. It is odorless.

Identification

(1) To 10 mL of a solution of Sodium Polyacrylate (1 in 500), add 1 mL of magnesium sulfate TS (0.5 mol/L), and shake. A white precipitate is formed.

(2) The residue on ignition of Sodium Polyacrylate responds to all the tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Free alkali Weigh 0.20 g of Sodium Polyacrylate, add 60 mL of water, and dissolve it while shaking well. Add 3 mL of a solution of calcium chloride dihydrate (3 in 40), heat on a water bath for about 20 minutes, cool, and filter. Wash the residue on the filter paper with water, combine the washings with the filtrate, and add water to make 100 mL. Refer to this solution as Solution A. Measure 50 mL of Solution A, and add 2 drops of phenolphthalein TS. No pink color develops.

(2) Sulfate Not more than 0.48% as SO_4.

Sample Solution 20 mL of Solution A prepared in Purity (1).

Control Solution 0.40 mL of 0.005 mol/L sulfuric acid.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Residual monomer Not more than 1.0%.

Weigh accurately about 1 g of Sodium Polyacrylate, transfer it into a 300-mL iodine flask, add 100 mL of water, and dissolve by allowing to stand for about 24 hours with occasional shaking. Add exactly 10 mL of potassium bromate–potassium bromide TS, shake well, add quickly 10 mL of hydrochloric acid, and immediately stopper tightly. Shake well, place 20 mL of potassium iodide TS in the neck of the iodine flask, and allow to stand in a dark place for 20 minutes. Loosen the stopper to allow the potassium iodide TS to flow down, immediately stopper tightly, shake well, and titrate with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test in the same manner, and calculate the content by the formula:

$$\text{Content (\%) of residual monomer} = \frac{0.0047 \times (a - b)}{\text{Weight (g) of the sample}} \times 100$$

a = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the blank test,

b = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in this test.

(6) Low molecular weight polymers Not more than 5.0%.

Dry a glass filter (1G4) at 105°C for 30 minutes, allow to cool in a desiccator, and weigh accurately. Weigh accurately about 2 g of Sodium Polyacrylate, add 200 mL of water, and dissolve it with occasional shaking. Add 50 mL of hydrochloric acid while stirring, warm in a water bath at 40°C for 30 minutes while stirring, and allow to stand for 24 hours. Filter the solution, add 1 drop of phenolphthalein TS to the filtrate, add sodium hydroxide solution (2 in 5) until the color of the filtrate changes to a slightly pink

color, and then add diluted hydrochloric acid (1 in 30) dropwise until the pink color disappears. Add 200 mL of water, then add 25 mL of a solution of calcium chloride dihydrate (3 in 40) dropwise while stirring, and warm in a water bath at about 40°C for 30 minutes while stirring. Filter this solution with suction through the glass filter, previously prepared, wash the residue three times with about 10 mL of water each time, dry at 105°C for 3 hours, and allow to cool in a desiccator. Weigh accurately, and calculate the content by the formula:

$$\text{Content (\%) of low molecular weight polymers} = \frac{\text{Weight (g) of the residue} \times 1.032}{\text{Weight (g) of the sample}} \times 100$$

Loss on Drying Not more than 10.0% (105°C, 4 hours).

Residue on Ignition Not more than 76.0% (calculated on the dried basis).

Sodium Polyphosphate

ポリリン酸ナトリウム

Content Sodium Polyphosphate, when dried, contains the equivalent of 53.0–80.0% of phosphorus(V) oxide (P_2O_5 = 141.94).

Description Sodium Polyphosphate occurs as a white powder, or as colorless to white glassy flakes or lumps.

Identification

(1) To 10 mL of a solution of Sodium Polyphosphate (1 in 100), add diluted acetic acid (1 in 20) to make it weakly acidic, and add 1 mL of silver nitrate solution (1 in 50). A white precipitate is formed.

(2) Sodium Polyphosphate responds to all the tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and very slightly turbid.

Test Solution Weigh 1.0 g of powdered Sodium Polyphosphate, add 20 mL of water, and dissolve it by heating.

(2) Chloride Not more than 0.21% as Cl (powder 0.10 g, Control Solution: 0.01 mol/L hydrochloric acid 0.60 mL).

(3) Orthophosphate Weigh 1.0 g of powdered Sodium Polyphosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(4) Sulfate Not more than 0.048% as SO_4.

Test Solution Weigh 0.40 g of powdered Sodium Polyphosphate, add 30 mL of water

and 2 mL of diluted hydrochloric acid (1 in 4), dissolve it by boiling for 1 minute, cool, and add water to make 50 mL.

Control Solution To 0.40 mL of 0.005 mol/L sulfuric acid, add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 50 mL.

(5) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Potassium Polyphosphate, add 5 mL of nitric acid and 25 mL of water, boil gently for 15 minutes with a watch glass covering it, and cool.

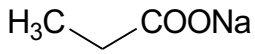
(6) Arsenic Not more than 3 μg/g as As (0.50 g of powdered sample, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 5.0% (110°C, 4 hours).

Assay Proceed as directed in the Assay for Potassium Polyphosphate.

Sodium Propionate

プロピオン酸ナトリウム



$C_3H_5NaO_2$ Mol. Wt. 96.06

Monosodium propanoate [137-40-6]

Content Sodium Propionate, when dried, contains not less than 99.0% of sodium propionate ($C_3H_5NaO_2$).

Description Sodium Propionate occurs as white crystals, crystalline powder, or granules. It is odorless or has a slight, characteristic odor.

Identification

(1) Proceed as directed in Identification (1) for Calcium Propionate.

(2) Sodium Propionate responds to all the tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and slightly turbid (1.0 g, water 20 mL).

(2) Free acid and free alkali Proceed as directed in Purity (2) for Calcium Propionate.

(3) Lead Not more than 5 μg/g as Pb (0.80 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 5.0% (105°C, 1 hour).

Assay Weigh accurately about 0.25 g of Sodium Propionate, previously dried, dissolve it in 40 mL of acetic acid for nonaqueous titration, and warm if necessary. Titrate with 0.1 mol/L perchloric acid (indicator: 2 drops of crystal violet–acetic acid TS). Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 9.606 mg of $C_3H_5NaO_2$

Sodium Pyrophosphate

Tetrasodium Pyrophosphate
Tetrasodium Diphosphate

ピロリン酸四ナトリウム

$Na_4P_2O_7 \cdot nH_2O$ (n = 10 or 0) Mol. Wt. decahydrate 446.06

anhydrous 265.90

Sodium diphosphate decahydrate [13472-36-1]

Sodium diphosphate [7722-88-5]

Definition Sodium Pyrophosphate occurs in two forms: the crystalline form (decahydrate) called Sodium Pyrophosphate (crystal) and the anhydrous form called Sodium Pyrophosphate (anhydrous).

Content Sodium Pyrophosphate, when dried, contains not less than 97.0% of sodium pyrophosphate ($Na_4P_2O_7$).

Description Sodium Pyrophosphate (crystal) occurs as colorless to white crystals or as a white crystalline powder. Sodium Pyrophosphate (anhydrous) occurs as a white powder or as lumps.

Identification

(1) To 10 mL of a solution of Sodium Pyrophosphate (1 in 100), add diluted acetic acid (1 in 20) to make it slightly acidic, and add 1 mL of silver nitrate solution (1 in 50). A white precipitate is formed.

(2) Sodium Pyrophosphate responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 9.9–10.7 (1.0 g, water 100 mL).

Purity Dry Sodium Pyrophosphate before performing the following tests.

(1) Clarity of solution Colorless and slightly turbid (1.0 g, water 20 mL).

(2) Chloride Not more than 0.21% as Cl (0.10 g, Control Solution: 0.01 mol/L hydrochloric acid 0.60 mL).

(3) Orthophosphate Weigh 1.0 g of Sodium Pyrophosphate, and add 2–3 drops of

silver nitrate solution (1 in 50). No brilliant yellow color develops.

(4) Sulfate Not more than 0.038% as SO_4 (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(5) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Pyrophosphate, add 5 mL of nitric acid and 25 mL of water, boil gently for 15 minutes with a watch glass covering it, and cool.

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying

Crystal: Not more than 42.0% (110°C, 4 hours).

Anhydrous: Not more than 5.0% (110°C, 4 hours).

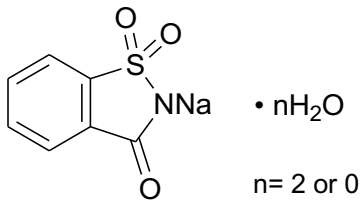
Assay Weigh accurately about 3 g of Sodium Pyrophosphate, previously dried, dissolve it in 75 mL of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 3–4 drops of methyl orange–xylene cyanol FF TS).

Each mL of 1 mol/L hydrochloric acid = 133.0 mg of $Na_4P_2O_7$

Sodium Saccharin

Soluble Saccharin

サッカリンナトリウム



$C_7H_4NNaO_3S \cdot nH_2O$ (n = 2 or 0) Mol. Wt. dihydrate 241.20

anhydrous 205.17

2-Sodio-1,2-benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide dehydrate [6155-57-3]

2-Sodio-1,2-benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide [128-44-9]

Content Sodium Saccharin, when dried, contains not less than 99.0% of sodium saccharin ($C_7H_4NNaO_3S$).

Description Sodium Saccharin occurs as colorless to white crystals or powder having an extremely sweet taste.

Identification

(1) To 10 mL of a solution of Sodium Saccharin (1 in 10), add 1 mL of diluted hydrochloric acid (1 in 4), allow to stand for 1 hour, and filter the white crystalline precipitate formed. Wash the residue on the filter paper thoroughly with water, dry at 105°C for 2 hours, and measure the melting point. It is 226–230°C.

(2) Proceed as directed in Identification (1) for Saccharin.

(3) Proceed as directed in Identification (2) for Saccharin.

(4) A solution of Sodium Saccharin (1 in 10) responds to all the tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution

Colorless and clear (powder sample 1.0 g, water 1.5 mL).

Colorless and clear (powder sample 1.0 g, ethanol (95) 70 mL).

(2) Free acid and free alkali Weigh 1.0 g of Sodium Saccharin, dissolve it in 10 mL of freshly boiled and cooled water, and add 1 drop of phenolphthalein TS. No pink color develops. Add 1 drop of 0.1 mol/L sodium hydroxide. A pink color develops.

(3) Lead Not more than 1 μg/g as Pb (4.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Benzoate and salicylate Weigh 0.5 g of Sodium Saccharin, dissolve it in 10 mL of water, and add 5 drops of acetic acid and 3 drops of a solution of iron(III) chloride hexahydrate (1 in 10). No precipitate is formed, and purple to red-purple color does not develop.

(6) *o*-Toluenesulfonamide Not more than 25 μg/g as *o*-toluenesulfonamide.

Test Solution Weigh 10 g of Sodium Saccharin, dissolve it in 50 mL of water, and proceed as directed in Purity (5) for Saccharin.

Loss on Drying Not more than 15.0% (120°C, 4 hours).

Assay Weigh accurately about 0.3 g of Sodium Saccharin, previously dried, dissolve it in 20 mL of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid (indicator: 2 drops of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 20.52 mg of $C_7H_4NNaO_3S$

Sodium Selenite

亜セレン酸ナトリウム

$Na_2SeO_3 \cdot 5H_2O$ Mol. Wt. 263.01

Disodium selenite pentahydrate [26970-82-1]

Content Sodium Selenite contains 98.5–101.5% of sodium selenite ($Na_2SeO_3 \cdot 5H_2O$).

Description Sodium Selenite occurs as a white crystalline powder.

Identification

(1) Dissolve 0.05 g of Sodium Selenite by adding 2.5 mL of water and 2.5 mL of 10% hydrochloric acid TS, and boil. When 0.05 g of L(+)-ascorbic acid is added, the resulting solution produces a red precipitate. When left to stand for a few minutes, the color of the precipitate changes to red-brown to black.

(2) Dissolve 0.05 g of Sodium Selenite by adding 5 ml of water and 1 ml of 10% hydrochloric acid TS. When 1 mL of a solution of barium chloride dihydrate (3 in 50) is added, no precipitate is produced.

(3) Sodium Selenite responds to all the tests for Sodium Salt in the Qualitative Tests.

pH 9.8–10.8 (2.0 g, water (carbon dioxide removed) 20 mL).

Purity

(1) Clarity of solution Colorless and clear (2.0 g, water (carbon dioxide removed) 20 mL).

(2) Chloride Not more than 0.005% as Cl.

Sample Solution Place 2.0 g of sodium selenite into a Nessler tube, and dissolve it in about 30 mL of water. Add 4 mL of nitric acid and mix.

Control Solution Use 0.30 mL of 0.01 mol/L hydrochloric acid.

(3) Sulfate Not more than 0.03% as SO_4 (0.8 g, Control solution: 0.005 mol/L sulfuric acid 0.50 mL).

(4) Lead Not more than 2 μg/g as Pb.

Standard Solution Measure exactly 2 mL of Lead Standard Stock Solution into a 100-mL volumetric flask, and add nitric acid (1 in 200) to volume.

Test Solution Weigh 1.00 g of sodium selenite into a 10-mL volumetric flask, dissolve it in nitric acid (1 in 200) to make 10 mL.

Standard Test Solutions Weigh 1.00 g of sodium selenite into each of three 10-mL volumetric flasks. To the flasks, add 0.5 mL, 1 mL, and 2 mL of the standard solution separately, and dissolve them by adding nitric acid (1 in 200) to make 10 mL of each solution.

Procedure Determine the emission intensity of lead in the test solution and standard test solutions by inductive coupled plasma-atomic emission spectrometry. Plot the values

obtained on a graph, with the amount of lead added (μg) in each solution on the x axis and emission intensity on the y axis, to prepare a regression line. Determine the amount of lead in the sample from the distance between the origin and the intersection of the regression line and the x axis.

(5) Iron Not more than 50 μg/g as Fe.

Standard Solution Measure exactly 5 mL of Iron Standard Stock Solution into a 100-mL volumetric flask, and add nitric acid (1 in 200) to volume.

Test Solution Weigh 1.00 g of sodium selenite into a 10-mL volumetric flask, dissolve it in nitric acid (1 in 200) to make 10 mL.

Standard Test Solutions Weigh 1.00 g of sodium selenite into each of three 10-mL volumetric flasks. To the flasks, add 0.5 mL, 1 mL, and 2 mL of the standard solution separately, and dissolve them by adding nitric acid (1 in 200) to make 10 mL of each solution.

Procedure Determine the emission intensity of iron in the test solution and standard test solutions by inductive coupled plasma-atomic emission spectrometry. Plot the values obtained on a graph, with the amount of iron added (μg) in each solution on the x axis and emission intensity on the y axis, to prepare a regression line.

Determine the amount of iron in the sample from the distance between the origin and the intersection of the regression line and the x axis.

(6) Arsenic Not more than 3 μg/g as As.

Standard Solution Measure exactly 3 mL of Arsenic Standard Stock Solution into a 100-mL volumetric flask, and add nitric acid (1 in 200) to volume.

Test Solution Weigh 1.00 g of sodium selenite into a 10-mL volumetric flask, dissolve it in nitric acid (1 in 200) to make 10 mL.

Standard Test Solutions Weigh 1.00 g of sodium selenite into each of three 10-mL volumetric flasks. To the flasks, add 0.5 mL, 1 mL, and 2 mL of the standard solution separately, and dissolve them by adding nitric acid (1 in 200) to make 10 mL of each solution.

Procedure Determine the emission intensity of arsenic in the test solution and standard test solutions by inductive coupled plasma-atomic emission spectrometry. Plot the values obtained on a graph, with the amount of arsenic added (μg) in each solution on the x axis and emission intensity on the y axis, to prepare a regression line. Determine the amount of arsenic in the sample from the distance between the origin and the intersection of the regression line and the x axis.

Assay Weigh accurately about 0.1 g of sodium selenite into a stoppered flask, and dissolve it in 100 mL of water. To this solution, add 3 g of potassium iodide and 5 mL of diluted hydrochloric acid (2 in 3), immediately stopper tightly, and allow to stand in a dark place for 5 minutes. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow-red. The endpoint is when the blue color produced disappears. Separately perform a blank test to make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 6.575 mg of $Na_2SeO_3 \cdot 5H_2O$

Sodium Stearoyl Lactylate

ステアロイル乳酸ナトリウム

[25383-99-7]

Definition Sodium Stearoyl Lactylate consists mainly of sodium salts of stearoyl lactylates. It is a mixture of sodium salts of stearoyl lactylic acids and minor proportions of related acids and other sodium salts of these acids.

Description Sodium Stearoyl Lactylate occurs as a white to pale yellow powder or brittle solid having a characteristic odor.

Identification

(1) Add 10 mL of diluted hydrochloric acid (1 in 4) to 2 g of Sodium Stearoyl Lactylate, heat in a water bath for 5 minutes, and filter. The filtrate imparts a yellow color to a flame. Neutralize the filtrate and add potassium hexahydroxoantimonate(V) TS. A white crystalline precipitate is formed.

(2) To the residue obtained by filtration in test (1), add 30 mL of sodium hydroxide (1 in 25), and heat while stirring for 30 minutes in a water bath at a temperature of not less than 95°C. After cooling, add 20 mL of diluted hydrochloric acid (1 in 4). Extract twice with two 30-mL portions of diethyl ether, and combine the diethyl ether layers, and wash with 20 mL of water. Add sodium sulfate to dehydrate, and filter. Heat the filtrate on a water bath to completely evaporate the diethyl ether. The melting point of the residue is 54–69°C.

(3) Sodium Stearoyl Lactylate responds to the test for Lactate in the Qualitative Tests.

Purity

(1) Acid value 60–130.

Weigh accurately about 1 g of Sodium Stearoyl Lactylate, add 25 mL of ethanol (neutralized), and dissolve it while warming. After cooling, add 5 drops of phenolphthalein TS, and immediately titrate with 0.1 mol/L sodium hydroxide to the first fain pink that persists for 30 seconds. Calculate the acid value by the formula:

$$\text{Acid value} = \frac{\text{Volume (mL) of 0.1 mol/L sodium hydroxide consumed} \times 5.611}{\text{Weight (g) of the sample}}$$

(2) Ester value 90–190 (Fats and Related Substances Tests).

To determine the ester value, use the acid value obtained in Purity (1). Measure the saponification value as directed in the Saponification Value Test in the Fats and Related Substances Tests, using about 1 g of Sodium Stearoyl Lactylate, weighed accurately. In the saponification value test, be careful not to let the precipitate adhere to the wall of

the flask when potassium hydroxide–ethanol TS is added. Perform the titration while the solution is hot.

(3) Total lactic acid 15–40% as lactic acid ($C_3H_6O_3$).

Proceed as directed in Purity (3) for Calcium Stearoyl Lactylate. To prepare a calibration curve, use 1, 2, 5, and 10 mL of Lithium Lactate Standard Solution, respectively.

(4) Sodium 2.5–5.0%.

Test Solution Place about 0.25 g of Sodium Stearoyl Lactylate, accurately weighed, into a beaker, add 10 mL of ethanol (95), and dissolve it while warming. Transfer this solution into a 25-mL volumetric flask, wash the beaker twice with two 5-mL portions of ethanol (95), and add the washings to the flask. Make up to volume with ethanol (95), and mix well. Transfer exactly 1 mL of the resulting solution into a 100-mL volumetric flask containing 10 mL of lanthanum oxide TS, make up to volume with water, and filter through a 5C filter paper.

Standard Solutions Dissolve 1.271 g of sodium chloride, previously dried at 130°C for 2 hours, in water to make exactly 500 mL. To exactly 10 mL of this solution, add water to make exactly 100 mL of a standard stock solution. Place exactly 2, 4, and 6 mL of the standard stock solution into separate 100-mL volumetric flasks, add 10 mL of lanthanum oxide TS to each, and dilute to volume with water. Prepare the standard solutions fresh.

Procedure Determine by the flame atomic absorption spectrophotometry using the operating conditions given below. Prepare a calibration curve using the standard solutions to determine the sodium concentration in the test solution, and calculate the sodium content by the formula:

$$\text{Sodium content (\%)} = \frac{\text{Concentration (μg/mL) of sodium in the test solution}}{\text{Weight (g) of the sample} \times 4}$$

Operating Conditions

Light source: Sodium hollow cathode lamp.

Wavelength: 589.0 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(5) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(6) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Sodium Sulfate

硫酸ナトリウム

$Na_2SO_4 \cdot nH_2O$ (n = 10 or 0)　　Mol. Wt. decahydrate 322.19

anhydrous 142.04

Sodium sulfate decahydrate　[7727-73-3]

Sodium sulfate　[7757-82-6]

Definition　Sodium Sulfate occurs in two forms: the crystalline form (decahydrate) called Sodium Sulfate (crystal) and the anhydrous form called Sodium Sulfate (anhydrous).

Content　Sodium Sulfate, when dried, contains not less than 99.0% of sodium sulfate (Na_2SO_4).

Description　Sodium Sulfate (crystal) occurs as colorless crystals or as a white crystalline powder. Sodium Sulfate (anhydrous) occurs as a white powder.

Identification　Sodium Sulfate responds to all the tests for Sodium Salt and for Sulfate in the Qualitative Tests.

Purity　For Sodium Sulfate (crystal), dry the sample before performing the test.

(1) Clarity of solution　Colorless and almost clear (1.0 g, water 10 mL.)

(2) Chloride　Not more than 0.11% as Cl (0.10 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution　To the specified amount of Sodium Sulfate, add 20 mL of diluted hydrochloric acid (1 in 4), boil gently for 15 minutes with a watch glass covering it, and allow to cool.

(4) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying

Crystal: 51.0–57.0% (105°C, 4 hours).

Anhydrous: Not more than 5.0% (105°C, 4 hours).

Assay　Weigh accurately about 0.4 g of Sodium Sulfate, previously dried, dissolve it in 200 mL of water, add 1 mL of hydrochloric acid, boil, and add gradually 30 mL of a solution of barium chloride dihydrate (1 in 6). Heat this solution in a water bath for 1 hour, cool, and filter through a filter paper for quantitative analysis (5C). Wash the residue on the filter paper with warm water until the washings are free of chloride. Dry the residue with the filter paper, ignite to constant weight, and accurately weigh as Barium Sulfate ($BaSO_4$).

$$\text{Content (\%) of sodium sulfate } (Na_2SO_4) = \frac{\text{Weight (g) of } BaSO_4 \times 0.6086}{\text{Weight (g) of the sample}} \times 100$$

Sodium Sulfite

Soda Sulfite

亜硫酸ナトリウム

$Na_2SO_3 \cdot nH_2O$ (n = 7 or 0) Mol. Wt. heptahydrade 252.15

anhydrous 126.04

Disodium sulfite heptahydrate [10102-15-5]

Disodium sulfite [7757-83-7]

Definition Sodium Sulfite occurs in two forms: the crystalline form (heptahydrate) called Sodium Sulfite (crystal) and the anhydrous form called Sodium Sulfite (anhydrous).

Content Sodium Sulfite, when calculated on the anhydrous basis, contains not less than 95.0% of sodium sulfite (Na_2SO_3).

Description Sodium Sulfite occurs as colorless to white crystals or as a white powder.

Identification Sodium Sulfite responds to all the tests for Sodium Salt and for Sulfite in the Qualitative Tests.

Purity In the case of Sodium Sulfite (crystal), use two times the quantity of the sample specified for each of the purity tests below.

(1) Clarity of solution Colorless and almost clear (0.50 g, water 10 mL).

(2) Lead Not more than 5 μg/g as Pb (0.80 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Sodium Sulfite, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 5 minutes with a watch glass covering it. Allow it to cool, and use as the sample solution. If the sample does not dissolve completely, evaporate it to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Gently boil for 5 minutes, and allow to cool.

(3) Arsenic Not more than 3 μg/g as As (on the anhydrous basis) (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Sodium Sulfite in 5 mL of water. Add 1 mL of sulfuric acid, heat on a hot plate until white fumes are evolved, and add water to make 5 mL.

Assay Weigh accurately a quantity of Sodium Sulfite equivalent to about 0.25 g of Sodium Sulfite (anhydrous), proceed as directed under Sulfite Determination, and

calculate the content by the formula:

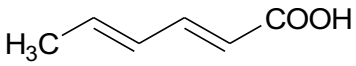
$$\text{Content (\%) of sodium sulfite } (Na_2SO_3) = \frac{a \times (50 - b)}{\text{Weight (g) of the sample} \times 10}$$

a = 12.61 (crystal) or 6.302 (anhydrous),

b = volume (mL) of 0.1 mol/L sodium thiosulfate consumed.

Sorbic Acid

ソルビン酸



$C_6H_8O_2$ Mol. Wt. 112.13

(2*E*,4*E*)-Hexa-2,4-dienoic acid [110-44-1]

Content Sorbic Acid, when calculated on the anhydrous basis, contains not less than 99.0% of sorbic acid ($C_6H_8O_2$).

Description Sorbic Acid occurs as colorless needles or as a white crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) To 1 mL of a solution (1 in 100) of Sorbic Acid in acetone, add 1 mL of water and 2 drops of bromine TS, and shake. The color of the solution immediately disappears.

(2) A solution (1 in 400,000) of Sorbic Acid in 2-propanol exhibits an absorption maximum at a wavelength of 252–256 nm.

Melting Point 132–135°C.

Purity

(1) Color of solution Weigh 0.20 g of Sorbic Acid, and dissolve it in 5.0 mL of acetone. The color of the solution is not darker than that of Matching Fluid C.

(2) Chloride Not more than 0.014% as Cl.

Sample Solution Weigh 1.50 g of Sorbic Acid, add 120 mL of water, and dissolve while boiling, and cool. Add water to make 120 mL, and filter. Use 40 mL of the filtrate as the sample solution.

Control Solution 0.20 mL of 0.01 mol/L hydrochloric acid.

(3) Sulfate Not more than 0.048% as SO_4.

Sample Solution 40 mL of the filtrate prepared in Purity (2).

Control Solution 0.50 mL of 0.005 mol/L sulfuric acid.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Water Content Not more than 0.50% (2 g, Volumetric Titration, Direct Titration).

Residue on Ignition Not more than 0.2%.

Assay Weigh accurately about 1 g of Sorbic Acid, and dissolve it in ethanol (neutralized) to make exactly 100 mL. Measure exactly 25 mL of this solution, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2–3 drops of phenolphthalein TS). Calculate on the anhydrous basis.

Each mL of 0.1 mol/L sodium hydroxide = 11.21 mg of $C_6H_8O_2$.

Sorbitan Esters of Fatty Acids

ソルビタン脂肪酸エステル

Definition Sorbitan Esters of Fatty Acids are esters of fatty acids and sorbitan.

Description Sorbitan Esters of Fatty Acids occur as white to yellow-brown powders, flakes, granules, or waxy lumps, or as white to yellow-brown liquids.

Identification

(1) Dissolve 0.5 g of the sample in 5 mL of ethanol (99.5) by heating, add 5 mL of diluted sulfuric acid (1 in 20), heat in a water bath for 30 minutes, and cool. Oil drops or a white to yellowish-white solid is deposited. Add 5 mL of diethyl ether to the separated oil drops or solid, and shake. It dissolves.

(2) Measure 2 mL of the liquid residue obtained when the oil drops or solid were removed in Identification (1), add 2 mL of freshly prepared 1,2-benzenediol solution (1 in 10), shake, add 5 mL of sulfuric acid, and shake. A red to red-brown color develops.

Purity

(1) Acid value Not more than 15 (Fats and Related Substances Tests).

(2) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

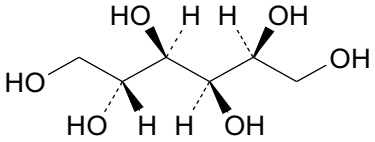
(4) Polyoxyethylene Weigh 1.0 g of the sample, dissolve it in 10 mL of dichloromethane, and add 20 mL of water. Warm, shake well, and cool. Add 10 mL of ammonium thiocyanate–cobalt(II) nitrate TS, and shake well. Centrifuge if necessary. The color of the dichloromethane layer does not change to blue.

Residue on Ignition Not more than 1.5%.

D-Sorbitol

Sorbitol
D-Sorbit

D-ソルビトール



$C_6H_{14}O_6$ Mol. Wt. 182.17

D-Glucitol [50-70-4]

Content D-Sorbitol, when dried, contains not less than 90.0% of D-sorbitol ($C_6H_{14}O_6$).

Description D-Sorbitol occurs as a white powder or as granules. It is odorless and has a cool, sweet taste.

Identification

(1) To 1 mL of a solution of D-Sorbitol (7 in 10), add 2 mL of iron(II) sulfate TS and 1 mL of sodium hydroxide solution (1 in 5). A blue-green color develops, but no turbidity appears.

(2) To 1 mL a solution of D-Sorbitol (1 in 100), add 1 mL of freshly prepared 1,2-benzenediol solution (1 in 10), shake well, add 2 mL of sulfuric acid, and shake. A red color develops immediately.

Purity

(1) Free acid Weigh 5 g of D-Sorbitol, dissolve it in 50 mL of freshly boiled and cooled water, add 1 drop of phenolphthalein TS and 0.5 mL of 0.01 mol/L sodium hydroxide, and shake. The color of the solution changes to pink that persists for not less than 30 seconds.

(2) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Nickel Weigh 0.50 g of D-Sorbitol, dissolve it in 5 mL of water, add 3 drops of a solution (1 in 100) of dimethylglyoxime in ethanol (95) and 3 drops of ammonia TS, and allow to stand for 5 minutes. The color of the solution does not change to pink.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Reducing sugars Not more than 0.68% as D-glucose.

Weigh 1.0 g of D-Sorbitol into a flask, and dissolve it in 25 mL of water. Add 40 mL of Fehling's TS, boil gently for 3 minutes, and allow to stand to precipitate cuprous oxide. After cooling, carefully filter the supernatant through a glass filter (1G4), leaving as much precipitate as possible in the flask. Discard the filtrate. Immediately add warm

water to the precipitate in the flask, wash, and carefully filter the washings through the glass filter, and discard the filtrate. Repeat the washing and filtering process until the washings are no longer alkaline. To the precipitate in the flask, immediately add 20 mL of iron(III) sulfate TS to dissolve, and filter through the glass filter. Wash the flask and the glass filter with water, and combine the washings with the filtrate. Heat the solution obtained to 80°C, and add 2.0 mL of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear immediately.

(6) Saccharide Not more than 4.4% as D-glucose.

Weigh 10 g of D-Sorbitol, dissolve it in 25 mL of water, add 8 mL of diluted hydrochloric acid (1 in 4), and heat under a reflux condenser in a water bath for 3 hours. Cool, and neutralize with sodium hydroxide solution (1 in 25), using 1 drop of methyl orange TS as the indicator. Add water to make 100 mL. Measure 10 mL of this solution, add 10 mL of water and 40 mL of Fehling's TS, boil gently for 3 minutes, and proceed as directed under Purity (5), using 13 mL of 0.02 mol/L potassium permanganate.

Loss on Drying Not more than 3.0% (not more than 0.7 kPa, 80°C, 3 hours).

Residue on Ignition Not more than 0.02% (5 g).

Assay Weigh accurately about 1 g each of D-Sorbitol and D-sorbitol for assay, previously dried, separately dissolve them in water to make exactly 50 mL of each. Use these solutions as the test solution and standard solution, respectively. Analyze 10 μL each of the test solution and the standard solution by liquid chromatography using the conditions given below. Measure the peak areas (A_T and A_S) of the D-sorbitol for the test solution and the standard solution, and determine the content by the formula:

$$\text{Content (\%) of D-sorbitol } (C_6H_{14}O_6) = \frac{\text{Weight (g) of D-sorbitol for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (4–8 mm internal diameter and 20–50 cm length).

Column packing material: 5- to-12-μm strongly acidic cation exchange resin for liquid chromatography.

Column temperature: A constant temperature of 40–85°C.

Mobile phase: Water.

Flow rate: A constant rate of 0.5–1.0 mL/min.

D-Sorbitol Syrup

D-Sorbit Syrup

D-ソルビトール液

Content D-Sorbitol Syrup contains not less than 50.0–75.0% of D-sorbitol ($C_6H_{14}O_6$ = 182.17).

Description D-Sorbitol Syrup is a colorless, clear, syrupy liquid. Colorless crystals may be deposited while cool. It is odorless and has a sweet taste.

Identification Proceed as directed in Identification (1) and (2) for D-Sorbitol.

Specific Gravity d_{25}^{25}: 1.285–1.315.

Purity

(1) Free Acid Proceed as directed in Purity (1) for D-Sorbitol.

(2) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Nickel Proceed as directed in Purity (3) for D-Sorbitol.

(4) Arsenic Not more than 3 μg/g As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Reducing sugars Not more than 0.68% as D-glucose.

Proceed as directed in Purity (5) for D-Sorbitol.

(6) Saccharide Not more than 6.8% as D-glucose.

Proceed as directed in Purity (6) for D-Sorbitol, using 20 mL of 0.02 mol/L potassium permanganate.

Residue on Ignition Not more than 0.02%.

Weigh accurately about 5 g of D-Sorbitol Syrup, add 2–3 drops of sulfuric acid, and heat gently to boil. Set it fire, incinerate, and cool. Using the substance obtained, proceed as directed under Residue on Ignition.

Assay Weigh accurately about 1 g of D-Sorbitol Syrup, and proceed as directed in the Assay for D-Sorbitol.

Spirulina Color

スピルリナ色素

Definition Spirulina Color is obtained from the entire part of the alga *Arthrospira platensis* (*Spirulina platensis*) and consists mainly of phycocyanin. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Spirulina Color is not less than 25 and in the range of 90–110% of the labeled value.

Description Spirulina is a blue powder or liquid having a slight characteristic odor.

Identification

(1) Weigh an amount of Spirulina Color equivalent to 0.4 g of spirulina color with a Color Value 25, and dissolve it in 100 mL of citrate buffer (pH 6.0). The solution is blue and emits red fluorescence.

(2) Heat a small amount of the solution obtained in Identification (1) at 90°C for 30 minutes. The fluorescence disappears.

(3) To 5 mL of the solution obtained in Identification (1), add 3.3 g of powdered ammonium sulfate in small portions to dissolve, and allow to stand. A blue precipitate is produced.

(4) To 5 mL of the solution obtained in Identification (1), add 1 mL of iron (III) chloride TS, and allow to stand for 20 minutes. The solution turns blue-green to dark purple.

(5) To 5 mL of the solution obtained in Identification (1), add 0.1 mL of sodium hypochlorite TS. The solution turns light yellow.

(6) A solution of Spirulina Color in citrate buffer (pH 6.0) exhibits an absorption maximum at a wavelength of 610–630 nm.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination, using the following operating conditions:

Operating Conditions

Solvent: Citrate buffer (pH 6.0).

Wavelength: Maximum absorption wavelength of 610–630 nm.

Starch Acetate

酢酸デンプン

[9045-28-7]

Definition Starch Acetate is obtained by esterifying starch with acetic anhydride or vinyl acetate.

Description Starch Acetate occurs as a white to off-white powder, or as flakes or granules. It has a faint odor.

Identification

(1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

(3) Proceed as directed in Identification (3) for Acetylated Distarch Adipate.

Purity

(1) Acetyl groups Not more than 2.5%.Proceed as directed in Purity (2) for Acetylated Distarch Adipate.

(2) Vinyl acetate Not more than 0.1 μg/g. This specification does not apply to pregelatinized starch. Proceed as directed in Purity (2) for Acetylated Distarch Phosphate.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (not more than 13.3 kPa, 120°C, 4 hours).

Starch Sodium Octenyl Succinate

オクテニルコハク酸デンプンナトリウム

Definition Starch Sodium Octenyl Succinate is obtained by esterifying starch with octenyl succinic anhydride.

Description Starch Sodium Octenyl Succinate occurs as a white to off-white powder, or as flakes or granules. It has a faint odor.

Identification

(1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

Purity

(1) Residual octenyl succinic acid Not more than 0.8%.

Test Solution Weigh accurately about 0.1 g of Starch Sodium Octenyl Succinate, and add 20 mL of methanol, and shake for 18 hours or more. Centrifuge the mixture at about 3000 rpm for 5 minutes, measure exactly 10 mL of the supernatant, and evaporate to dryness under vacuum at 40°C. Dissolve the residue by adding water and make

exactly 5 mL.

Standard Solutions Weigh accurately about 20 mg of octenyl succinic anhydride, add 10 mL of potassium hydroxide (7 in 1250), heat at 80°C for 3 hours. After cooling, add 8 mL of diluted phosphoric acid (1 in 200), and dilute this solution to exactly 20 mL with water. Measure exactly 2 mL of this solution, and add water to make exactly 20 mL. Place exactly 1 mL, 2 mL, 5 mL, and 10 mL of the resulting solution into four separate 20-mL volumetric flasks, and dilute each to volume with water.

Procedure Analyze 20 μL each of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Measure the sum of the peak areas of two main peaks for each standard solution, and prepare a calibration curve for octenyl succinic anhydride from the sums obtained and the concentrations of octenyl succinic anhydride in the standard solutions. Measure the sum of the peak areas of two main peaks for the test solution. Determine the concentration of octenyl succinic anhydride (μg/mL) in the test solution from the calibration curve, and calculate residual octenyl succinic acid in the sample product by the following formula:

$$\text{Content (\%) of residual octenyl succinic acid } (C_{12}H_{20}O_4) = \frac{\text{Octenyl succinic anhydride concentration (μg/mL) in the test solution} \times 1.086}{\text{Dry basis weight (g) of the sample} \times 1000}$$

Operating conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 205 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Packing material: 5 μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 1:1 mixture of diluted phosphoric acid (1 in 1000)/acetonitrile.

Flow rate: Adjust the retention time of the main peak to about 9 minutes.

(2) Octenyl succinic groups Not more than 3.0%.

Test Solution Weigh accurately about 20 mg of Starch Sodium Octenyl Succinate, dissolve it in 10 mL of potassium hydroxide (7 in 1250), stopper, and heat at 80°C for 3 hours. After cooling, add 8 mL of diluted phosphoric acid (1 in 200), dilute this solution to exactly 20 mL with water.

Procedure Analyze the test solution by liquid chromatography using operating conditions directed in Purity (1). Measure the sum of the peak areas of two main peaks of the test solution, and determine the concentration of octenyl succinic anhydride (μg/mL) in the test solution from the calibration curve prepared in Purity (1). Calculate the content (%) of the total octenyl succinic acid in the sample product using the following formula, and determine the content (%) of octenyl succinic groups.

Content (%) of total octenyl succinic acid ($C_{12}H_{20}O_4$)

$$= \frac{\text{Octenyl succinic anhydride concentration (μg/mL) in the test solution} \times 1.086}{\text{Dry basis weight (g) of the sample} \times 500}$$

Content (%) of octenyl succinic groups

$$= \left[\begin{array}{c}\text{Content (\%) of} \\ \text{total octenyl succinic acid}\end{array}\right] - \left[\begin{array}{c}\text{Content (\%) of} \\ \text{residual octenyl succinic acid}\end{array}\right]$$

(3) Lead Not more than 2 μg/g as Pb (2 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Atomic Absorption Spectrophotometry).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (not more than 13.3 kPa, 120°C, 4 hours).

Stevia Extract

ステビア抽出物

Definition Stevia Extract is obtained by extraction from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni and consists mainly of steviol glycosides.

Content Stevia Extract, when calculated on the dried basis, contains not less than 80.0% of the total of the four steviol glycosides (stevioside, rebaudioside A, rebaudioside C and dulcoside A).

Description Stevia Extract occurs as a white to light yellow powder, or as flakes or granules. It is odorless or has a slight characteristic odor. It has a strong sweet taste.

Identification Analyze the test solution and the standard solutions prepared for the assay by liquid chromatography using the operating conditions given in the Assay. The retention time of main peak of the test solution corresponds to that of either of the stevioside peak and the rebaudioside A peak from the standard solutions.

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1 μg/g as As (1.5 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 6.0% (105°C, 2 hours).

Residue on Ignition Not more than 1.0%.

Assay

Test Solution Dissolve 50 mg of Stevia Extract, weighed accurately, in a 7 : 3 mixture of acetonitrile/water to make exactly 100 mL.

Standard Solutions Weigh accurately about 50 mg each of stevioside for assay and rebaudioside A for assay, previously dried, and dissolve them separately in a 7 : 3 mixture of acetonitrile/water to make exactly 100 mL of each.

Procedure Analyze 10 μL each of the test solution, the standard solutions, and the mixture of four steviol glycosides by liquid chromatography using the operating conditions given below. Measure the peak areas of stevioside and rebaudioside A from the standard solutions and express as A_{s1} and A_{s2}, respectively. Measure the peak area of each of stevioside, rebaudioside A, rebaudioside C, and dulcoside A from the test solution and express A_x. Determine the total content of the four steviol glycosides by the following formula. Identify each peak from the test solution by comparing it with the retention time of each steviol glycoside from the mixture of four steviol glycosides. The factor (f_x), the ratio of the formula weight of each to the formula weight of stevioside, is 1.00 for steviside, 1.18 for rebaudioside C, and 0.98 for dulcoside A.

$$\text{The content (\%) of each steviol glycoside (except rebaudioside A)} = \frac{\text{Weight (mg) of stevioside for assay}}{\text{Dry basis weight (mg) of the sample}} \times \frac{A_x \times f_x}{A_{s1}} \times 100$$

$$\text{The content (\%) of rebaudioside A} = \frac{\text{Weight (mg) of rebaudioside A for assay}}{\text{Dry basis weight (mg) of the sample}} \times \frac{A_x}{A_{s2}} \times 100$$

$$\text{The total content (\%) of the four steviol glycosides} = \left[\begin{matrix}\text{Contents (\%)}\\ \text{of stevioside}\end{matrix}\right] + \left[\begin{matrix}\text{Contents (\%) of}\\ \text{rebaudioside A}\end{matrix}\right] + \left[\begin{matrix}\text{Contents (\%) of}\\ \text{rebaudioside C}\end{matrix}\right] + \left[\begin{matrix}\text{Contents (\%) of}\\ \text{dulcoside A}\end{matrix}\right]$$

Operating Conditions

Detector: Ultraviolet absorption spectrophotometer (wavelength: 210 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 25 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 17:8 mixture of phosphate buffer (0.01 mol/L, pH 2.6)/acetonitrile.

Flow rate: 1.0 mL/minute.

Column selection: Use a column that is capable of well separating stevioside and rebaudioside A when a 1:1 mixture of the stevioside for assay standard solution and rebaudioside A for assay standard solution is chromatographed according to the above operating conditions.

Steviol Glycosides

ステビオール配糖体

Definition Steviol Glycosides are obtained by extraction from the leaves of *Stevia rebaudiana* (Bertoni) Bertoni and consist mainly of steviol glycosides.

Content Steviol Glycosides, when calculated on the dried basis, contain not less than 80.0% of the total of the four steviol glycosides (stevioside, rebaudioside A, rebaudioside C and dulcoside A), and not less than 95.0% of the total of the nine steviol glycosides (stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside, and steviolbioside).

Description Steviol Glycosides occur as white powders, flakes, or crystals. They are odorless or have a slight characteristic odor. They have a strong sweet taste.

Identification Proceed as directed in Identification for Stevia Extract.

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1 μg/g as As (1.5 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 6.0% (105°C, 2 hours).

Residue on Ignition Not more than 1.0%.

Assay

Test Solution Weigh accurately 50 mg of the sample, dissolve it in a 7 : 3 mixture of acetonitrile/water to make exactly 100 mL.

Standard Solutions Weigh accurately about 50 mg each of stevioside for assay and rebaudioside A for assay, previously dried, and dissolve them separately in a 7 : 3 mixture of acetonitrile/water to make exactly 100 mL of each.

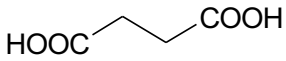
Procedure Analyze 10 μL each of the test solution, the standard solutions, and the mixture of nine steviol glycosides by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract. Measure the peak areas of stevioside and rebaudioside A from the standard solutions and express as A_{s1} and A_{s2}, respectively. Measure the peak area of each of the nine steviol glycosides and express A_x. Determine the total content (%) of the four steviol glycosides by proceeding as directed in the Assay for Stevia Extract, and then determine the total content (%) of the nine steviol glycosides by the following formula. Identify each peak of the test solution by

comparing it with the retention time of each steviol glycoside in the mixture of nine steviol glycosides. The factor (f_x), the ratio of the formula weight of each to the formula weight of stevioside, is 1.00 for rebaudioside B, 1.40 for rebaudioside D, 1.16 for rebaudioside F, and 0.80 for rubusoside and steviolbioside. For rebaudioside C and dulcoside A, use the ratios given in Assay for Stevia Extract.

The total content (%) of the nine steviol glycosides
= sum of the contents (%) of the nine steviol glycosides

Succinic Acid

コハク酸



$C_4H_6O_4$ Mol. Wt. 118.09

Butanedioic acid [110-15-6]

Content Succinic Acid contains not less than 99.0% of succinic acid ($C_4H_6O_4$).

Description Succinic Acid occurs as colorless to white crystals or as a white crystalline powder. It is odorless and has a characteristic acid taste.

Identification Adjust the pH of a solution of 5 mL of Succinic Acid (1 in 20) to about 7 with ammonia TS. Add 2–3 drops of a solution of iron(III) chloride hexahydrate (1 in 10). A brown precipitate is formed.

Melting Point 185–190°C.

Purity

(1) Lead Not more than 2 μg/g as Pb (5.0 g, Method 1, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Readily oxidizable substances Weigh 1.0 g of Succinic Acid, dissolve it in 25 mL of water and 25 mL of diluted sulfuric acid (1 in 20), and add 4.0 mL of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 3 minutes.

Residue on Ignition Not more than 0.025% (5 g).

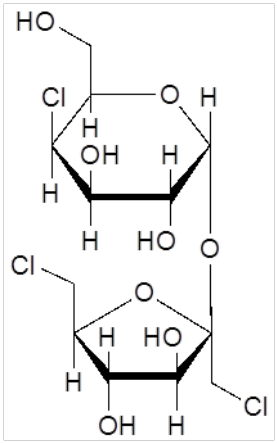
Assay Weigh accurately about 1 g of Succinic Acid, and dissolve it in water to make exactly 250 mL. Measure exactly 25 mL of this solution, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2–3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide = 5.904 mg of $C_4H_6O_4$

Sucralose

Trichlorogalactosucrose

スクラロース



$C_{12}H_{19}Cl_3O_8$ Mol. Wt. 397.63

1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside

[56038-13-2]

Content Sucralose, when calculated on the anhydrous basis, contains 98.0–102.0% of Sucralose ($C_{12}H_{19}Cl_3O_8$).

Description Sucralose occurs as a white to light grayish white crystalline powder. It is odorless and has a sweet taste.

Identification Determine the absorption spectrum of Sucralose as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum of Sucralose. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Specific Rotation $[\alpha]_D^{20}$: +84.0 to +87.5° (1.0 g, water 10 mL, on the anhydrous basis).

Purity

(1) Lead Not more than 1 μg/g as Pb (10.0 g, Method 1, Control Solution: Lead Standard Solution 10.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (1.0 g, Control Solution: Arsenic Standard Solution 6.0 mL, Apparatus C).

Test Solution Weigh the specified amount of Sucralose into a platinum, quartz, or porcelain crucible. Add 10 mL of a solution (1 in 10) of magnesium nitrate hexahydrate in ethanol (95), and ignite and burn the ethanol. Heat gradually, and incinerate at 450–550°C. If carbonized matter still remains, moisten it with a small quantity of a solution (1 in 50) of magnesium nitrate hexahydrate in ethanol (95), heat again, and incinerate at 450–550°C. After cooling, add 3 mL of hydrochloric acid to the residue,

dissolve it by heating on a water bath, and make up to exactly 10 mL with water.

Control Solution To the specified amount of Arsenic Standard Solution, add 3 mL of hydrochloric acid, and make up to exactly 10 mL with water.

(3) Other chlorinated disaccharides Not more than 0.5%.

Test Solution Dissolve 1.0 g of Sucralose in 10 mL of methanol.

Control Solution Measure 0.5 mL of the test solution, and add methanol to make 100 mL.

Procedure Analyze 5 μL each of the test solution and the control solution by thin-layer chromatography using a 7:3 mixture of sodium chloride solution (1 in 20)/acetonitrile as the developing solvent. Use a thin-layer plate that has been coated with octadecylsilanized silica gel for thin-layer chromatography as the solid support and then dried at 110°C for one hour. Stop the development when the developing solvent has ascended to a point 15 cm above the starting line, and air-dry to remove the solvent. Spray with 15% sulfuric acid–methanol TS, and heat at 125°C for 10 minutes. The main spot from the test solution is at the same position as the spot from the control solution and any other single spot from the test solution is not more intense than that in the control solution.

(4) Chlorinated monosaccharides Not more than 0.16% as D(–)-fructose.

Test solution Weigh 2.5 g of Sucralose, and add methanol to make exactly 10 mL.

Control Solution A Weigh 10.0 g of D(–)-mannitol, and add water to make exactly 100 mL.

Control Solution B Weigh exactly 10.0 g of D(–)-mannitol and 40 mg of D(–)-fructose, and add water to make exactly 100 mL.

Procedure Apply 1 μL each of the test solution and Control Solutions A and B onto a thin-layer chromatographic plate coated with a 0.25-mm thick layer of silica gel, and air-dry. Repeat this procedure four more times. Spray the plate with *p*-anisidine–phthalic acid TS, and heat at 98–102°C for about 10 minutes to fix the color. The spot from the test solution is not more intense than the spot from Control Solution B. If any spot is observed in Control Solution A, prepare a second plate, and repeat the procedure once more.

(5) Triphenylphosphine oxide Not more than 0.015%.

Test Solution Weigh accurately about 0.1 g of Sucralose, dissolve it in a 67:33 mixture of acetonitrile/water to make exactly 10 mL.

Standard Solution Weigh 0.100 g of triphenylphosphine oxide, dissolve it in a 67:33 mixture of acetonitrile/water to make exactly 10 mL. Measure exactly 1 mL of the resulting solution, and add a 67:33 mixture of acetonitrile/water to make exactly 100 mL. Then measure exactly 1 mL of the resulting solution, and add a 67:33 mixture of acetonitrile/water to make exactly 100 mL.

Procedure Analyze 25 μL each of the test solution and the standard solution by liquid chromatography using the conditions given below. Measure the peak areas (As

and A_T) of triphenylphosphine oxide (TPPO) for the test solution and the standard solution, and calculate the content of TPPO in Sucralose from the formula:

$$\text{Amount (\%) of TPPO } (C_{18}H_{15}OP) = \frac{1}{\text{Weight (g) of the sample} \times 1000} \times \frac{A_T}{A_S}$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 220 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 15 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 67:33 mixture of acetonitrile/water.

Flow rate: 1.5 mL/min.

(6) Methanol Not more than 0.10%.

Test solution Weigh accurately about 2 g of Sucralose, add water to make exactly 10 mL, and mix.

Control Solution Measure 2.0 g of methanol, add water to make exactly 100 mL, and mix. Measure exactly 1 mL of this solution, add water to make exactly 100 mL, and mix.

Procedure Analyze 1 μL each of the test solution and the control solution by gas chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of methanol for the test solution and the control solution, and calculate the content of methanol by the formula:

$$\text{Amount (\%) of methanol} = \frac{2.0}{\text{Weight (g)} \times 1000} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube about (2–4 mm internal diameter and 2 m length).

Column packing material: 150- to 180-μm styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of 140–160°C.

Injection port temperature: 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the peak of methanol appears about 4 minutes after injection.

Residue on Ignition Not more than 0.7%.

Water Content Not more than 2.0% (1 g, Volumetric Titration, Direct Titration).

Assay Weigh accurately about 1g of Sucralose, and dissolve it in water to make exactly 100 mL. Measure exactly 10 mL of this solution, add 10 mL of sodium hydroxide solution (1 in 10), and gently heat under reflex condenser for 30 minutes. Cool, neutralize with 10% nitric acid TS, and titrate with 0.1 mol/L silver nitrate. The endpoint is confirmed using a silver electrode as the indicator electrode and a silver–silver chloride electrode as the reference electrode. Perform a blank test in the same manner, and make any necessary correction and calculate on the dried basis.

Each mL of 0.1 mol/L silver nitrate = 13.25 mg of $C_{12}H_{19}Cl_3O_8$

Sucrose Esters of Fatty Acids

ショ糖脂肪酸エステル

Definition Sucrose Esters of Fatty Acids are categorized into two types: esters of sucrose with fatty acids and sucrose acetate isobutyrate.

Description Sucrose Esters of Fatty Acids occur as white to yellow-brown powders or lumps, or as colorless to red-brown, viscous resins or liquids. They are odorless or have a slight, characteristic odor.

Identification

(1) To 1 g of the sample, add 25 mL of 3.5% (w/v) potassium hydroxide–ethanol TS, and heat under a reflux condenser on a water bath for 1 hour. Add 50 mL of water to the solution, and distill until about 30 mL of liquid remains in the flask. After cooling, add 10 mL of diluted hydrochloric acid (1 in 4) to the residual liquid, shake well, add sodium chloride to make a saturated solution, and extract twice with 30 mL of diethyl ether each time. Collect and combine the diethyl ether layers. Wash the diethyl layer with 20 mL of sodium chloride saturated solution, dehydrate with 2 g of sodium sulfate, and evaporate the diethyl ether. Completely remove the diethyl ether by letting in air, and cool the residue to 10°C. In the case of esters of sucrose with fatty acids, an oil drop or colorless to light yellow-brown solid is formed. In the case of sucrose acetate isobutyrate, a liquid with odors of acetic acid and isobutyric acid remains.

(2) In a test tube, place 2 mL of the aqueous layer that has remained after the diethyl ether layer is removed in Identification (1), warm in a water bath until the odor of diethyl ether disappears, and cool. Add 1 mL of anthrone TS gently down the inside of the test tube. The boundary surface of the two layers turns blue to green.

Purity

(1) Acid value Not more than 6.0.

Test Solution Weigh accurately about 3 g of the sample, and dissolve it in 60 mL of a 2:1 mixture of 2-propanol/water.

Procedure Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests.

(2) Lead Not more than 2.0 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Free sucrose Not more than 5.0%.

Test Solution Weigh accurately about 40 mg of the sample into a centrifuge tube, add 1 mL of the internal standard, 1 mL of *N*-*N*-dimethylformamide, 0.4 mL of *N*-*O* bis(trimethylsilyl)acetamide, and 0.2 mL of trimethylchlorosilane, shake vigorously, and allow to stand for 5 minutes at room temperature.

Internal Standard Place 0.25 g octacosane in a 50-mL volumetric flask, dissolve it in 25 mL of tetrahydrofuran, and add tetrahydrofuran to make exactly 50 mL.

Standard Solutions Weigh accurately about 50 mg of sucrose, and add *N*-*N*-dimethylformamide to make exactly 10 mL. Take 1 mL, 2 mL, and 5 mL of this solution, add *N*-*N*-dimethylformamide to each to make exactly 10 mL of solutions. To 1 mL of each solution, add 1 mL of the internal standard, and proceed as directed for the test solution to prepare silylated sucrose standard solutions.

Procedure Analyze 1 μL each of the test solution and the standard solutions by gas chromatography using the operating conditions given below. Measure the peak area of sucrose in the test solution to determine the amount of free sucrose by the Internal Standard Method from the calibration curve. Calculate the content by the formula:

$$\text{Content (\%) of free sucrose} = \frac{\text{Amount (mg) of free sucrose in the test solution}}{\text{Weight (mg) of the sample}} \times 100$$

*Operating Condition*s

Detector: Flame ionization detector.

Column: A fused silica tube (0.32 mm internal diameter and 30 m length) coated by a 0.25-μm thick layer of dimethylpolysiloxane.

Column temperature: Maintain the temperature at 100°C for 1 minute, raise at a rate of 12°C/minute to 300°C, and maintain at 300°C for 45 minutes.

Injection port temperature: 280°C.

Carrier gas: Helium.

Flow rate: Adjust so that the peak of a sucrose derivative appears about 19 minutes after injection.

Injection method: Splitless.

(5) Dimethyl sulfoxide Not more than 2.0 μg/g as dimethyl sulfoxide.

This specification does not apply to sucrose acetate isobutyrate.

Test Solution Weigh accurately about 5 g of the sample, dissolve it in tetrahydrofuran to make exactly 25 mL.

Standard Solutions Weigh accurately about 0.1 g of dimethyl sulfoxide, and dissolve it in tetrahydrofuran to make exactly 100 mL. Measure exactly 1 mL of this solution, and add tetrahydrofuran to prepare a standard stock solution of exactly 100 mL. Transfer exactly 0.5 mL, 1 mL, 2 mL, and 5 mL of the standard stock solution into separate 50-mL volumetric flasks, and dilute each with tetrahydrofuran to volume.

Procedure Analyze 3 μL each of the test solution and the standard solutions by gas chromatography using the conditions below. Measure the peak heights or peak areas of dimethyl sulfoxide for the standard solutions, and prepare a calibration curve on a logarithmic paper. Measure the peak height or peak area of dimethylformamide for the test solution, and determine its amount from the calibration curve.

Operating Conditions

Detector: Flame photometric detector (with sulfur filter).

Column: A glass tube (3 mm internal diameter and 2 m length).

Column packing material

Liquid phase: 10% Polyethylene glycol 20M of the amount of support and 3% potassium hydroxide of the amount of support.

Support: 180- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of 150–170°C.

Injection port temperature: 210°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the peak of dimethyl sulfoxide appears about 3 minutes after injection.

(6) Dimethyl formamide Not more than 1.0 μg/g as *N*-*N*-dimethyl formamide.

Test Solution Weigh accurately about 2 g of the sample, and dissolve it in tetrahydrofuran to make exactly 20 mL.

Standard Solutions Weigh accurately about 0.1 g of *N*-*N*-dimethyl formamide, and dissolve it in tetrahydrofuran to make exactly 100 mL. Measure exactly 1 mL of this solution, and add tetrahydrofuran to prepare a standard stock solution of exactly 100 mL. Transfer exactly 0.5 mL, 1 mL, and 2 mL of the standard stock solution into separate 100-mL volumetric flasks, and dilute each with tetrahydrofuran to volume.

Procedure Analyze 1 μL each of the test solution and the standard solutions by gas chromatography using the conditions below. Measure the peak areas for the standard solutions, and prepare a calibration curve. Measure the peak area of *N*-*N*-dimethyl formamide in the test solution, and determine its amount from the calibration curve.

Operating Conditions

Detector: Nitrogen phosphorous detector.

Column: A fused silica tube (0.32 mm internal diameter and 30 m length) coated with a 0.5-μm thick layer of polyethylene glycol.

Column temperature: Maintain the temperature at 40°C for 2 minutes, raise at a rate of 20°C/minute to 160°C, and then maintain at 160°C for 2 minutes.

Injection port temperature: 180°C.

Carrier gas: Helium.

Flow rate: Adjust so that the peak of *N*-*N*-dimethyl formamide appears about 6 minutes after injection.

Injection method: Splitless.

(7) Other solvents

The specifications given below do not apply to sucrose acetate isobutyrate.

2-Butanone Not more than 10 μg/g.

Ethyl acetate, 2-propanol, and propylene glycol Not more than 0.035% as the sum of these three solvents.

Methanol Not more than 10 μg/g.

2-Methyl-1-propanol Not more than 10 μg/g.

(i) 2-Butanone, ethyl acetate, 2-propanol, methanol, and 2-methyl-1-propanol

Standard Solutions Weigh accurately about 0.2 g each of 2-butanone, ethyl acetate, 2-propanol, methanol, and 2-methyl-1-propanol in a volumetric flask, mix them, and dissolve the mixture in water to make exactly 50 mL. Refer to this solution as Standard Solution A. Transfer exactly 5 mL and 10 mL of Standard Solution A into separate 20-mL volumetric flasks, and dilute each with water to volume. Refer to the solutions obtained as Standard Solutions B and C, respectively.

Test Solution Weigh 1.00 g of the sample into a vial, and add exactly 5 μL of water.

Standard Additions Test Solutions Prepare three vials containing 1.00 g each of the sample. To each vial, add a 5 μL-potion of Solutions A, B, and C, respectively, to prepare standard additions test solutions.

Procedure Determine the solvent contents by headspace gas chromatography for the test solution and the standard additions test solutions under the conditions below.

Measure the peak area of each solvent for each solution. Prepare a linear regression by plotting the values obtained on a graph, with the added amount of each solvent on the abscissa and the peak area on the ordinate. Determine the amount of each solvent in the sample from the distance between the coordinate origin and the intersection point of the regression line and the abscissa.

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.53 mm internal diameter and 30 m length) coated with a 1.5-μm thick layer of dimethyl polysiloxane.

Column temperature: 40°C.

Injection port temperature: 110°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the peak of 2-methyl-1-propanol appears about 5 minutes after equilibrium injection.

Injection method: Splitless.

Headspace sampler

Equilibrium temperature in the vial: 80°C.

Equilibrium time in the vital: 40 minutes.

Amount of injection: 1.0 mL.

(ii) Propylene glycol

Test Solution Weigh accurately about 1 g of the sample, add 0.1 mL of the internal standard, and dissolve the mixture in pyridine to make exactly 10 mL. Measure exactly 0.5 mL of this solution, add 0.25 mL of 1,1,1,3,3,3-hexamethyldisilazane and 0.1 mL of trimethylchlorosilane, and shake vigorously. Allow it to stand for 30 minutes at room temperature, and centrifuge. Use the supernatant as the test solution.

Internal standard To 25 mg of ethylene glycol, add pyridine to make exactly 50 mL.

Standard Solutions Weigh accurately about 25 mg of propylene glycol, and add pyridine to make exactly 50 mL. Transfer exactly 40 μL, 0.2 mL. 0.5 mL, and 1 mL of this solution into separate 10-mL volumetric flasks, add 0.1 mL of the internal standard to each, and dilute with pyridine to volume. Using these solutions, proceed in the same manner as the preparation of the test solution.

Procedure Analyze 1 μL each of the test solution and the standards solutions by gas chromatography using the following operating conditions. Prepare a calibration curve, and determine the amount of propylene glycol by the internal standard method.

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.32 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of dimethyl polysiloxane.

Column temperature: Maintain the temperature at 60°C for 5 minutes, raise at a rate of 20°C/minute to 250°C, and maintain at 250°C for 5 minutes.

Injection port temperature: 230°C.

Carrier gas: Helium.

Flow rate: Adjust so that the peak of a propylene glycol derivative appears about 8 minutes after injection.

Injection method: Splitless.

Water Content Not more than 4.0% (0.5 g, Volumetric Titration, Back Titration).

Residue on Ignition Not more than 2.0%.

Sulfuric Acid

硫酸

H_2SO_4 Mol. Wt. 98.08

Sulfuric acid [7664-93-9]

Content Sulfuric Acid contains not less than 94.0% of sulfuric acid (H_2SO_4).

Description Sulfuric Acid is a colorless or slightly brownish, clear or almost clear, viscous liquid.

Identification

(1) A diluted solution of Sulfuric Acid (1 in 100) is strongly acidic.

(2) A diluted solution of Sulfuric Acid (1 in 100) responds to all the tests for Sulfate in the Qualitative Tests.

Purity

(1) Chloride Not more than 0.005% as Cl (2.0 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(2) Nitrate Not more than 10 μg/g as NO_3.

Add 5 g of Sulfuric Acid gradually to 8 mL of water, add 1 mL of a solution (1 in 500) of brucine *n*-hydrate in sulfuric acid and sulfuric acid to make 25 mL, shake well, and warm at about 80°C for 10 minutes. The color of the solution is not darker than that of the solution prepared as follows: To 0.50 mL of Nitrate Standard Solution, add 8 mL of water, and then add 5 mL of sulfuric acid gradually. Add 1 mL of a solution (1 in 500) of brucine *n*-hydrate in sulfuric acid and sulfuric acid to make 25 mL. Shake well, and warm at about 80°C for 10 minutes.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Test Solution Heat the specified amount of Sulfuric Acid, weighed exactly, until white fumes are no longer evolved. To the residue, add 10 mL of diluted hydrochloric acid (1 in 4), and evaporate to dryness. Warm the residue with a little amount of diluted nitric acid (1 in 100), and allow to cool. Make up to exactly 10 mL with diluted nitric acid (1 in 100).

Control Solution Measure exactly the specified amount of Lead Standard Solution, and make up to exactly 10 mL with diluted nitric acid (1 in 100).

(4) Iron Not more than 0.010% as Fe (0.10 g, Method 2, Control Solution: Iron

Standard Solution 1.0 mL).

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(6) Readily oxidizable substances Not more than 40 μg/g as SO_2.

Add 8 g of Sulfuric Acid to 10 mL of cold water while cooling, and add 0.10 mL of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 5 minutes.

Residue on Ignition Not more than 0.02% (10 g).

Assay To 50 mL of water, add about 2 g of Sulfuric Acid, weighed accurately, cool, and add water to make exactly 100 mL. Measure exactly 25 mL of this solution, and titrate with 0.5 mol/L sodium hydroxide (indicator: 1–2 drops of bromothymol blue TS).

Each mL of 0.5 mol/L sodium hydroxide = 24.52 mg of H_2SO_4

Talc

タルク

Definition Talc is natural, hydrated magnesium silicate that is carefully selected. It occasionally contains a small amount of aluminum silicate.

Description Talc occurs as a white to grayish white, fine crystalline powder. It has a smooth feel and is odorless.

Identification Mix 0.2 g of Talc, 0.9 g of sodium carbonate, and 1.3 g of potassium carbonate, transfer the mixture into a platinum or nickel crucible, and fuse completely by heating. After cooling, transfer the contents into a beaker with about 5 mL of hot water, add hydrochloric acid until effervescence no longer occurs, add another 10 mL of hydrochloric acid, and evaporate to dryness on a water bath. After cooling, add 20 mL of water, boil, and filter. A gelatinous substance remains, and the filtrate responds to the test for Magnesium Salt.

pH 7.5–9.5.

Test Solution Weigh 10.0 g of Talc, add 100 mL of water, heat for 2 hours on a water bath with occasional shaking while replenishing the lost water, and cool. Filter with suction using a filter holder (47 mm diameter) equipped with a membrane filter (0.45 μm pore size). If the filtrate is turbid, repeat the filtration with suction through the same filter. Wash the container and the residue on the filter with water, combine the washings with the filtrates, and add water to make 100 mL.

Purity

(1) Water-soluble substances Not more than 0.20%.

Evaporate 50 mL of the test solution for the pH test to dryness, dry the residue at 105°C for 2 hours, and weigh.

(2) Hydrochloric acid-soluble substances　Not more than 2.0%.

Weigh 1.0 g of Talc, add 20 mL of diluted hydrochloric acid (1 in 4), warm for 15 minutes at 50°C while shaking, cool, and filter. Wash the container and the residue on the filter paper with a small amount of water, combine the washings with the filtrate, and add water to make 20 mL. Measure 10 mL of this solution, add 1 mL of diluted sulfuric acid (1 in 20), evaporate to dryness, and ignite at 550°C to constant weight and weigh the residue.

(3) Water-soluble iron　Make 20 mL of the test solution for the pH test weakly acidic with hydrochloric acid, and add 1 drop of a freshly prepared solution of potassium hexacyanoferrate(II) trihydrate (1 in 10). No blue color develops.

(4) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution　To the specified amount of Talc, add 20 mL of diluted hydrochloric acid (1 in 4), cover with a watch glass, and boil gently for 15 minutes while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and allow to cool.

(5) Arsenic　Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B)

Test Solution　To the specified amount of Talc, add 5 mL of diluted sulfuric acid (3 in 50), heat gradually to boiling while shaking well, cool rapidly, and filter. Wash the residue with 5 mL of diluted sulfuric acid (3 in 50), then with 10 mL of water, and combine the washings with the filtrate, and evaporate on a water bath to make 5 mL.

Loss on Ignition　Not more than 6.0% (550°C, constant weight).

Tamarind Color

タマリンド色素

Definition　Tamarind Color is obtained from the roasted seeds of the tamarind tree *Tamarindus indica* L. by extraction with an alkaline solution and neutralization. It may contain dextrin or lactose.

Color Value　The Color Value ($E_{1cm}^{10\%}$) of Tamarind Color is not less than 20 and is in the range of 90–110% of the labeled value.

Description　Tamarind Color occurs as a red-brown to dark brown powder, as lumps, or as a paste or liquid. It has a slight, characteristic odor.

Identification

(1) Weigh an amount of Tamarind Color equivalent to 2.5 g of tamarind color with a

Color Value 20, and dissolve it in 100 mL of water. The resulting solution is red-brown to dark brown.

(2) To 5 mL of the solution obtained in Identification (1), add 2–3 drops of hydrochloric acid, and allow to stand. A red-brown to dark brown precipitate is produced.

(3) To 5 mL of the solution obtained in Identification (1), add 2 mL of iron(III) chloride hexahydrate solution (1 in 50). A dark brown color is produced.

(4) Weigh an amount of Tamarind Color equivalent to 1 g of tamarind color with a Color Value 20, and dissolve it in 100 mL of sodium hydroxide solution (1 in 250). To 5 mL of this solution, add 10 mL of diluted hydrochloric acid (9 in 1000) and 0.1 mL of zinc chloride TS (pH3.0), and shake. Stopper, warm at 50°C for 20 minutes, and centrifuge at 3000 rpm for 10 minutes if necessary. A red-brown to dark brown precipitate is produced.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value Determination Proceed as directed under Color Value Determination. Prepare a test solution as directed given below. Weigh accurately a specified amount of Tamarind Color, and add water to make exactly 100 mL. Refer to this as the sample solution. Dilute the sample solution exactly with a 1:1 mixture of citrate buffer (pH 7.0)/water. Centrifuge at 3000 rpm for 10 minutes if necessary. Use the supernatant as the test solution. Determine the color value using the following operation conditions.

Operating Conditions

Reference: water.

Wavelength: 500 nm.

Tamarind Seed Gum

タマリンドシードガム

Definition Tamarind Seed Gum is obtained from the seeds of the tamarind tree *Tamarindus indica* L. and consists mainly of polysaccharides. It may contain sucrose, glucose, lactose, dextrin, or maltose.

Description Tamarind Seed Gum occurs as a white to light brown powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Add gradually 2 g of Tamarind Seed Gum to 100 mL of sodium hydroxide solution

(1 in 125), and dissolve it by vigorous stirring. To 5 mL of this solution, add 3 mL of a saturated solution of sodium sulfuric acid. White lumps are produced.

(2) To the solution obtained in Identification (1), add gently a few drops of iodine–potassium iodide TS. Dark blue-green lumps are produced on the solution surface, and the color disappears on stirring.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Protein Not more than 3.0 %.

Weigh accurately about 0.5 g of Tamarind Seed Gum, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

Each mL of 0.005 mol/L sulfuric acid = 0.8754 mg of protein

Loss on Drying Not more than 14.0 % (105°C, 5 hours).

Ash Not more than 5.0 % (on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid For total plate count and the enumeration of yeasts and molds, prepare as follows: Mix 1 g of Tamarind Seed Gum with 200 mL of phosphate buffer, 0.1% peptone solution, or sodium chloride-peptone buffer to disperse uniformly.

Pre-enrichment Culture For the *Escherichia coli* test, prepare as follows: Mix 1 g of Tamarind Seed Gum with 200 mL of lauryl sulfate broth to disperse uniformly, and incubate at 35 ± 1°C for 48 ± 2 hours. For the *Salmonella* test, prepare five cultures in the following manner, and perform the test for each fluid: Mix 1 g of Tamarind Seed Gum with 200 mL of lactose broth to disperse uniformly, and incubate at 35 ± 1°C for 24 ± 2 hours.

Tannase

タンナーゼ

Definition Tannase includes enzymes that hydrolyze depside linkages in tannins. It is derived from the culture of filamentous fungi (limited to *Aspergillus niger*, *Aspergillus niger* var. *awamori*, *Aspergillus oryzae*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Tannase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Tannase complies with the Tannase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Tannase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 1.0 g of Tannase, add citrate buffer (0.05 mol/L) at pH 5.5 to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold or 100-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Weigh 0.320 g of tannic acid *n*-hydrate, dissolve it in about 10 mL of citrate buffer (0.05 mol/L) at pH 5.5 while warming or shaking, and add the citrate buffer (0.05 mol/L) at pH 5.5 to make 100 mL.

Procedure Add 1 mL of the sample solution to 4 mL of the substrate solution, equilibrated at 30°C for about 10 minutes, shake well, and incubate at 30°C. Both 10

minutes and 20 minutes later, measure 1 mL each of this solution, add 9 mL of a 1:4 mixture of water/ethanol (99.5) to each solution, and shake well. Dilute each solution to exactly 10 times its original volume with a 1:4 mixture of water/ethanol (99.5). Measure the absorbance of them at a wavelength of 310 nm against a 1:4 mixture of water/ethanol (99.5). The absorbance value obtained 10 minutes later is higher than that obtained 20 minutes later.

Tara Gum

タラガム

Definition Tara Gum is obtained from the seeds of the tara tree *Caesalpinia spinosa* (Molina) Kuntze and consists mainly of polysaccharides. It may contain sucrose, glucose, lactose, dextrin, or maltose.

Description Tara Gum occurs as a white to light yellow powder having almost no odor.

Identification

(1) Proceed as directed in Identification (1) for Carob Been Gum. A viscous liquid is formed. Heat 100 mL of this liquid on a water bath for about 10 minutes, and cool to room temperature. The liquid is more viscous than before heating:

(2) Proceed as directed in Identification (2) for Carob Been Gum.

Purity

(1) Acid-insoluble substances Not more than 5.0 %.

Proceed as directed in Purity (4) for Semirefined Carrageenan.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Protein Not more than 3.5%.

Weigh accurately about 0.2 g of Tara Gum, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

Each mL of 0.005 mol/L sulfuric acid = 0.7984 mg of protein

(5) Starch Dissolve 0.10 g of Tara Gum in 10 mL of water by heating with stirring. After cooling, add 2 drops of iodine TS. No blue color develops.

Loss on Drying Not more than 15.0 % (105°C, 5 hours).

Ash Not more than 1.5 % (550°C, 1 hour).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 10,000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative pet test.

Sample Fluid For total plate count and the enumeration of yeasts and molds, prepare as follows: Mix 1 g of Tara Gum with 200 mL of phosphate buffer, 0.1% peptone solution, or sodium chloride-peptone buffer to disperse uniformly.

Pre-enrichment Culture For the *Escherichia coli* test, prepare as follows: Mix 1 g of Tara Gum with 200 mL of lauryl sulfate broth to disperse uniformly, and incubate at 35 ± 1°C for 48 ± 2 hours. For the *Salmonella* test, prepare five cultures in the following manner and perform the test for each fluid: Mix 1 g of Tara Gum with 200 mL of lactose broth to disperse uniformly, and incubate at 35 ± 1°C for 24 ± 2 hours.

Tar color preparations

タール色素の製剤

Identification To tar color preparations given in Column 1 in the following table, apply the corresponding procedures specified in Column 2 in the table. Perform the test as directed under Other Coloring Matters in the Coloring Matter Tests, and compare the spots obtained between each sample and the corresponding reference standard. Both spots correspond to each other in the tone of color and Rf value

Column 1	Column 2
Food Red No. 2 Food Red No. 3 Food Red No. 40 Food Red No. 102 Food Red No. 104 Food Red No. 105 Food Yellow No. 4 Food Yellow No. 5 Food Blue No. 2	*Test Solution* A solution of the sample equivalent to 0.1% of the tar color under test (when an insoluble matter remains, centrifuge at 3000–3500 rpm to remove it). *Procedure* Develop as directed in Other Coloring Matters in the Coloring Matter Preparations Tests.

Food Red No. 106	*Test Solution* A solution of the sample equivalent to 0.03% of the tar color under test (when an insoluble matter remains, centrifuge by 3000–3500 rpm to remove it). *Procedure* Develop as directed in Other Coloring Matters in the Coloring Matter Preparations Tests.
Food Green No. 3 Food Blue No. 1	*Test Solution* A solution of the sample equivalent to 0.05% of the tar color under test (when an insoluble matter remains, centrifuge by 3000–3500 rpm to remove it). *Procedure* Develop as directed in Other Coloring Matters in the Coloring Matter Preparations Tests.
Food Red No. 2 Aluminum Lake Food Red No. 40 Aluminum Lake Food Yellow No. 4 Aluminum Lake Food Yellow No. 5 Aluminum Lake Food Green No. 3 Aluminum Lake Food Blue No. 1 Aluminum Lake	*Test Sample* Weigh a quantity of the sample equivalent to 0.5 g of the tar color aluminum lake under test into a centrifuge tube, add 50 mL of water, shake well, and centrifuge for about 10 minutes at 3000–3500 rpm. Remove the supernatant, add 50 mL of water to the residue, shake well, and centrifuge again. Repeat this procedure three times. Use the residue as the test sample. *Procedure* Develop as directed in Other Coloring Matter Lakes (1) in the Coloring Matter Preparations Tests.
Food Red No. 3 Aluminum Lake	*Test Sample* Weigh a quantity of the sample equivalent to 0.5 g of the tar color aluminum lake under test into a centrifuge tube, add 50 mL of water, shake well, and centrifuge for about 10 minutes at 3000–3500 rpm. Remove the supernatant, add 50 mL of water to the residue, shake well, and centrifuge again. Repeat this procedure three times. Use the residue as the test sample. *Procedure* Develop as directed in Other Coloring Matter Lakes (2) in the Coloring Matter Preparations Tests.

Food Blue No. 2 Aluminum Lake	*Test Sample* Weigh a quantity of the sample equivalent to 0.5 g of the tar color aluminum lake under test into a centrifuge tube, add 50 mL of water, shake well, and centrifuge for about 10 minutes at 3000–3500 rpm. Remove the supernatant, add 50 mL of water to the residue, shake well, and centrifuge again. Repeat this procedure three times. Use the residue as the test sample. *Procedure* Develop as directed in Other Coloring Matter Lakes (3) in the Coloring Matter Preparations Tests.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (Coloring Matter Preparations Tests, Heavy metals).

(2) Manganese Apply this test to preparations containing Food Red No. 106, Food Green No. 3, and Food Blue No. 1.

When the content of the coloring matter is more than 50%: Not more than 50 μg/g as Mn. When the content of the coloring matter is not more than 50%: Not more than 25 μg/g as Mn. (Coloring Matter Preparations Tests, Manganese and Chromium (1)).

(3) Chromium Apply this test to preparations containing Food Red No. 106, Food Green No. 3, and Food Blue No. 1.

When the content of the coloring matter is more than 50%: Not more than 50 μg/g as Cr. When the content of the coloring matter is not more than 50%: Not more than 25 μg/g as Cr. (Coloring Matter Preparations Tests, Manganese and Chromium (2))

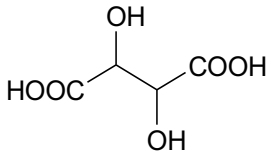
(4) Arsenic Not more than 3 μg/g as As.

In the case of tar color preparations not containing tar color aluminum lakes, proceed as directed in the test for Arsenic in the Coloring Matter Tests. In the case of tar color preparations containing tar color aluminum lakes, proceed as directed in the test for Arsenic in the Coloring Matter Aluminum Lake Tests.

DL-Tartaric Acid

dl-Tartaric Acid

DL-酒石酸



$C_4H_6O_6$ Mol. Wt. 150.09

2,3-Dihydroxybutanedioic acid [133-37-9]

Content DL-Tartaric Acid, when dried, contains not less than 99.5% of DL-tartaric acid ($C_4H_6O_6$).

Description DL-Tartaric Acid occurs as colorless crystals or as a white crystalline powder. It is odorless and has an acid taste.

Identification

(1) A solution of DL-Tartaric Acid (1 in 10) has no optical rotation.

(2) A solution of DL-Tartaric Acid (1 in 10) is acidic.

(3) DL-Tartaric Acid responds to all the tests for Tartrate in the Qualitative Tests.

Melting Point 200–206°C (decomposition).

Purity

(1) Sulfate Not more than 0.048% as SO_4 (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Readily oxidizable substances Dissolve 1.0 g of DL-Tartaric Acid in 25 mL of water and 25 mL of diluted sulfuric acid (1 in 20). Add 4.0 mL of 0.02 mol/L potassium permanganate, keeping the solution at 20°C. The pink color of the solution does not disappear within 3 minutes.

Loss on Drying Not more than 0.5% (3 hours).

Residue on Ignition Not more than 0.1% (2 g).

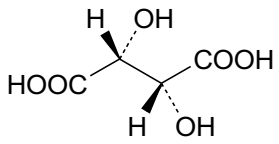
Assay Weigh accurately about 1.5 g of DL-Tartaric Acid, previously dried, and dissolve it in water to make exactly 250 mL. Measure exactly 25 mL of this solution, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2–3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide = 7.504 mg of $C_4H_6O_6$

L-Tartaric Acid

Tartaric Acid
***d*-Tartaric Acid**
L(+)-Tartaric Acid

L-酒石酸



$C_4H_6O_6$ Mol. Wt. 150.09

(2*R*,3*R*)-2,3-Dihydroxybutanedioic acid [87-69-4]

Content L-Tartaric Acid, when dried, contains not less than 99.5% of L-tartaric acid ($C_4H_6O_6$).

Description L-Tartaric Acid occurs as colorless crystals or as a white, fine crystalline powder. It is odorless and has an acid taste.

Identification

(1) A solution of L-Tartaric Acid (1 in 10) is dextrorotatory.

(2) Proceed as directed in Identification (2) and (3) for DL-Tartaric Acid.

Specific Rotation $[\alpha]_D^{20}$: +11.5 to +13.5° (previously dried, 10 g, water, 50 mL).

Purity

(1) Sulfate Not more than 0.048% as SO_4 (0.50 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Oxalate Dissolve 1.0 g of L-Tartaric Acid in 10 mL of water, and add 2 mL of a solution of calcium chloride dihydrate (2 in 25). No turbidity appears.

Loss on Drying Not more than 0.5% (3 hours).

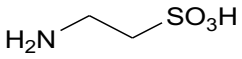
Residue on Ignition Not more than 0.1% (2 g).

Assay Proceed as directed in the Assay for DL-Tartaric Acid.

Each mL of 0.1 mol/L sodium hydroxide = 7.504 mg of $C_4H_6O_6$

Taurine (Extract)

タウリン(抽出物)



$C_2H_7NO_3S$ Mol. Wt. 125.15

2-Aminoethanesulfonic acid [107-35-7]

Definition Taurine (Extract) is obtained from the visceral organs or meat of fish, shellfish, or mammals and consists mainly of taurine.

Content Taurine (Extract), when dried, contains not less than 98.5% of taurine ($C_2H_7NO_3S$).

Description Taurine (Extract) occurs as a white crystalline powder. It is odorless.

Identification

(1) To 5 mL of a solution of Taurine (Extract) (1 in 20), add 5 drops of 10% hydrochloric acid and 5 drops of sodium nitrite solution (1 in 10). The solution effervesces, emitting a colorless gas.

(2) To 0.5 g of Taurine (Extract), add 7.5 mL of sodium hydroxide TS (1 mol/L), heat gradually, and evaporate to dryness. Next, heat at 500°C for 2 hours to decompose. To the residue, add 5 mL of water, shake, and filter. To this mixture, add 1 drop of sodium pentacyanonitrosylferrate(III) TS. A red-purple color develops.

Purity

(1) Clarity of solution Colorless and clear (0.5 g, water 20 mL).

(2) Chloride Not more than 0.011% as Cl (1.0 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Sulfate Not more than 0.014% as SO_4 (1.5 g, Control Solution: 0.005 mol/L sulfuric acid 0.45 mL).

(4) Ammonium Not more than 0.020% as NH_4.

Weigh 0.10 g of Taurine (Extract) into a flask, dissolve it in 70 mL of water, and add 1 g of magnesium oxide. Connect the flask to distillation equipment, and place a receiver containing 10 mL of boric acid solution (1 in 200) so that the lower end of the condenser is immersed in the solution in the receiver. Distill at a rate of 5–7 mL/minute until 30 mL of distillate is obtained. To the distillate obtained, add water to make 50 mL. Measure 30 mL of this solution in a Nessler tube, add 6.0 mL of phenol–sodium pentacyanonitrosylferrate(III) TS, and mix. Add 4 mL of sodium hypochlorite–sodium hydroxide TS and water to make 50 mL, mix, and allow to stand for 60 minutes. The

color of the resulting solution is not darker than that of a control solution prepared in the same manner as the sample, using 2.0 mL of Ammonium Standard Solution in place of the sample.

(5) Readily carbonizable substances　Dissolve 0.10 g of Taurine (Extract) in 1 mL of sulfuric acid for the readily carbonizable substances test. No color develops.

(6) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(7) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 2, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more than 0.2% (105°C, 2 hours).

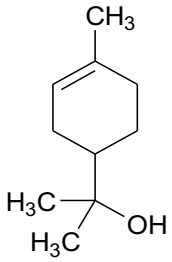
Residue on Ignition　Not more than 0.5% (1 g).

Assay　Weigh accurately about 0.2 g of Taurine (Extract), previously dried, dissolve it in 50 mL of water, and add 5 mL of formaldehyde solution. Titrate with 0.1 mol/L sodium hydroxide (indicator: 3 drops of phenolphthalein TS). Separately, perform a blank test in the same manner, and make any necessary correction.

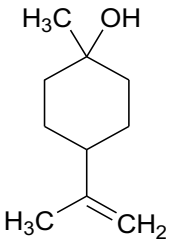
Each mL of 0.1 mol/L sodium hydroxide solution = 12.52 mg of $C_2H_7NO_3S$

Terpineol

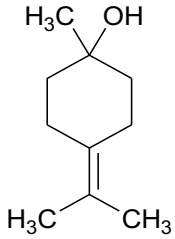
テルピネオール



α-Terpineol



β-Terpineol



γ-Terpineol

$C_{10}H_{18}O$　　Mol. Wt. 154.25

Mixture of 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol (α-terpineol),

1-methyl-4-(1-methylethenyl)cyclohexan-1-ol (β-terpineol), and

1-methyl-4-(1-methylethylidene)cyclohexan-1-ol (γ-terpineol)

Content　Terpineol contains not less than 97.0% of terpineol ($C_{10}H_{18}O$).

Description　Terpineol is a colorless or light yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Terpineol as directed in the Liquid Film Method under Infrared Spectrophotometry. It exhibits absorptions at about 3380 cm^{-1}, 2965 cm^{-1}, 2925 cm^{-1}, 2835 cm^{-1}, 1385 cm^{-1}, 1377 cm^{-1}, 1150 cm^{-1}, and 1135 cm^{-1}.

Refractive Index n_D^{20}: 1.482–1.484.

Specific Gravity d_{20}^{20}: 0.932–0.938.

Purity Clarity of solution Clear (1.0 mL, 70% (vol) ethanol 2.0 mL).

Assay Weigh 5.0 g of Terpineol and 20.0 g of xylene, transfer into a flask, add 10 mL of acetic anhydride and 1 g of sodium acetate. Boil gently under a reflux condenser for 6 hours, and cool. Add 10 mL of water, heat in a water bath for 15 minutes with occasional shaking, and cool. Transfer the contents into a separating funnel and separate the aqueous layer. Wash the oil layer with sodium carbonate solution (1 in 8) until the washings is alkaline, and wash with sodium chloride solution (1 in 10) until the washings is neutral. Transfer into a dry container, add about 2 g of sodium sulfate, shake, allow to stand for about 30 minutes, and filter. Weigh accurately about 5 g of the filtrate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests. In the test, boil the solution for 4 hours before titrating. Separately, perform a blank test in the same manner, and calculate the content by the formula:

$$\text{Content (\%) of terpineol } (C_{10}H_{18}O) = \frac{154.2 \times (a - b) \times 0.5}{[S - (a - b) \times 0.02102] \times 5/25 \times 1000} \times 100$$

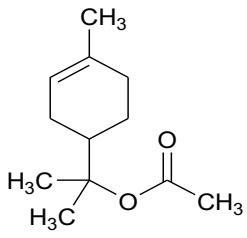
a = volume (mL) of 0.5 mol/L hydrochloric acid consumed in the blank test,

b = volume (mL) of 0.5 mol/L hydrochloric acid consumed in this test,

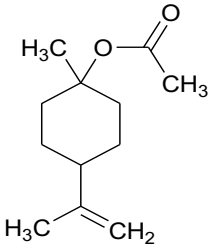
S = weight (g) of the filtrate.

Terpinyl Acetate

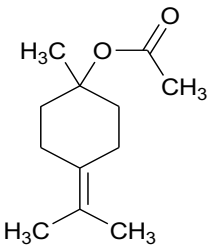
酢酸テルピニル



α-Terpinyl Acetate



β-Terpinyl Acetate



γ-Terpinyl Acetate

$C_{12}H_{20}O_2$ Mol. Wt. 196.29

Mixture of 2-(4-methylcyclohex-3-en-1-yl)propan-2-yl acetate (α-terpinyl acetate), 1-

methyl-4-(1-methylethenyl)cyclohexyl acetate (β-terpinyl acetate), and 1-methyl-4-(1-methylethylidene)cyclohexyl acetate (γ-terpinyl acetate)　[8007-35-0]

Content　Terpinyl Acetate contains not less than 97.0% of terpinyl acetate ($C_{12}H_{20}O_2$).

Description　Terpinyl Acetate is a colorless to light yellow, clear liquid having a characteristic odor.

Identification　Determine the absorption spectrum of Terpinyl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry. The spectrum exhibits absorption at about 2970 cm^{-1}, 2935 cm^{-1}, 1730 cm^{-1}, 1360 cm^{-1}, 1270 cm^{-1}, 1220 cm^{-1}, and 1135 cm^{-1}.

Refractive Index　n_D^{20}: 1.464–1.467.

Specific Gravity　d_{20}^{20}: 0.956–0.965.

Purity

(1) Acid value　Not more than 1.0 (Flavoring Substances Tests).

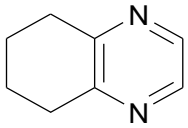
(2) Clarity of solution　Clear (1.0 mL, 70% (vol) ethanol 5.0 mL).

Assay　Weigh accurately about 0.7 g of Terpinyl Acetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests. In the test, use 20 mL of 0.5 mol/L ethanolic potassium hydroxide, and boil the mixture for 2 hours before titrating.

Each mL of 0.5 mol/L ethanolic potassium hydroxide = 98.14 mg of $C_{12}H_{20}O_2$

5,6,7,8-Tetrahydroquinoxaline

5,6,7,8-テトラヒドロキノキサリン



$C_8H_{10}N_2$　　Mol. Wt.　134.18

5,6,7,8-Tetrahydroquinoxaline [34413-35-9]

Content　5,6,7,8-Tetrahydroquinoxaline contains not less than 98.0% of 5,6,7,8-tetrahydroquinoxaline ($C_8H_{10}N_2$).

Description　5,6,7,8-Tetrahydroquinoxaline occurs as a colorless to light yellow, clear liquid having a characteristic odor.

Identification　Determine the absorption spectrum of 5,6,7,8-Tetrahydroquinoxaline, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

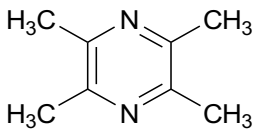
Refractive Index　n_D^{20}: 1.540–1.550.

Specific gravity d_{25}^{25}: 1.078–1.088.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavor Substance Tests. Use operating conditions (4).

2,3,5,6-Tetramethylpyrazine

2,3,5,6-テトラメチルピラジン



$C_8H_{12}N_2$ Mol. Wt. 136.19

2,3,5,6-Tetramethylpyrazine [1124-11-4]

Content 2,3,5,6-Tetramethylpyrazine contains not less than 95.0% of 2,3,5,6-tetramethylpyrazine ($C_8H_{12}N_2$).

Description 2,3,5,6-Tetramethylpyrazine occurs as white crystals or powder having a characteristic odor.

Identification Determine the absorption spectrum of 2,3,5,6-Tetramethylpyrazine as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 85–90°C.

Assay Using a solution (1 in 10) of 2,3,5,6-Tetramethylpyrazine in ethanol (95) as the test solution, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay in the Flavoring Substances Tests. Use operating conditions (1).

Thaumatin

タウマチン

Definition Thaumatin is obtained from the seeds of *Thaumatococcus daniellii* (Benn.) Benth. & Hook. f. and consists mainly of thaumatin.

Content Thaumatin, when dried, contains not less than 94% of thaumatin.

Description Thaumatin occurs as a light yellow-brown to gray-brown, odorless powder or as flakes. It has an intensely sweet taste.

Identification

(1) To 2 mL of a solution of Thaumatin (1 in 100), add 2 mL of ninhydrin–acetic acid

TS and 2 mL of hydrazinium sulfate solution (13 in 25,000), and heat in a water bath. A blue-purple color develops.

(2) A solution of Thaumatin (1 in 100,000) is sweet.

Specific Absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 278 nm): 11.5–13.0 (0.1 g, water, 200 mL).

Purity

(1) Aluminum Not more than 100 μg/g as Al.

Test Solution Weigh accurately about 2 g of Thaumatin, and gently heat to carbonize. After cooling, add a small quantity of sulfuric acid, carefully heat until a white smoke no longer appears, and intensely heat at 450–550°C to incinerate. Add 0.2 mol/L hydrochloric acid to make exactly 25 mL.

Standard Solutions Measure exactly a certain volume of Aluminum Standard Stock Solution, and add water to prepare plural solutions with stepwise concentrations ranging 2.0–10.0 μg/mL of aluminum (Al = 26.98).

Procedure Measure the absorbance of the test solution and the standard solutions as directed under Flame Atomic Absorption Spectrophotometry using the operating conditions given below. Determine the aluminum content in the test solution, using a calibration curve obtained from the absorbance of the standard solutions.

Operating Conditions

Light source: Aluminum hollow cathode lamp.

Wavelength of analytical line: 309.3 nm.

Supporting gas: Nitrous oxide.

Combustible gas: Acetylene.

(2) Carbohydrate Not more than 3.0%.

Test Solution Weigh accurately about 0.5 g of Thaumatin, dissolve it in water, previously adjusted to pH 3 with hydrochloric acid, and make exactly 50 mL. To 0.10 mL of this solution, add exactly 6 mL of cysteine–sulfuric acid TS, heat in a water bath for 3 minutes, and cool with cold water for 5 minutes.

Standard Solutions Prepare plural solutions of D(+)-glucose solutions with different concentrations ranging from 10 to 100 μg/mL. Using 0.10 mL each of these solutions, prepare standard solutions as directed for the test solution.

Procedure Measure the absorbance of the test solution and the standard solutions at a wavelength of 400 nm, determine the content of carbohydrate (as D-glucose) in the test solution, using a calibration curve obtained from the absorbance of the standard solutions. As the control solution, use a solution prepared without sample in the same manner as for the test solution.

(3) Lead Not more than 3 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 6.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (1.0 g, Control Solution: Arsenic Standard Solution 6.0 mL, Apparatus C).

Test Solution Weigh the specified amount of Thaumatin into a crucible made of platinum, quartz, or porcelain. Add 10 mL of a 1 in 10 solution of magnesium nitrate hexahydrate in ethanol (95), ignite the ethanol, heat gradually, and incinerate at 450–550°C. If carbonized matter still remains, moisten with a small quantity of a 1 in 50 solution of magnesium nitrate in ethanol (95), heat again, and incinerate at 450–550°C. After cooling, add 3 mL of hydrochloric acid, heat on a water bath to dissolve the residue, and add water to make exactly 10 mL.

Control Solution To the specified amount of Arsenic Standard Solution, add 3 mL of hydrochloric acid, and make up to exactly 10 mL with water.

Loss on Drying Not more than 9.0% (105°C, 3 hours).

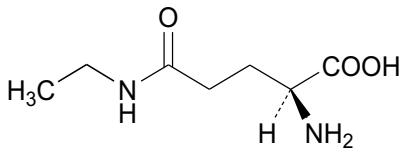
Residue on Ignition Not more than 2.0%.

Assay Weigh accurately about 0.15 g of Thaumatin, previously dried, and proceed as directed in the Kjeldahl Method under Nitrogen Determination. Calculate the thaumatin content by the formula:

$$\text{Content (\%) of thaumatin} = \frac{\text{Volume (mL) of 0.1 mol/L sodium hydroxide consumed} \times 1.401 \times 6.25}{\text{Weight (g) of the sample} \times 1000} \times 100$$

L-Theanine

L-テアニン



$C_7H_{14}N_2O_3$ Mol. Wt. 174.20

(2*S*)-2-Amino-4-(*N*-ethylcarbamoyl)butanoic acid [3081-61-6]

Content L-Theanine, when calculated on the dried basis, contains 98.0–102.0% of L-theanine ($C_7H_{14}N_2O_3$).

Description L-Theanine occurs as a white crystalline powder. It is odorless and has a slightly characteristic and sweet taste.

Identification

(1) To 5 mL of a solution of L-Theanine (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) Dissolve about 1 g of L-Theanine in 10 mL of diluted hydrochloric acid (1 in 2), heat on a water bath under a reflux condenser for 6 hours, and add water to make 20 mL. Transfer 5 mL of this solution into a test tube, and add 2 g of sodium hydroxide. Suspend a litmus paper (red) moistened with water in the test tube, cover the mouth of the test tube, and heat in a water bath for 5 minutes. The litmus paper turns blue.

Specific Rotation $[\alpha]_D^{20}$: +7.7 to +8.5° (2.5 g, water, 50 mL, on the dried basis).

pH 5.0–6.0 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.021% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.5% (105°C, 3 hours).

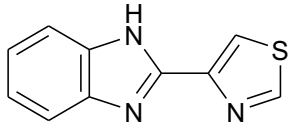
Residue on Ignition Not more than 0.2%.

Assay Weigh accurately about 0.35 g of L-Theanine, and proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 17.42 mg of $C_7H_{14}N_2O_3$

Thiabendazole

チアベンダゾール



$C_{10}H_7N_3S$ Mol. Wt. 201.25

2-(1,3-Thiazol-4-yl)-1*H*-benzo[*d*]imidazole [148-79-8]

Content Thiabendazole, when dried, contains not less than 98.0% of thiabendazole ($C_{10}H_7N_3S$).

Description Thiabendazole occurs as a white to whitish powder. It is odorless.

Identification

(1) Dissolve 5 mg of Thiabendazole in 5 mL of diluted hydrochloric acid (1 in 100), add 3 mg of *p*-phenylenediamine dihydrochloride and about 0.1 g of zinc dust, and allow to

stand for 2 minutes. An odor of hydrogen sulfide is evolved. To this solution, add 0.5 mL of ammonium iron(III) sulfate–sulfuric acid TS (1 in 35). A blue to blue-purple color develops.

(2) Dissolve 5 mg of Thiabendazole in 1000 mL of diluted hydrochloric acid (1 in 100). The solution exhibits absorption maxima at wavelengths of 298–306 nm and 239–247 nm, and an absorption minimum at a wavelength of 254–262 nm.

Melting Point 296–303°C (decomposition).

Purity Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Loss on Drying Not more than 0.5% (reduced pressure, 24 hours).

Residue on Ignition Not more than 0.2%.

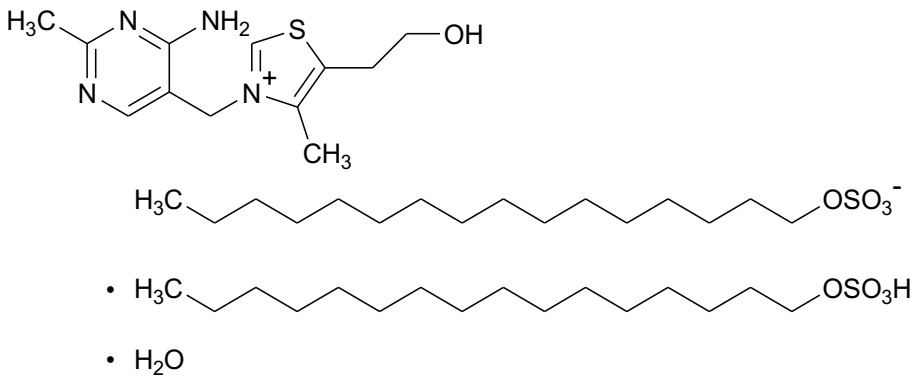
Assay Weigh accurately about 0.2 g of Thiabendazole, previously dried, add 10 mL of acetic acid for nonaqueous titration, dissolve it by warming, and cool. Add 50 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid (indicator: 1 mL of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Separately, perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 20.12 mg of $C_{10}H_7N_3S$

Thiamine Dicetylsulfate

Vitamin B_1 Dicetylsulfate

チアミンセチル硫酸塩



$C_{44}H_{84}N_4O_9S_3 \cdot H_2O$ Mol. Wt. 927.37

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium dihexadecylsulfate monohydrate

Content Thiamine Dicetylsulfate, when dried, contains 96.0–102.0% of thiamine dicetylsulfate ($C_{44}H_{84}N_4O_9S_3 \cdot H_2O$).

Description Thiamine Dicetylsulfate occurs as colorless to white crystals or as a white crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) To 0.1 g of Thiamine Dicetylsulfate, add 20 mL of potassium chloride–hydrochloric acid TS, boil gently for about 30 minutes, cool, and filter. To 1 mL of the filtrate, add 1 mL of lead(II) acetate TS and 1 mL of sodium hydroxide solution (1 in 10). A yellow color develops. The solution turns brown when heated on a water bath, and then a black-brown precipitate is formed on standing.

(2) To 1 mL of the filtrate obtained in Identification (1), add 5 mL of sodium hydroxide solution (1 in 50) and 0.5 mL of a freshly prepared solution of potassium hexacyanoferrate(III) (1 in 10), then add 5 mL of 2-methyl-1-propanol, and shake vigorously for 2 minutes. Allow to stand, and examine under ultraviolet light. The 2-methyl-1-propanol layer emits a blue-purple fluorescence, which disappears when the solution is acidic but reappears when it is made alkaline.

(3) To 1 g of Thiamine Dicetylsulfate, add 30 mL of water and 15 mL of hydrochloric acid, boil under a reflux condenser for about 4 hours, and cool. Extract twice with 15 mL of diethyl ether each time, combine the diethyl ether extracts, wash with water, and remove the diethyl ether by evaporating on a water bath. Dry the residue at 100°C for 15 minutes, and cool. Measure the melting point of the residue. It is 46–56°C.

Purity

(1) Chloride Not more than 0.057% as Cl.

Test Solution Weigh 0.25 g of Thiamine Dicetylsulfate, add 30 mL of water, shake well, and allow to stand for 10 minutes. Dissolve it by adding 6 mL of diluted nitric acid (1 in 10), filter, and wash with water. Combine the washings with the filtrate, and add water to make 50 mL.

Control Solution To 0.40 mL of 0.01 mol/L hydrochloric acid, add 6 mL of diluted nitric acid (1 in 10) and water to make 50 mL.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Loss on Drying Not more than 2.0% (24 hours).

Residue on Ignition Not more than 0.3%.

Assay

Test Solution Weigh accurately about 0.14 g of Thiamine Dicetylsulfate, previously dried, add 40 mL of potassium chloride–hydrochloric acid TS, and heat on a water bath for 30 minutes with occasional shaking. After cooling, filter, wash with 50 mL of water, combine the washings with the filtrate, and add water to make exactly 100 mL. Measure exactly 2 mL of this solution, add exactly 5 mL of a solution (1 in 1000) of methyl benzoate in methanol, and add the mobile phase (the same solution used in the Assay for Thiamine Hydrochloride) to make exactly 100 mL.

Standard Solution Weigh accurately about 50 mg of Thiamine Hydrochloride

Reference Standard (the water content should be measured previously in the same manner as for Thiamine Hydrochloride), dissolve it in 40 mL of potassium chloride–hydrochloric acid TS, and add water to make exactly 100 mL. Measure exactly 2 mL of this solution, add exactly 5 mL of a solution (1 in 1000) of methyl benzoate in methanol, and add the mobile phase to make exactly 100 mL.

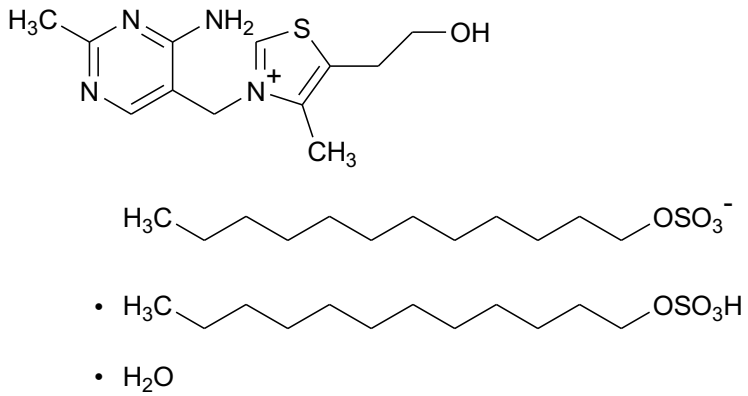
Procedure Proceed as directed in the Assay for Thiamine Hydrochloride, using the test solution and the standard solution. Calculate the content by the formula:

$$\text{Content (\%) of thiamine dicetylsulfate } (C_{44}H_{84}N_4O_9S_3 \cdot H_2O) = \frac{\text{Anhydrous basis weight (g) of Thiamine Hydrochloride Reference Standard}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 2.750 \times 100$$

Thiamine Dilaurylsulfate

Vitamin B_1 Dilaurylsulfate

チアミンラウリル硫酸塩



$C_{36}H_{68}N_4O_9S_3 \cdot H_2O$ Mol. Wt. 815.16

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium didodecylsulfate monohydrate

Content Thiamine Dilaurylsulfate, when dried, contains 98.0–102.0% of thiamine dilaurylsulfate ($C_{36}H_{68}N_4O_9S_3 \cdot H_2O$).

Description Thiamine Dilaurylsulfate occurs as colorless to white crystals or as a white crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Proceed as directed in Identification (1) and (2) for Thiamine Dicetylsulfate.

(2) Proceed as directed in Identification (3) for Thiamine Dicetylsulfate. The melting point is 20–28°C.

Purity

(1) Chloride Not more than 0.057% as Cl.

Proceed as directed in Purity (1) for Thiamine Dicetylsulfate.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Loss on Drying Not more than 2.0% (24 hours).

Residue on Ignition Not more than 0.3%.

Assay

Test Solution Weigh accurately about 0.12 g of Thiamine Dilaurylsulfate, previously dried, add 40 mL of potassium chloride–hydrochloric acid TS, and heat on a water bath for 30 minutes with occasional shaking. After cooling, filter, wash with 50 mL of water, combine the washings with the filtrate, and add water to make exactly 100 mL. Measure exactly 2 mL of this solution, add exactly 5 mL of a solution (1 in 1000) of methyl benzoate in methanol, and add the mobile phase (the same solution used in the Assay for Thiamine Hydrochloride) to make exactly 100 mL.

Standard Solution Weigh accurately about 50 mg of Thiamine Hydrochloride Reference Standard (the water content should be measured previously in the same manner as for Thiamine Hydrochloride), dissolve it in 40 mL of potassium chloride–hydrochloric acid TS, and add water to make exactly 100 mL. Measure exactly 2 mL of this solution, add exactly 5 mL of a solution (1 in 1000) of methyl benzoate in methanol, and add the mobile phase to make exactly 100 mL.

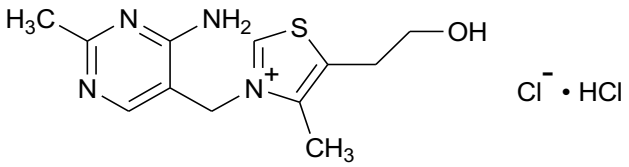
Procedure Proceed as directed in the Assay for Thiamine Hydrochloride, using the test solution and the standard solution. Calculate the content by the formula:

$$\text{Content (\%) of thiamine dilaurylsulfate } (C_{36}H_{68}N_4O_9S_3 \cdot H_2O) = \frac{\text{Anhydrous basis weight (g) of Thiamine Hydrochloride Reference Standard}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 2.417 \times 100$$

Thiamine Hydrochloride

Vitamin B_1 Hydrochloride

チアミン塩酸塩



$C_{12}H_{17}ClN_4OS \cdot HCl$ Mol. Wt. 337.27

3-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride monohydrochloride [67-03-8]

Content Thiamine Hydrochloride, calculated on the anhydrous basis, contains 98.0–102.0% of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$).

Description Thiamine Hydrochloride occurs as white to yellowish-white, fine crystals or crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) To 1 mL of a solution of Thiamine Hydrochloride (1 in 500), add 1 mL of lead(II) acetate TS and 1 mL of sodium hydroxide solution (1 in 10). A yellow color develops. The solution turns brown when heated on a water bath, and then a black-brown precipitate is formed on standing.

(2) To 5 mL of a solution of Thiamine Hydrochloride (1 in 500), add 2.5 mL of sodium hydroxide solution (1 in 25) and 0.5 mL of a freshly prepared solution of potassium hexacyanoferrate(III) (1 in 10), then add 5 mL of 2-methyl-1-propanol, shake vigorously for 2 minutes, and allow to stand. Examine under ultraviolet light. The 2-methyl-1-propanol layer emits a blue-purple fluorescence. The fluorescence disappears when the solution is acidic but reappears when the solution is made alkaline.

(3) Thiamine Hydrochloride responds to all the tests for Chloride in the Qualitative Tests.

pH 2.7–3.4 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution Weigh 1.0 g of Thiamine Hydrochloride, and dissolve it in water to make 10 mL. The solution is clear and not darker in color than a solution prepared by adding water to 1.5 mL of 1/60 mol/L potassium dichromate solution to make 1000 mL.

(2) Sulfate Not more than 0.011% as SO_4 (1.5 g Control Solution: 0.005 mol/L sulfuric acid 0.35 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead

Standard Solution 4.0 mL, Flame Method).

Water Content Not more than 5.0% (0.50 g, Volumetric Titration, Direct Titration).

Residue on Ignition Not more than 0.2%.

Assay

Test Solution and Standard Solution Weigh accurately about 0.1 g each of Thiamine Hydrochloride and Thiamine Hydrochloride Reference Standard (the water content should be previously measured in the same manner as for Thiamine Hydrochloride), dissolve each in the mobile phase prepared as directed in the operating conditions to make exactly 50 mL. To exactly 10 mL each of the solutions, add exactly 5 mL of a solution (1 in 50) of methyl benzoate in methanol, and then add the mobile phase to make two solutions of exactly 50 mL each. Use these solutions as the test solution and the standard solution, respectively.

Procedure Analyze 10 μL each of these solutions by liquid chromatography using the conditions given below. Calculate the peak area ratio of thiamine to methyl benzoate for the test solution and the standard solution, and express as Q_T and Q_S, respectively. Calculate the content by the formula:

$$\text{Content (\%) of thiamine hydrochloride } (C_{12}H_{17}ClN_4OS\cdot HCl) = \frac{\text{Anhydrous basis weight (g) of Thiamine Hydrochloride Reference Standard}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 254 nm).

Column: A stainless steel tube (about 4 mm internal diameter and 15–30 cm length).

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

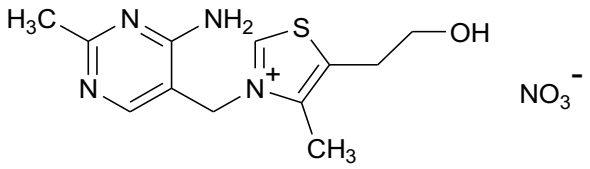
Mobile phase: Dissolve 1.1 g of sodium 1-octanesulfonate in 1000 mL of diluted acetic acid (1 in 100). To 600 mL of this solution, add 400 mL of a 3:2 mixture of methanol/acetonitrile.

Flow rate: Adjust the retention time of thiamine to about 12 minutes.

Thiamine Mononitrate

Vitamin B_1 Mononitrate

チアミン硝酸塩



$C_{12}H_{17}N_5O_4S$ Mol. Wt. 327.36

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium nitrate [532-43-4]

Content Thiamine Mononitrate, when dried, contains 98.0–102.0% of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$).

Description Thiamine Mononitrate occurs as white to yellowish-white crystals or crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Proceed as directed in Identification (1) and (2) for Thiamine Hydrochloride.

(2) Thiamine Mononitrate responds to all the tests for Nitrate in the Qualitative Tests.

pH 6.5–8.0 (1.0 g, water 50 mL).

Purity

(1) Chloride Not more than 0.057% as Cl (0.25 g, Control Solution: 0.01 mol/L hydrochloric acid 0.40 mL).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Loss on Drying Not more than 1.0% (105°C, 2 hours).

Residue on Ignition Not more than 0.2%.

Assay Weigh accurately about 0.1 g each of Thiamine Mononitrate, previously dried, and Thiamine Hydrochloride Reference Standard (the water content should be measured in the same manner as for Thiamine Hydrochloride). Proceed as directed in the Assay for Thiamine Hydrochloride, and calculate the content by the formula:

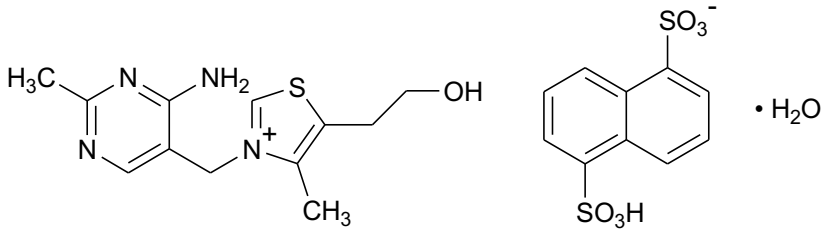
Content (%) of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$)

$$= \frac{\text{Anhydrous basis weight (g) of Thiamine Hydrochloride Reference Standard}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 0.9706 \times 100$$

Thiamine Naphthalene-1,5-disulfonate

Vitamin B_1 Naphthalene-1,5-disulfonate

チアミンナフタレン-1,5-ジスルホン酸塩



$C_{22}H_{24}N_4O_7S_3 \cdot H_2O$ Mol. Wt. 570.66

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium naphthalene-1,5-disulfonate monohydrate

Content Thiamine Naphthalene-1,5-disulfonate, when dried, contains 98.0–102.0% of thiamine naphthalene-1,5-disulfonate ($C_{22}H_{24}N_4O_7S_3$ = 552.65).

Description Thiamine Naphthalene-1,5-disulfonate occurs as a white, fine crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Proceed as directed in Identification (1) and (2) for Thiamine Hydrochloride.

(2) Dissolve 10 mg of Thiamine Naphthalene-1,5-disulfonate in 100 mL of diluted hydrochloric acid (1 in 10,000). To 5 mL of the solution, add diluted hydrochloric acid (1 in 10,000) to make 100 mL. The solution exhibits an absorption maximum at a wavelength of 225–227 nm.

Purity

(1) Chloride Not more than 0.057% as Cl.

Proceed as directed in Purity (1) for Thiamine Dicetylsulfate.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Loss on Drying Not more than 5.0% (105°C, 2 hours).

Residue on Ignition Not more than 0.2%.

Assay

Test Solution Weigh accurately about 0.16 g of Thiamine Naphthalene-1,5-disulfonate, previously dried, add 30 mL of diluted hydrochloric acid (1 in 1000), and dissolve by heating on a water bath. After cooling, add diluted hydrochloric acid (1 in 1000) to make exactly 50 mL. Measure exactly 10 mL of this solution, add 50 mL of diluted hydrochloric acid (1 in 1000), and add methanol to make exactly 100 mL. Measure exactly 25 mL of the second solution, add exactly 5 mL of a solution (1 in 200) of methyl benzoate in methanol, and add water to make exactly 50 mL.

Standard Solution Weigh accurately about 0.1 g of Thiamine Hydrochloride Reference Standard (the water content should be measured previously in the same manner as for Thiamine Hydrochloride), dissolve it in diluted hydrochloric acid (1 in 1000) to make exactly 50 mL. Then proceed in the same manner as the preparation of the test solution.

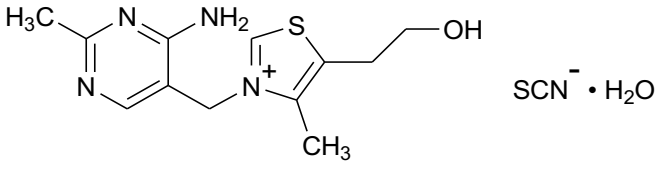
Procedure Proceed as directed in the Assay for Thiamine Hydrochloride, using the test solution and the standard solution. Calculate the content by the formula:

$$\text{Content (\%) of thiamine naphthalene-1,5-disulfonate } (C_{22}H_{24}N_4O_7S_3) = \frac{\text{Anhydrous basis weight (g) of Thiamine Hydrochloride Reference Standard}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 1.639 \times 100$$

Thiamine Thiocyanate

Vitamin B_1 Rhodanate

チアミンチオシアン酸塩



$C_{13}H_{17}N_5OS_2 \cdot H_2O$ Mol. Wt. 341.45

3-[(4-Amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium thiocyanate monohydrate [130131-60-1]

Content Thiamine Thiocyanate, when dried, contains 98.0–102.0% of thiamine

thiocyanate ($C_{13}H_{17}N_5OS_2$ = 323.44).

Description Thiamine Thiocyanate occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Proceed as directed in Identification (1) and (2) for Thiamine Hydrochloride.

(2) Thiamine Thiocyanate saturated solution responds to all the tests for Thiocyanate in the Qualitative Tests.

Purity

(1) Chloride Not more than 0.057% as Cl.

Test Solution Weigh 0.25 g of Thiamine Thiocyanate, add 1.5 mL of water, 0.3 g of ammonium nitrate, and 0.9 mL of sodium hydroxide solution (2 in 5), and then add 3 mL of hydrogen peroxide gradually dropwise while shaking. Heat on a water bath for 30 minutes with occasional shaking, cool, and add 3 mL of diluted nitric acid (2 in 3) and water to make 50 mL. Add 0.1 mL of a solution of dextrin hydrate (1 in 50) and 0.5 mL of silver nitrate solution (1 in 50), and allow to stand for 5 minutes.

Control Solution Prepare as directed for the test solution using 0.40 mL of 0.01 mol/L hydrochloric acid.

Procedure The test solution is not higher in turbidity than the control solution.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Loss on Drying Not more than 6.0% (105°C, 2 hours).

Residue on Ignition Not more than 0.2%.

Assay

Test Solution Weigh accurately about 0.1 g of Thiamine Thiocyanate, previously dried, and dissolve it in diluted hydrochloric acid (1 in 10,000) to make exactly 200 mL. Measure exactly 2 mL of this solution, add exactly 5 mL of a solution (1 in 50) of methyl benzoate in methanol, and add the mobile phase (the same solution used in the Assay for Thiamine Hydrochloride) to make exactly 50 mL.

Standard Solution Weigh accurately about 0.1 g of Thiamine Hydrochloride Reference Standard (the water content should be measured previously as directed for Thiamine Hydrochloride), and proceed in the same manner as the preparation of the test solution.

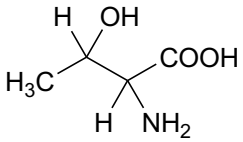
Procedure Proceed as directed in the Assay for Thiamine Hydrochloride, using the test solution and the standard solution. Calculate the content by the formula:

Content (%) of thiamine thiocyanate ($C_{13}H_{17}N_5OS_2$)

$$= \frac{\text{Anhydrous basis weight (g) of Thiamine Hydrochloride Reference Standard}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 0.9590 \times 100$$

DL-Threonine

DL-トレオニン



$C_4H_9NO_3$ Mol. Wt. 119.12

2-Amino-3-hydroxybutanoic acid [80-68-2]

Content DL-Threonine, when calculated on the dried basis, contains 98.0–102.0% of DL-threonine ($C_4H_9NO_3$).

Description DL-Threonine occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and has a slightly sweet taste.

Identification

(1) To 5 mL of a solution of DL-Threonine (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) To 5 mL of a solution of DL-Threonine (1 in 10), add 0.5 g of potassium periodate, and heat in a water bath. An evolved gas changes the color of a litmus paper (red) moistened with water to blue.

(3) A solution of DL-Threonine (1 in 25) has no optical rotation.

pH 5.0－6.5 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.021% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Allothreonine

Test Solution Weigh 0.10 g of DL-Threonine, and dissolve it in water to make 50 mL.

Procedure Analyze 5 μL of the test solution by paper chromatography using a 5:3:1:1 mixture of 1-butanol/2-butanone/water/ammonia TS as the developing solvent. No control solution is used. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the developing solvent has ascended to a point about 30 cm above the starting line. Air-dry the filter paper, then dry at 100°C for 20 minutes, spray with a solution of ninhydrin in acetone (1 in 50), and dry at 100°C for 5 minutes. Examine the chromatogram in daylight. Only one spot is observed.

Loss on Drying Not more than 0.2% (105°C, 3 hours).

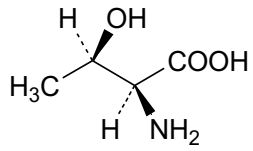
Residue on Ignition Not more than 0.1%.

Assay Proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 11.91 mg of $C_4H_9NO_3$

L-Threonine

L-トレオニン



$C_4H_9NO_3$ Mol. Wt. 119.12

(2*S*,3*R*)-2-Amino-3-hydroxybutanoic acid [72-19-5]

Content L-Threonine, when calculated on the dried basis, contains 98.0–102.0% of L-threonine ($C_4H_9NO_3$).

Description L-Threonine occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and has a slightly sweet taste.

Identification

(1) Proceed as directed in Identification (1) for DL-Threonine.

(2) To 0.5 g of L-Threonine, add 5 mL of water, dissolve it by warming, and proceed as directed in Identification (2) for DL-Threonine.

Specific Rotation $[\alpha]_D^{20}$: –26.0 to –29.0° (3 g, water, 50 mL, on the dried basis).

pH 5.0–6.5 (0.2 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.021% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of L-Threonine, and dissolve it in 5 mL of diluted hydrochloric acid (1 in 4).

(5) Allothreonine Proceed as directed in Purity (5) for DL-Threonine.

Loss on Drying Not more than 0.2% (105°C, 3 hours).

Residue on Ignition Not more than 0.1%.

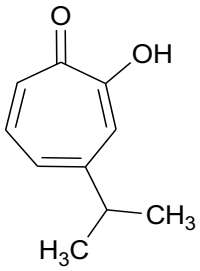
Assay Proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 11.91 mg of $C_4H_9NO_3$

Thujaplicin (Extract)

Hinokitiol (Extract)

ツヤプリシン (抽出物)



$C_{10}H_{12}O_2$ Mol. Wt. 164.20

2-Hydroxy-4-(1-methylethyl)cyclohepta-2,4,6-trien-1-one [499-44-5]

Definition Thujaplicin (Extract) is obtained from the trunks, branches, or roots of the tree *Thujopsis dolabrata* (L. f.) Siebold & Zucc. and consists mainly of thujaplicins.

Content Thujaplicin (Extract), when dried, contains 98.0–102.0% of β-thujaplicin ($C_{10}H_{12}O_2$ = 164.20).

Description Thujaplicin (Extract) occurs as white to yellow crystals, crystalline powder, or lumps. It has a characteristic odor.

Identification Dissolve 0.1 g of Thujaplicin (Extract) in 10 mL of ethanol (95), and add 1 drop of iron(III) chloride TS. A dark red color develops.

Purity

(1) Clarity of solution　Clear (1.0 g, ethanol (95) 5.0 mL).

(2) Lead　Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying　Not more than 0.5 % (1 g, 1.7–2.0 kPa, 4 hours).

Residue on Ignition　Not more than 0.05 %.

Ignite a platinum, quartz or porcelain crucible for about 30 minute according to the specified operating conditions, allow it to cool in a desiccator, and weigh it accurately. Transfer about 2 g of Thujaplicin (Extract), and weigh accurately the crucible containing the sample. Heat gradually to almost carbonize or sublime the sample at the lowest possible temperature, and allow to cool. Moisten the residue with sulfuric acid, incinerate completely, and ignite in an electric muffle at 450–550°C for 3 hours. Allow the crucible to cool in the desiccator, and weigh it accurately. If the percentage obtained does not meet the standard value, ignite the residue to constant weight.

Assay

Test Solution　Weigh accurately about 0.2 g of Thujaplicin (Extract), previously dried, add exactly 1 mL of the internal standard solution, and add ethanol (95) to make exactly 100 mL.

Standard Solution　Weigh accurately about 0.2 g of β-thujaplicin for assay, previously dried, add exactly 1 mL of the internal standard solution, and add ethanol (95) to make exactly 100 mL.

Internal Standard　Add ethanol (99.5) to 1.0 g of diphenyl ether to make exactly 5 mL.

Procedure　Analyze 0.5 μL each of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratios (Q_T and Q_S) of β-thujaplicin to diphenyl ether for the test solution and the standard solution. Calculate the content of β-thujaplicin by the formula:

$$\text{Content (\%) of β-thujaplicin } (C_{10}H_{12}O_2) = \frac{\text{Weight (g) β-thujaplicin for assay}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Upon injection at 100°C, raise the temperature at a rate of 10°C per minute to 250°C.

Injection port temperature: 250°C.

Carrier gas: Helium.

Flow rate: Adjust so that the peak of β-thujaplicin appears about 7 minutes after injection.

Injection method: Split.

Split ratio: 1 : 10.

Titanium Dioxide

二酸化チタン

TiO_2 Mol. Wt. 79.87

Titanium dioxide [13463-67-7]

Content Titanium Dioxide, when dried, contains not less than 99.0% of titanium dioxide (TiO_2) to a sample from which aluminum oxide and silicon dioxide are removed.

Description Titanium Dioxide occurs as a white powder. It is odorless and tasteless.

Identification To 0.5 g of Titanium Dioxide, add 5 mL of sulfuric acid, and heat gently until fumes of sulfuric acid are evolved. After cooling, add water gradually to make about 100 mL, and filter. To 5 mL of the filtrate, add hydrogen peroxide TS. A yellow-red to orange-red color develops.

Purity

(1) Water-soluble substances Not more than 0.25%.

Weigh 4.0 g of Titanium Dioxide, add 50 mL of water, shake, and allow to stand overnight. Add 2 mL of ammonium chloride solution (1 in 10), and shake. If a precipitate is not formed, add another 2 mL of ammonium chloride solution (1 in 10), and allow to stand. After the precipitate is formed, add water to make 200 mL, and filter while shaking. Discard 10 mL of the initial filtrate, transfer 100 mL of the subsequent filtrate into a platinum crucible, previously weighed, evaporate to dryness, ignite to constant weight, and weigh the residue.

(2) Hydrochloric acid-soluble substances Not more than 0.50%.

Weigh 5.0 g of Titanium Dioxide, add 100 mL of diluted hydrochloric acid (1 in 20), shake, heat on a water bath for 30 minutes with occasional stirring, and filter. Wash the residue three times with 10 mL of diluted hydrochloric acid (1 in 20) each time, combine the washings with the filtrate, evaporate to dryness, ignite to constant weight, and weigh

the residue.

(3) Lead Not more than 10 μg/g as Pb (4.0 g, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Test Solution To the specified amount of Titanium Dioxide, add 50 mL of diluted hydrochloric acid (1 in 20), and boil gently for 20 minutes with a watch glass covering it. Centrifuge the mixture to precipitate the insoluble matter, filter the supernatant to remove the insoluble matter, and wash the residue on the filter paper and the container used for centrifugation three times with 10 mL of hot water each time. Add the washings to the filtrate through the filter paper. Wash again the filter paper with 10–15 mL of hot water, and add the washings to the filtrate. After cooling, add water to make 100 mL. Use this solution as the sample solution. To 10 mL of the sample solution, add hydrochloric acid (25% of the volume of the sample solution), and heat gently to dryness. Warm the residue with a little amount of diluted nitric acid (1 in 100), and cool. Add diluted nitric acid (1 in 100) again to make exactly 10 mL.

Control Solution Measure exactly the specified amount of Lead Standard Solution, and add diluted nitric acid (1 in 100) to make exactly 10 mL.

(4) Arsenic Not more than 1 μg/g as As (10 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of Titanium Dioxide into a 250-mL beaker, add 50 mL of diluted hydrochloric acid (1 in 20), and heat to boiling with a watch glass covering it. Boil it gently for an additional 15 minutes. Centrifuge the mixture to precipitate the insoluble matter, filter the supernatant, and wash the residue on the filter paper and the container used for centrifugation three times with 10 mL of hot water each time. Add the washings to the filtrate through the filter paper. Wash the filter paper with 10–15 mL of hot water, and add the washings to the filtrate. After cooling, add water to make 100 mL. Use this solution as the sample solution. Use 15 mL of the sample as the test solution.

(5) Aluminum oxide and silicon dioxide Not more than 2.0%.

Test Solution Weigh accurately about 0.5 g of dried Titanium Dioxide into a platinum or nickel crucible, mix with 5 g of potassium hydroxide and 2 g of boric acid, melt the mixture completely by heating, and allow to cool. Place the crucible in a 250-mL polypropylene or polytetrafluoroethylene beaker, add 150 mL of hot water, and move the crucible into it while warming if necessary to dissolve or suspend the solid matter in the crucible. Remove the crucible from the water and wash it with a little amount of water, and add the washings to the beaker. Add 50 mL of hydrochloric acid to the beaker, and shake. Transfer the content into a polypropylene volumetric flask to make up to 250 mL. Use this solution as the sample solution. Dilute the sample solution with diluted hydrochloric acid (1 in 20) to four times the original volume.

Standard Solutions Measure exactly the appropriate volumes of Aluminum Standard Stock Solution and Silicon Standard Stock Solution, and add diluted hydrochloric acid (1 in 20) to prepare at least three standard solutions with graded concentrations in range of 0.2–10 μg.

Procedure Measure emission intensities of the test solution and the standard solutions as directed in Inductive Coupled Plasma-Atomic Emission Spectrometry. Prepare a calibration curve from the emission intensities of the standard solutions, and determine the aluminum concentration, C_A (μg/mL), and the silicon concentration, C_B (μg/mL), in the test solution to calculate the sum of the contents of aluminum oxide and silicon dioxide by the formula:

$$\text{Sum (\%) of aluminum oxide and silicon dioxide} = \frac{C_A \times 1.889 + C_B \times 2.139}{\text{Weight (g) of the sample} \times 10}$$

Loss on Drying Not more than 0.5% (105°C, 3 hours).

Loss on Ignition Not more than 0.5% (dried substance, 775–825°C).

Assay Prepare a test solution by diluting the sample solution prepared in Purity (5) with diluted hydrochloric acid (1 in 20) exactly to 1000 times its volume. Prepare at least three standard solutions with graded concentrations containing titanium at 0.2–2 μg/mL by diluting Titanium Standard Solution, exactly measured, with diluted hydrochloric acid (1 in 20). Measure emission intensities of the test solution and the standard solutions as directed under Inductive Coupled Plasma-Atomic Emission Spectrometry. Prepare a calibration curve from the emission intensities of the standard solutions, and determine the titanium concentration, C (μg/mL), in the test solution to calculate the titanium dioxide content by the formula:

$$\text{Content (\%) of titanium dioxide} = \frac{C \times 25 \times 1.668}{M \times (100 - a)} \times 100$$

C = titanium concentration (μg/mL) in the test solution,

M = weight (g) of the sample,

a = the sum (%) of aluminum oxide and silicon dioxide.

d-α-Tocopherol

α-Vitamin E

d-α-トコフェロール

[59-02-9]

Definition *d*-α-Tocopherol is obtained by isolation from vegetable fats and oils, which are produced from oil seeds, or mixed tocopherols (products consisting mainly of *d*-*α*-, *d*-*β*-, *d*-*γ*-, and *d*-δ-tocopherols, obtained from vegetable fats and oils). It consists mainly of *d*-α-tocopherol. It may contain edible fats or oils.

Content *d*-α-Tocopherol contains not less than 40% of total tocopherols, of which not

less than 50% consists of *d*-α-tocopherol.

Description *d*-α-Tocopherol is a light yellow to red-brown, clear viscous liquid having a slight characteristic odor.

Identification Dissolve 50 mg of *d*-α-Tocopherol in 10 mL of ethanol (99.5), add 2 mL of nitric acid, and heat at about 75°C for 15 minutes. An orange to red color develops.

Specific Rotation $[\alpha]_D^{20}$: not less than +24°.

Weigh accurately an amount of *d*-α-Tocopherol equivalent to about 0.1 g of the total tocopherols, transfer into a separating funnel, and dissolve it in 50 mL of diethyl ether. Add 2 g of potassium hexacyanoferrate(III) dissolved in 20 mL of sodium hydroxide solution (1 in 125), and shake for 3 minutes. Wash the diethyl ether layer with four 50-mL portions of water, and collect the diethyl ether layer. Dehydrate the diethyl ether layer by adding about 2 g of anhydrous sodium sulfate, and filter. Evaporate the diethyl ether from the filtrate, immediately dissolve the residue in 5 mL of 2,2,4-trimethylpentane, and measure the optical rotation. Calculate the specific rotation of this solution, using the concentration (g/mL) of the total tocopherols in the solution determined as directed in the Assay.

Purity

(1) Acid value Not more than 5.0.

Proceed as directed in Purity (1) for Tocotrienol.

(2) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay

Test Solution Weigh accurately an amount of *d*-α-Tocopherol equivalent to about 50 mg of the total tocopherols, transfer into a brown volumetric flask, and dissolve it in hexane to make exactly 100 mL.

Standard Solution Weigh accurately about 50 mg each of *d*-α-tocopherol for assay, *d*-β-tocopherol for assay, *d*-γ-tocopherol for assay, and *d*-δ-tocopherol for assay into separate brown 100-mL volumetric flasks, and dissolve each in hexane to make standard stock solutions. Transfer exactly an appropriate amount of each of the standard stock solutions into a volumetric flask so that the relative proportion of tocopherols in the resulting solution is almost the same as that in the sample. Mix them to prepare a standard solution.

Procedure Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas of *d*-α-tocopherol, *d*-β-tocopherol, *d*-γ-tocopherol, and *d*-δ-tocopherol for the test solution and the standard solution, and express as $A_{T\alpha}$, $A_{T\beta}$, $A_{T\gamma}$, and $A_{T\delta}$ for the test solution and $A_{S\alpha}$, $A_{S\beta}$, $A_{S\gamma}$, and $A_{S\delta}$ for the standard solution, respectively. Calculate the content of the total tocopherols using the following formula, and then determine the

percentage of *d*-α-tocopherol in the total tocopherols.

$$\text{Content (\%) of total tocopherols} = \left(\frac{A_{T\alpha}}{A_{S\alpha}} \times S_{\alpha} + \frac{A_{T\beta}}{A_{S\beta}} \times S_{\beta} + \frac{A_{T\gamma}}{A_{S\gamma}} \times S_{\gamma} + \frac{A_{T\delta}}{A_{S\delta}} \times S_{\delta}\right) \times \frac{1}{\text{Weight (g) of the sample}} \times 100$$

S_{α} = amount (g) of *d*-α-tocopherol in 100 mL of the standard solution,

S_{β} = amount (g) of *d*-β-tocopherol in 100 mL of the standard solution,

S_{γ} = amount (g) of *d*-γ-tocopherol in 100 mL of the standard solution,

S_{δ} = amount (g) of *d*-δ-tocopherol in 100 mL of the standard solution.

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 292 nm).

Column: A stainless steel tube of 3–6 mm internal diameter and 15–25 cm length.

Column packing material: 5- to 10-μm silica gel for liquid chromatography.

Column temperature: A constant room temperature.

Mobile phase: A 200:1 mixture of hexane/2-propanol.

Flow rate: Adjust the retention time of *d*-α-tocopherol to about 5 minutes.

d-γ -Tocopherol

γ-Vitamin E

d-γ - トコフェロール

Definition *d*-γ-Tocopherol is obtained by isolation from vegetable fats and oils, which are produced from oil seeds, or mixed tocopherols (products consisting mainly of *d*-α-, *d*-β-, *d*-γ-, and *d*-δ-tocopherols, obtained from vegetable fats and oils). It consists mainly of *d*-γ-tocopherol. It may contain edible fats or oils.

Content *d*-γ-Tocopherol contains not less than 40% of total tocopherols, of which not less than 70% consists of *d*-γ- tocopherol.

Description *d*-γ-Tocopherol is a light yellow to red-brown, clear viscous liquid having a slight, characteristic odor.

Identification Dissolve 50 mg of *d*-γ-Tocopherol in 10 mL of ethanol (99.5), add 2 mL of nitric acid, and heat at about 75°C for 15 minutes. An orange to red color develops.

Specific Rotation $[\alpha]_D^{20}$: Not less than +20°. Proceed as directed in Specific Rotation for *d*-α-Tocopherol.

Purity

(1) Acid value Not more than 5.0.

Proceed as directed in Purity (1) for Tocotrienol.

(2) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay Proceed as directed in the Assay for *d*-α-Tocopherol.

d-δ-Tocopherol

δ-Vitamin E

***d*-δトコフェロール**

Definition *d*-δ-Tocopherol is obtained by isolation from vegetable fats and oils, which are produced from oil seeds, or mixed tocopherols (products consisting mainly of *d*-α-, *d*-β-, *d*-γ-, and *d*-δ-tocopherols, obtained from vegetable fats and oils). It consists mainly of *d*-δ-tocopherol. It may contain edible fats or oils.

Content *d*-δ-Tocopherol contains not less than 40% of total tocopherols, of which not less than 60% consists of *d*-δ-tocopherol.

Description *d*-δ-Tocopherol is a light yellow to red-brown, clear viscous liquid having a slight, characteristic odor.

Identification Dissolve 50 mg of *d*-δ-Tocopherol in 10 mL of ethanol (99.5), add 2 mL of nitric acid, and heat at about 75°C for 15 minutes. An orange to red color develops.

Specific Rotation $[\alpha]_D^{20}$: Not less than +20°.

Proceed as directed in Specific Rotation for *d*-α-Tocopherol.

Purity

(1) Acid value Not more than 5.0.

Proceed as directed in Purity (1) for Tocotrienol.

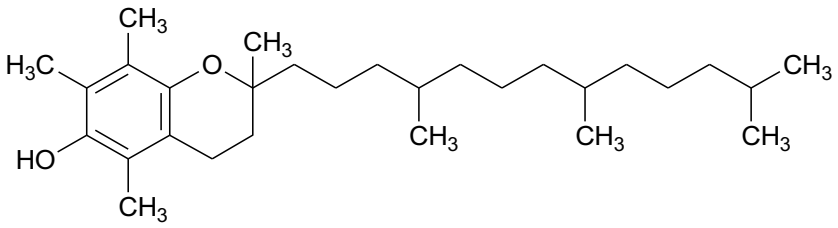
(2) Lead Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(3) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay　Proceed as directed in the Assay for *d*-α-Tocopherol.

dl-α-Tocopherol

***dl*-α-トコフェロール**



$C_{29}H_{50}O_2$　　Mol. Wt. 430.71

2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol

Content　*dl*-α-Tocopherol contains 96.0–102.0% of *dl*-α-tocopherol ($C_{29}H_{50}O_2$).

Description　*dl*-α-Tocopherol is a light yellow to red-brown, clear, viscous liquid. It is odorless.

Identification　Proceed as directed in Identification for *d*-α-Tocopherol.

Specific Absorbance　$E_{1cm}^{1\%}$ (292 nm) : 71.0－76.0.

Weigh accurately about 0.1 g of *dl*-α-Tocopherol, and dissolve it in ethanol (99.5) to make exactly 100 mL. Measure exactly 5 mL of this solution, add ethanol (99.5) to make exactly 100 mL, and measure the absorbance.

Refractive Index　n_D^{20}: 1.503–1.507.

Purity

(1) Clarity of solution　Clear (0.10 g, ethanol (99.5) 10 mL).

(2) Lead　Not more than 2 μg/g as Pb (5.0 g, Method 2, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(3) Arsenic　Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay

Test Solution and Standard Solution　Weigh accurately about 50 mg each of *dl*-α-Tocopherol and *dl*-αTocopherol Reference Standard into separate brown 50-mL volumetric flasks, and add ethanol (99.5) to make two solutions of exactly 50 mL each. Use them as the test solution and as the standard solution, respectively.

Procedure　Analyze 20 μL each of these solutions by liquid chromatography using

the operating conditions given below. Measure peak heights (H_T and H_S) of *dl*-α-Tocopherol for the test solution and the standard solution, and obtain the content by the formula:

$$\text{Content (\%) of } dl\text{-}\alpha\text{-tocopherol}(C_{29}H_{50}O_2) = \frac{\text{Weight (g) of } dl\text{-}\alpha\text{-Tocopherol Reference Standard}}{\text{Weight (g) of the sample}} \times \frac{H_T}{H_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 292 nm).

Column: A stainless steel tube of 4.6 mm internal diameter and 15 cm length.

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of around 35°C.

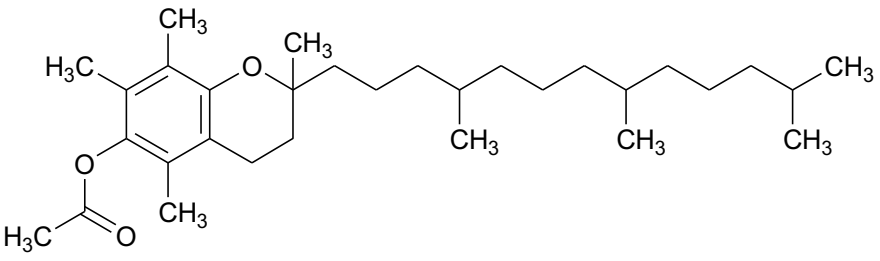
Mobile phase: A 49:1 mixture of methanol/water.

Flow rate: Adjust the retention time of *dl*-α-tocopherol to about 10 minutes.

Column selection: The column should be capable of resolving the peaks of *dl*-α-tocopherol and tocopherol acetate, in this order, with a resolution rate of 2.6 or more when 20 μL of a solution containing 50 mg each of *dl*-α-Tocopherol and tocopherol acetate in 50 mL of ethanol (99.5) is chromatographed using the conditions given above. The relative standard deviation of peak heights of *dl*-α-tocopherol is 0.8% or less in five repetitive tests conducted on the standard solution using the given operating conditions.

All-*rac*-α-Tocopheryl Acetate

トコフェロール酢酸エステル



$C_{31}H_{52}O_3$ Mol. Wt. 472.74

2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl acetate [7695-91-2]

Content All-*rac*-α-Tocopheryl Acetate contains 96.0–102.0% of *all-rac*-α-tocopheryl acetate ($C_{31}H_{52}O_3$).

Description All-*rac*-α-Tocopheryl Acetate occurs as a colorless to yellow, clear viscous liquid. It is odorless.

Identification

(1) Dissolve 50 mg of *all-rac*-α-Tocopheryl Acetate in 10 mL of ethanol (99.5), add 2 mL of nitric acid, and heat at about 75°C for 15 minutes. An orange to red color develops.

(2) Determine the absorption spectrum of *all-rac*-α-Tocopheryl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry. Compare the spectrum obtained with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) A solution (1 in 10) of *all-rac*-α-Tocopheryl Acetate in ethanol (95) has no optical rotation.

Specific Absorbance $E_{1cm}^{1\%}$ (284 nm): 41.0–45.0. Dissolve about 10 mg of *all-rac*-α-Tocopheryl Acetate, accurately weighed, in ethanol (99.5) to make exactly 100 mL. Measure the absorbance of the resulting solution.

Refractive Index n_D^{20}: 1.494-1.499.

Specific Gravity d_{20}^{20}: 0.952–0.966.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) α-Tocopherol

Test Solution Weigh exactly 0.10 g of *all-rac*-α-Tocopheryl Acetate, and dissolve it in 10 mL of hexane, measured exactly.

Control Solution Weigh exactly 50 mg g of *dl*-α-Tocopherol Reference Standard, and dissolve it in hexane to make exactly 100 mL. To 1 mL of this solution, measured exactly, add hexane to make exactly 10 mL.

Procedure Analyze 10 μL each of the test solution and the control solution by thin-layer chromatography using a 19:1 mixture of toluene/acetic acid as the developing solution. Use a thin-layer plate coated with silica gel for thin-layer chromatography, as the solid support, and then dried at 110°C for 1 hour. Stop the development when the solvent front ascends to a point about 10 cm above the starting line, and air-dry the plate. Spray uniformly with a solution (1 in 500) of iron(III) chloride hexahydrate in ethanol (99.5), then with a solution (1 in 200) of 2,2′-dipyrizil in ethanol (99.5), and allow to stand for 2–3 minutes. A spot from the test solution is not larger in size or darker in color than the corresponding spot from the control solution.

Assay

Test Solution Weigh accurately about 50 mg of *all-rac*-α-Tocopheryl Acetate, and dissolve it in ethanol (99.5) to make exactly 50 mL.

Standard Solution Weigh accurately about 50 mg Tocopheryl Acetate Reference Standard, and dissolve it in ethanol (99.5) to make exactly 50 mL.

Procedure Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions give below. Measure the peak heights (H_T and H_S) of *all-rac*-α-tocopheryl acetate for the test solution and the standard solution, and determine the content by the formula:

$$\text{Content (\%) of } \textit{all-rac}\text{-α-tocopheryl acetate } (C_{31}H_{52}O_3) = \frac{\text{Weight (g) of Tocopheryl Acetate Reference Standard}}{\text{Weight (g) of the sample}} \times \frac{H_T}{H_S} \times 100$$

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 284 nm).

Column: A stainless steel tube (4.6 mm of internal diameter and 15 cm length).

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature of about 35°C.

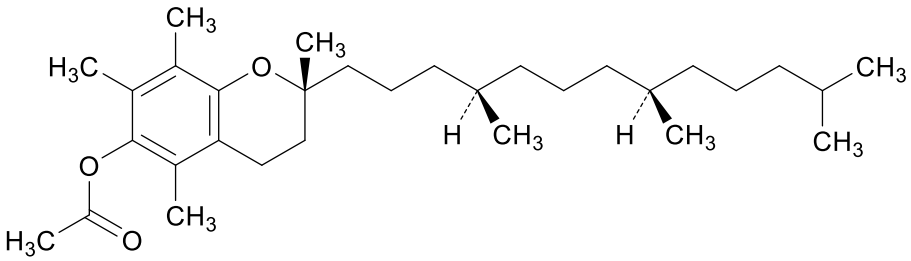
Mobile phase: A 49:1 mixture of methanol/water.

Flow rate: Adjust the retention time of *all-rac*-α-tocopheryl acetate to about 12 minutes.

Column selection: Use a column that is capable of eluting *dl*-α-tocopherol and *all-rac*-α-tocopheryl acetate in the order listed and of giving a resolution of not less than 2.6 when 20-μL portions of the solutions prepared by dissolving 50 mg each of *all-rac*-α-Tocopheryl Acetate and *dl*-α-Tocopherol Reference Standard separately in 50 mL of ethanol (99.5) are chromatographed. When the standard solution is chromatographed 5 times under the above conditions, the relative standard deviation of the peak height of *all-rac*-α-tocopheryl acetate is not more than 0.8%.

R,R,R-α-Tocopheryl Acetate

***d*-α-トコフェロール酢酸エステル**



$C_{31}H_{52}O_3$ Mol. Wt. 472.74

(2*R*)-2,5,7,8,-Tetramethyl-2-[(4*R*,8*R*)-4,8,12,-trimethyltridecyl]chroman-6-yl acetate

Content *R,R,R*-α-Tocopheryl Acetate contains 96.0–102.0% of *R,R,R*-α-tocopheryl acetate ($C_{31}H_{52}O_3$).

Description *R,R,R*-α-Tocopheryl Acetate occurs as a colorless to yellow, clear viscous liquid and may solidify when cooled. It is odorless or has a slight characteristic odor.

Identification Proceed as directed under Identification (1) and (2) for *all-rac*-α-Tocopheryl Acetate.

Specific Absorbance $E_{1cm}^{1\%}$(284 nm): 41.0–45.0.

Proceed as directed in Specific Absorbance for *all*-rac-α-Tocopheryl Acetate.

Refractive Index n_D^{20}: 1.494–1.499.

Specific Rotation $[\alpha]_D^{20}$: Not less than +24° (*d*-α-tocopherol equivalent).

Weigh accurately about 0.22 g of *R,R,R*-α-Tocopheryl Acetate into an eggplant-shaped flask, and add 50 mL of diluted sulfuric acid (3 in 50) in ethanol (99.5) to dissolve it. Reflux under a reflux condenser for three hours. After cooling, add 100 mL of water, and extract three times with 50 mL of diethyl ether each time. Combine the diethyl ether layers in a separating funnel, and add 50 mL of water. Mix gently by turning the funnel upside down 2 to 3 times, and allow to stand. Remove the water layer. Wash the diethyl ether layer three times with 50 mL of water each time by shaking increasingly vigorously. Remove the water layer, and add 40 mL of potassium hexacyanoferrate(III)–sodium hydroxide (0.2 mol/L) TS (1 in 10). Shake vigorously for 3 minutes, and remove the water layer. Wash the diethyl ether layer four times with 50 mL of water each time, and transfer into an Erlenmeyer flask. Wash the funnel twice with 10 mL of diethyl ether each time, and add the diethyl ether into the Erlenmeyer flask. Dry the diethyl ether layer with sodium sulfate, and decant the diethyl ether extract into an eggplant-shaped flask. Wash the remaining sodium sulfate twice with 10 mL of diethyl ether each time, and add the washings into the eggplant-shaped flask. Evaporate to 7–8 mL under reduced pressure in a 40°C water bath. Remove the solvent under reduced pressure without applying heat. Immediately add exactly 10 mL of 2,2,4-trimethylpentane to dissolve the residue. Measure the specific rotation of the resulting solution.

$$[\alpha]_D^{20} = \frac{1000 \times \alpha}{M \times P \times 0.911}$$

α = the angle (°) of rotation, in degrees, of the plane of polarization,

M = the amount (g) of the sample,

P = the content (%) of *R,R,R*-α- tocopheryl acetate in the sample,

0.911 = the conversion factor of *d*-α-tocopherol.

Specific Gravity d_{20}^{20}: 0.952–0.966.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) α-Tocopherol

Proceed as directed under Purity (2) for *all-rac*-α-Tocopheryl Acetate.

Assay Proceed as directed under the Assay for *all-rac*-α-Tocopheryl Acetate.

Tocotrienol

トコトリエノール

Definition Tocotrienol is obtained by isolating and purifying bran oil from the rice plant *Oryza sativa* L. or oil from the oil palm *Elaeis guineensis* Jacq. It consists mainly of tocotrienols. It may contain edible fats or oils.

Content Tocotrienol contains not less than 25% of total tocotrienols.

Description Tocotrienol is a yellow to red-brown, viscous liquid having a slight, characteristic odor.

Identification Dissolve 50 mg of Tocotrienol in 10 mL of ethanol (99.5), add 2 mL of nitric acid, and heat at about 75°C for 15 minutes. An orange to red color develops.

Specific Gravity d_{20}^{20}: 0.94–0.99.

Purity

(1) Acid value Not more than 5.0.

Test Solution Weigh accurately about 2.5 g of Tocotrienol, and add 50 mL of a 1:1 mixture of ethanol (95)/diethyl ether to which 0.02 mol/L ethanolic potassium hydroxide has been previously added until the faint pink color of the solution persists for 30 seconds (indicator: 2–3 drops of phenolphthalein TS).

Procedure Add a few drops of phenolphthalein TS to the test solution, and titrate with 0.02 mol/L ethanolic potassium hydroxide to the first faint pink color that persists for 30 seconds. Determine the acid value, using the formula given below.

$$\text{Acid value} = \frac{\text{Volume (mL) of 0.02 mol/L ethanolic potassium hydroxide consumed} \times 5.611}{\text{Weight (g) of the sample} \times 5}$$

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead

Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 1.5 μg/g as As (1.0 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay

Test Solution Weigh accurately an appropriate amount of Tocotrienol equivalent to about 25 mg of total tocotrienols into a brown volumetric flask, dissolve it in hexane to make exactly 100 mL.

Standard Solutions Weigh accurately about 50 mg each of *d*-α-, *d*-β-, *d*-γ-, and *d*-δ-tocopherols for assay in separate brown 100-mL volumetric flasks, and dilute each with hexane to volume to prepare standard stock solutions. Prepare a standard solution, using the standard stock solutions so that it contains *d*-α-, *d*-β-, *d*-γ-, and *d*-δ-tocopherols at almost the same ratio as that of the corresponding tocotrienols in the sample (the approximate ratio of tocotrienols in the sample should be determined by conducting preliminary tests).

Procedure Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas ($A_{T\alpha}$, $A_{T\beta}$, $A_{T\gamma}$, and $A_{T\delta}$) of individual tocotrienols for the test solution and the peak areas ($A_{S\alpha}$, $A_{S\beta}$, $A_{S\gamma}$, and $A_{S\delta}$) of individual tocophenols for the standard solution. Determine the content of each tocotrienol, using the formula given below. The relative retention times of corresponding *d*-α-tocotrienol, *d*-β-tocotrienol, *d*-γ-tocotrienol, and *d*-δ-tocotrienol to *d*-α-tocopherol, *d*-β-tocopherol, *d*-γ-tocopherol, and *d*-δ-tocopherol are about 1.1–1.3.

Operating Conditions

Detector: Ultraviolet absorption spectrophotometer (wavelength: 292 nm).

Column: A stainless steel tube of 3–6 mm internal diameter and 15–25 cm length.

Column packing material: 5- to 10-μm silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 197:2:1 mixture of hexane/1,4-dioxane/2-propanol.

Flow rate: Adjust the retention time of *d*-α-tocopherol to about 7–8 minutes.

$$\text{Content (\%) of total tocotrienols} = \left(\frac{A_{T\alpha}}{A_{S\alpha}} \times S_\alpha + \frac{A_{T\beta}}{A_{S\beta}} \times S_\beta + \frac{A_{T\gamma}}{A_{S\gamma}} \times S_\gamma + \frac{A_{T\delta}}{A_{S\delta}} \times S_\delta\right) \times \frac{1}{\text{Weight (g) of the sample}} \times 100$$

S_α = amount (g) of *d*-α-tocopherol in 100 mL of the standard solution,

S_β = amount (g) of *d*-β-tocopherol in 100 mL of the standard solution,

S_γ = amount (g) of *d*-γ-tocopherol in 100 mL of the standard solution,

S_δ = amount (g) of *d*-δ-tocopherol in 100 mL of the standard solution.

Tomato Color

トマト色素

Definition Tomato Color is obtained from the fruits of the tomato plant *Lycopersicon esculentum* Mill. (*Solanum lycopersium* L.) and consists mainly of lycopene. It may contain edible fats or oils.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Tomato Color is not less than 300 and is in the range of 95–115% of the labeled value.

Description Tomato Color occurs as a brown to dark red powder, as lumps, or as a paste or liquid. It has a slight, characteristic odor.

Identification

(1) Weigh an amount of Tomato Color equivalent to 0.1 g of tomato color with a Color Value 300, and dissolve it in 100 mL of ethyl acetate. An orange color develops.

(2) A solution of Tomato Color in hexane exhibits absorption maxima at wavelengths of 438–450 nm, 465–475 nm, and 495–505 nm.

(3) Weigh an amount of Tomato Color equivalent to 0.1 g of tomato color with a Color Value 300, and dissolve it in 10 mL of ethyl acetate. Use this solution as the test solution. Analyze a 5-μL portion of the test solution by thin-layer chromatography using a 7:3 mixture of hexane/acetone as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. A yellow-red spot of lycopene is observed at an R_f value of about 0.7–0.8. This color immediately disappears when the spot is sprayed with sodium nitrite solution (1 in 20) followed by sulfuric acid TS (0.5 mol/L).

Purity

(1) Lead Not more than 1 μg/g as Pb (4.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Color Value

Test Solution Weigh accurately an appropriate amount of Tomato Color, dissolve it in 25 mL of a 1:1 mixture of acetone/cyclohexane, and add hexane to make exactly 100 mL. Measure exactly 2 mL of this solution, and add hexane to make exactly 100 mL. Centrifuge the solution obtained if necessary, and use the supernatant as the test solution.

Procedure Conduct the test according to the operating conditions given below, as directed under Color Value Determination .

Operating Conditions

Solvent: Hexane.

Wavelength: Maximum absorption wavelength of 465–475 nm.

Tragacanth Gum

トラガントガム

[9000-65-1]

Definition Tragacanth Gum is obtained from the exudate of tragacanth (*Astracantha gummifera* (Labill.) Podl. (*Astragalus gummifer* Labill.)) and consists mainly of polysaccharides.

Description Tragacanth Gum occurs as a white to whitish powder or as white to light yellowish-white, translucent, flattened or lamellated fragments. It is odorless.

Identification

(1) To 1 g of powdered Tragacanth Gum, add 50 mL of water. An almost homogeneous, somewhat turbid viscous solution is formed.

(2) Place about 1.0 g of powdered Tragacanth Gum in a watch glass containing 2–3 drops of a 1:1 mixture of water/glycerol and 1 drop of iodine TS. Mix well with the end of a small glass rod, taking care to prevent air bubble formation. Allow to stand for 10 minutes or more and swell it. Apply small amount of swelled sample to a slide glass with the end of a small glass rod, add 1 drop of a 1:1 mixture of water/glycerol. Cover it with a cover glass, being careful not to allow air bubbles to be trapped, and examine by an optical microscope. A few blue granules of starch are found. For microscopic examination, use 10 or 40 times scale as an objective and 10 times scale as an eyepiece.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 2.0%.

Previously, dry a glass filter (1G3) for 30 minutes at 110°C, cool in a desiccators, and weigh accurately. Accurately weigh about 2 g of powdered Tragacanth Gum, add 95 mL of methanol to moisten and swell the powder. Add 60 mL of hydrochloric acid and boiling chips, and heat under a reflux condenser in a water bath for 3 hours with occasional

shaking. While warm, filter by suction with a glass filter (1G3), previously dried for 30 minutes at 110°C, cooled in a desiccators, and accurately weighed. Wash the residue well first with warm water and then with 40 mL of methanol again. Dry the residue together with the glass filter for 2 hours at 105°C. Allow to cool in a desiccator, and weigh accurately.

(2) Karaya gum Weigh accurately about 1.0 g of Tragacanth Gum, add 20 mL of water, and heat until a homogeneous mucilage is formed. Add 5 mL of hydrochloric acid, and boil for 5 minutes. No light red to red color is produced.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 17.0% (105°C, 5 hours).

Ash Not more than 4.0%.

Acid-insoluble Ash Not more than 0.5%.

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative pet test.

Sample Fluid Prepare as directed in Method 2 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture For the *Escherichia coli* test, prepare as directed in Method 2. For the *Salmonella* test, prepare as follows: Mix 1 g of Tragacanth Gum with 100 mL of lactose broth to disperse uniformly, and incubate at 35 ± 1°C for 24 ± 2 hours.

Transglucosidase

トランスグルコシダーゼ

Definition Transglucosidase includes enzymes that hydrolyze glucosidic linkages in maltose and oligosaccharides and transfers glucosyl groups. It is derived from the culture of filamentous fungi (limited to *Aspergillus niger* and *Aspergillus usamii*) or bacteria (limited to *Sulfolobus solfataricus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Transglucosidase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Transglucosidase complies with the Transglucosidase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Transglucosidase Activity Test Perform the test using either of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 1.0 g of Transglucosidase, and add acetic acid–sodium hydroxide buffer (0.01 mol/L, pH 4.0, containing acarbose) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold or 100-fold dilution by adding the same buffer to the resulting solution.

Substrate Solution Weigh 1.00 g of D(+)-maltose monohydrate, and dissolve it in acetic acid–sodium hydroxide buffer (0.01 mol/L, pH 4.0, containing acarbose) to make 25 mL.

Test Solution Add 0.5 mL of the sample solution to 0.5 mL of the substrate solution, equilibrated at 50°C for 10 minutes, and mix. Incubate the mixture at 50°C for 60 minutes, and heat it in a water bath for 10 minutes, and allow to cool. To this solution, add 9 mL of sulfuric acid TS (5.5 mmol/L), and mix gently.

Control Solution Add 0.5 mL of the sample solution to 0.5 mL of the substrate solution, equilibrated at 50°C for 60 minutes, mix them, and immediately shake the solution. Heat it in a water bath for 10 minutes, allow to cool, add 9 mL of sulfuric acid TS (5.5 mmol/L), and mix gently.

Standard Solution Dissolve 0.100 g of panose in sulfuric acid TS (0.005 mol/L) to

make 100 mL.

Procedure Filter the test solution, the control solution, and the standard solution through separate membrane filters (0.45 μm pore size), and analyze the filtrates by liquid chromatography. A peak is observed at the retention time of panose for the test solution. The peak area is larger than that of the panose peak of the control solution.

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube (7.8 mm internal diameter and 30 cm length).

Column packing material: 9-μm H-form cation exchange resin for liquid chromatography.

Column temperature: 60°C.

Mobile phase: Sulfuric acid TS (0.005 mol/L)

Flow rate: 0.7 mL/min.

Method 2

Proceed as directed in Method 2 of α-Glucosidase Activity Test in the monograph for α-Glucosidase.

Transglutaminase

トランスグルタミナーゼ

Definition Transglutaminase includes enzymes that catalyze the acyl transfer reaction in which the γ-carboxamide group of glutamine residues in proteins and peptides acts as an acyl donor, and the primary amine group in amine compounds or the ε-amino group of lysine residues in proteins or peptides acts an acyl receptor. It is derived from the liver of animals or the culture of actinomycetes (limited to *Streptoverticillium mobaraense* and species of the genus *Streptomyces*) or bacteria (limited to species of the genus *Bacillus*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Transglutaminase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Transglutaminase complies with the Transglutaminase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the

preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Transglutaminase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 0.10 g of Transglutaminase, add Tris buffer (0.2 mol/L) at pH6.0 to dissolve it or disperse it uniformly, and make 10 mL. As necessary, prepare a 10-fold or 100-fold dilution by adding the same buffer (0.2 mol/L, pH6.0) to the resulting solution.

Substrate Solution Weigh 4.048 g of benzyloxycarbonyl-L- glutaminylglycine, 2.780 g of hydroxylammonium chloride, 1.229 g of glutathione (reduced form), 0.295 g of calcium chloride dehydrate, and 9.688 g of 2-amino-2-hydroxy methyl-1,3-propanediol, and dissolve them in water, then adjust the pH of this solution to 6.0, and make 400 mL.

Test Solution Equilibrate 0.2 mL of the sample solution at 37°C for 1 minutes, add 2 mL of the substrate solution, equilibrated at 37°C for 10 minutes, and shake well immediately. Incubate the mixture at 37°C for 10 minutes. Add 2 mL of iron(III) chloride (for the transglutaminase activity test), and shake well immediately. Centrifuge this solution at 3000 rpm, and use the supernatant as the test solution.

Control Solution Equilibrate 2 mL of the substrate solution at 37°C for 10 minutes, add 2 mL of iron(III) chloride (for the transglutaminase activity test), and shake well immediately. Then add 0.2 mL of the sample solution, shake well, and centrifuge this solution. Use the supernatant as the control solution.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 525 nm. The absorbance value of the test solution is higher than that of the control solution.

Trehalose Phosphorylase

トレハロースホスホリラーゼ

Definition Trehalose Phosphorylase includes enzymes that phosphorolyze trehalose. It is derived from the culture of bacteria (*Paenibacillus* sp. and species of the genus *Plesiomonas*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Trehalose Phosphorylase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Trehalose Phosphorylase complies with the Trehalose Phosphorylase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Trehalose Phosphorylase Activity Test Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution Weigh 1.0 g of Trehalose Phosphorylase, add phosphate buffer (0.05 mol/L) at pH7.0 or water to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, or 100-fold, or 1000-fold dilution by adding the same buffer or water to the resulting solution.

Substrate Solution Weigh 3.78 g of trehalose dihydrate, and dissolve it in phosphate buffer (0.05 mol/L) at pH7.0 to make 500 mL.

Test Solution Add 0.01 mL of the sample solution to 0.5 mL of the substrate solution,

equilibrated at 50°C for 5 minutes, shake immediately. Incubate the mixture at 50°C for 15 minutes. Heat it in a water bath for 3 minutes, and allow it to cool. Add 2 mL of TS for D-glucose determination (containing mutarotase), mix, and warm at 37°C for 10 minutes.

Control Solution Add 0.01 mL of the sample solution to 0.5 mL of the substrate solution, heat the mixture immediately in a water bath for 3 minutes, and allow to cool. Add 2 mL of TS for D-glucose determination (containing mutarotase), mix, and warm at 37°C for 10 minutes.

Procedure Measure the absorbance of the test solution and the control solution at a wavelength of 505 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Tricalcium Phosphate

Calcium Phosphate, Tribasic
Tertiary Calcium Phosphate

リン酸三カルシウム

Definition Tricalcium Phosphate consists of a mixture of calcium phosphates having an approximate composition of $10CaO \cdot 3P_2O_5 \cdot H_2O$.

Content Tricalcium Phosphate, when dried, contains the equivalent of 98.0–103.0% of tricalcium phosphate ($Ca_3(PO_4)_2 = 310.18$).

Description Tricalcium Phosphate occurs as a white powder.

Identification

(1) Moisten Tricalcium Phosphate with silver nitrate solution (1 in 50). A yellow color develops.

(2) To 0.1 g of Tricalcium Phosphate, add 5 mL of diluted acetic acid (1 in 4), boil, cool, and filter. To the filtrate, add 5 mL of a solution of ammonium oxalate monohydrate (1 in 30). A white precipitate is formed.

Purity

(1) Clarity of solution Slightly turbid.

Weigh 2.0 g of Tricalcium Phosphate, add 15 mL of water and 5.0 mL of hydrochloric acid, and dissolve by heating for 5 minutes in a water bath.

(2) Carbonate Weigh 2.0 g of Tricalcium Phosphate, add 5 mL of water, and boil. After cooling, add 2 mL of hydrochloric acid. Little or no effervescence occurs.

(3) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Tricalcium Phosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it. Allow to cool, and add 30 mL of water. Use the resulting solution as the sample solution. If the sample does not dissolve completely, evaporate the solution to dryness, and add 20 mL of diluted hydrochloric acid (1 in 4) to the residue. Boil gently for 5 minutes with a watch glass covering it. Allow to cool, and add 30 mL of water.

In Method 5 of the Lead Limit Test, use 50 mL of a solution of diammonium hydrogen citrate (1 in 2) and 1mL of bromothymol blue TS as the indicator instead of 1 mL of thymol blue TS, and then add ammonia solution until the color of the solution changes from yellow to yellow-green.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

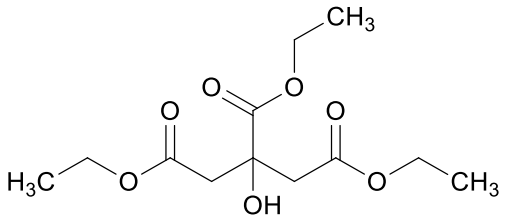
Loss on Drying Not more than 10.0% (200°C, 3 hours).

Assay Weigh accurately about 0.3 g of Tricalcium Phosphate, previously dried, dissolve it in 10 mL of diluted hydrochloric acid (1 in 4), and add water to make exactly 200 mL. Proceed as directed in Method 2 in Calcium Salt Determination, using this solution as the test solution.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.068 mg of $Ca_3(PO_4)_2$

Triethyl Citrate

クエン酸三エチル



$C_{12}H_{20}O_7$ Mol. Wt. 276.28

1,2,3-Triethyl 2-hydroxypropane-1,2,3-tricarboxylate [77-93-0]

Content Triethyl Citrate contains not less than 99.0% of triethyl citrate ($C_{12}H_{20}O_7$).

Description Triethyl Citrate occurs as a colorless oily liquid. It is odorless or has a slight characteristic odor.

Identification Determine the absorption spectrum of Triethyl Citrate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.440–1.444.

Specific Gravity d_{25}^{25}: 1.135–1.139.

Purity

(1) Free acids Not more than 0.02% as citric acid.

Weigh exactly 32.0 g of Triethyl Citrate, add 30 mL of ethanol (95), and titrate with 0.1 mol/L potassium hydroxide. The amount of 0.1 mol/L potassium hydroxide consumed is not more than 1.0 mL. Before the test, prepare ethanol (95) by adding 0.1 mol/L potassium hydroxide until the color of ethanol (95) is yellow-green using a few drops of bromothymol blue as the indicator.

(2) Lead Not more than 2 μg/g as Pb (5.0 g, Method 1, Control Solution: Lead Standard Solution 10 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.5 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Water Content Not more than 0.25% (5 g, Volumetric Titration, Direct Titration).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under Flavor Substance Tests. Use operating conditions (1). The column temperature should be raised to 230°C from 150°C at a rate of 5°C/minute and maintained for 24 minutes.

Trimagnesium Phosphate

Magnesium Phosphate, Tribasic
Tertiary Magnesium Phosphate

リン酸三マグネシウム

$Mg_3(PO_4)_2 \cdot nH_2O$ (n = 8, 5, or 4) Mol. Wt. octahydrate 406.98

tetrahydrate 334.92

Trimagnesium phosphate octahydrate [13446-23-6]

Trimagnesium phosphate pentahydrate

Trimagnesium phosphate tetrahydrate [13465-22-0]

Definition Trimagnesium Phosphate occurs as several crystalline compounds (octa-, penta-, and tetrahydrates).

Content Trimagnesium Phosphate, when ignited, contains 98.0–101.5% of trimagnesium phosphate anhydrous ($Mg_3(PO_4)_2$ = 262.86).

Description Trimagnesium Phosphate occurs as a white crystalline powder.

Identification

(1) Dissolve 0.2 g of Trimagnesium Phosphate in 10 mL of 10% nitric acid TS. To this solution, add a few drops of ammonium molybdate TS. A yellow precipitate is produced.

When ammonium TS is added, the precipitate dissolves, and a white precipitate is produced.

(2) Dissolve 0.1 g of Trimagnesium Phosphate by adding 0.7 mL of acetic acid TS (1 mol/L) and 20 mL of water. Add 1 mL of iron(III) chloride TS, allow to stand for 5 minutes, and filter. The filtrate responds to all the tests for Magnesium Salts in the Qualitative Tests.

Purity

(1) Clarity of solution Turbid.

Test Solution Weigh 2.0 g of Trimagnesium Phosphate, add 16 mL of water and 4.0 mL of 10% hydrochloric acid TS, and dissolve it by heating for 5 minutes on a water bath.

(2) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Trimagnesium Phosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Dissolve the specified amount of Trimagnesium Phosphate in 5 mL of 10% hydrochloric acid TS.

(4) Fluoride Not more than 5.0 μg/g as F.

Test Solution Weigh 1.0 g of Trimagnesium Phosphate in a beaker, and dissolve it in 10 mL of diluted hydrochloric acid (1 in 10). Heat the solution, boil for 1 minute, and transfer into a polyethylene beaker, and immediately cool with ice. Add 15 mL of a solution of trisodium citrate dihydrate (1 in 4) and 10 mL of a solution of disodium dihydrogen ethylenediaminetetraacetate dihydrate (1 in 40), and mix them. Adjust the pH of the mixture to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5). Transfer it into a 100-mL volumetric flask, and add water to make up to volume. Take 50 mL of the obtained solution in a polyethylene beaker.

Control Solution Weigh 2.210 g of sodium fluoride, previously dried at 110°C for 2 hours, in a polyethylene beaker, add 200 mL of water, and dissolve it by stirring. Transfer this solution into a 1000-mL volumetric flask, and add water to make up to volume. Transfer the solution into a polyethylene beaker, and use as the control stock solution. Place exactly 5 mL of the control stock solution into a 1000-mL volumetric flask, and add water to make up to volume. Transfer exactly 1 mL of this solution into a polyethylene beaker, add 15 mL of a solution of trisodium citrate dihydrate (1 in 4) and 10 mL of a solution of disodium dihydrogen ethylenediaminetetraacetate dihydrate (1 in 40), and mix. Adjust the pH of the mixture to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5). Transfer the resulting solution into a 100-

mL volumetric flask, and add water to make up to volume. Transfer 50 mL of the last solution into a polyethylene beaker.

Procedure Measure the electric potentials of the test solution and the control solution, using a potentiometer with a fluorine-ion indicator electrode and a silver/silver chloride reference electrode. The electric potential of the test solution is not lower than that of the control solution.

Loss on Ignition

Tetrahydrate: 15–23% (1.0g, 425°C, 3 hours).

Pentahydrate: 20–27% (1.0g, 425°C, 3 hours).

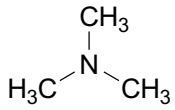
Octahydrate: 30–37% (1.0g, 425°C, 3 hours).

Assay Weigh accurately about 0.3 g of Trimagnesium Phosphate, previously ignited, and dissolve it by adding 50 mL of water and 5 mL of diluted hydrochloric acid (2 in 3). Then add 40 mL of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate, and heat in a water bath at 50°C for 30 minutes. After cooling, add about 10 mL of ammonium buffer (pH10.7), and titrate with 0.1 mol/L zinc acetate (indicator: 5 drops of eriochrome black T TS). The endpoint is when the blue color of the solution changes to blue-purple. Separately, perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate = 8.762 mg of $Mg_3(PO_4)_2$

Trimethylamine

トリメチルアミン



C_3H_9N Mol. Wt. 59.11

Trimethylamine [75-50-3]

Content Trimethylamine contains not less than 98.0% of trimethylamine (C_3H_9N).

Description Trimethylamine occurs as a colorless gas having a characteristic odor.

Identification Perform a test as directed in the Assay. A molecular ion peak (*m/z* 59), a standard peak (*m/z* 58), and fragment peaks (*m/z* 15, *m/z* 30, and *m/z* 42) are observed in the mass spectrum of the main peak.

Assay To 1 mL of water cooled to 0–4°C, add 0.1 g of Trimethylamine, previously cooled to −20°C, and dissolve it. Analyze the resulting solution by gas chromatography using operating conditions given below. Determine the peak area percentage of the component to be determined by normalizing the sum of the areas of all peaks, excluding those from water, that appear within 40 minutes after injection as 100.

Operating conditions

Detector: Mass spectrometer (electron impact ionization method)

Mass scanning range: m/z 10.00–300.00.

Column: Use a fused silica tube (0.25–0.53 mm internal diameter and 30–60 m length) coated with a 0.25–1 μm thick layer of dimethylpolysiloxane or polyethylene glycol for gas chromatography.

Column temperature: Maintain the temperature for 5 minutes at 50°C, and raise to 230°C at a rate of 5°C/minute.

Injection port temperature: 125–175°C.

Carrier gas: Helium.

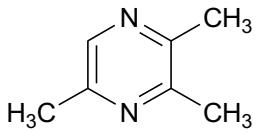
Flow rate: Adjust so that the peak of the component to be determined appears between 3–20 minutes after injection.

Injection method: Sprit

Split ratio: 1:30–1:250. Adjust so that any component does not exceed the acceptable level of the column used.

2,3,5-Trimethylpyrazine

2,3,5-トリメチルピラジン



$C_7H_{10}N_2$ Mol. Wt. 122.17

2,3,5-Trimethylpyrazine [14667-55-1]

Content 2,3,5-Trimethylpyrazine contains not less than 98.0% of 2,3,5-trimethylpyrazine ($C_7H_{10}N_2$).

Description 2,3,5-Trimethylpyrazine is a colorless to yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of 2,3,5-Trimethylpyrazine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.500–1.509.

Specific Gravity d_{25}^{25}: 0.960–0.990.

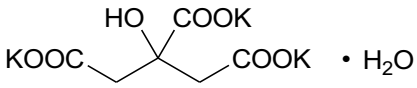
Assay Proceed as directed in the Peak Area Percentage Method in the Gas

Chromatographic Assay in the Flavoring Substances Tests. Use operating conditions (1).

Tripotassium Citrate

Potassium Citrate

クエン酸三カリウム



$C_6H_5K_3O_7 \cdot H_2O$ Mol. Wt. 324.41

Tripotassium 2-hydroxypropane-1,2,3-tricarboxylate monohydrate [6100-05-6]

Content Tripotassium Citrate, when calculated on the dried basis, contains not less than 99.0% of tripotassium citrate ($C_6H_5K_3O_7$ = 306.39).

Description Tripotassium Citrate occurs as colorless crystals or as a white crystalline powder. It is odorless.

Identification Tripotassium Citrate responds to all the tests for Potassium Salt and to test (2) for Citrate in the Qualitative Tests.

pH 7.6–9.0 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 20 mL).

(2) Sulfate Not more than 0.024% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 6.5% (200°C, 2 hours).

Assay Weigh accurately about 0.2 g of Tripotassium Citrate, add 30 mL of acetic acid for nonaqueous titration, and dissolve it by warming. Cool, and titrate with 0.1 mol/L perchloric acid. Usually, a potentiometer is used to confirm the endpoint. When crystal violet–acetic acid TS (1 mL) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Separately, perform a blank test in the same manner, and make any necessary correction. Calculate the content on the dried basis.

Each mL of 0.1 mol/L perchloric acid = 10.21 mg of $C_6H_5K_3O_7$

Tripotassium Phosphate

Potassium Phosphate, Tribasic
Tertiary Potassium Phosphate

リン酸三カリウム

$K_3PO_4 \cdot nH_2O$ (n = 3, 1 ½ , 1, or 0) Mol. Wt. trihydrate 266.31

anhydrous 212.27

Tripotassium phosphate trihydrate

Tripotassium phosphate sesquihydrate

Tripotassium phosphate monohydrate

Tripotassium phosphate [7778-53-2]

Content Tripotassium Phosphate, when ignited, contains not less than 97.0% of tripotassium phosphate (K_3PO_4).

Description Tripotassium Phosphate occurs as colorless to white crystals or lumps, or as a white powder.

Identification A solution of Tripotassium Phosphate (1 in 20) responds to all the tests for Potassium Salt and for Phosphate in the Qualitative Tests.

pH 11.5–12.5 (1.0 g, water 100 mL).

Purity

(1) Clarity of solution Colorless and very slightly turbid (1.0 g, water 20 mL).

(2) Chloride Not more than 0.011% as Cl (1.0 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.40 mL).

(4) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Tripotassium Phosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Ignition Not more than 23.0% (120°C for 2 hours, then 300–400°C for 1 hour).

Assay Weigh accurately about 2 g of Tripotassium Phosphate, previously ignited,

dissolve it in 50 mL of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 3–4 drops of methyl orange–xylene cyanol FF TS).

Each mL of 1 mol/L hydrochloric acid = 106.1 mg of K_3PO_4

Trisodium Citrate

Sodium Citrate

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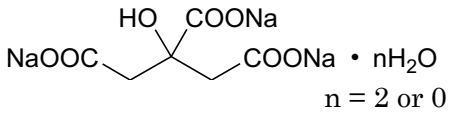


$C_6H_5Na_3O_7 \cdot nH_2O$ (n = 2 or 0) Mol. Wt. dihydrate 294.10

anhydrous 258.07

Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate [6132-04-3]

Trisodium 2-hydroxypropane-1,2,3-tricarboxylate [68-04-2]

Definition Trisodium Citrate occurs in two forms: the crystal form (dihydrate) called Trisodium Citrate (crystal) and the anhydrous form called Trisodium Citrate (anhydrous).

Content Trisodium Citrate, when dried, contains not less than 99.0% of trisodium citrate ($C_6H_5Na_3O_7$).

Description Trisodium Citrate occurs as colorless crystals or as a white powder. It is odorless and has a cool, salty taste.

Identification Trisodium Citrate responds to all the tests for Sodium Salt and to test (2) for Citrate in the Qualitative Test.

pH 7.6–9.0 (1.0 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, water 20 mL).

(2) Sulfate Not more than 0.024% as SO_4 (1.0 g, Control Solution: 0.005 mol/L sulfuric acid 0.50 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 3, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying

Crystal 10.0–13.0% (180°C, 2 hours).

Anhydrous　Not more than 1.0% (180°C, 2 hours).

Assay　Weigh accurately about 0.2 g of Trisodium Citrate, previously dried, add 30 mL of acetic acid for nonaqueous titration, and dissolve it by warming. After cooling, titrate with 0.1 mol/L perchloric acid. Usually, a potentiometer is used to confirm the endpoint. When crystal violet–acetic acid TS (1 mL) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Separately, perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 8.602 mg of $C_6H_5Na_3O_7$

Trisodium Phosphate

Sodium Phosphate, Tribasic
Tertiary Sodium Phosphate

リン酸三ナトリウム

$Na_3PO_4 \cdot nH_2O$ (n = 12, 6, or 0)　　Mol. Wt. dodecahydrate 380.12
anhydrous 163.94

Trisodium phosphate dodecahydrate　[10101-89-0]

Trisodium phosphate hexahydrate

Trisodium phosphate　[7601-54-9]

Definition　Trisodium Phosphate occurs in two forms: the crystalline form (dodeca- and hexahydrates) called Trisodium Phosphate (crystal) and the anhydrous form called Trisodium Phosphate (anhydrous).

Content　Trisodium Phosphate, when dried, contains 97.0–103.0% of trisodium phosphate (Na_3PO_4).

Description　Trisodium Phosphate (crystal) occurs as colorless to white crystals or crystalline powder. Trisodium Phosphate (anhydrous) occurs as a white powder or as granules.

Identification　A solution of Trisodium Phosphate (1 in 20) responds to all the tests for Sodium Salt and for Phosphate in the Qualitative Tests.

pH　11.5–12.5 (1.0 g, water 100 mL).

Purity　For Trisodium Phosphate (crystal), dry the sample before the tests are performed.

(1) Clarity of solution　Colorless and very slightly turbid (0.50 g, water 20 mL).

(2) Chloride　Not more than 0.071% as Cl (0.30 g, Control Solution: 0.01 mol/L hydrochloric acid 0.60 mL).

(3) Sulfate　Not more than 0.058% as SO_4 (0.50 g, Control Solution: 0.005 mol/L

sulfuric acid 0.60 mL).

(4) Lead Not more than 4 μg/g as Pb (1.0 g, Method 5, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Sample Solution To the specified amount of Trisodium Phosphate, add 20 mL of diluted hydrochloric acid (1 in 4), and boil gently for 15 minutes with a watch glass covering it while stirring occasionally. Centrifuge the mixture to precipitate the insoluble matter, and filter the supernatant to remove the insoluble matter. Wash the residue on the filter paper and the container used for centrifugation with 5 mL of hot water, combine the washings with the filtrate, and cool.

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying

Crystal Not more than 58.0% (120°C, 2 hours, then 200°C, 5 hours).

Anhydrous Not more than 5.0% (200°C, 5 hours).

Assay Weigh accurately about 2 g of Trisodium Phosphate, previously dried, dissolve it in 50 mL of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 3–4 drops of methyl orange–xylene cyanol FF TS).

Each mL of 1 mol/L hydrochloric acid = 81.97 mg of Na_3PO_4

Trypsin

トリプシン

Definition Trypsin is a proteolytic enzyme derived from pancreas of animals or internal organs of fishes or crustaceans. It may contain lactose or dextrin.

Enzyme Activity Trypsin has an enzyme activity of not less than 600,000 units per gram.

Description Trypsin occurs as a white to yellow-brown powder or as granules, or as a light brown to brown liquid or paste.

Identification When tested by enzyme activity determination, Trypsin shows activity.

Purity

(1) Sulfate Not more than 48% as SO_4.

Test Solution Weigh 1.0 g of Trypsin, dissolve it in water to make 1000 mL. Use 50 mL of the solution as the test solution.

Control Solution Use 50 mL of 0.005 mol/L sulfuric acid.

(2) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4 mL, Flame Method). In the preparation of the test solution, if the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100), proceed as directed in

Method 3 under the Lead Limit Test.

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella test.*

Enzyme Activity Determination

(i) *Substrate Solution*

Dissolve 85.7 mg of α-*N*-benzoyl-L-arginine ethyl ester hydrochloride in water to make exactly 100 mL. Measure exactly 10 mL of this solution, and add phosphate buffer (pH 7.6) to make exactly 100 mL.

(ii) *Sample Solution*

Weigh accurately an amount of Trypsin equivalent to 5000–6000 units and dissolve it in hydrochloric acid TS (0.001 mol/L) to make exactly 100 mL.

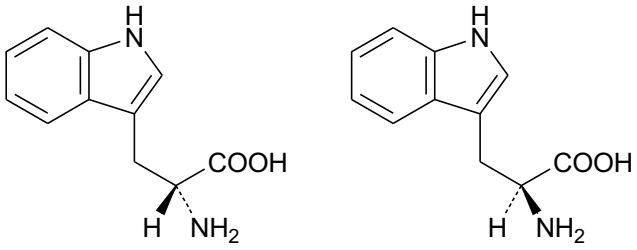
(iii) *Procedure*

Measure exactly 0.20 mL of 0.001 mol/L hydrochloric acid, add 3.0 mL of the substrate solution, and mix. Adjust the absorbance to 0.050 at a wavelength of 253 nm at 25 ± 0.1°C, using water as the reference. Measure exactly 0.20 mL of the sample solution, add 3.0 mL of the substrate solution, and mix. Measure the absorbance at 30-second intervals for 5 minutes under the same conditions as given above in this section. Plot the time (seconds) against the absorbance on a graph. Determine the change (ΔA) in absorbance per minute in a range in which the time-absorbance curve is straight. Calculate the enzyme activity by the formula given below. One unit of the enzyme activity is the quantity of enzyme that changes the absorbance by 0.003 per minute when a test is performed under the conditions given in this section.

$$\text{Enzyme Activity of Trypsin (units/g)} = \frac{\Delta A \times 100}{0.003 \times \text{Weight (g) of the sample} \times 0.2} \times 1000$$

DL-Tryptophan

DL-トリプトファン



$C_{11}H_{12}N_2O_2$ Mol. Wt. 204.23

(2*RS*)-2-Amino-3-(1*H*-indol-3-yl)propanoic acid [54-12-6]

Content DL-Tryptophan, when calculated on the dried basis, contains 98.0－102.0% of DL-tryptophan ($C_{11}H_{12}N_2O_2$).

Description DL-Tryptophan occurs as white to yellowish-white crystals or crystalline powder. It is odorless or has a slight odor and has a slightly sweet taste.

Identification

(1) To 5 mL of a solution of DL-Tryptophan (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

(2) To 0.2 g of DL-Tryptophan, add 100 mL of water, and dissolve it by warming. To 10 mL of this solution, add 5 mL of *p*-dimethylaminobenzaldehyde TS and 2 mL of diluted hydrochloric acid (1 in 4), and heat in a water bath for 5 minutes. A red-purple to blue-purple color develops.

(3) To 0.2 g of DL-Tryptophan, add 100 mL of water, and dissolve it by warming. The solution has no optical rotation.

pH 5.5–7.0.

To 0.20 g of DL-Tryptophan, add 100 mL of water, and dissolve it by warming. Measure the pH of the resulting solution.

Purity

(1) Clarity of solution Weigh 0.50 g of DL-Tryptophan, and dissolve it in 10 mL of sodium hydroxide solution (1 in 50). The solution is almost clear and not darker in color than that of Matching Fluid C.

(2) Chloride Not more than 0.021% as Cl.

Test Solution Weigh 0.50 g of DL-Tryptophan, dissolve it in 6 mL of diluted nitric acid (1 in 10), and add water to make 50 mL.

Control Solution Use 0.30 mL of 0.01 mol/L hydrochloric acid.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution Weigh the specified amount of DL-Tryptophan, add 5 mL of diluted hydrochloric acid (1 in 20), and dissolve it by heating.

Loss on Drying Not more than 0.3% (105°C, 3 hours).

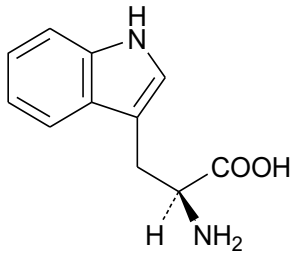
Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.3 g of DL-Tryptophan, and proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 20.42 mg of $C_{11}H_{12}N_2O_2$

L-Tryptophan

L-トリプトファン



$C_{11}H_{12}N_2O_2$ Mol. Wt. 204.23

(2*S*)-2-Amino-3-(1*H*-indol-3-yl)propanoic acid [73-22-3]

Content L-Tryptophan, when calculated on the dried basis, contains 98.0–102.0% of L-tryptophan ($C_{11}H_{12}N_2O_2$).

Description L-Tryptophan occurs as white to yellowish-white crystals or crystalline powder. It is odorless or has a slight odor and has a slightly bitter taste.

Identification

(1) Proceed as directed in Identification (1) and (2) for DL-Tryptophan.

(2) To 1.0 g of L-Tryptophan, add 100 mL of water, and dissolve it by warming. The solution is levorotatory. It is dextrorotatory when made alkaline by adding sodium hydroxide solution (1 in 5).

Specific Rotation $[\alpha]_D^{20}$: −30.0 to −33.0°.

Weigh accurately about 0.5 g of L-Tryptophan, add about 40 mL of water, dissolve it by warming, and cool, and then add water to make exactly 50 mL. Measure the angular rotation of this solution, and calculate on the dried basis.

pH 5.5–7.0.

Dissolve 1.0 g of L-Tryptophan in 100 mL of water by warming, and measure the pH

of the resulting solution.

Purity

(1) Clarity of solution Weigh 0.50 g of L-Tryptophan, and dissolve it in 10 mL of sodium hydroxide solution (1 in 50). The solution is almost clear and not darker in color than Matching Fluid C.

(2) Chloride Not more than 0.021% as Cl.

Test Solution Dissolve 0.50 g of L-Tryptophan in 6 mL of diluted nitric acid (1 in 10), add water to make 50 mL.

Control Solution Use 0.30 mL of 0.01 mol/L hydrochloric acid.

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Test Solution To the specified amount of L-Tryptophan, add 3 mL of hydrochloric acid TS (1 mol/L) and 2 mL of water, and dissolve it by heating.

Loss on Drying Not more than 0.3% (105°C, 3 hours).

Residue on Ignition Not more than 0.1%.

Assay Weigh accurately about 0.3 g of L-Tryptophan, and proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 20.42 mg of $C_{11}H_{12}N_2O_2$

Turmeric Oleoresin

Curcumin

ウコン色素

Definition Turmeric Oleoresin is obtained from the rhizomes of the turmeric plant *Curcuma longa* L. and consists mainly of curcumin. It may contain edible fats or oils.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Turmeric Oleoresin is not less than 1500 and is in the range of 90–110 % of the labeled value.

Description Turmeric Oleoresin occurs as a yellow to dark red-brown powder, as lumps, or as a paste or liquid. It has a characteristic odor.

Identification

(1) Weigh an amount of Turmeric Oleoresin equivalent to 0.1 g of turmeric oleoresin with a Color Value 1500, dissolve it in 200 mL of ethanol (95). A yellow color with a light-green fluorescence develops.

(2) A solution of Turmeric Oleoresin in ethanol (95) exhibits an absorption maximum at a wavelength of 420–430 nm.

(3) Weigh an amount of Turmeric Oleoresin equivalent to 1 g of turmeric oleoresin with a Color Value 1500, dissolve it in 100 mL of ethanol (95), and add hydrochloric acid until the color of the solution turns slightly orange. Use this solution as the test solution. Add boric acid to the test solution. A red-orange color develops.

(4) Weigh an amount of Turmeric Oleoresin equivalent to 1 g of turmeric oleoresin with a Color Value 1500, dissolve it in 100 mL of ethanol (95), centrifuge the solution at 3000 rpm for 10 min. Use the supernatant as the test solution. Analyze a 5-μl portion of the test solution by thin-layer chromatography using a 4:4:2:1 mixture of ethanol (95)/3-methyl-1-butanol/water/ammonia solution as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. Examine both in daylight and under ultraviolet light (around 366 nm). Two or more yellow spots are observed at R_f values of 0.40–0.85. All spots show yellow fluorescence in UV-light.

Purity

(1) Lead Not more than 2 μg /g as Pb (2.0 g, Method 2, Control solution: Lead Standard Solution 4.0 mL, Flame method).

(2) Arsenic Not more than 3 μg /g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B)

Color Value Proceed as directed under Color Value Determination.

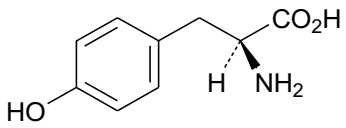
Operating Conditions

Solvent: Ethanol (95).

Wavelength: Maximum absorption wavelength of 420–430nm.

L-Tyrosine

L-チロシン



$C_9H_{11}NO_3$ Mol.Wt.181.19

(2*S*)-2-Amino-3-(4-hydroxyphenyl)propanoic acid [60-18-4]

Content L-Tyrosine, when calculated on the dried basis, contains 98.0–102.0% of L-tyrosine ($C_9H_{11}NO_3$).

Description L-Tyrosine occurs as white crystals or crystalline powder. It is odorless, and is tasteless or has a very slight characteristic taste.

Identification

(1) To 5 mL of a saturated solution of L-Tyrosine, add 1 mL of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A blue-purple color develops.

(2) To 5 mL of a saturated solution of L-Tyrosine, add 1 mL of a solution of iron(III) chloride hexahydrate (1 in 20), and heat. A dark red color develops.

Specific Rotation $[\alpha]_D^{20}$: –10.5 to –12.5° (5 g, hydrochloric TS (1 mol/L), 100 mL, dried basis).

pH 5.0–6.5 (a saturated solution).

Purity

(1) Clarity of solution Colorless and almost clear (1.0 g, 1 mol/L hydrochloric acid 20 mL).

(2) Chloride Not more than 0.10% as Cl (70 mg, Control Solution: 0.01 mol/L hydrochloric acid 0.20 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

Residue on Ignition Not more than 0.1%.

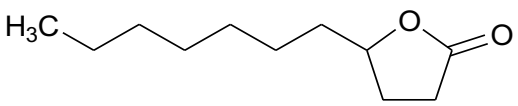
Assay Weigh accurately about 0.3 g of L-Tyrosine, and proceed as directed in the Assay for L-Asparagine.

Each mL of 0.1 mol/L perchloric acid = 18.12 mg of $C_9H_{11}NO_3$.

γ-Undecalactone

Undecalactone
Undecano-1,4-lactone

γ-ウンデカラクトン



$C_{11}H_{20}O_2$ Mol. Wt. 184.28

5-Heptyldihydrofuran-2(3*H*)-one [104-67-6]

Content γ-Undecalactone contains not less than 98.0% of γ-undecalactone ($C_{11}H_{20}O_2$).

Description γ-Undecalactone is a colorless to light yellow, clear liquid having a peach-like odor.

Identification Determine the absorption spectrum of γ-Undecalactone as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.448–1.453.

Specific Gravity d_{25}^{25}: 0.941–0.944.

Purity Acid value Not more than 5.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4)

Urease

ウレアーゼ

Definition Urease includes enzymes that hydrolyze urea. It is derived from the culture of bacteria (limited to *Lactobacillus fermentum* and species of the genus *Arthrobacter*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Urease occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Urease complies with the Urease Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid　Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture　Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Urease Activity Test　Perform the test using the method given below. If the identification test cannot be performed by the given method, appropriate replacement of the sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Sample Solution　Weigh 1.0 g of Urease, add water or acetate buffer (0.1 mol/L, pH 4.0, containing ethanol) to dissolve it or disperse it uniformly, and make 100 mL. As necessary, prepare a 10-fold, 100-fold, or 1000-fold dilution by adding water or the same buffer to the resulting solution.

Substrate Solution　Dissolve 0.6 g of urea in water to make 100 mL. Prepare fresh before use.

Test Solution　To 0.5 mL of the sample solution, add 2.5 mL of acetate buffer (0.1 mol/L, pH 4.0, containing ethanol), equilibrate the mixture at 37°C for 5 minutes, add 1.0 mL of the substrate solution, equilibrated at 37°C, and shake immediately. Incubate the mixture at 37°C for 30 minutes, add 4 mL of trichloroacetic acid solution (1 in 10), and shake. To 2 mL of this solution, add water to make 20 mL. To 4 mL of the resulting solution, add 2 mL of phenol–sodium pentacyanonitrosylferrate(III) TS, and shake gently. Add 2 mL of sodium hypochlorite–sodium hydroxide TS (for urease activity test), shake and warm the mixture at 37°C for 30 minutes, and cool to room temperature.

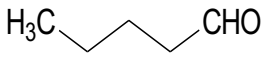
Control Solution　To 0.5 mL of the sample solution, add 2.5 mL of acetate buffer (0.1 mol/L, pH 4.0, containing ethanol), equilibrate the mixture at 37°C for 35 minutes, add 4 mL of trichloroacetic acid solution (1 in 10), shake, and add 1.0 mL of the substrate solution. To 2 mL of this solution, add water to make 20 mL. To 4 mL of the resulting solution, add 2 mL of phenol–sodium pentacyanonitrosylferrate(III) TS, and shake gently.　Add 2 mL of sodium hypochlorite–sodium hydroxide TS (for urease activity test), shake, warm the mixture at 37°C for 30 minutes, and cool to room temperature.

Procedure　Measure the absorbance of the test solution and the control solution at a wavelength of 640 nm. The absorbance value of the test solution is higher than that of the control solution. If the test solution and the control solution are turbid, centrifuge them, and use the supernatants.

Valeraldehyde

Pentanal

バレルアルデヒド



$C_5H_{10}O$ Mol. Wt. 86.13

Pentanal [110-62-3]

Content Valeraldehyde contains not less than 95.0% of valeraldehyde ($C_5H_{10}O$).

Description Valeraldehyde occurs as a colorless to pale yellow, clear liquid having a characteristic odor.

Identification Determine the absorption spectrum of Valeraldehyde, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Refractive Index n_D^{20}: 1.390–1.400.

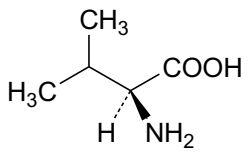
Specific Gravity d_{25}^{25}: 0.805–0.820.

Purity Acid value Not more than 5.0 (Flavoring Substance Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavor Substance Tests. Use operating conditions (3).

L-Valine

L-バリン



$C_5H_{11}NO_2$ Mol. Wt. 117.15

(2*S*)-2-Amino-3-methylbutanoic acid [72-18-4]

Content L-Valine, when calculated on the dried basis, contains 98.0–102.0% of L-valine ($C_5H_{11}NO_2$).

Description L-Valine occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and has a slight, characteristic taste.

Identification To 5 mL of a solution of L-Valine (1 in 1000), add 1 mL of ninhydrin solution (1 in 1000), and heat for 3 minutes. A purple color develops.

Specific Rotation $[\alpha]_D^{20}$: +26.5 to +29.0° (4 g, hydrochloric acid TS (6 mol/L), 50 mL, on the dried basis).

pH 5.5–7.0 (0.5 g, water 20 mL).

Purity

(1) Clarity of solution Colorless and clear (0.50 g, water 20 mL).

(2) Chloride Not more than 0.021% as Cl (0.50 g, Control Solution: 0.01 mol/L hydrochloric acid 0.30 mL).

(3) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 2, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Loss on Drying Not more than 0.3% (105°C, 3 hours).

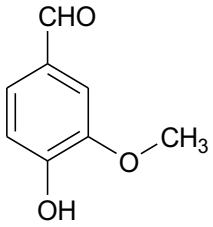
Residue on Ignition Not more than 0.1%.

Assay Proceed as directed in the Assay for DL-Alanine.

Each mL of 0.1 mol/L perchloric acid = 11.71 mg of $C_5H_{11}NO_2$

Vanillin

バニリン



$C_8H_8O_3$ Mol. Wt. 152.15

4-Hydroxy-3-methoxybenzaldehyde [121-33-5]

Content Vanillin contains not less than 97.0% of vanillin ($C_8H_8O_3$).

Description Vanillin occurs as white to light yellow needles or crystalline powder having a vanilla-like odor and taste.

Identification Determine the absorption spectrum of Vanillin as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 81–84°C.

Assay Using a solution (1 in 10) of Vanillin in acetone as the test solution, proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests. Use operation conditions (4).

Vegetable Sterol

Phytosterol

植物性ステロール

Definition Vegetable Sterol is obtained from oil seeds and consists mainly of phytosterols. There are two types of Vegetable Sterols: High Concentration Free Sterol and Low Concentration Free Sterol.

High Concentration Free Sterol

Content High Concentration Free Sterol contains not less than 85.0% of free phytosterols.

Description High Concentration Free Sterol occurs as white to yellowish-white crystals, powder, flakes, or granules. It is odorless or has a slight, characteristic odor.

Identification Dissolve 5 mg of High Concentration Free Sterol in 2 mL of hexane, add 1 mL of acetic anhydride and 1 drop of sulfuric acid, and agitate. A red-purple color immediately forms in the lower layer of the mixture, which changes through blue to green.

Purity

(1) Acid value Not more than 5.0.

Test Solution Weigh accurately about 2.5 g of High Concentration Free Sterol, add 50 mL of a 1:1 mixture of ethanol (99.5)/toluene, and dissolve it by warming.

Procedure Immediately after the preparation of the test solution, proceed as directed in Acid Value under the Fats and Related Substances Tests.

(2) Clarity of solution Slightly turbid.

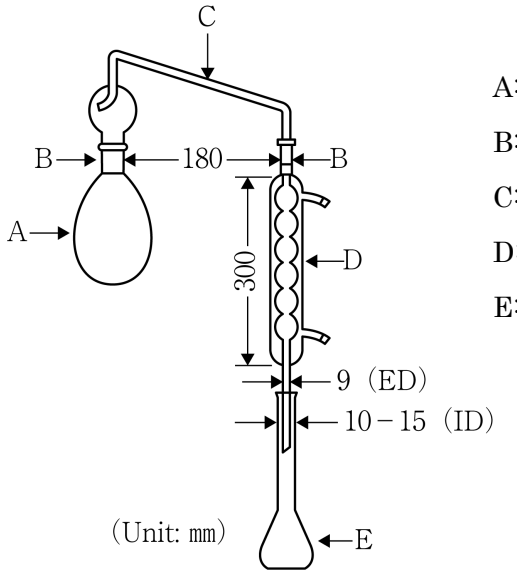
Test Solution Weigh 0.50 g of High Concentration Free Sterol into a stoppered flask, add 50 mL of ethanol (99.5), and heat in a water bath for 15 minutes. Allow it to stand at 20–40°C for 2 hours.

(3) Lead Not more than 1 μg/g as Pb (4.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(5) Total amount of 1-propanol, hexane, and methanol Not more than 50 μg/g.

(i) Apparatus Use the apparatus as illustrated in the figure.



A: Eggplant-shaped flask (100 mL)

B: Ground glass joint

C: Delivery tube with a spray trap

D: Condenser

E: Wide-mouth volumetric flask (25 mL)

(ii) Method

Test Solution Weigh accurately about 10 g of High Concentration Free Sterol in eggplant-shaped flask A, add 10 mL of 1-butanol, mix well, and add a few boiling chips. Place exactly 2 mL of the internal standard solution in wide-mouth volumetric flask E, and set up the apparatus. Moisten the joint parts with 1-butanol. Heat flask A at 180°C, and continue distillation until about 9 mL of distillate is obtained in about 1 hour. To flask E containing the distillate, add 1-butanol to make exactly 25 mL.

Internal Standard Solution A solution (3 in 10,000) of 2-butanol in 1-butanol.

Standard Solution Weigh accurately about 0.5 g each of 1-propanol, hexane, and methanol, and add 1-butanol to make exactly 100 mL. To exactly 1 mL of this solution, add 1-butanol to make exactly 100 mL. Then measure exactly 10 mL of the second solution and 2 mL of the internal standard solution, and add 1-butanol to make exactly 25 mL.

Procedure Analyze 2 μL each of the test solution and the standard solution by gas chromatography using the operating conditions below. Determine the peak area ratios of 1-propanol, hexane, and methanol to 2-butanol for each solution, and express as Q_{T1}, Q_{T2}, and Q_{T3} for the test solution and as Q_{S1}, Q_{S2}, and Q_{S3} for the standard solution. Calculate each amount by the following formulae, and obtain the sum of these substances.

$$\text{Amount (μg/g) of 1-propanol} = \frac{\text{Weight (g) of 1-propanol}}{\text{Weight (g) of the sample}} \times \frac{Q_{T1}}{Q_{S1}} \times 1000$$

$$\text{Amount (μg/g) of hexane} = \frac{\text{Weight (g) of hexane}}{\text{Weight (g) of the sample}} \times \frac{Q_{T2}}{Q_{S2}} \times 1000$$

$$\text{Amount (μg/g) of methanol} = \frac{\text{Weight (g) of methanol}}{\text{Weight (g) of the sample}} \times \frac{Q_{T3}}{Q_{S3}} \times 1000$$

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 60 m length) coated with a 1.40-μm thick layer of 25% phenyl/75% methylpolysiloxane for gas chromatography.

Column temperature: Upon injection at 50°C, maintain the temperature at 50°C for 3 minutes, and raise at a rate of 5°C/minute to 110°C and then at a rate of 15°C/minute to 200°C, and maintain at 200°C for 4 minutes.

Injection port temperature: A constant temperature of about 150°C.

Detection temperature: A constant temperature of about 150°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention time of 2-butanol to about 12 minutes.

Injection method: Split.

Split ratio: 1 : 20.

Loss on Drying Not more than 3.0% (105°C, 2 hours).

Residue on Ignition Not more than 0.5%.

Assay

Test Solution and Standard Solution Weigh accurately about 80 mg of High Concentration Free Sterol and about 25 mg of stigmasterol for assay, dissolve them separately by adding exactly 20 mL of the internal standard solution, and add ethyl acetate to make exactly 50 mL each. Use them as the test solution and the standard solution, respectively. As the internal standard solution, use a solution prepared by dissolving 50 mg of 5α-cholestane in ethyl acetate to make exactly 50 mL.

Phytosterol Mixture Dissolve brassicasterol, campesterol, stigmasterol for assay, β-sitosterol, and sitostanol in ethyl acetate so that each concentration is about 0.1 mg/mL.

Procedure Analyze 2 μL each of the test solution, the standard solution, and the phytosterol mixture by gas chromatography using the operating conditions given below. Determine the ratio (Q_T) of the total peak area of 6 phytosterols (brassicasterol, campesterol, campestanol, stigmasterol, β-sitosterol, and sitostanol) to the peak area of 5α-cholestane for the test solution and the ratio (Q_S) of the peak area of stigmasterol to the peak area of 5α-cholestane for the standard solution. Calculate the content of free phytosterol by the following formula.

To identify each phytosterol in the test solution, confirm that each retention time corresponds to the retention time of each substance in the phytosterol mixture. A substance whose peak corresponds to a relative retention time of about 0.96 to the

retention time of stigmasterol is campesterol.

$$\text{Content (\%) of free phytosterols} = \frac{\text{Weight (mg) of stigmastrol for assay}}{\text{Weight (mg) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: 280°C.

Injection port temperature: 290°C.

Carrier gas: Helium.

Flow rate: Adjust the retention time of stigmasterol to about 12 minutes.

Injection method: Split.

Split ratio: 1 : 50.

Low Concentration Free Sterol

Content Low Concentration Free Sterol contains less than 85.0% of free phytosterols and 85.0–102.0% as the total phytosterols.

Description Low Concentration Free Sterol occurs as white to yellow crystals, powder, flakes, granules, wax-like lumps, or paste. It is odorless or a slight, characteristic odor.

Identification Dissolve 5 mg of Low Concentration Free Sterol in 2 mL of hexane, add 1 mL of acetic anhydride and 1–2 drop of sulfuric acid, and agitate. A red-purple color immediately forms in the lower layer of the mixture, which changes through blue to green.

Purity

(1) Acid value Not more than 5.0.

Test Solution Weigh accurately about 2.5 g of Low Concentration Free Sterol, add 50 mL of a 1:1 mixture of ethanol (99.5)/toluene, and dissolve it by warming.

Procedure Immediately proceed as directed in Acid Value under the Fats and Related Substances Tests.

(2) Lead Not more than 1 μg/g as Pb (4.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic

Standard Solution 3.0 mL, Apparatus B).

(4) Total amount of 1-propanol, hexane, and methanol Not more than 50 μg/g.

Proceed as directed in Purity (5) for High Concentration Free Sterol.

Loss on Drying Not more than 3.0% (105°C, 2 hours)

Residue on Ignition Not more than 0.5%.

Assay

(1) Free phytosterols

Test Solution Weigh accurately about 70 mg of Low Concentration Free Sterol, add exactly 10 mL of the internal standard solution to dissolve it, add hexane to make exactly 25 mL. Refer to this as the sample solution. Pour 2 mL of a 1:1 mixture of hexane/acetone and then 6 mL of hexane into a silica gel mini column (500 mg), and discard the effluent. Pour exactly 10 mL of the sample solution and then 6 mL of a 95:5 mixture of hexane/ethyl acetate, and discard the effluent. Pour 10 mL of a 1:1 mixture of hexane/acetone, and collect the effluent into an eggplant-shape flask. If a precipitate is observed outside of the column outlet, wash it with a 1:1 mixture of hexane/acetone, and add the washings to the flask. Distil away the solvent under reduced pressure. To the residue, add 10 mL of a 3:2 mixture of ethyl acetate/hexane to dissolve it.

Standard Solution Weigh accurately about 25 mg of stigmasterol for assay, add exactly 20 mL of the internal standard solution to dissolve it, and add ethyl acetate to make 50 mL.

Internal Standard Dissolve 50 mg of cholestanol by adding hexane to make exactly 50 mL.

Procedure Proceed as directed in the Procedure under Assay for High Concentration Free Sterol to determine 6 phytosterols, and calculate the content of free phytosterols by the following formula. Determine the ratio (Q_T) of the total peak area of 6 phytosterols (brassicasterol, campesterol, campestanol, stigmasterol, β-sitosterol, and sitostanol) to the peak area of cholestanol for the test solution and the ratio (Q_S) of the peak area of stigmasterol to the peak area of cholestanol for the standard solution.

$$\text{Content (\%) of free phytosterols} = \frac{\text{Weight (mg) of stigmastrol for assay}}{\text{Weight (mg) of the sample} \times 2} \times \frac{Q_T}{Q_S} \times 100$$

(2) Total phytosterols

Test Solution Weigh accurately about 150 mg of Low Concentration Free Sterol in an eggplant-shape flask, and add 70 mL of ethanol (99.5), 10 mL of potassium hydroxide solution (9 in 10), and a few boiling chips. Heat under a reflux condenser in a boiling water bath for 60 minutes to hydrolyze it. Immediately cool, add exactly 20 mL of the internal standard solution, and transfer the mixture to a separating funnel (A). Wash the flask twice with 25 mL of water each time, then twice with 35 mL of diethyl ether each time, and add the washings to the separating funnel. Agitate it, and allow to stand.

Transfer the water layer to a separating funnel (B), add 50 mL of diethyl ether, agitate, and allow it to stand. Transfer the water layer to the eggplant-shape flask and the diethyl ether layer to separating funnel A. Transfer the water layer in the flask to separating funnel B, wash the flask once with 10 mL of water and twice with 25 mL of diethyl ether each time, collect the washings to separating funnel B, agitate, and allow to stand. Remove the water layer in separating funnel B, and add the diethyl ether layer to separating funnel A. Wash separating funnel B twice with 25 mL of water each time, and add the washings to separating funnel A. Invert the separating funnel gently a few times, allow it to stand, and remove the water layer. Wash the diethyl ether layer in the separating funnel several times with 50 mL of water each time until the washing no longer produce a color with phenolphthalein TS. Transfer the diethyl ether to a 300-mL eggplant-shape flask, wash the separating funnel twice with 10 mL of diethyl ether each time, and add the washings to the flask. Distill the solvent in the flask under reduced pressure, and dissolve the residue in 50 mL of a 3:2 mixture of ethyl acetate/hexane.

Standard Solution Weigh accurately about 25 mg of stigmasterol for assay, add exactly 20 mL of the internal standard solution to dissolve it, and add ethyl acetate to make 50 mL.

Internal Standard Solution Dissolve 50 mg of chlestanol by adding hexane to make exactly 50 mL.

Procedure Proceed as directed in the Procedure of Assay (1) to determine the total content of 6 phytosterols, and designate it as the content of phytosterols in the hydrolysate. Calculate the content of the total phytosterols by the formula:

$$\text{Content (\%) of phytosterols in the hydrolysate} = \frac{\text{Weight (mg) of stigmasterol for assay}}{\text{Weight (mg) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

$$\text{Content (\%) of total phytosterols} = \text{Content of free phytosterols} + \left\{\left(\begin{array}{c}\text{Content of phytosterols in the hydrolysate} \\ - \text{Content of free phytosterols}\end{array}\right) \times 1.64\right\}$$

Vegetable Tannin

植物タンニン

Definition Vegetable Tannin, one of the substances belonging to the "Tannin (Extract)" category, is obtained from nutgalls or the seed pods of Tara and consists mainly of tannin

and tannic acid.*

Content Vegetable Tannin, when dried, contains the equivalent of not less than 96% of tannic acid.

Description Vegetable Tannin occurs as a yellowish-white to light brown powder having a slight, characteristic odor. It has a strong astringent taste.

Identification

(1) To 5 mL of a solution of Vegetable Tannin (1 in 20), add two drops of iron(III) chloride hexahydrate (1 in 10). A bluish-black color is formed, and a precipitate is produced on standing.

(2) To three 5-mL portions of a solution of Vegetable Tannin (1 in 20), separately add a drop of albumin TS, a drop of gelatin TS, or 1 mL of starch TS. Each solution produces a precipitate.

(3) Dissolve 1 g of Vegetable Tannin in 100 mL of water, add 5 mL of diluted hydrochloric acid (1 in 2), and heat at 80 to 90°C for 2 hours. Use this solution as the test solution. Separately, dissolve 0.1 g of gallic acid monohydrate in 100 mL of water, and use this solution as the control solution. Analyze a 5-μL portion each of the test solution and the control solution by thin-layer chromatography using a 5:4:1 mixture of ethyl formate/toluene/formic acid as a developing solvent. Use a thin-layer plate coated with fluorescent silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. Examine under ultraviolet light (around 254 nm). A spot is observed at an R_f value of about 0.35 for each solution and emits blue-purple fluorescence under ultraviolet light.

(4) Dissolve 50 mg of Vegetable Tannin in 3 mL of water, add 1 mL of calcium hydroxide TS, and shake thoroughly. No yellow or red color develops.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 2, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Gum or dextrin Dissolve 3.0 g of Vegetable Tannin in 15 mL of hot water. The solution is clear or slightly turbid. Cool and filter this solution, and add 5 mL of ethanol (95) to 5 mL of the filtrate. No turbidity occurs.

(4) Resinous substances To 5 mL of the filtrate obtained in Purity (3), add 10 mL of

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* "Tannin (Extract)" is defined in the List of Existing Food Additives as a substance that is obtained from the fruits of the Japanese persimmon (*Diospyros kaki* Thunberg); the seed pods of Tara (*Caesalpinia spinosa*); nutgalls of *Rhus javanica* Linné and other species of the genus *Rhus*; nutgalls of *Quercus infectoria* Oliver and other species of the genus *Quercus*; or silver wattle bark. It consists mainly of tannin and tannic acid.

water. No turbidity appears.

Loss on Drying Not more than 7.0% (105°C, 2 hours).

Residue on Ignition Not more than 1.0%.

Assay

Test Solution and Control Solution Weigh 0.100 g of Vegetable Tannin and 1 mg of gallic acid monohydrate, and add a 4:1 mixture of water/methanol to each to make two solutions of exactly 100 mL. Use them as the test solution and as the control solution, respectively.

Procedure Analyze 10 μL each of them by liquid chromatography using the operating conditions given below. Confirm that the peak of gallic acid appears at a retention time of 2.2–2.5 minutes after the injection of the control solution. Measure the total area of all peaks that appear within 30 minutes of the injection of the test solution to normalize to 100, and measure the total area of all peaks that appear in 10–25 minutes as the peak area of tannic acid. Determine the peak area percentage of tannic acid from both total areas, and calculate the content of Vegetable Tannin.

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength: 280nm).

Column: A stainless steel tube (4 mm internal diameter and 25 cm length).

Column packing material: 7-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: Room temperature.

Mobile phase

A: 0.1% (w/v) phosphoric acid.

B: 0.1% (w/v) phosphoric acid in methanol.

Concentration gradient (A/B): Run a linear gradient from 80/20 to 0/100 in 30 minutes.

Flow rate: 1.0 mL/min.

Vitamin A Esters of Fatty Acids

Retinol Fatty Acid Esters

ビタミン A 脂肪酸エステル

Definition Vitamin A Esters of Fatty Acids are categorized into two types: a vitamin A ester of acetic acid or a vitamin A ester mainly of palmitic acid.

Content 1 g of Vitamin A Esters of Fatty Acids contains the equivalent of not less than 450 mg of vitamin A and the equivalent of 90–120% of the labeled content of vitamin A.

Three hundred mg of vitamin A is equivalent to one million international units.

Description Vitamin A Esters of Fatty Acids occur as light yellow to reddish light-yellow crystals or oily substances having a slight, characteristic odor.

Identification

(1) Prepare a test solution by dissolving an amount of the sample equivalent to 1500 units of vitamin A in 5 mL of petroleum ether. Analyze a 5-μL portion of the test solution by thin-layer chromatography using a 4:1 mixture of cyclohexane/diethyl ether as the developing solvent. Use a thin-layer plate coated with fluorescent silica gel for thin-layer chromatography as the solid support and then dried at 105°C for 2 hours. Stop the development when the solvent front has ascended to a point about 10 cm above the starting line, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm). Spots corresponding to vitamin A, vitamin A ester of acetic acid, and vitamin A ester of palmitic acid are observed at R_f values of about 0.09, 0.45, and 0.62, respectively.

(2) Dissolve 50 mg of the sample in 2-propanol for vitamin A determination to prepare a solution containing about 3 μg of vitamin A per mL. The solution exhibits an absorption maximum at a wavelength of 324–328 nm.

Purity

(1) Acid value Not more than 2.8.

Weigh accurately about 2 g of the sample, and proceed as directed in the Acid Value Test in the Fats and Related Substances Tests.

(2) Absorbance ratio

Test Solution Weigh accurately an amount of the sample equivalent to about 60 mg of vitamin A, and dissolve it in 2-propanol for vitamin A determination to make exactly 100 mL. Measure exactly 1 mL of this solution, add 2-propanol for vitamin A determination to make exactly 200 mL.

Procedure Measure the absorbance of the test solution at wavelengths of 300 nm, 310 nm, 320 nm, 326 nm, 330 nm, 340 nm, and 350 nm, respectively. Calculate the ratio of the absorbance at each wavelength to the absorbance (A) at 326 nm when the absorbance is expressed as 1000. Each absorbance ratio is within ± 0.030 of each value given in the table.

Wavelength (nm)	Ratios of Absorbance	
	Vitamin A ester of acetic acid	Vitamin A ester of palmitic acid
300	0.578	0.590
310	0.815	0.825
320	0.948	0.950
326	1.000	1.000

330	0.972	0.981
340	0.786	0.795
350	0.523	0.527

Assay From the absorbance (A) at 326 nm of the test solution prepared in Purity (2), calculate by the formula:

$$\text{Content (mg) of vitamin A} = \frac{A \times V}{M \times 100} \times 0.570$$

V = total number of milliliters of the test solution,

M = number of grams of the sample in V mL of the test solution.

Vitamin A in Oil

ビタミン A 油

Definition Vitamin A in Oil is a fatty oil obtained from the fresh liver, pyloric appendage, or other parts of aquatic animals; a vitamin A (retinol) concentrate of the fatty oil; vitamin A esters of fatty acids (retinol fatty acid ester); or a product prepared by dissolving any of the former three substances in edible fats or oils.

Content 1 g of Vitamin A in Oil contains the equivalent of not less than 30 mg of vitamin A and the equivalent of 90–120% of the labeled content of vitamin A. Three hundred mg of vitamin A is equivalent to one million international units.

Description Vitamin A in Oil occurs as a light yellow to reddish light-yellow oily substance having a slight, characteristic odor.

Identification Proceed as directed in Identification (1) and (2) for Vitamin A Esters of Fatty Acids.

Purity

(1) Acid value Not more than 2.8.

Proceed as directed under Acid Value in the Fats and Related Substances Tests, using about 2 g of Vitamin A in Oil, weighed accurately.

(2) Absorbance ratio If the sample contains vitamin A esters of fatty acids, proceed as directed in Purity (2) for Vitamin A Esters of Fatty Acids.

Assay

Test Solution Weigh accurately an amount of Vitamin A in Oil that is equivalent to not less than 0.15 mg of vitamin A and that contains not more than 1 g of fat or oil,

transfer into a flask, and add 30 mL of ethanol (aldehyde-free) and 1 mL of a solution (1 in 10) of pyrogallol in ethanol (95). Add 3 mL of potassium hydroxide solution (9 in 10), and heat under a reflux condenser on a water bath for 30 minutes to saponify. Cool quickly to ordinary temperature, add 30 mL of water, and transfer into separating funnel A. Wash the flask with 10 mL of water and then with 40 mL of diethyl ether for vitamin A determination, add the washings to separating funnel A, shake well, and allow to stand. Transfer the aqueous layer into separating funnel B, wash the flask with 30 mL of diethyl ether for vitamin A determination, add the washings to separating funnel B, and shake to extract. Transfer the aqueous layer into the flask, transfer the diethyl ether layer into separating funnel A, transfer the aqueous layer from the flask above into separating funnel B, add 30 mL of diethyl ether for vitamin A determination, and shake to extract. Transfer the diethyl ether layer into separating funnel A, add 10 mL of water, invert the separating funnel gently 2 or 3 times, allow to stand, and remove the separated aqueous layer. Wash three times with 50 mL of water each time, shaking stronger. Wash repeatedly with 50 mL of water each time until the washings no longer shows a color with phenolphthalein TS, and allow to stand for 10 minutes. Remove water as much as possible, transfer the diethyl ether layer into an Erlenmeyer flask, wash the separating funnel twice with 10 mL of diethyl ether for vitamin A determination each time, and add the washings to the Erlenmeyer flask. Add 5 g of sodium sulfate, shake, and transfer the diethyl ether extract into an eggplant-shaped flask by decantation. Wash the remaining sodium sulfate more than twice with 10 mL of diethyl ether for vitamin A determination each time, and add the washings to the eggplant-shaped flask. Concentrate the diethyl ether extract to about 1 mL while shaking in a water bath at 45°C, using an aspirator. Immediately add 2-propanol for vitamin A determination to dissolve, and dilute exactly to obtain a solution containing about 3 μg of vitamin A per mL.

Procedure Measure the absorbance (A_1, A_2, and A_3) of the test solution at wavelengths of 310 nm, 325 nm, and 334 nm, respectively, and calculate the content by the formula:

$$\text{Content (mg/g) of vitamin A} = E^{1\%}_{1\text{cm}}\ (325\ \text{nm}) \times 0.549$$

$$E^{1\%}_{1\ \text{cm}}\ (325\ \text{nm}) = \frac{A_2}{M} \times \frac{V}{100} \times f$$

$$f = 6.815 - 2.555 \times \frac{A_1}{A_2} - 4.260 \times \frac{A_3}{A_2}$$

f = correction factor,

V = total number of milliliters of the test solution,

M = number of grams of the sample in V mL of the test solution.

When the sample contains vitamin A esters of fatty acids, proceed as directed in the Assay for Vitamin A Esters of Fatty Acids.

Storage Standards Store in a hermetic, light-resistant container under inert gas.

Welan Gum

ウェランガム

Definition Welan Gum is obtained from the culture fluid of *Sphingomonas sp.* and consists mainly of polysaccharides. It may contain sucrose, glucose, lactose, dextrin, and maltose.

Description Welan Gum occurs as a white to brown powder having a slight odor.

Identification

(1) Add 1 g of Welan Gum to 100 mL of water while stirring. A viscous solution is produced.

(2) To 1 mL of the solution obtained in (1), add water to make 10 mL. To 2 mL of this solution, add 5 mL of acetone, and shake well. A white cotton-like precipitate is produced.

(3) To a solution obtained by dispersing 1 g of calcium hydroxide in 9 mL of water, add 10 mL of the solution obtained in (1), and shake well. A viscous solution is produced without forming gel.

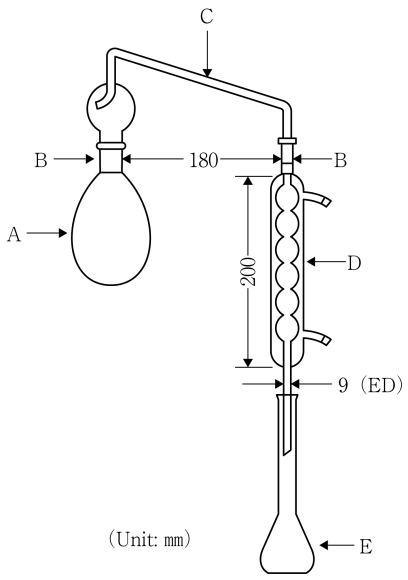
Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) 2-Propanol Not more than 0.50%.

(i) Apparatus Use the apparatus as illustrated in the figure.



A: Eggplant-shaped flask (300 mL)

B: Ground-glass joint

C: Delivery tube with a spray trap

D: Condenser

E: Volumetric flask (100 mL)

(ii) Method

Test Solution Weigh accurately about 2 g of Welan Gum in eggplant-shaped flask A, add 200 mL of water, a few boiling chips, and about 1 mL of silicon resin, and mix well. Place exactly 4 mL of the internal standard solution in volumetric flask E, and assemble the apparatus. Moisten the joint with water. Distill at a rate of 2 to 3 mL/minute, taking care not to allow bubbles to enter delivery tube C, and collect about 90 mL of distillate. To the distillate, add water to make exactly 100 mL. Use 2-methyl-2-propanol solution (1 in 1000) as the internal standard solution.

Standard Solution Weigh accurately about 0.5 g of 2-propanol, and add water to make exactly 50 mL. Measure exactly 5 mL of this solution, and add water to make exactly 50 mL. Next, measure exactly 10 mL of the second solution and 4 mL of the internal standard solution in a 100-mL volumetric flask, and add water to volume.

Procedure Analyze 2.0 μL each of the test solution and the standard solution by gas chromatography using the operating conditions below. Determine the peak area ratios (Q_T and Q_S) of 2-propanol to 2-methyl-2-propanol for the test solution and the standard solution, respectively. Obtain the amount of 2-propanol by the formula:

$$\text{Amount (\%) of 2-propanol} = \frac{\text{Weight (g) 2-propanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 2$$

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube (3 mm internal diameter and 2 m length).

Column packing material: 180- to 250-μm styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Injection port temperature: A constant temperature of about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention time of 2-propanol to about 10 minutes.

Loss on Drying Not more than 15.0% (105°C, 2 hours).

Ash Not more than 16.0% (on the dried basis).

Microbial limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

yeasts and molds: Not more than 500 per gram.

Escherichia coli and *Salmonella*: Negative per test.

Sample Fluids Prepare for total plate count and the enumeration of yeasts and molds in the following manner: Mix 1 g of Welan Gum with 200 mL of phosphate buffer,

0.1% peptone solution, or sodium chloride-peptone buffer to disperse it uniformly.

Pre-enrichment Culture Prepare for the *Escherichia coli* test in the following manner: Mix 1 g of Welan Gum with 300 mL of lauryl sulfate broth to disperse it uniformly and incubate the mixture at 35 ± 1°C for 48 ± 2 hours. For the *Salmonella* test, prepare five pre-enrichment cultures in the following manner, and perform the same test for each fluid: Mix 1 g of the Welan Gum with 300 mL of fluid lactose broth to disperse it completely, and incubate at 35 ± 1°C for 24 ± 2 hours.

Xanthan Gum

キサンタンガム

[11138-66-2]

Definition Xanthan Gum is obtained from the culture fluid of *Xanthomonas campestris* and consists mainly of polysaccharides. It may contain glucose, lactose, dextrin, or maltose.

Content Xanthan Gum, when dried, contains 72.0–108.0% of xanthan gum.

Description Xanthan Gum occurs as a white to brownish powder having a slight odor.

Identification Place 300 mL of water into a 500-mL beaker, heat to 80°C, and add a mixture of 1.5 g of Xanthan Gum and 1.5 g of carob bean gum powder while magnetically stirring at high speed. Stir at 60°C or higher until the mixture dissolve, and continue to stir for at least 30 minutes at 60°C or higher. Allow to cool to room temperature for 2 hours. Then cool the mixture at a temperature under 4°C, and an elastic gel is formed. A 1% solution of Xanthan Gum prepared in the same manner without carob bean gum does not form an elastic gel.

Purity

(1) Total nitrogen Not more than 1.5% (about 0.2 g, Semi-micro Kjeldahl Method).

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) 2-Propanol Not more than 0.05%.

(i) Apparatus Use the apparatus illustrated in Purity (7) for Semirefined Carrageenan.

(ii) Method

Test Solution Weigh accurately about 2 g of Xanthan Gum in an eggplant-shaped flask (A), add 200 mL of water, a few boiling chips, and about 1 mL of silicon resin, and stir well. Place exactly 4 mL of the internal standard solution in a volumetric flack (E),

and set up the apparatus. Moisten the joint parts with water. Distill it at a rate of 2 to 3 mL/minute, taking care not to allow bubbles to be trapped in the delivery tube with a spray trap (C), and collect about 90 mL of distillate. To the distillate, add water to make exactly 100 mL. Use 2-methyl-2-propanol solution (1 in 1000) as the internal standard solution.

Standard Solution Weigh accurately about 0.5 g of 2-propanol, and add water to make exactly 50 mL. Measure exactly 5 mL of this solution, and add water to make exactly 50 mL. Then place exactly 2 mL of the second solution and 8 mL of the internal standard solution into a 200-mL volumetric flask, and add water to volume.

Procedure Analyze 2.0 μL each of the test solution and the standard solution by gas chromatography using the operating conditions below. Determine the peak area ratios (Q_T and Q_S) of 2-propanol to 2-methyl-2-propanol for the test solution and the standard solution. Obtain the content of 2-propanol by the formula:

$$\text{Amount (\%) of 2-propanol} = \frac{\text{Weight (g) of 2-propanol}}{\text{Weight (g) the sample}} \times \frac{Q_T}{Q_S} \times 0.2$$

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube (3 mm internal diameter and 2 m length).

Column packing material: 180- to 250-μm styrene–divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of about 120°C.

Injection port temperature: A constant temperature of about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the retention time of 2-propanol to about 10 minutes.

Loss on Drying Not more than 15.0% (105°C, 150 minutes).

Ash Not more than 16.0% (Use the sample dried at 105°C for 4 hours).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid For total plate count and the enumeration of yeasts and molds, prepare as follows: Mix 1 g of Xanthan Gum with 200 mL of phosphate buffer, 0.1% peptone solution, or sodium chloride-peptone buffer to disperse uniformly.

Pre-enrichment Culture For the *Escherichia coli* test, prepare as follows: Mix 1 g of

Xanthan Gum with 200 mL of lauryl sulfate broth to disperse uniformly, and incubate at 35 ± 1°C for 48 ± 2 hours. For the *Salmonella* test, prepare five cultures, and perform the test for each culture: Mix 1 g of Xanthan Gum with 200 mL of lactose broth to disperse uniformly, and incubate at 35 ± 1°C for 24 ± 2 hours.

Assay Dry a glass filter (1G4) under reduced pressure at 80°C for 30 minutes, allow to cool in a desiccator, and weigh accurately. Weigh accurately about 0.5 g of dried Xanthan Gum, add 10 mL of potassium hydroxide solution (1 in 25) to dissolve, and add 90 mL of water. To this solution, add 15 mL of diluted hydrochloric acid (1 in 3) and 300 mL of ethanol (99.5), and stir vigorously. Allow to stand for 2 hours, and centrifuge with 4000 rpm for 10 minutes, and remove the supernatant. Add ethanol (99.5) again, and repeat the same procedures until the supernatant is free of chlorides. Filter the precipitate obtained through the glass filter, with ethanol (99.5). Wash the residue with acetone, dry under the reduced pressure at 80°C for 1.5 hours, allow to cool in a desiccator, and weigh accurately. Calculate the content by the formula:

$$\text{Content (\%) of xanthan gum} = \frac{\text{Weight (g) of the residue}}{\text{Weight (g) of the sample}} \times 100$$

Xylanase

キシラナーゼ

Definition Xylanase includes enzymes that degrade xylan. It is derived from the culture of filamentous fungi (limited to *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus niger*, *Disporotrichum dimorphosporum*, *Humicola insolens*, *Rasamsonia emersonii*, *Trichoderma koningii, Trichoderma longibrachiatum*, *Trichoderma reesei,* and *Trichoderma viride*) or actinomycetes (limited to *Streptomyces avermitilis*, *Streptomyces thermoviolaceus*, and *Streptomyces violaceoruber*). It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Description Xylanase occurs as white to dark brown granules, powder, or paste, or as a colorless to dark brown liquid. It is odorless or has a characteristic odor.

Identification Xylanase complies with the Xylanase Activity Test.

Purity

(1) Lead Not more than 5 μg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution, proceed as directed in Method 3.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under the Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Xylanase Activity Test Perform the test using one of the methods given below. If the identification test cannot be performed by any given method, appropriate replacement of the substrate, sample dilution factor, buffer solution, and temperature for reaction is acceptable where this is scientifically justifiable.

Method 1

Sample Solution Weigh 0.50 g of Xylanase, add acetate buffer (0.01 mol/L) at pH 4.5 or water to dissolve it or disperse it uniformly, and make 5 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, 10,000-fold, or 100,000-fold dilution by adding the same buffer or water to the resulting solution.

Substrate Solution Add 4.0 g of xylan or arabinoxylan gradually to 50 mL of sodium hydroxide TS (1 mol/L) while stirring to dissolve it, and add 2 drops of phenolphthalein–sodium carbonate TS. Neutralize this solution with hydrochloric acid TS (1 mol/L), and add 100 mL of acetate buffer (pH 4.5) and water to make 200 mL.

Test Solution Transfer 2 mL of the substrate solution into a test tube, equilibrate it at 40°C for 5 minutes, add 1 mL of the sample solution, and shake well. Incubate the mixture at 40°C for 30 minutes, add 0.5 mL of diluted sulfuric acid (3 in 50), shake well, and allow to stand for 10 minutes. To this solution, add 1 drop of phenolphthalein–sodium carbonate TS, neutralize the solution with sodium hydroxide TS (1 mol/L), and add water to make 5 mL. Add 5 mL of copper TS (for xylanase/dextranase activity test), and shake. Stopper the test tube loosely, heat in a water bath for 20 minutes with occasional shaking, and cool rapidly to 20–30°C. To the resulting solution, add 2 mL of potassium iodide solution (1 in 40), shake, then add 1.5 mL of diluted sulfuric acid (3 in 50), and immediately shake vigorously. When the solution has become clear, use it as the test solution.

Control Solution Transfer 2 mL of the substrate solution into a test tube, add 0.5 mL of diluted sulfuric acid (3 in 50), shake well, then add 1 mL of the sample solution, and shake well. To this solution, add 1 drop of phenolphthalein–sodium carbonate TS, neutralize with sodium hydroxide TS (1 mol/L), and add water to make 5 mL. Proceed as directed for the test solution.

Procedure Titrate each of the test solution and the control solution with 0.005 mol/L sodium thiosulfate. When the solution turns light yellow, add 1 mL of starch TS, and continue titration until the blue color disappears. The amount of 0.005 mol/L sodium thiosulfate consumed by the test solution is less than that of 0.005 mol/L sodium thiosulfate consumed by the control solution.

Method 2

Sample Solution Weigh 0.50 g of Xylanase, add acetic acid–sodium hydroxide buffer (0.025 mol/L) at pH 4.7 to dissolve it or disperse it uniformly, and make 50 mL. As necessary, prepare a 10-fold, 100-fold, 1000-fold, or 10,000-fold dilution by adding the same buffer to the resulting solution.

Test Solution Equilibrate 1 mL of the sample solution at 40°C for 5 minutes, add 100 mg of azurine cross-linked wheat arabinoxylan, and allow the mixture to incubate at 40°C for 10 minutes, and add 10 mL of a 2% (w/v) solution of 2-amino-2-hydroxymethyl-1,3-propanediol, and immediately shake. Allow this solution to stand at room temperature for 5 minutes, stir it, and filter through a filter paper.

Control Solution To 1 mL of the sample solution, add 10 mL of a 2% (w/v) solution of 2-amino-2-hydroxymethyl-1,3-propanediol, shake well, add 100 mg of azurine-crosslinked wheat arabinoxylan, allow the mixture to stand for 10 minutes, and filter through a filter paper.

Procedure Measure the absorbance of the test solution and control solution at a wavelength of 590 nm. The absorbance value of the test solution is higher than that of the control solution.

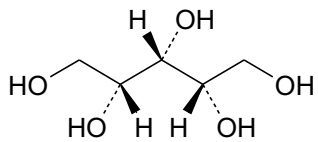
Method 3 Proceed as directed in Method 1 of the Hemicellulase Activity Test for Hemicellulase.

Method 4 Proceed as directed in Method 2 of the Hemicellulase Activity Test for Hemicellulase.

Xylitol

Xylit

キシリトール



$C_5H_{12}O_5$ Mol. Wt. 152.15

meso-Xylitol [87-99-0]

Content Xylitol, when calculated on the anhydrous basis, contains not less than 98.5% of xylitol ($C_5H_{12}O_5$).

Description Xylitol occurs as white crystals or crystalline powder. It is odorless and has a cool, sweet taste.

Identification

(1) Dissolve 5 g of Xylitol in 10 mL of a 1:1 mixture of hydrochloric acid/formaldehyde. Heat at 50°C for 2 hours, and add 25 mL of ethanol (95). Crystals are deposited. Collect the crystals by filtration, add 10 mL of water, and dissolve it by warming. Add 50 mL of ethanol (95) to produce crystals. Collect the deposited crystals by filtration, recrystallize twice from ethanol (95), and dry at 105°C for 2 hours. The melting point is 195–201°C.

(2) Determine the absorption spectrum of Xylitol, previously dried in a phosphorus(V) oxide desiccator under reduced pressure for 24 hours, as directed in the Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum or the Xylitol Reference Standard spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Melting Point 92–96°C.

pH 5.0–7.0 (1.0 g, water 10 mL).

Purity

(1) Clarity of solution Clear (1.0 g, water 2.0 mL).

(2) Lead Not more than 1 μg/g as Pb (4.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(3) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(4) Nickel Not more than 2.0 μg/g as Ni.

Weigh 50.0 g of Xylitol, dissolve it in a 1:1 mixture of water/acetic acid TS (1 mol/L) to make 500 mL. Use this solution as Solution A.

Test Solution Transfer 100 mL of Solution A into a separating funnel, add 2.0 mL of ammonium pyrrolidine dithiocarbamate solution (1 in 100) and 10 mL of 4-methyl-2-pentanon, shake, and collect the 4-methyl-2-pentanon layer.

Control Solution Transfer 100 mL of Solution A into each of 3 separating funnels. To the funnels, add 0.5, 1.0, and 1.5 mL of Nickel Standard Solution, respectively, and then proceed as directed for the test solution.

Procedure Perform the test on the test solution and the control solutions as directed under Flame Atomic Absorption Spectrophotometry using the operating conditions below, and determine the content of Nickel using the Standard Addition Method.

Operating Conditions

Light source: Nickel hollow cathode lump.

Wavelength of analytical line: 232.0 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(5) Other sugar-alcohols Not more than 1.0%.

Calculate each content (%) of L-arabinitol, galactitol, D(–)-mannitol, and D-sorbitol as

directed in the Assay. The total of these contents is the content (%) of other sugar-alcohols.

Control Solution Weigh accurately about 10 mg of each sugar-alcohol standards, and dissolve it in water to make exactly 100 mL.

(6) Reducing sugars Not more than 0.2% as D-glucose.

Weigh 1.0 g of Xylitol, transfer into a flask, and dissolve it in 25 mL of water. Add 40 mL of Fehling's TS, boil gently for 3 minutes, and allow to stand to form a precipitate of cuprous oxide. Filter the supernatant through a glass filter (1G4). Add immediately warm water in the flask, wash the precipitate, filter the washings through the same glass filter, and discard the filtrate. Repeat the washing and filtering process until the washings are no longer alkaline. Immediately add 20 mL of ferric sulfate TS to the precipitate in the flask, and dissolve. Filter through the above glass filter, wash with water, and combine the washings with the filtrate. Heat to 80°C, and add 0.6 mL of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear immediately.

Water Content Not more than 0.50% (1 g, Volumetric Titration, Direct Titration).

Residue on Ignition Not more than 0.1%.

Assay

Test Solution Weigh accurately about 2 g of Xylitol, and dissolve it in water to make exactly 100 mL. Measure exactly 1 mL of this solution, and add exactly 1 mL of the internal standard solution. Evaporate in a water bath at about 60°C under reduced pressure to dryness. Add 1.0 mL of pyridine (dehydrated) and 1.0 mL of acetic anhydride. Heat under a reflux condenser in a water bath for 1 hour, and cool. Use the resulting solution as the test solution. As the internal standard solution, use a solution prepared by diluting about 0.2 g of *meso*-erythritol, accurately weighed, with water to exactly 25 mL.

Control Solution Weigh accurately about 0.2 g of Xylitol Reference Standard, dissolve it in water to make exactly 10 mL. Measure exactly 1 mL of this solution, and proceed as directed for the test solution.

Procedure Analyze both the test solution and the control solution by gas chromatography using the operating conditions below. Determine the peak area ratios (Q_T and Q_S) of xylitol derivative to erythritol derivative for the test solution and the control solution, respectively. Determine the content of xylitol by the formula below, and calculate on the anhydrous basis.

$$\text{Content (\%) of xylitol } (C_5H_{12}O_5) = \frac{\text{Weight (g) of Xylitol Reference Standard} \times 10}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of 14% cyanopropyl phenyl–86% dimethylpolysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 180°C for 2 minutes, thereafter raise at a rate of 10°C/minute to 220°C, and then maintain at 220°C for 15 minutes.

Injection port temperature: 250°C.

Carrier gas: Helium.

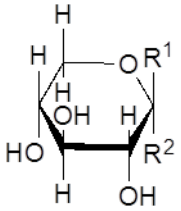
Flow rate: Adjust so that the peak of the erythritol derivative appears about 6 minutes after injection.

Injection method: Sprit.

Split ratio: 1:20.

D-Xylose

D-キシロース



α-D-Xylopyranose: $R^1 = H$, $R^2 = OH$

β-D-Xylopyranose: $R^1 = OH$, $R^2 = H$

$C_5H_{10}O_5$ Mol. Wt. 150.13

D-Xylopyranose [58-86-6]

Content D-Xylose, when dried, contains not less than 98.0% of D-xylose ($C_5H_{10}O_5$).

Description D-Xylose occurs as colorless to white crystals or as a white crystalline powder. It is odorless and has a sweet taste.

Identification

(1) Add 2–3 drops of a solution of D-Xylose (1 in 20) to 5 mL of boiling Fehling's TS. A red precipitate is formed.

(2) Dissolve 1 g of D-Xylose in 25 mL of freshly boiled and cooled water. The solution is dextrorotatory.

(3) To 1 g of D-Xylose, add 3 mL of water, dissolve it by warming, add 3 mL of a 5:2 mixture of diluted hydrochloric acid (1 in 4)/a solution (1 in 40) of diphenylamine in ethanol (95), and heat in a water bath for 5 minutes. A yellow to light orange color

develops.

(4) Dissolve 0.5 g of D-Xylose in 20 mL of water, add 30 mL of phenylhydrazinium chloride–sodium acetate TS and 10 mL of diluted acetic acid (1 in 20), heat in a water bath for about 2 hours to form a precipitate, and recrystallize the precipitate from water. The melting point is 160–163°C.

Purity

(1) Clarity of solution Colorless and almost clear (4.0 g, water 20 mL).

(2) Free acid Weigh 1.0 g of D-Xylose, dissolve it in 10 mL of freshly boiled and cooled water, add 1 drop of phenolphthalein TS, and add 1 drop of 0.2 mol/L sodium hydroxide. The color of the solution is pink.

(3) Sulfate Not more than 0.005% as SO_4.

Test Solution Weigh 1.0 g of D-Xylose, and dissolve it in 30 mL of water.

Control Solution Use 0.10 mL of 0.005 mol/L sulfuric acid.

(4) Lead Not more than 2 μg/g as Pb (2.0 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(5) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(6) Other saccharide

Test Solution Weigh 0.5 g of D-Xylose, and dissolve it in water to make 1000 mL.

Procedure Analyze 0.1 mL of the test solution by paper chromatography using a 6:4:3 mixture of 1-butanol/pyridine/water as the developing solvent. No control solution is used. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the solvent front has ascended to a point about 15 cm above the point on which the test solution was applied, and mark the front point. After the filter paper is air-dried, again develop with the same developing solvent, and stop the development when the solvent front reaches the point marked. Repeat the developing process once more, spray the filter paper with the color developing reagent, dry at 100–125°C for 5 minutes, and observe from above in daylight. Only one pink spot is observed. Prepare a color developing reagent as follows: Weigh 0.93 g of aniline and 1.66 g of phthalic anhydride, and dissolve them in 100 mL of water saturated 1-butanol.

Loss on Drying Not more than 1.0% (105°C, 3 hours).

Residue on Ignition Not more than 0.05% (5 g).

Assay Weigh accurately about 1 g of D-Xylose, previously dried, and dissolve it in water to make exactly 500 mL. Measure exactly 10 mL of this solution, transfer into a ground-glass stoppered flask, and add exactly 50 mL of a solution of sodium metaperiodate (1 in 400). Add 1 mL of sulfuric acid, and heat in a water bath for 15 minutes. After cooling, add 2.5 g of potassium iodide, shake well, allow to stand in a dark and cold place for 15 minutes, and titrate with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add starch TS near the endpoint of the titration, when the solution is pale yellow. The endpoint is when the blue color of the solution disappears. Separately, perform a blank

test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 1.877 mg of $C_5H_{10}O_5$

Yeast Cell Wall

酵母細胞壁

Definition Yeast Cell Wall is obtained from the yeast *Saccharomyces cerevisiae, Saccharomyces bayanus, or Saccharomyces pastorianus* and consists mainly of polysaccharides.

Description Yeast Cell Wall occurs as a whitish to brownish-red powder or suspension having a slight, characteristic odor.

Identification

(1) If the sample is a powder, prepare a suspension by magnetically stirring 1 g of it with 100 mL of water at a high speed. If the sample is a suspension, use as is for testing. Examine the suspension sample with a 200–400 power microscope. Egg-shaped or flat single cells with a long axis diameter of 1 to 12 μm or their fragments, are observed.

(2) To 1 g of a powder sample or a previously dried suspension sample, add 50 mL of phosphate buffer (pH 6.8), magnetically stir at a high speed, and allow to stand for 30 minutes. It swells.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g of a powder sample or previously dried suspension sample, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g of a powder sample or previously dried suspension sample, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Total nitrogen Not more than 5.6% (on the dried basis, about 1.0 g, Semi-micro Kjeldahl Method).

(4) Starch To 1.0 g of a powder sample or previously dried suspension sample, add 1 drop of iodine TS, and examine microscopically. Little or no blackish-purple stained particles are observed.

Loss on Drying

Powder sample: Not more than 8.0% (120°C, 2 hours).

Suspension sample: Not more than 92.0% (120°C, 2 hours).

Ash Not more than 10.0% (1.0 g of a powder sample or previously dried suspension sample).

Microbial Limits Proceed as directed in the Microbial Limit Tests (excluding the Method Suitability Test).

Total plate count: Not more than 5000 per gram.

Yeasts and molds: Not more than 500 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 1 for total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture Prepare as directed in Method 1, respectively, for the *Escherichia coli* test and the *Salmonella* test.

Yucca Foam Extract

ユッカフォーム抽出物

Definition Yucca Foam Extract is obtained from *Yucca brevifolia* Engelm. or *Yucca schidigera* Roezl ex Ortgies and consists mainly of saponins.

Content Yucca Foam Extract, when calculated on the anhydrous basis, contains not less than 3.0% of yucca saponins.

Description Yucca Foam Extract occurs as a yellow to brown powder or as a brown liquid having a characteristic odor.

Identification

(1) Weigh an amount of Yucca Foam Extract equivalent to 0.6 g on the anhydrous basis, add 10 mL of a 9:1 mixture of methanol/water, shake vigorously, and filter. Analyze 1 μL of the filtrate as the test solution by thin-layer chromatography using a 40:16:8:1 mixture of ethyl acetate/ethanol (95)/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate for yucca foam extract, previously dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 8 cm above the starting line. Air-dry the plate, spray with 4-methoxybenzaldehyde–sulfuric acid TS, and heat at 110°C for 10 minutes. At least four yellow-green to blue-green spots are observed at R_f values of about 0.4–0.6.

(2) Measure 3 mL of Solution A prepared in the Assay, evaporate the solvent, and dissolve the residue in 0.1 mL of ethyl acetate. Use this solution as the test solution. Use Solution B, prepared in the Assay, as the control solution. Analyze 2 μL each of the test

solution and the control solution by thin-layer chromatography using a 2:1 mixture of hexane/ethyl acetate as the developing solvent. Use a thin–layer plate for yucca foam extract, previously dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 8 cm above the starting line, and air-dry the plate. Spray with 4-methoxybenzaldehyde-sulfuric acid TS, and heat at 110°C for 10 minutes. The spot from the test solution corresponds in color tone and R_f value to the yellow-green to blue-green spot from the control solution.

pH 3.5–5.0 (1.0 g on the anhydrous basis, water 100 mL).

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Method 2, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1.5 μg/g as As (1.0 g on the anhydrous basis, Method 3, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Water Content

Liquid sample: Not more than 60% (0.1 g, Volumetric Titration, Direct Titration).

Powder sample: Not more than 8.0% (0.1 g, Volumetric Titration, Direct Titration).

Residue on Ignition Not more than 5.0% (2 g on the anhydrous basis).

Assay

Test Solution Weigh accurately an amount of Yucca Foam Extract equivalent to about 0.2 g on the anhydrous basis, and dissolve it in 5 mL of water. Pour this solution into a glass tube of 15 mm internal diameter packed with 20 mL of styrene–divinylbenzene absorption resin. Wash the resin with 100 mL of water and 100 mL of a 3:2 mixture of water/methanol at a flow rate of not more than 2 mL/minute. Next, elute with 100 mL of a 9:1 mixture of methanol/water, and evaporate the solvent in the eluate. Dissolve the residue in ethanol (95) to make exactly 20 mL. Measure exactly 10 mL of this solution, add 10 mL of hydrochloric acid TS (2 mol/L), and heat under a reflux condenser for 3 hours in a water bath. After cooling, extract twice with 80 mL of diethyl ether each time, and combine the diethyl ether phases. Then wash the diethyl ether phase with 20 mL of water. Dehydrate with 20 g of anhydrous sodium sulfate, and evaporate the diethyl ether. Dissolve the residue in ethyl acetate to make exactly 50 mL of solution. Refer to the resulting solution as Solution A. Measure exactly 1 mL of Solution A, and add ethyl acetate to make exactly 10 mL.

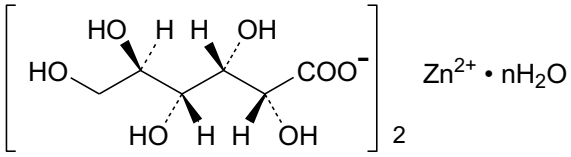
Standard Solution and Blank Test Solution Weigh accurately an amount of sarsasapogenin for assay equivalent to about 5 mg on the anhydrous basis, dissolve it in ethyl acetate to make exactly 5 mL. Refer to this solution as Solution B. Measure exactly 1 mL of Solution B, and add ethyl acetate to make exactly 200 mL. Use this solution as the standard solution. As the blank test solution, use ethyl acetate.

Procedure Measure 2 mL each of the test solution, the standard solution, and the blank test solution. To each, add 1 mL of 0.5% 4-methoxybenzaldehyde–ethyl acetate TS and 1 mL of a 1:1 mixture of sulfuric acid/ethyl acetate, and shake gently for exactly 10 minutes in a 60°C water bath. After cooling for exactly 10 minutes in a water bath at room temperature, immediately measure the absorbance (A_T, A_S and A_0) of the test solution, standard solution, and blank test solution at 430 nm, using ethyl acetate as the reference solution. Determine the yucca saponin content by the formula:

$$\text{Content (\%) of yucca saponin} = \frac{\text{Weight (g) of sarsasapogenin for assay}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T - A_0}{A_S - A_0} \times 2.10 \times 100$$

Zinc Gluconate

グルコン酸亜鉛



n = 3 or 0

$C_{12}H_{22}O_{14}Zn \cdot nH_2O$ (n = 3 or 0) Mol. Wt. trihydrate 509.72

anhydrous 455.67

Monozinc bis(D-gluconate)trihydrate

Monozinc bis(D-gluconate) [4468-02-4]

Content Zinc Gluconate, when calculated on the anhydrous basis, contains 97.0–102.0% of zinc gluconate ($C_{12}H_{22}O_{14}Zn$).

Description Zinc Gluconate occurs as a white crystalline powder or as granules.

Identification

(1) A solution of Zinc Gluconate (1 in 20) responds to all the tests for Zinc Salt in the Qualitative Tests.

(2) Measure 5 mL of a solution of Zinc Gluconate (1 in 10) in warm water, and proceed as directed in Identification (2) for Glucono-δ-Lactone.

Purity

(1) Lead Not more than 2 μg/g as Pb (2.0 g, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Test Solution To the specified amount of Zinc Gluconate, add 40 mL of diluted

hydrochloric acid (1 in 4), boil for 10 minutes with a watch glass covering it, and allow to cool. Use the resulting solution as the sample solution. To the sample solution, add 10 mL of diammonium hydrogen citrate solution (1 in 2). Add ammonia solution until the color of solution changes yellow to green, using 1 mL of thymol blue TS as the indicator. After cooling, add 5 mL of ammonium pyrrolidine dithiocarbamate solution (3 in 100), and add ammonium solution until the produced white precipitate dissolves. Transfer the solution to a separating funnel, wash the container with a small amount of water, and add the washings to the funnel to make about 150 mL. Add exactly 10 mL of buthylacetate, shake for 5 minutes, and allow the solution to stand or centrifuge it. Use the buthylacetate layer as the test solution.

Control Solution Measure the specified volume of Lead Standard Solution, and prepare in the same manner as for the sample solution.

(2) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

(3) Reducing sugars Not more than 1.0% as D-glucose.

Weigh 1.0 g of Zinc Gluconate, transfer into a 250-mL Erlenmeyer flask, dissolve it in 10 mL of water, and add 25 mL of copper(II) citrate TS (alkaline). Cover with a small beaker, boil gently for exactly 5 minutes, and cool quickly to room temperature. To this solution, add 25 mL of diluted acetic acid (1 in 10), exactly 10 mL of 0.05 mol/L iodine, 10 mL of diluted hydrochloric acid (1 in 4), and 3 mL of starch TS, in that order. Titrate the excess iodine with 0.1 mol/L sodium thiosulfate. The volume of the sodium thiosulfate solution consumed is not less than 6.3 mL.

Water Content Not more than 11.6% (0.2 g, Volumetric Titration, Direct Titration).

Assay Weigh accurately about 0.7 g of Zinc Gluconate, add 100 mL of water, and dissolve it by warming if necessary. Add 5 mL of ammonium buffer (pH 10.7), and titrate with 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 0.1 mL of eriochrome black T TS) until the color of the solution changes to blue. Calculate the content on the anhydrous basis.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 22.79 mg of $C_{12}H_{22}O_{14}Zn$

Zinc Sulfate

硫酸亜鉛

$ZnSO_4 \cdot 7H_2O$ Mol. Wt. 287.55

Zinc sulfate heptahydrate [7446-20-0]

Content Zinc Sulfate contains 98.0–102.0% of zinc sulfate ($ZnSO_4 \cdot 7H_2O$).

Description Zinc Sulfate occurs as colorless crystals or as a white crystalline powder. It is odorless.

Identification Zinc Sulfate responds to all the tests for Zinc Salt and for Sulfate in the Qualitative Tests.

Purity

(1) Free acid Weigh 0.25 g of Zinc Sulfate, dissolve it in 5 mL of water, and add 1 drop of methyl orange TS. No red color develops.

(2) Lead Not more than 2 μg/g as Pb (2.0 g, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

Test Solution Add 40 mL of diluted hydrochloric acid (1 in 4) to the specified amount of Zinc Sulfate, boil for 10 minute with a watch glass covering it, and allow to cool. Use this solution as the sample solution. To the sample solution, add 10 mL of diammonium hydrogen citrate solution (1 in 2) and 1 mL of thymol blue TS as the indicator, and then add ammonia solution until the solution changes from yellow to green. After cooling, add 5 mL of a solution of ammonium pyrrolidine dithiocarbamate (3 in 100), and ammonia solution until the produced white precipitate dissolves. Transfer this solution into a separating funnel, wash the container with a little amount of water, and add the washings to the funnel to make about 150 mL. Add exactly 10 mL of butyl acetate, shake for 5 minutes, and allow to stand or centrifuge. Use the butyl acetate layer as the test solution.

Control Solution Using the specified amount of Lead Standard Solution, proceed as directed for the sample solution.

(3) Alkali metal and alkali-earth metals Not more than 0.50%.

Weigh 2.0 g of Zinc Sulfate, dissolve it in 150 mL of water, and add ammonium sulfide TS until the precipitate is no longer formed. Add water to make 200 mL, and filter through a dry filter paper. Discard the initial 20 mL of filtrate, measure the subsequent 100 mL of filtrate, evaporate to dryness, ignite at 450–550°C to constant weight, and weigh the residue.

(4) Arsenic Not more than 3 μg/g as As (0.50 g, Method 1, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Assay Weigh accurately about 0.4 g of Zinc Sulfate, add 100 mL of water, and dissolve it by warming if necessary. Add 5 mL of ammonium buffer (pH 10.7) , and titrate with 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 0.1 mL of eriochrome black T TS) until the color of the solution changes to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate = 14.38 mg of $ZnSO_4 \cdot 7H_2O$