C. REAGENTS, SOLUTIONS, AND OTHER REFERENCE MATERIALS

C. REAGENTS, SOLUTIONS, AND OTHER REFERENCE MATERIALS

Unless otherwise specified, the reagents, test solutions (TS), volumetric solutions, standard solutions, reference standards, stationary phases and packing materials for chromatography, thermometers, filter papers, filters, sieves, and gas measuring instruments, and measurement instruments to be used in the tests specified in JSFA-IX shall meet the specifications given in the corresponding sections below. The Reference Infrared Absorption Spectra are given in section 11.

Reagents that meet the Japanese Industrial Standards are given a Japanese Industrial Standard Number (JIS number). In addition, if these reagents are graded, they are accompanied by the corresponding grade category, like "Special Grade," "First Grade," or "pH Standard Solution Grade" in the square brackets.

When a reagent name in this publication is different from that in the Japanese Industrial Standards, the JIS name is given in square brackets following the reagent name in Section C. Certified reference materials mean those that have complied with JIS Q0034 and have been accompanied by a certificate specified by JIS Q0031. Standard solutions and standard gases that are specified by the Measurement Act of Japan (Act No. 207, 1951) mean those that have complied with JIS Q0034 and have been accompanied by a certificate based on Article 144, Paragraph 1 of the Measurement Act.

Glass containers to store reagents, TS, volumetric solutions, and standard solutions shall be extremely low in solubility and in alkalinity and contain as little lead and arsenic as possible.

1. Reagents and Test Solutions (TS)

Absorbing Solution for Arsine Dissolve 0.50 g of *N,N*-silver diethyldithiocarbamate in pyridine to make 100 mL. Store in a tightly stoppered, light-resistant bottle in a cold place.

ABTS TS Dissolve 41 mg of diammonium 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonate) in a small amount of water, and add water to make 10 mL. Prepare fresh before use.

Acarbose $C_{25}H_{43}NO_{18}$ Use a product suitable for the corresponding enzyme activity tests.

Acetaldehyde CH_3CHO [K8030] [75-07-0]

Acetate Buffer Dissolve 82 g of sodium acetate in 140 mL of water. Add 25 mL of acetic acid and water to make 250 mL. Adjust the pH to 5.51 ± 0.03 with acetic acid or sodium acetate trihydrate solution (2 in 15).

Acetate Buffer (1 mol/L)

Solution 1 Dissolve 82 g of sodium acetate in water to make 1000 mL.

Solution 2 To 60 g of acetic acid, add water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Acetate Buffer (1 mol/L, pH 5.0) Dissolve 88.8 g of sodium acetate trihydrate in 1800 mL of water. Adjust the pH to 5.0 with acetic acid and dilute with water to exactly 2000 mL.

Acetate Buffer (0.2 mol/L)

Solution 1 Dissolve 16.4 g of sodium acetate in water to make 1000 mL.

Solution 2 To 12.0 g of acetic acid, add water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Acetate Buffer (0.2 mol/L, pH 6.0, containing calcium chloride and sodium chloride) Dissolve 27.2 g of sodium acetate trihydrate in 900 mL of water, and adjust the pH to 6.0 with diluted acetic acid (1 in 100). Add 75 mg of calcium chloride dihydrate and 0.6 g of sodium chloride to dissolve them, and add water to make 1000 mL.

Acetate Buffer (0.1 mol/L)

Solution 1 Dissolve 8.2 g of sodium acetate in water to make 1000 mL.

Solution 2 To 6.0 g of acetic acid, add water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Acetate Buffer (0.1 mol/L, pH 4.0, containing ethanol)

Solution 1 To 6.0 g of acetic acid, add 200 mL of ethanol (99.5) and water to make 1000 mL.

Solution 2 Dissolve13.6 g of sodium acetate trihydrate in water, and add 200 mL of ethanol (99.5) and water to make 1000 mL.

Mix both solutions, and adjust the pH to 4.0.

Acetate Buffer (0.1 mol/L, pH 4.3, containing polyoxyethylene(10) octylphenyl ether)

Solution 1 Dissolve 8.2 g of sodium acetate in water to make 1000 mL.

Solution 2 To 6.0 g of acetic acid, add water to make 1000 mL.

Mix both solutions, adjust the pH to 4.3, and add polyoxyethylene(10) octylphenyl ether by 0.1% (w/v).

Acetate Buffer (0.1 mol/L, pH 5.0, containing polyoxyethylene(23) lauryl ether) To 500 mL of acetate buffer (1 mol/L, pH 5.0), add 3.5 L of water and 7.5 mL of polyoxyethylene(23) lauryl ether TS. Adjust the pH to 5.0 with sodium hydroxide solution of an appropriate concentration, and dilute with water exactly to 5000 mL.

Acetate Buffer (0.1 mol/L, pH 6.0, containing albumin) Dissolve 0.1 g of bovine serum albumin and 0.33 g of sodium azide in 500 mL of water, add 100 mL of acetate buffer (1 mol/L) at pH 6.0 and water to make 1000 mL.

Acetate Buffer (0.1 mol/L, pH 6.0, containing calcium chloride)

Solution 1 Dissolve 6.0 g of acetic acid and 0.74 g of calcium chloride dihydrate in water to make 1000 mL.

Solution 2 Dissolve 8.2 g of sodium acetate and 0.74 g of calcium chloride dihydrate in water to make 1000 mL.

Mix both solutions, and adjust the pH to 6.0.

Acetate Buffer (0.1 mol/L, pH 6.0, containing polyoxyethylene(10) octylphenyl ether and sodium chloride) Dissolve 11.7 g of sodium chloride in water, and add 100 mL of acetate buffer (1 mol/L) at pH 6.0, 2 mL of a solution of polyoxyethylene(10) octylphenyl ether (1 in 20), and water to make 1000 mL.

Acetate Buffer (0.05 mol/L)

Solution 1 Dissolve 4.1 g of sodium acetate in water to make 1000 mL.

Solution 2 To 3.0 g of acetic acid, add water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Acetate Buffer (0.05 mol/L, pH 6.0, containing calcium chloride) Mix 50 mL of acetate buffer (1 mol/L) at pH 6.0 and 20 mL of calcium chloride TS (1 mol/L), and add water to make 1000 mL.

Acetate Buffer (0.02 mol/L)

Solution 1 Dissolve 1.64 g of sodium acetate in water to make 1000 mL.

Solution 2 To 1.20 g of acetic acid, add water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Acetate Buffer (0.02 mol/L, pH 5.0, containing albumin) Dissolve 25 mg of bovine serum albumin by adding 10 mL of acetate buffer (1 mol/L) at pH 5.0 and 490 mL of water. Store in a cold place and use within one month.

Acetate Buffer (0.01 mol/L)

Solution 1 Dissolve 0.82 g of sodium acetate in water to make 1000 mL.

Solution 2 To 0.60 g of acetic acid, add water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Acetate Buffer (0.01 mol/L, pH 5.5, containing calcium chloride) Mix 10 mL of acetate buffer (1 mol/L) at pH 5.5 and 10 mL of calcium chloride TS (1 mol/L), and add water to make 1000 mL.

Acetate Buffer (0.01 mol/L, pH 5.5, containing magnesium chloride and calcium chloride) Dissolve 1.0 g of magnesium chloride hexahydrate and 0.74 g of calcium chloride dihydrate in water, and 10 mL of acetate buffer at pH 5.5 and 10 mL of a solution of polyoxyethylene(23) lauryl ether (3 in 20). Adjust the pH to 5.5 with hydrochloric acid TS (2 mol/L) or sodium hydroxide TS (1 mol/L), add water to make 1000 mL.

Acetate Buffer (0.01 mol/L, pH 6.0, containing polyoxyethylene(10) octylphenyl ether) Dissolve 10 mL of acetate buffer (1 mol/L) at pH 6.0 and 1 mL of a solution of

polyoxyethylene(10) octylphenyl ether (1 in 20) in water to make 1000 mL.

Acetate Buffer (0.005 mol/L)

Solution 1 Dissolve 0.41 g of sodium acetate in water to make 1000 mL.

Solution 2 To 0.30 g of acetic acid, add water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Acetate Buffer (pH 4.0) Dissolve 2.95 g sodium acetate in 900 mL of water, add a few drops of acetic acid to adjust the pH to 4.0, and add water to make 1000 mL.

Acetate Buffer (pH 4.5)

Solution 1 To 6.0 g of acetic acid, add water to make 1000 mL.

Solution 2 Dissolve 8.2 g of sodium acetate in water to make 1000 mL.

Mix both solutions, and adjust the pH to 4.5 with either Solution 1 or 2.

Acetate Buffer (pH 5.4)

Solution 1 To 5.78 mL of acetic acid, add water to make 1000mL.

Solution 2 Dissolve 8.5 g of sodium acetate in water to make 1000 mL.

Mix 176 volumes of Solution 1 and 824 volumes of Solution 2. Adjust the pH to 5.4 with either of the solutions 1 and 2.

Acetate Buffer (pH 5.5) Dissolve 10 g of sodium acetate trihydrate by adding 10 mL of acetate buffer (1 mol/L) and water to make 1000 mL. Adjust the pH to 5.5 if necessary.

Acetate Buffer (pH 5.6, containing zinc sulfate) Dissolve 0.60 g of acetic acid, 12.3 g of sodium acetate trihydrate, and 0.29 g of zinc sulfate heptahydrate in water to make 1000 mL. Confirm the pH to be 5.6 before use.

Acetate Buffer (pH 5.6, containing zinc sulfate and albumin) To 20 mL of a solution of bovine serum albumin (1 in 100), add acetate buffer (pH 5.6, containing zinc sulfate) to make 1000 mL. Prepare fresh before use.

Acetic Acid CH_3COOH [K8355, Special Grade] [64-19-7]

Acetic Acid–Citric Acid–Sodium Hydroxide Buffer (pH 4.2) Dissolve 60 g of acetic acid and 6.3g of citric acid monohydrate in 700 mL of water, adjust the pH to 4.2 with sodium hydroxide TS (2 mol/L), and add water to make 1000 mL.

Acetic Acid for Nonaqueous Titration Measure 1000 mL of acetic acid, add 5 g of chromium(VI) oxide, and allow to stand overnight. Filter, and distill the filtrate. To the distillate obtained at 115°C or above, add 20 g of acetic anhydride, and redistill. Use the fraction obtained at a constant boiling temperature of 117–118°C.

Acetic Acid–Potassium Chloride–Zinc Sulfate TS Dissolve 70 g of potassium chloride and 20 g of zinc sulfate heptahydrate in 700 mL of water, and add 200 mL of acetic acid and water to make 1000 mL.

Acetic Acid–Sodium Acetate Buffer (pH 4.5) for Iron Limit Test Dissolve 75.4 mL of acetic acid and 111 g of sodium acetate trihydrate in water to make 1000 mL.

Acetic Acid–Sodium Hydroxide Buffer (2 mol/L) To 120 g of acetic acid, add 500 mL of water, adjust the pH to the corresponding value specified in this publication with sodium

hydroxide TS (1 mol/L), and add water to make 1000 mL.

Acetic Acid–Sodium Hydroxide Buffer (1mol/L)

Solution 1 To 60 g of acetic acid, add water to make 1000 mL.

Solution 2 Dissolve 40 g of sodium hydroxide in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Acetic Acid–Sodium Hydroxide Buffer (0.5 mol/L) To 30 g of acetic acid, add 600 mL of water, adjust the pH to the corresponding value specified in this publication with sodium hydroxide TS (1 mol/L), and add water to make 1000 mL.

Acetic Acid–Sodium Hydroxide Buffer (0.4 mol/L, pH 6.0, containing calcium chloride) Dissolve 24 g of acetic acid and 7.4 g of calcium chloride dihydrate in 600 mL of water. Adjust the pH to 6.0 with sodium hydroxide TS (1 mol/L), and add water to make 1000 mL.

Acetic Acid–Sodium Hydroxide Buffer (0.2 mol/L)

Solution 1 To 12 g of acetic acid, add water to make 1000 mL.

Solution 2 Dissolve 8.0 g of sodium hydroxide in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Acetic Acid–Sodium Hydroxide Buffer (0.1 mol/L)

Solution 1 To 6.0 g of acetic acid, add water to make 1000 mL.

Solution 2 Dissolve 4.0 g of sodium hydroxide in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Acetic Acid–Sodium Hydroxide Buffer (0.1 mol/L, pH 4.3, containing sodium chloride) Dissolve 2.8 g of acetic acid and 2.9 g of sodium chloride dihydrate in 900 mL of water. Adjust the pH to 4.3 with sodium hydroxide TS (2 mol/L), and add water to make 1000 mL.

Acetic Acid–Sodium Hydroxide Buffer (0.05 mol/L) To 3.0 g of acetic acid, add 800 mL of water, adjust the pH to the corresponding value specified in this publication with sodium hydroxide TS (1 mol/L), and add water to make 1000 mL.

Acetic Acid–Sodium Hydroxide Buffer (0.05 mol/L, pH 5.8, containing sodium chloride) Dissolve 2.8 g of acetic acid and 12.9 g of sodium chloride in 900 mL of water. Adjust the pH to 5.8 with sodium hydroxide TS (2 mol/L), and add water to make 1000 mL.

Acetic Acid–Sodium Hydroxide Buffer (0.025 mol/L) To 1.5 g of acetic acid, add 900 mL of water, adjust the pH to the corresponding value specified in this publication with sodium hydroxide TS (1 mol/L), and add water to make 1000 mL.

Acetic Acid–Sodium Hydroxide Buffer (0.02 mol/L) To 1.2 g of acetic acid, add 900 mL of water, adjust the pH to the corresponding value specified in this publication with sodium hydroxide TS (1 mol/L), and add water to make 1000 mL.

Acetic Acid–Sodium Hydroxide Buffer (0.01 mol/L, pH 4.0, containing acarbose)

Dissolve 0.26 g of acarbose in 50 mL of acetic acid–sodium hydroxide buffer (0.02 mol/L) at pH 4.0, and add water to make 100 mL.

Acetic Acid TS (6 mol/L) Dissolve 360 g of acetic acid in water to make 1000 mL.

Acetic Acid TS (1 mol/L) Dissolve 6 g of acetic acid in water to make 100 mL.

Acetic Acid TS (0.75 mol/L) Dissolve 45 g of acetic acid in water to make 1000 mL.

Acetic Acid TS (0.1 mol/L) Dissolve 6 g of acetic acid in water to make 1000 mL.

Acetic Anhydride $(CH_3CO)_2O$ [K8886, Special Grade] [108-24-7]

Acetic Anhydride–Pyridine TS To 25 g of acetic anhydride, add pyridine (dehydrated) to make 100 mL. Prepare fresh before use.

Acetone CH_3COCH_3 [K8034, Special Grade] [67-64-1]

Acetone (dehydrated) CH_3COCH_3 [67-64-1] A colorless, clear liquid.

Content Not less than 99.5% of acetone (CH_3COCH_3).

Specific gravity d_{20}^{20}: 0.788–0.792.

Water Not more than 0.001%.

Assay Analyze 0.2-μL portions of acetone by gas chromatography using the operating conditions given below. Measure the peak area of each peak to determine the percentage of the main peak by the peak area percentage method.

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.53 mm internal diameter and 30 m length) coated with a 5.0 μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 40°C for 5 minutes, raise at 5°C/minute to 90°C, and maintain the temperature at 90°C for 2 minutes.

Injection port temperature: 150°C.

Detector temperature: 150°C.

Carrier gas: Helium.

Flow rate: 5 mL/min.

Acetonitrile CH_3CN [K8032, Special Grade] [75-05-8]

Acetonitrile (for HPLC) CH_3CN [75-05-8] A colorless, clear liquid.

Content Not less than 99.8%.

Identification Determine the absorption spectrum of acetonitrile (for HPLC) as directed in the Liquid Film Method under Infrared Spectrophotometry. The spectrum exhibits absorptions at 3000 cm^{-1}, 2250 cm^{-1}, 1440 cm^{-1}, 1380 cm^{-1}, 1040 cm^{-1}, 920 cm^{-1}, and 750 cm^{-1}.

Density 0.780–0.783 g/mL (20°C).

Absorbance Determine the absorbance against water. It is not more than 0.05 at 200 nm, not more than 0.02 at 220 nm, and not more than 0.005 at 240 nm.

Assay Analyze 0.2-μL portions of acetonitrile by gas chromatography using operating conditions given below. Measure the peak area of each peak to determine the percentage of the main peak by the peak area percentage method.

Operating conditions

Detector: Flame-ionization detector.

Column: A fused silica tube (0.25 mm internal diameter and about 30 m length) coated with a 0.25-μm thick layer of polyethylene glycol for gas chromatography.

Column temperature: 60°C.

Injection port temperature: 110°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 1.2 mL/min.

Injection method: Split.

Split ratio: 1:200.

Loss on drying Not more than 0.1% (0.1 g, reduced pressure, 24 hours).

Acetylacetone $C_5H_8O_2$ [K8027]

Acetylacetone TS Mix 1 mL of acetylacetone and 50 mL of sodium carbonate TS (0.5 mol/L). Prepare fresh before use.

Acetylene C_2H_2 [Dissolved Acetylene, K1902] [74-86-2]

***N*-Acetyl-DL-methionine** $CH_3SCH_2CH_2CH(NHCOCH_3)COOH$ Use a product suitable for the corresponding enzyme activity tests.

2-Acetyl-4-tetrahydroxybutylimidazole $C_9H_{14}N_2O_5$ [94944-70-4] Grayish-white crystals or crystalline powder. Freely soluble in methanol or in ethanol (95), and sparingly soluble in water.

Melting point 234–236°C.

Purity Dissolve 10.0 mg of 2-acetyl-4-tetrahydroxybutylimidazole in 100 mL of methanol. Analyze this solution by liquid chromatography using the operating conditions given below. No peaks other than the peak of 2-acetyl-4-tetrahydroxybutylimidazole are observed.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 280 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 15 cm length).

Packing material of column: 5-μm octadecylsilanized silica gel for liquid chromatography.

Mobile phase: A 60:45 mixture of methanol/0.2% (w/v) phosphoric acid.

Flow rate: 0.6 mL/min.

2-Acetyl-4-tetrahydroxybutylimidazole 2,4-Dinitrophenylhydrazone $C_{15}H_{18}N_6O_8$ Add 1 mL of hydrochloric acid to 0.50 g of 2,4-dinitrophenylhydrazine, mix, and add 10 mL of ethanol (95) to dissolve by heating in a water bath. To the resulting solution, add 0.1 g of 2-acetyl-4-tetrahydroxybutylimidazole and dissolve it. Leave this solution to cool to room temperature, and collect the crystals of 2-acetyl-4-tetrahydroxybutylimidazole 2,4-dinitrophenylhydrazone. Repeat the recrystallization at least twice with 5 mL of ethanol (95) to which 1 drop of hydrochloric acid is added. Dry the crystals produced in a

desiccator for 24 hours at room temperature. Store in cool place and use within one year.

Purity Related substances Determine 2-acetyl-4-tetrahydroxybutylimidazole by liquid chromatography using the operating conditions specified in Procedure in Purity (8) (ii) for Caramel III in the Monographs. Continue the chromatography for four times the retention time of the main peak, and measure the peak area of each peak. When determined using the peak area percentage method, the area percentage of the main peak is not less than 98%.

***N*-Acetyl-DL-tryptophan** $C_{13}H_{14}N_2O_3$ Use a product suitable for the corresponding enzyme activity tests.

Acriflavine Hydrochloride $C_{27}H_{28}Cl_4N_6$ [8063-24-9] A dark red-brown crystalline powder. A solution of acriflavine hydrochloride (1 in 100) is reddish brown.

When 30 mL of water is added to 1 mL of this solution, the solution turns yellow, emitting fluorescence. On the subsequent addition of 1 mL of hydrochloric acid, the fluorescence disappears. When sodium hydrogen carbonate solution (1 in 20) is added to a solution of acriflavine hydrochloride (1 in 10), an effervescence occurs.

Acrylate Resin for Adsorption Porous resin made as adsorbent.

Active Carbon Use medicinal carbon in the Japanese Pharmacopoeia.

Adenosine 3′-Monophosphate Sodium Salt $C_{10}H_{14}N_5O_7P \cdot 2Na$ [4958-39-8] Use a product suitable for the corresponding enzyme activity tests.

Adenosine 5′-Monophosphate Sodium Salt $C_{10}H_{14}N_5O_7P \cdot mNa \cdot nH_2O$ [149022-20-8] Use a product suitable for the corresponding enzyme activity tests.

Adipic Acid $HOOC(CH_2)_4COOH$ [124-04-9] "Adipic Acid"

Advantame-acid $C_{23}H_{28}N_2O_7$ *N*-[3-(3-Hydroxy-4-methoxyphenyl)propyl]-L-α-aspartyl-L-phenyl Alanine A white to yellow powder.

Content Not less than 94% of advantame-acid ($C_{23}H_{28}N_2O_7$) on anhydrous basis.

Purity (1) Chlorides Not more than 1.0% as Cl.

Prepare a test solution by dissolving about 10 mg of advantame-acid, accurately weighed, in a 7:3 mixture of water/acetonitrile and making exactly 100 mL. Prepare two standard solutions. First, add water to about 16 mg of sodium chloride, accurately weighed, to disslove and make exactly 100 mL. Designate this as Standard Solution A. Prepare Standard Solution B by dilutuing exactly measured 2 mL of Standard Solution A with water to make exactly 20 mL. Anlyze 30-μL potions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Measure the peak areas of chloride anion for Standard Solutions A and B to make a calibration curve. Measure the peak area of chloride anion for the test solution. Determine the concentration of chlorides in the test solution from the clibration curve and calculate the amount of chlorides by the formula:

$$\text{Amount (\%) of chlorides} = \frac{\text{Concentration (g/mL) of chlorides in the test solution}}{\text{Weight (g) of the sample}} \times 10{,}000$$

Operating conditions

Detector: Electric conductivity detector.

Column: A polyether ketone tube (about 4.6 mm internal diameter and about 15 cm length).

Column packing material: 6-μm strongly basic anion-exchange resin.

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

Mobile phase: A mixture prepared by dissolving 201.62 mg of sodium hydrogen carbonate and 264.98 mg of sodium carbonate in 1000 mL of water.

Flow rate: Adjust the retention time of chloride anion to about 7 minutes.

(2) Sodium Not more than 5.0% as Na.

Prepare a test solution by dissolving about 10 mg of advantame-acid, accurately weighed, in a 7:3 mixture of water/acetonitrile and making exactly 100 mL. Prepare two standard solutions. First, add water to about 6 mg of sodium chloride, accurately weighed, to disslove and make exactly 100 mL. Designate this as Standard Solution A. Prepare Standard Solution B by dilutuing exactly measured 2 mL of Standard Solution A with water to make exactly 20 mL. Anlyze 30-μL potions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Measure the peak areas of sodium for Standard Solutions A and B to make a calibration curve. Measure the peak area of sodium cation for the test solution. Determine the concentration of sodium cation in the test solution from the clibration curve and calculate the amount of sodium by the formula:

$$\text{Amount (\%) of sodium} = \frac{\text{Concentration (g/mL) of sodium in the test solution}}{\text{Weight (g) of the sample}} \times 10{,}000$$

Operating conditions

Detector: Electric conductivity detector.

Column: A polyether ketone tube (about 4.6 mm internal diameter and about 15 cm length).

Column packing material: 3-μm weakly acidic cation-exchange resin for liquid chromatography.

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

Mobile phase: A solution prepared by adding 1.25 mL of methanesulfonic acid (24 in 125) and 1000 mL of water to 77.58 mg of L-histidine.

Flow rate: Adjust the retention time of sodium cation to about 4 minutes.

Water Not more that 1.0% (0.1 g, Volumetric Titration, Direct Titration).

Assay Prepare a test solution by dissolving 10 mg of advantame-acid in a 7:3 mixture of water/acetonitrile and making exactly 50 mL. Anlyze 20-μL potions of the test solution by liquid chromatography using the operating conditions given below. Continue chromatography for about six times the retention time of advantame-acid. Measure the peak areas, and determine the area percentage, C (%), of the main peak by normalizing

the sum of the peak areas of all components in the test solution to 100. Calculate the content by the formula:

$$\text{Content (\%) of advantame-acid } (C_{28}H_{28}N_2O_7) = (100 - \text{chlorides amount} - \text{sodium amount} - \text{water}) \times \frac{\text{C (\%)}}{100}$$

Operating conditions

Follow the conditions given in the Assay for Advantame in the Monographs.

Advantame for Assay $C_{24}H_{30}N_2O_7 \cdot H_2O$ [714229-20-6] A white to yellowish white powder.

Content Not less than 99.0% of advantame ($C_{24}H_{30}N_2O_7$) when calculated on the anhydrous basis.

Identification Determine the infrared absorption spectrum of advantame for assay as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorption bands at about 3405 cm^{-1}, 3320 cm^{-1}, 2945 cm^{-1}, 1717 cm^{-1}, 1661 cm^{-1}, 1582 cm^{-1}, 1376 cm^{-1}, 1242 cm^{-1}, 1131 cm^{-1}, and 703 cm^{-1}.

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

Purity Related substances Not more than 1.0% as advantame-acid.

Prepare a test solution by dissolving about 0.1 g of advantame for assay, accurately weighed, in a 7:3 mixture of water/acetonitrile and making exactly 100 mL. Prepare a standard solution as follows: Add a 7:3 mixture of water/acetonitrile to about 0.1 g of advantame-acid, accuretaly weighed, to dissolve it and make exactly 100 mL. To exactly measured 2 mL of this solution, add a 7:3 mixture of water/acetonitrile to make exactly 20 mL. To exactly measured 2 mL of the second solution, add a 7:3 mixture of water/acetonitrile to make exactly 20 mL. Analyze 20 μL potions of the test solution and the standard solution 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_T) of the peak areas of all components, other than advantame, in the test solution and the peak area (A_S) of advantame-acid in the standard solution. Calculate the amount of the related substances by the formula:

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

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

Operating Conditions

Follow the operating conditions given in Purity (3) for Advantame in the Monographs.

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

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

Assay Weigh accurately about 0.5 g of advantame for assay, dissolve it in 100 mL of ethanol, and titrate with 0.1 mol/L sodium hydroxide. To confirm the endpoint, use a potentiometer with a glass indicator electrode and a silver-silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes. Perform a blank test to make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide = 45.85 mg of $C_{24}H_{30}N_2O_7$

Agar [K8263, Special Grade] [9002-18-0]

L-Alanyl-prolyl-glycine $C_{10}H_{17}N_3O_4$ Use a product suitable for the corresponding enzyme activity tests.

Albumin (egg-derived) Ovalbumin Use a product suitable for the corresponding enzyme activity tests.

Albumin TS Take carefully the egg white from a fresh chicken egg. Shake well with 100 mL of water, and filter. Prepare fresh before use.

Aldehyde Dehydrogenase A white powder. Enzyme activity equivalent to not less than 2 units per milligram.

Enzyme activity determination

(i) Sample Solution Dissolve about 20 mg of aldehyde dehydrogenase, accurately weighed, in 1 mL of water, add an ice-cold solution of bovine serum albumin (1 in 100) to make exactly 200 mL.

(ii) Procedure Dissolve 20.0 mg of β-nicotinamide adenine dinucleotide in water to make exactly 1 mL. Add 0.20 mL of the resulting solution, 0.10 mL of pyrazole solution (17 in 2500), and 0.10 mL of the sample solution to 2.50 mL of pyrophosphate buffer (pH 9.0), stir, stopper tightly, and allow to stand at 25 ± 1°C for 2 minutes. To this solution, add 0.01 mL of diluted acetaldehyde (3 in 1000), stir, and stopper. Every 30 seconds, measure the absorbance at 340 nm as directed under Ultraviolet-visible Spectrophotometry, and calculate a change (Δ A) in absorbance per minute from the straight line region of the absorbance-straight curve, and determine the enzyme activity by the formula given below. One unit is an amount of enzyme that oxidizes 1 μmol of acetaldehyde per minute when enzyme activity measurement is done using the operating conditions given in the procedure.

$$\text{Enzyme Activity (unit/mg) of aldehyde dehydrogenas} = \frac{2.91 \times \Delta A \times 200}{6.3 \times \text{amount (g) of the sample} \times 0.10 \times 1000}$$

Aldehyde Dehydrogenase TS Dissolve an amount of aldehyde dehydrogenase equivalent to 70 units in 10 mL of water. Prepare fresh before use.

Alizarin Red S $C_{14}H_5O_2(OH)_2SO_3Na \cdot H_2O$ [K8057, Special Grade] [130-22-3]

Allyl Isothiocyanate for Assay C_4H_5NS [57-06-7] A colorless to yellow-brown clear liquid having a lachrymatory and pungent odor.

Content Not less than 99.0%.

Assay Analyze 1 μL of allyl isothiocyanate for assay by gas chromatography using the operating conditions given below. Determine the content of allyl isothiocyanate from the area of the allyl isothiocyanate peak and the sum of all peak areas.

Operating conditions

Detector: Thermal conductivity detector.

Column: A glass or stainless tube (3 mm internal diameter and 2 m length).

Column packing material

Liquid phase: Methylphenylsilicone polymer (20% of the support).

Support: 180–250 μm diatomaceous earth for gas chromatography.

Column temperature: 120°C.

Detector temperature: 250°C.

Injection port temperature: 200°C.

Carrier gas: Helium.

Flow rate: 20 mL/minute.

Measurement time: 3 times the retention time of the main peak.

Aluminum(III) Chloride Hexahydrate $AlCl_3 \cdot 6H_2O$ [K8114, Special Grade] [7784-13-6]

Aluminum Potassium Sulfate Dodecahydrate $AlK(SO_4)_2 \cdot 12H_2O$ [K8255, Special Grade] [7784-24-9]

Amidoblack 10B $C_{22}H_{14}N_6O_9S_2Na_2$ Use a product suitable for the corresponding enzyme activity tests.

Amidoblack TS Dissolve 0.1 g of amidoblack 10B in 50 mL of a 1:4 mixture of ethanol (95)/water.

Amidol TS Dissolve 0.50 g of 2,4-diaminophenol dihydrochloride and 10.0 g of sodium hydrogen sulfite in water to make 50 mL, and filter. Prepare fresh before use.

Amidosulfuric Acid (Reference Material) $HOSO_2NH_2$ [Reference Material for Volumetric Analysis, Amidosulfuric Acid, K8005] [5329-14-6]

In addition to Reference Material for Volumetric Analysis specified in JIS K8005, a certified reference material capable of being used for volumetric analysis is usable.

4-Aminoantipyrine $C_{11}H_{13}N_3O$ [4-amino-2,3-dimethyl-1-phenyl-5-pyrazolone, K8048, Special Grade] [83-07-8]

4-Aminoantipyrine TS (0.009 mol/L) Dissolve 1.83 g of 4-aminoantipyrine in water to make 1000 mL. Store in a glass bottle, protected from light, at 30°C. Use after leaving for 24 hours.

4-Aminobenzenesulfonic Acid $C_6H_7NO_3S$ [121-57-3] A white to slightly light powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 245–251 nm): Not less than 850. Weigh accurately about 10 mg of 4-aminobenzenesulfonic acid, previously dried in a vacuum desiccator for 24 hours, and dissolve it in ammonium acetate TS (0.02mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to

make exactly 100 mL. This solution exhibits an absorption maximum at a wavelength of 245–251 nm. Measure the absorbance of this solution at the maximum between 245–251 nm against ammonium acetate TS (0.02 mol/L) to determine the specific absorbance.

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add the mobile phase to 5 mg of 4-aminobenzenesulfonic acid to make exactly 50 mL. Analyze 10 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 20 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the mobile phase in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 250 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.

Mobil phase: A 4:1 mixture of ammonium acetate–tetra-*n*-butylammonium bromide TS/acetonitrile (for HPLC).

Flow rate: 1.0 mL/min.

2-Aminobenzoic Acid $C_7H_7NO_2$ [118-92-3] A white to brown powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 335 nm): Not less than 0.55. Weigh accurately about 0.2 g of 2-aminobenzoic acid, and dissolve it in ethanol (95) to make exactly 100 mL. Measure the absorbance of this solution at the maximum at around 335 nm against ethanol (95).

Purity Clarity of solution Almost clear (1 g, Ethanol (95) 20mL).

Assay Weigh accurately about 0.3 g of 2-aminobenzoic acid, dissolve it in 15 mL of ethanol (99.5), titrate the resulting solution with 0.1 mol/L sodium hydroxide (indicator: 3 drops of phenolphthalein). The endpoint is when the faint red color persists for about 30 second.

Each mL of 0.1 mol/L sodium hydroxide = 13.71 mg of $C_7H_7NO_2$

2-Amino-2-hydroxymethyl-1,3-propanediol $H_2NC(CH_2OH)_3$ [K9704, Special Grade] [77-86-1]

4-Amino-5-methoxy-2-methylbenzenesulfonic Acid $C_8H_{11}NO_4S$ [6471-78-9] A white to pale yellow powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 247–253 nm): Not less than 362. Weigh accurately 10 mg of 4-amino-5-methoxy-2-methylbenzenesulfonic acid, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A.

Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits absorption maxima in the ranges 209–215 nm, 247–253 mm, and 288–294 nm, respectively. Measure the absorbance of this solution at the maximum between 247–253 nm against ammonium acetate TS (0.02 mol/L) to determine the specific absorbance.

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add the mobile phase to 10 mg of 4-amino-5-methoxy-2-methylbenzenesulfonic acid to make exactly 25 mL. Analyze 10 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 20 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the mobile phase in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 290 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.

Mobil phase: A 3:1 mixture of ammonium acetate–tetra-*n*-butylammonium bromide TS/acetonitrile (for HPLC).

Flow rate: 1.0 mL/min.

Water Not more than 5.0% (50 mg, Coulometric Titration) The node solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

1-Amino-2-naphthol-4-sulfonic Acid $C_{10}H_5(NH_2)(OH)SO_3H$ [K8050, Special Grade] [116-63-2]

1-Amino-2-naphthol-4-sulfonic Acid TS Weigh 0.2 g of 1-amino-2-naphthol-4-sulfonic acid, and add 195 mL of sodium hydrogen sulfite solution (3 in 20) and 5 mL of sodium sulfite solution (1 in 5) to dissolve, and filter if necessary. Stopper tightly, and store in a dark, cold place. Use within 10 days of preparation.

2-Amino-5-sulfobenzoic Acid $C_7H_7NO_5S$ [3577-63-7] White to slightly reddish yellow crystals, powder, or lumps.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 256–262 nm): 522–638. Weigh accurately about 10 mg of 2-amino-5-sulfobenzoic acid, and dissolve it in ammonium acetate TS (0.02 mol/L) to make 100 mL. Refer to this solution as Solution A. Add ammonium acetate TS (0.02 mol/L) to 5 mL of Solution A, exactly measured, to make 50 mL. This solution exhibits an absorption maximum at a wavelength of 256–262 nm. Measure the absorbance (A_B) of this solution at the maximum in the range 256–262

nm against ammonium acetate TS (0.02 mol/L) to determine the specific absorbance by the formula:

$$E_{1cm}^{1\%} = A_B \times \frac{10}{\text{Weight (g) of the sample}} \times \frac{100}{100 - \text{Loss on drying (\%)}}$$

Purity (1) Clarity of solution Clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Analyze 20 μL each of Solution A prepared for the specific absorbance measurement and ammonium acetate TS (0.02 mol/L) by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 30 minutes of injection. The area percentage of the main peak of Solution A is not less than 95.0% of the total area of all the peaks, excluding those from the ammonium acetate TS in Solution A.

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 octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: An 80:20 mixture of ammonium acetate–tetra-*n*-butylammonium bromide TS/acetonitrile (for HPLC).

Flow rate: 1.0 mL/min.

Loss on drying Not more than 2.0% (50 mg, 135°C, 6 hours).

Ammonia Solution NH_4OH [K8085, Special Grade or K 9903] [1336-21-6]

Ammonia Solution (28) NH_4OH [K8085, Special Grade, Content 28%] [1336-21-6]

Ammonia Solution–Ammonium Chloride TS Add 57 mL of ammonia solution to7.0 g of ammonium chloride, and dilute to 100 mL with water. Store in a tightly stoppered polyethylene bottle.

Ammonia TS To 400 mL of ammonia solution (28), add water to make 1000 mL.

Ammonium Acetate CH_3COONH_4 [K8359, Special Grade] [631-61-8]

Ammonium Acetate–Tetra-*n*-butyl Ammonium Bromide TS Dissolve 1.54 g of ammonium acetate and 3.22 g of tetra-*n*-butyl ammonium bromide in water to make 1000 mL.

Ammonium Acetate TS (0.1 mol/L) Dissolve 7.7 g of ammonium acetate in water to make 1000 mL.

Ammonium Acetate TS (0.02 mol/L) Dissolve 1.54 g of ammonium acetate in water to make 1000 mL.

Ammonium Acetate TS (0.01 mol/L) Dissolve 0.77 g of ammonium acetate in water to make 1000 mL.

Ammonium Amidosulfate $NH_4OSO_2NH_2$ [K8588, Special Grade] [7773-06-0]

Ammonium Buffer (pH 10.0) Dissolve 5.4 g of ammonium chloride by adding 21 mL of ammonia solution (28) and water to make exactly 100 mL.

Ammonium Buffer (pH 10.7) Dissolve 67.5 g of ammonium chloride in 570 mL of ammonia solution (28), and add freshly boiled and cooled water to make 1000 mL.

Ammonium Carbonate $(NH_4)_2CO_3$ [K8613, Special Grade] [506-87-6]

Ammonium Carbonate TS Dissolve 20 g of ammonium carbonate by adding 20 mL of ammonia TS and water to make 100 mL.

Ammonium Chloride NH_4Cl [K8116, Special Grade] [12125-02-9]

Ammonium Iron(II) Sulfate Hexahydrate $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ [K8979, Special Grade] [7783-85-9]

Ammonium Iron(III) Sulfate Dodecahydrate $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$ [K8982, Special Grade] [7783-83-7]

Ammonium Iron(III) Sulfate–Hydrochloric Acid TS Dissolve 50 mg of ammonium iron(III) sulfate dodecahydrate in 50 mL of hydrochloric acid. Prepare fresh before use.

Ammonium Iron(III) Sulfate–Nitric Acid TS Dissolve 10 g of ammonium iron(III) sulfate dodecahydrate by adding 10 mL of diluted nitric acid (1 in 3) and 80 mL of water.

Ammonium Iron(III) Sulfate–Sulfuric Acid TS Dissolve 14 g of ammonium iron(III) sulfate dodecahydrate in 100 mL of water while shaking well. Filter, and add 10 mL of sulfuric acid. Store in a brown bottle.

Ammonium Iron(III) Sulfate–Sulfuric Acid (1 in 35) TS Dissolve 15 g of ammonium iron(III) sulfate dodecahydrate in 90 mL of water. Filter, and add 10 mL of diluted sulfuric acid (1 in 35).

Ammonium Iron(III) Sulfate TS for Oxyethylene Determination Dissolve 8 g of ammonium iron(III) sulfate in water to make 100 mL.

Ammonium Molybdate–Iron(II) Sulfate TS Dissolve 10 g of hexaammonium heptamolybdate tetrahydrate in 800 mL of water, add 32 mL of sulfuric acid, then make up to 1000 mL with water. Dissolve 7.32 g of iron(II) sulfate heptahydrate by adding the first solution to make 100 mL. Prepare fresh before use.

Ammonium Molybdate–Sulfuric Acid TS (for phytase activity test) Mix 100 mL of a solution of hexaammonium heptamolybdate tetrahydrate (3 in 250), 100 mL of diluted sulfuric acid, and 200 mL of acetone, and immediately cool in icy water. Prepare fresh before use.

Ammonium Molybdate TS Dissolve 6.5 g of powdered molybdenum(VI) oxide in a mixture of 14 mL of water and 14.5 mL of ammonia solution, and cool. Add gradually the solution to a cooled mixture of 32 mL of nitric acid and 40 mL of water while stirring. Allow to stand for 48 hours, and filter through a glass-fiber filter under reduced pressure. This solution cannot withstand long storage. The solution is usable when it meets the following test: When 2 mL of a solution of disodium hydrogenphosphate dodecahydrate (1 in 8) is added to 5 mL of the prepared solution, an abundant yellow precipitate is formed immediately or after slight warming. Store protected from light. If a precipitate

is formed during storage, use the supernatant.

Ammonium Nitrate NH_4NO_3 [K8545, Special Grade] [6484-52-2]

Ammonium Oxalate Monohydrate $H_4NOOCCOONH_4 \cdot H_2O$ [K8521, Special Grade] [6009-70-7]

Ammonium Peroxodisulfate $(NH_4)_2S_2O_8$ [K8252, Special Grade] [7727−54−0]

Ammonium Pyrrolidine Dithiocarbamate $C_5H_{12}N_2S_2$ [5108-96-3](for atomic absorption spectrophotometry)

Ammonium Sodium Hydrogenphosphate Tetrahydrate $NaNH_4HPO_4 \cdot 4H_2O$ [7783-13-3] White crystals or granules. Easily effloresce in air.

Identification Allow 1 g of ammonium sodium hydrogenphosphate tetrahydrate to adhere to the end of platinum wire moistened with water. Fuse it by a burner, and cool. A colorless transparent globe is produced.

Ammonium Sulfate $(NH_4)_2SO_4$ [K8960, Special Grade] [7783-20-2]

Ammonium Sulfide TS $(NH_4)_2S$ [Ammonium Sulfide Solution (Colorless), K8943, First Grade] Store in a small, completely filled, light-resistant bottle.

Ammonium Thiocyanate NH_4SCN [K9000, Special Grade] [1762-95-4]

Ammonium Thiocyanate–Cobalt(II) Nitrate TS Weigh 17.4 g of ammonium thiocyanate and 2.8 g of cobalt(II) nitrate hexahydrate, mix, and add water to make 100 mL.

Ammonium Vanadate(V) NH_4VO_3 [K8747, Special Grade] [7803-55-6]

α-Amylase Sample Diluent Use an appropriate one.

1. Dissolve 0.84 g of calcium carbonate and 0.29 g of sodium chloride in water to make 100 mL. Dilute it to 500 times its original volume with water.
2. Dissolve 0.34 g of calcium sulfate dihydrate, 0.53 g of boric acid, 0.14 g of sodium tetraborate decahydrate in water, add 0.5 mL of a solution of polyoxyethylene(10) octylphenyl ether (1 in 10) to make 1000 mL.
3. Dissolve 25 mg of polyoxyethylene(23) lauryl ether, 4.41 g of calcium chloride dihydrate in water to make 1000 mL.
4. Cooled Sodium Chloride Solution (3 in 500).
5. To about 800 mL of water, add 5 mL of calcium acetate solution (0.2 mol/L), 20 mL of sodium acetate solution (1 mol/L), and 50 mL of sodium chloride (2 mol/L). Adjust the pH to 6.0 with acetic acid TS (0.1 mol/L), and make 1000 mL with water.
6. Phosphate Buffer (0.02 mol/L) at pH 7.0.
7. Dissolve 0.29 g of calcium chloride dihydrate in 800 mL of water, add 5 mL of sodium chloride solution (2 mol/L), 2 mL of acetate buffer (1 mol/L) at pH 6.0, and water to make 1000 mL.
8. Dissolve 1.46 g of sodium chloride in 250 mL of phosphate buffer (0.1 mol/L) at pH 7.0.
9. Dissolve 1.0 g of bovine serum albumin (for enzyme) in 100 mL of maleic acid TS (0.05 mol/L, pH 5.6).
10. Phosphate Buffer (0.1 mol/L) at pH 7.0.

11. Dissolve 0.15 g of calcium chloride dihydrate in 800 mL of water, and add 50 mL at pH 6.0 acetate buffer (1 mol/L) and water to make 1000 mL.

β-Amylase Sample Diluent Use an appropriate one.

1. Dissolve 1.0 g of egg albumin and 0.35 of L-cysteine hydrochloride monohydrate in acetate buffer (0.05 mol/L) at pH 6.0 to make 1000 mL.

2. Dissolve 0.84 g of calcium carbonate and 0.29 g of sodium chloride in water to make 100 mL. Dilute it to 500 times its original volume with water.

Amylose Use a product suitable for the corresponding enzyme activity tests.

Amylose TS Mix well 1.2 g of amylose with 100 mL of dimethyl sulfoxide, warm at 70°C for 20 minutes. Centrifuge (10,000 × g, 10 minutes) this solution to remove insoluble matters, store at 25°C.

Aniline $C_6H_5NH_2$ [K8042, Special Grade] [62-53-3]

Aniline Azo Schaeffer's Salt $C_{16}H_{11}N_2NaO_4S$ [1934-20-9] Monosodium 6-hydroxy-5-(phenylazo)-2-naphthalenesulfonate. A yellow-red to reddish yellow powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 480–486 nm): Not less than 450. Weigh accurately 10 mg of aniline azo Schaeffer's salt, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate solution TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits an absorption maximum at a wavelength of 480–486 nm. Measure the absorbance of this solution at the maximum between 480–486 nm against ammonium acetate solution TS (0.02 mol/L) to determine the specific absorbance.

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate solution TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution by adding ammonium acetate TS (0.02 mol/L) to 5 mg of aniline azo Schaeffer's salt and making up to exactly 25 mL. Analyze 10 μL each of the test solution and ammonium acetate TS (0.02 mol/L) by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 40 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the ammonium acetate TS (0.02 mol/L) in the test solution.

Operating conditions

Detector: Visible spectrophotometer (wavelength 485 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: Ammonium acetate TS (0.02 mol/L).

B: Acetonitrile (for HPLC).

Concentration gradient (A/B): Maintain at 65/35 for 10 minutes, run a linear gradient from 65/35 to 10/90 in 10 minutes, and maintain at 10/90 for 20 minutes.

Flow rate: 1.0 mL/minutes.

***p*-Anisidine** $CH_3OC_6H_4NH_2$ [104-94-9] White to light brown crystals or crystalline powder.

Melting point 57–60°C.

***p*-Anisidine–Phthalic Acid TS** Dissolve 1.23 g of *p*-anisidine and 1.66 g of phthalic acid in methanol to make 100 mL. Store in a tightly-stoppered, light-resistant container in a cold place.

Anthraquinone $C_{14}H_8O_2$ [84-65-1] A light yellow to light yellow-brown powder.

Clarity of solution Almost clear (0.1 g, Heat in a water bath, Toluene 20 mL).

Melting point 282–288°C.

Anthrone $C_{14}H_{10}O$ [90-44-8] Light yellow crystals or crystalline powder.

Identification Determine the absorption spectrum of anthrone as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 1660 cm^{-1}, 1600 cm^{-1}, 1470 cm^{-1}, 1400 cm^{-1}, 1310 cm^{-1}, 1170 cm^{-1}, 930 cm^{-1}, and 710 cm^{-1}.

Melting point 154–160°C.

Purity (1) Related substances Weigh 0.1 g of anthrone into a 200-mL volumetric flask, dissolve it in 100 mL of diluted sulfuric acid (2 in 3), and add the same sulfuric acid (2 in 3) to make 200 mL. Refer to this solution as Solution A. Dissolve 0.50 g of D(+)-glucose in water to make exactly 100 mL. To 10 mL of this solution, exactly measured, add water to make 100 mL. Transfer 1 mL of this solution into a 50-mL ground-glass stoppered, flat bottom test tube, and add exactly 25 mL of Solution A. Use the resulting solution as the test solution. Prepare a blank test solution by transferring 1 mL of water into a 50-mL ground-glass stoppered, flat bottom test tube and adding exactly 25 mL of Solution A. Shake well both the test solution and blank test solution, heat them in a water bath for 10 minutes, and cool in icy water. Measure the absorbance of the test solution as directed under Ultraviolet-visible Spectrophotometry at 625 nm against the blank test solution. Similarly, measure the absorbance of the blank test solution against water. The absorbance of the test solution is not less than 0.70 and the absorbance of the blank test solution is 0.05.

(2) Anthraquinone Not more than 1.0%. Dissolve 0.50 g of anthrone in acetonitrile to make 100 mL, and dilute 20 mL of the resulting solution up to 200 mL with acetonitrile. Use the resulting solution as the test solution. Dissolve 50 mg of anthraquinone in 80 mL of acetonitrile, and add acetonitrile to make 100 mL. To 2 mL of this solution, measured exactly, add 20 mL of the test solution, and add acetonitrile to make 200 mL. Use the resulting solution as the control solution.

Analyze 10 μL each of the test solution and the control solution by liquid

chromatography using the operating conditions given below. Measure the peak areas of anthraquinone for the test solution and the control solution, and refer to them as A_1 and A_2, respectively. A_1 is not larger than $A_2 - A_1$

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 254).

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

Column packing material: 5-μm phenyl-bonded silica gel for liquid chromatograph.

Column temperature: A constant temperature of 30–40°C.

Mobile phase: Add 140 mL of water to 60 mL of acetonitrile, and add 2.5 mL of tetrabutylammonium hydroxide–methanol TS, and adjust the pH to 3.0 with diluted phosphoric acid (1 in 2).

Flow rate: 1.0 mL/min.

Anthrone TS Dissolve 50 mg–0.2 g of anthrone in 100 mL of sulfuric acid. Prepare fresh before use.

Antimony Tartrate–Molybdic Acid TS To 50 mL of sulfuric acid TS (2.5 mol/L), add 5 mL of potassium antimonyl tartrate TS, 15 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 25), and 30 mL of L(+)-ascorbic acid solution (11 in 625), and stir well. Prepare fresh before use.

Aqua Regia Mix 3 parts hydrochloric acid and 1 part nitric acid by volume. Prepare fresh before use.

Arabic Gum Use a product suitable for the corresponding enzyme activity tests.

Arabic Gum TS Weigh 17.9 g of sodium chloride and 0.41 g of potassium dihydrogen phosphate, dissolve them by adding 400 mL of water and 540 mL of glycerol. To this solution, adding 6.0 g of Arabic gum by adding little by little while stirring, and add water to make 1000 mL.

Arabinan A polysaccharide that consists mainly of arabinose. Use a product suitable for the corresponding enzyme activity tests.

Arabinogalactan Use a product suitable for the corresponding enzyme activity tests.

L-Arabinose for Assay $C_5H_{10}O_5$ [87-72-9] White crystals or powder.

Specific rotation $[\alpha]_D^{20}$: +103.0° to +105.5° (2 g, water 50 mL, on the dried basis). Allow the test solution to stand for 24 hours before measurement.

Purity Related substances Prepare a test solution by dissolving 1.0 g of L-arabinose for assay in 25 mL of water. Prepare a control solution by diluting 1 mL of the test solution, measured exactly, with water to make exactly 100 mL. Analyze 10 μL each of these solutions by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Use the conditions specified in the Assay for L-Arabinose in the Monographs.

Arabinoxylan Use a product suitable for the corresponding enzyme activity tests.

L-Arabitol $C_5H_{12}O_5$ [7643-75-6] White crystals or crystalline powder.

Clarity of solution Clear (1 g, water 20 mL).

Melting point 102–104°C.

Water Not more than 0.5% (1 g, Volumetric Titration, Direct Titration).

Residue on ignition Not more than 0.1% (2 g).

L-Arginine Hydrochloride $H_2N(HN)CNH(CH_2)_3CH(NH_2)COOH \cdot HCl$ [1119-34-2] White crystals or crystalline powder. Freely soluble in water.

Content Not less than 99.0%.

Purity Other amino acids Prepare a test solution: Dissolve 0.10 g of L-arginine hydrochloride in water to make exactly 10 mL. Apply 5 μL of the test solution, in 2–6 mm circular spots, 10-mm from either side of the plate on the starting line 20 mm from the bottom of the thin-layer plate, at 10-mm or more intervals. Dry the plate. Wind a filter paper on the inside wall, moister the filter with an appropriate developing solvent, and fill the developing chamber with the solvent up to 10 mm deep. Tightly seal the chamber, allow it to stand for about 1 hour at room temperature to saturate the chamber with the vapor of the developing solvent. Use an appropriate one of the three types of solvents: a 10:10:5:2 mixture of 1-butanol/acetone/water/ dicyclohexylamine, a 67:33 mixture of 1-propanol/ammonia solution, or a 2:1:1:1 mixture of ethanol (99.5)/water/ammonia solution/1-butanol. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110ºC for 1 hour.

Place the plate into the chamber, with care, without allowing it to contact with the inside wall, and seal tightly, and allow to stand at room temperature to develop the chromatogram. Remove the plate from the chamber when the solvent front has ascended to a point about 10 cm above the starting line, and mark the location of the solvent front quickly. Air-dry the plate at 100°C for 30 minutes, and leave it to cool. Spray the plate with a solution of ninhydrin in acetone (1 in 50), and heat at 80°C for 10 minutes to allow color to develop. More than 1 spot is not detected.

Assay Weigh accurately about 0.1 g of L-arginine hydrochloride, previously dried, dissolve it in 2 mL of formic acid, add 15 mL of 0.1 mol/L perchloric acid, and heat on a water bath for 30 minutes. After cooling, add 45 mL of acetic acid, and titrate the excess of perchloric acid with 0.1 mol/L sodium acetate. Use a potentiometer to confirm the endpoint. To confirm the endpoint, use a potentiometer with a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes. Perform a blank test to make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 10.53 mg of $C_6H_{14}N_4O_2 \cdot HCl$

Argon Ar [K1105, Second Grade] [7440-37-1]

Arsenic Trioxide TS Dissolve 1 g of diarsenic trioxide in 30 mL of sodium hydroxide

solution (1 in 40) while heating. Cool, and add acetic acid gradually to make 100 mL.

L(+)-Ascorbic Acid $C_6H_8O_6$ [K9502] [50-81-7]

L-Ascorbic Acid 2-Glucoside for Assay $C_{12}H_{18}O_{11}$ [129499-78-1] White, oderless crystals or crystalline powder having an acid taste.

Content Not less than 99.9% of L-ascorbic acid 2-glucoside ($C_{12}H_{18}O_{11}$) on the dried basis.

Identification (1) To 5 mL of a solution of L-ascorbic acid 2-glucoside for assay (1 in 50), add one drop of potassium permanganate solution (1 in 300). The color of the solution disappears immediately. To 5 mL of a solution of L-ascorbic acid 2-glucoside for assay (1 in 50), add one to two drops of 2,6-dichloroindophenol sodium salt TS. The color of solution disappears immediately.

(2) To 5 mL of boiled Fehling's TS, add 2–3 drops of a solution of L-ascorbic acid 2-glucoside for assay (5 in 40), and heat for about 5 minutes. A red precipitate is formed.

(3) Determine the absorption spectrum of L-ascorbic acid 2-glucoside for assay as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3300 cm^{-1}, 1770 cm^{-1}, 1700 cm^{-1}, 1110 cm^{-1} and 1060 cm^{-1}.

Purity (1) Clarity of solution Clear (1.0g, Water 50 mL).

(2) Free ascorbic acid and free D-glucose Prepare a test solution as follows: Dissolve 0.50 g of L-ascorbic acid 2-glucoside for assay in the mobile phase specified in the operating conditions to make exactly 25 mL. Prepare a standard solution as follows: Dissolve 0.50 g of L(+)-ascorbic acid in the mobile phase to make exactly 25 mL, take exactly 1.0 mL of this solution, and add the mobile phase to make exactly 100 mL. Use the resulting solution as the L-ascorbic acid standard stock solution. Each 1.0 mL of this solution contains 0.2 mg of L-ascorbic acid. Separately, dissolve 0.50 g of D(+)-glucose in the mobile phase to make exactly 25 mL. Take exactly 1.0 mL of this solution, and add the mobile phase to make exactly 100 mL. Use the resulting solution as the D-glucose standard stock solution. Each 1.0 mL of this solution contains 0.2 mg of D-glucose. Place exactly 10 mL each of the L-ascorbic acid standard stock solution and D-glucose standard stock solution into a volumetric flask, and add the mobile phase to make exactly 100 mL of a solution. Use this as the mixed standard solution.

Analyze 10 μL each of the test solution and the mixed standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas of L-ascorbic acid and D-glucose for each solution. Each of the peak areas from the test solution corresponding to the retention times of L-ascorbic acid and D-glucose is not greater than each of the peak areas of corresponding peaks of L-ascorbic and D-glucose in the standard solution.

Operating conditions

Detector: Differential refractometer.

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

Column packing material: 5–10 μm amino-bonded silica gel for liquid

chromatography.

Column temperature: 40°C.

Mobile phase: A 3:2 mixture of acetonitrile/a solution (5.44 in 1000) of potassium dihydrogen phosphate in 0.5% (vol) phosphoric acid.

Flow rate: A constant rate about 0.7 mL/minute.

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

Assay Weigh accurately about 1 g of the sample, dissolve it in 30 mL of water, and add two drops of phenolphthalein TS. Titrate with 0.2 mol/L sodium hydroxide solution to the first light red color that persists about for 30 seconds.

Each mL of 0.2 mol/L sodium hydroxide = 67.65 mg of $C_{12}H_{18}O_{11}$

L(+)-Ascorbic Acid TS To 70 mg of L(+)-ascorbic acid, add 1.5 g of metaphosphoric acid and 4 mL of acetic acid, and dilute to 100 mL with water.

Asparaginase (*A. niger*-derived) for Enzyme Activity Determination Obtained from the filamentous fungi, *A. niger* ASP-72, in which asparaginase productivity is improved by amplifying the asparaginase gene intrinsically occurring in *Aspergillus niger.* Occurs as a clear, yellow to brown liquid or as pale gray or slightly yellowish white granules. Has an enzyme activity. One unit of this substance corresponds to the amount of the enzyme required to liberate 1 μmol of ammonia in one minute at pH 5.0 and at 37°C when L-asparagine as the substrate is used.

Asparaginase (*A. oryzae*-derived) for Enzyme Activity Determination Obtained from the filamentous fungi, *A. oryzae* NZYM-SP, in which asparaginase productivity is improved by amplifying the asparaginase gene intrinsically occurring in *Aspergillus oryzae.* Occurs as a light brown liquid or as white to grayish white granules. Has an enzyme activity. One unit of this substance is equivalent to the amount of the enzyme required to liberate 1 μmol of ammonia in one minute at pH 7.0 and at 37°C when L-asparagine as the substrate is used.

L-Asparagine Monohydrate $C_4H_8N_2O_3 \cdot H_2O$ [K8021] [5794-13-8]

L-α-Aspartyl-D-phenylalanine Methyl Ester $C_{14}H_{18}N_2O_5$ [22839-65-2] A white, crystalline powder. Soluble in water.

Melting point 142.0–145.0°C.

Purity Other amino acids or peptide compounds Use a solution of L-α-aspartyl-D-phenylalanine methyl ester (1 in 1000) as the test solution. Analyze 2 μL of the test solution by thin-layer chromatography using a 32:15:3:1 mixture of chloroform/methanol/water/acetic acid as the developing solvent. The control solution is not used. Use a thin-layer plate coated with silica gel for thin-layer chromatography and dried at 110°C for 1 hour. When the solvent front has ascended to a point about 10 cm above the starting line, stop the development, air-dry the plate, then dry at 80°C for 30 minutes. Spray with ninhydrin TS, dry at 80°C for 10 minutes, and examine in daylight. Only one spot is observed.

Azocasein Use a product suitable for the corresponding enzyme activity tests.

Azocollagen Use a product suitable for the corresponding enzyme activity tests.

Azoxystrobin for Assay $C_{22}H_{17}N_3O_5$ [131860-33-8] A white powder.

Content Not less than 99.0% of azoxystrobin ($C_{22}H_{17}N_3O_5$).

Identification Determine the infrared absorption spectrum of azoxystrobin for assay as directed in the Paste Method or Disk Method under Infrared Spectrophotometry. It exhibits absorptions at about 2230 cm^{-1}, 1625 cm^{-1}, 1587 cm^{-1}, 1201 cm^{-1}, 1155 cm^{-1}, 840 cm^{-1}.

Melting point 115–119°C.

Assay Weigh accurately about 20 mg of azoxystrobin for assay and about 4 mg of 1,4-BTMSB-d_4, and add 2 mL of deuterated acetonitrile to dissolve them together. Transfer the resulting solution to an NMR tube of 5 mm in external diameter, and stopper tightly. Measure ^1H NMR spectra using an NMR spectrometer with a proton resonance frequency of 400 MHz or more under the following operating conditions. Assuming the signal of 1,4-BTMSB-d_4 as δ 0.23 ppm, when the signal area intensities at around δ 3.40–3.80 ppm, δ 6.43 ppm, and δ 8.28 ppm are designated as A_1 (corresponding to 6 hydrogen atoms), A_2 (corresponding to 1 hydrogen atom), and A_3 (corresponding to 1 hydrogen atom), respectively. Confirm that each of $(A_1/6)/A_2$, $(A_1/6)/A_3$, and A_2/A_3 is 1.0. Then, assuming the signal area intensity of 1,4-BTMSB-d_4 as 18.00, when the sum of A_1, A_2, and A_3, the sum of the number of hydrogens, and the purity of 1,4-BTMSB-d_4 are designated as I, N, and P(%), respectively, determine the content of azoxystrobin by the following formula. If the signal from Azoxystrobin for Assay is overlapped with the signal from a contaminant, do not use its signal area intensity for the assay.

$$\text{Content (\%) of azoxystrobin } (C_{22}H_{17}N_3O_5) = \frac{\text{Weight (mg) of 1,4-BTMSB-}d_4 \times \text{I} \times \text{P}}{\text{Weight (mg) of the sample} \times \text{N}} \times 1.781$$

Operating conditions

Spinning: Off.

^{13}C decoupling: Present.

Acquisition time: Not less than 4 seconds.

Spectral range: At least 20 ppm including between −5 ppm and 15 ppm.

Flip angle: 90°.

Delay time: Not less than 64 seconds.

Dummy scans: Not less than 1.

Number of accumulation: Not less than 8.

Azurine-Crosslinked Wheat Arabinoxylan This is wheat arabinoxylan to which azurine is crosslinked. Use a product suitable for the corresponding enzyme activity tests.

BANASS–Brilliant Yellow TS Dissolve 0.10 g of 4,4′-bis(4-amino-1-naphthylazo)-2,2′-stilbenesulfonic acid and 20 mg of brilliant yellow in 3 mL of sodium hydroxide (1 in 250),

add 7 mL of water, and make up to 100 mL with methanol. Store in a brown glass bottle.

Barbital Sodium $C_8H_{11}N_2NaO_3$ 5,5-Diethyl Barbituric Acid Sodium Salt. Use a product suitable for the corresponding enzyme activity tests.

Barbital Sodium–Hydrochloric Acid Buffer (0.1 mol/L)

Solution 1 Dissolve 20.6 g of barbital sodium in water to make 1000 mL.

Solution 2 Dissolve 9 mL of hydrochloric acid in water to make 1000 mL.

Mix Solutions 1 and 2, and adjust the pH to the corresponding value specified in this publication.

Barbital Sodium–Hydrochloric Acid Buffer (pH5.0, containing sodium acetate and sodium chloride) Dissolve 5.9 g of barbital sodium and 2.3 g of sodium acetate in 400 mL of water, and mix this solution with 80 mL of sodium chloride solution (85 in 1000). Adjust the pH to 5.0 with hydrochloric acid TS (1 mol/L), and add water to make 1000 mL.

Barium Carbonate $BaCO_3$ [K1415] [513-77-9] A white powder.

Content Not less than 99.0%.

Purity (1) Sodium Not more than 0.01%. Prepare the test solution by dissolving 1.0 g of barium carbonate in diluted hydrochloric acid (1 in 10) to make 100 mL. Prepare the control solution: To 1.0 g of Barium Carbonate, add 1 mL of each of Sodium Standard Solution (0.1 mg/mL), Potassium Standard Solution (0.1 mg/mL), and Calcium Standard Solution (0.1 mg/mL) and 5 mL of Strontium Standard Solution (1.0 mg/mL), then add diluted hydrochloric acid (1 in 10) to dissolve, and make 100 mL. Measure the absorbance of the test solution and the control solution, using the operating conditions given below. The absorbance of the test solution does not exceed the difference in absorbance between the control solution and the test solution.

Operating conditions

Light source: Sodium hollow cathode lamp.

Analytical line: 589.0 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(2) Potassium Not more than 0.01%. Measure the absorbances of the test solution and the control solution prepared in (1), using the operating conditions given below. The absorbance of the test solution does not exceed the difference in absorbance between the control solution and the test solution.

Operating conditions

Light source: Potassium hollow cathode lamp.

Analytical line: 766.5 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(3) Calcium Not more than 0.01%. Measure the absorbances of the test solution and the control solution prepared in (1), using the operating conditions given below. The

absorbance of the test solution does not exceed the difference in absorbance between the control solution and the test solution.

Operating conditions

Light source: Calcium hollow cathode lamp.

Analytical line: 422.7 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(4) Strontium Not more than 0.5%. Measure the absorbance of the test solution and the control solution prepared in (1), using the operating conditions given below. The absorbance of the test solution does not exceed the difference in absorbance between the control solution and the test solution.

Operating conditions

Light source: Strontium hollow cathode lamp.

Analytical line: 460.7 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(5) Barium hydroxide Not more than 0.02%. To 5 g of barium carbonate, add 50 mL of water (carbon dioxide-removed). Shake for 5 minutes, and filter through a filter paper for quantitative analysis (5C). Titrate this solution with 0.05 mol/L hydrochloric acid (indicator: bromothymol blue TS).

Each mL of 0.05 mol/L hydrochloric acid = 4.284 mg of $Ba(OH)_2$

Assay Weigh accurately about 1 g of barium carbonate, and add 50 mL of water and 40 mL of 1 mol/L hydrochloric acid. Boil and cool the mixture. Titrate with 1 mol/L sodium hydroxide (indicator: bromothymol blue TS). Separately perform a blank test to make any necessary correction.

Each mL of 1 mol/L hydrochloric acid = 98.67 mg of $BaCO_3$

Barium Chloride Dihydrate $BaCl_2 \cdot 2H_2O$ [K8155, Special Grade] [10326-27-9]

Barium Hydroxide Octahydrate $Ba(OH)_2 \cdot 8H_2O$ [K8577, Special Grade] [12230-71-6]

Barium Oxide BaO [1304-28-5] A white to light yellow powder. Absorbs moisture and carbon dioxide in the air. Soluble in hydrochloric acid and slightly soluble in water. A solution of barium oxide is alkaline.

Content Not less than 90.0%.

Assay To 30 mL of water, add about 0.5 g of barium oxide, weighed accurately, then add 20 mL of diluted hydrochloric acid (1 in 4) to dissolve. After cooling, titrate with 0.02 mol/L potassium permanganate. Conduct a blank test to make any necessary correction. Determine the content (C) of barium peroxide.

Each mL of 0.02 mol/L potassium permanganate = 8.466 mg of BaO_2

Then, weigh accurately about 2.0 g of barium oxide into a 300-mL ground-glass stoppered Erlenmeyer flask containing100 mL of carbon dioxide-removed water, and titrate with 1 mol/L hydrochloric acid (indicator: 2–3 drops of phenolphthalein TS). Determine the

content of barium oxide by the formula:

$$\text{Content (\%) of barium oxide} = \frac{76.66 \times v}{\text{Weight (g) of the sample} \times 100} \times 100 - C \times 0.9055$$

v = volume (mL) of 1 mol/L hydrochloric acid consumed.

Basic Bismuth Nitrate $Bi_5H_9N_4O_{22}$ [1304-85-4] A white fine crystalline powder. Causes a moistened litmus paper (blue) to turn red.

Residue on ignition 79.0–82.0%.

Benzene C_6H_6 [K8858, Special Grade] [71-43-2]

1,2-Benzenediol [120-80-9] White to yellow-brown crystals.

Content Not less than 99.0%.

Identification Determine the absorption spectrum of 1,2-benzenediol as directed in the Disk Method under Infrared Spectrophotometry. It exhibits the absorptions at wavenumbers of about 3400 cm^{-1}, 1639 cm^{-1}, 1451 cm^{-1}, 1270 cm^{-1}, 1231 cm^{-1}, 1173 cm^{-1}, 1049 cm^{-1}, 848 cm^{-1}, and 662 cm^{-1}.

Congealing point 23–26°C.

Assay Prepare a test solution by dissolving 1 g of 1,2-benzenediol in ethanol (99.5) and making up to 10 mL. Analyze 1 μL of the test solution by gas chromatography using the operating conditions given below. Determine the content of 1,2-benzenediol from the peak area of 1,2-benzenediol in the test solution and the sum of the areas of all the peaks, other than the ethanol peak.

Operating conditions

Detector: Flame-ionization detector

Column: A fused silica tube (0.25 mm internal diameter and 39 m length) coated with a 0.25-μm thick layer of polyethylene glycol for gas chromatography.

Column temperature: Upon injection at 200°C, raise the temperature at a rate of 10°C/minute to 250°C, and maintain at 250°C for 15 minute.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 1.0 mL/minute.

Injection method: Split.

Split ratio: 1:140.

Measurement time: 20 minutes.

α-*N*-Benzoyl-L-arginine Ethyl Ester Hydrochloride $C_{15}H_{22}N_4O_3 \cdot HCl$ [2645-08-1] A white crystalline powder.

Melting point 128–133°C.

Purity Dissolve 0.10 g of *N*-benzoyl-L-arginine ethyl ester hydrochloride in water to make exactly 10 mL. Use this solution as the test solution. Analyze 10 μL of the test solution by thin-layer chromatography using a 4:1:1 mixture of 1-butanol/acetic

acid/water 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 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, and allow to stand in iodine vapor for 30 seconds. Only one spot is observed.

Benzyl Alcohol $C_6H_5CH_2OH$ [100-51-6] A colorless, transparent liquid having a characteristic odor. Very soluble in diethyl ether and sparingly soluble in water.

Content Not less than 99.0%. Analyze 0.5 μL of benzyl alcohol by gas chromatography using the operating conditions given below. Determine the content of benzyl alcohol from the peak area of benzyl alcohol and the sum of the areas of all the peaks.

Operating conditions

Detector: Flam-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 polyethylene glycol for gas chromatography.

Column temperature: 130°C.

Injection port temperature: 180°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 1.33 mL/minute.

Injection method: Split.

Split ratio: 1:100.

Measurement time: 30 minutes.

5-Benzyl-3,6-dioxo-2-piperazineacetic Acid $C_{13}H_{14}N_2O_4$ [5262-10-2] A white to gray crystalline powder. Slightly soluble in acidic water, freely soluble in neutral to alkaline water, and soluble in dimethyl sulfoxide.

Melting point 242–246°C.

Purity Other amino or imino compounds Analyze 10 μL of a solution of 5-benzyl-3,6-dioxo-2-piperazineacetic acid (1 in 1000) by thin-layer chromatography, using a 32:15:3:1 mixture of chloroform/methanol/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 dried at 110°C for 1 hour. When the solvent front has ascended to a point about 10 cm above the starting line, stop the development, and air-dry the plate for 30 minutes. Prepare a beaker filled with chlorine gas: Place about 3 g of bleaching powder in a beaker, add 1 mL of hydrochloric acid cautiously to generate chlorine gas, and leave it tightly sealed for 30 seconds. Transfer the air-dried plate to the beaker filled with chlorine gas, seal the beaker tightly, and allow to stand for 20 minutes. Take the plate out of the beaker, allow to stand for 10 minutes, spray with ethanol (95), and air-dry. Spray it with potassium iodide–starch TS, and examine in daylight. Only one spot is observed.

Benzyloxycarbonyl-L-glutaminyl Glycine $C_{15}H_{19}N_3O_6$ Use a product suitable for the corresponding enzyme activity tests.

Description White crystals or crystalline powder.

Melting point 180–188°C.

Loss on drying Not more than 0.5% (0.5 g, reduced pressure, desiccant phosphorus(V) oxide, room temperature, 16 hours).

Betaine for Assay $C_5H_{11}NO_2 \cdot H_2O$ [590-47-6] White, hygroscopic, deliquescent crystals having a slight odor and a sweet, slightly bitter taste.

Identification Determine the absorption spectrum of betaine for assay, 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.

Purity Related substances Prepare a test solution by dissolving about 1 g of betaine for assay, previously dried, in water to make exactly 100 mL. Prepare a control solution by diluting 1 mL of the test solution, measured exactly, with water to make exactly 100 mL. Analyze 10 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for about two times the retention time of the main peak and measure the peak areas. Exclude the solvent peak from the measurement. The sum of the areas of all the peaks from the test solution, other than the main peak, is not greater than the area of the main peak from the control solution.

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 for liquid chromatography.

Column temperature: 70°C.

Mobile phase: Water.

Flow rate: Adjust the retention time of betaine to about 9 minutes.

Loss on drying 12.0–14.6% (105°C, reduced pressure, 3 hours).

2,2′- Bipyridyl $(C_5H_4N)_2$ [K8486, Special Grade] [366-18-7]

4,4′-Bis(4-amino-1-naphthylazo)-2,2′-stilbenesulfonic Acid $C_{34}H_{26}N_6O_6S_2$ [5463-64-9] Black granules with a metallic luster.

A solution prepared by dissolving this substance in sodium hydroxide solution (1 in 2500) exhibits an absorption maximum at a wavelength of about 516 nm.

Bis(3-methyl-1-phenyl-5-pyrazolone) $C_{20}H_{18}N_4O_2$ [K9545, Special Grade] [7477-67-0]

Bismuth(III) Acetate $(CH_3CO_2)_3Bi$ Use a product suitable for the corresponding enzyme activity tests.

Bismuth Nitrate Pentahydrate $Bi(NO_3)_3 \cdot 5H_2O$ [K8566, Special Grade] [10035-06-0]

Bismuth Nitrate TS Dissolve 5 g of bismuth nitrate pentahydrate by adding 25 mL of

water and 25 mL of acetic acid, and add water to make exactly 250 mL.

Bis[(+)-tartrato]diantimonate(III) Dipotassium Trihydrate $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$ [K 8533, Special Grade] [16039-64-8]

***N,O*-Bis(trimethylsilyl)acetamide** $CH_3C[NSi(CH_3)_3]OSi(CH_3)_3$ [10416-59-8] A colorless liquid.

Refractive index n_D^{20}: 1.414–1.418.

Specific gravity d_{20}^{20}: 0.825–0.835.

Boiling point 71.0–73.0°C (4.7 kPa).

***N,O*-Bis-(trimethylsilyl)trifluoracetamide** $CF_3CO[Si(CH_3)_3]N[Si(CH_3)_3]$ [25561-30-2] A colorless or slightly light yellow, clear liquid.

Content Not less than 97.0%.

Identification Determine the absorption spectrum of *N,O*-bis-(trimethylsilyl)trifluoracetamide as directed in the Liquid Film Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2960 cm^{-1}, 1750 cm^{-1}, 1330 cm^{-1}, 1250 cm^{-1}, 1200 cm^{-1}, 1150 cm^{-1}, 940 cm^{-1}, 850 cm^{-1}, 760 cm^{-1}, 640 cm^{-1}, and 500 cm^{-1}.

Assay Analyze 1 μL of *N,O*-bis-(trimethylsilyl)trifluoracetamide by the gas chromatography using the operating conditions given below. Determine the purity from the peak area of *N,O*-bis-(trimethylsilyl)trifluoracetamide and the sum of the areas of all the peaks.

Operating conditions

Detector: Thermal conductivity detector.

Column: A fused silica tube (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of (50% phenyl) methyl polysiloxane.

Column temperature: 80°C.

Injection port temperature: 200°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 1.33 mL/minute.

Injection method: Split.

Split ratio: 1:100.

Measurement time: Three times the retention time of the main peak.

Bixin $C_{25}H_{30}O_4$ [6983-79-5]

Content Not less than 70.0%.

Description A strong-red crystalline powder.

Identification Dissolve 5.0 mg of bixin in acetone to make exactly 25 mL. Refer to this as Solution A. A solution prepared by making 1 mL of Solution A up to 50 mL with acetone exhibits absorption maxima in the ranges 452–460 nm and 482–490 nm, respectively.

Assay Analyze 10 μL of Solution A by liquid chromatography using the operation

conditions given below. Continue the chromatography for two times the retention time of the main peak. Determine the peak area ratio of the area of the main peak to the sum of the areas of all the peaks.

Operating conditions

Detector: Visible spectrophotometer (wavelength: 460 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 250 mm length).

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

Column temperature: 40°C.

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

Flow rate: Adjust the retention time of the main peak to about 20 minutes.

Bleaching Powder $CaCl_2O_2$ [7778-54-3, High-Test Hypochlorite] A white or whitish powder having a chlorine odor.

Content Equivalent to not less than 60% effective chlorine.

Identification To 0.5 g of bleaching powder, add 5 mL of water, and shake. When dipped into the resulting solution, a red litmus paper turns blue and fades.

Assay Weigh accurately about 5 g of bleaching powder into a mortar, and grind well with 50 mL of water. Transfer it into a 500-mL volumetric flask, and add water to the volume. Shake well, measure exactly 50 mL of this solution into an iodine flak, and add 10 mL of potassium iodide solution and 10 mL of 10% hydrochloric acid TS. Titrate liberated iodine with 0.1 mol/L sodium thiosulfate. Add 3 mL of starch TS near the endpoint when the solution is light yellow. The endpoint is when the solution becomes colorless. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 3.4543 mg of Cl

Borate Buffer (0.02 mol/L)

Solution 1 Dissolve 1.24 g of boric acid in water to make 1000 mL.

Solution 2 Dissolve 7.63 g of sodium tetraborate decahydrate in water to make 1000 mL.

Mix Solutions 1 and 2, and adjust the pH to the value specified in the corresonding section of this publication.

Boric Acid H_3BO_3 [K8863, Special Grade] [10043-35-3]

Boric Acid–Sodium Hydroxide Buffer Mix 12.36 g of boric acid and 4.00 g of sodium hydroxide, and dissolve the mixture in water to make 1000 mL.

Boric Acid–Sodium Hydroxide Buffer (0.2 mol/L) Dissolve 12.4 g of boric acid in water, adjust the pH to the value specified in the corresponding section of this publication with sodium hydroxide TS (1 mol/L), and make up to 1000 mL with water.

Boron Trifluoride BF_3 [7637-07-2] A colorless gas having a pungent odor.

Boiling point −100.3°C.

Melting point −127.1°C.

Boron Trifluoride–Methanol TS Dissolve 14 g of boron trifluoride in methanol to make

100 mL.

Bovine Serum Albumin Obtained from bovine serum and contains not less than 95% of albumin.

Bovine Serum Albumin (for enzyme) Use a product suitable for the corresponding enzyme activity tests.

Branched Dextrin High molecular dextrin, which is produced by removing low-molecular components from starch hydrolysate. Use a product suitable for the corresponding enzyme activity tests.

Brassicasterol $C_{28}H_{46}O$ [474-67-9] White crystalline powder.

Identification Proceed as directed in Identification for Campesterol. The relative retention time of the main peak of the test solution to the retention time of stigmasterol of standard solution is about 0.85.

Melting point 130−139°C.

Purity Proceed as directed in Purity for Campesterol.

Brilliant Green $C_{27}H_{34}N_2O_4S$ [633-03-4] Fine yellow crystals with luster. Soluble in water and in ethanol (95). Exhibits an absorption maximum at 623 nm.

Brilliant Yellow $C_{26}H_{18}N_4Na_2O_8S_2$ [3051-11-4] An orange-brown powder. Soluble in water. A solution of brilliant yellow in sodium hydroxide solution (1 in 2,500) exhibits an absorption maximum at about 492 nm.

Bromine Br_2 [K8529, Special Grade] [7726-95-6]

Bromine–Potassium Bromide TS for Oxyethylene Determination Add 1 mL of bromine to 300 mL of acetic acid saturated by 5 g of potassium bromide.

Bromine TS Set up a glass bottle with a stpper to which vaseline is applied. Add 2-3 mL of bromine to the bottle, and add 100 mL of cold water. Stopper tightly, and shake. Use the water phase. Store in a cold place, protected from light.

Bromocresol Green $C_{21}H_{14}Br_4O_5S$ [K8840, Special Grade] [76-60-8]

Bromocresol Green–Methyl Red Mixture TS Mix equal volumes of bromocresol green TS and methyl red TS.

Bromocresol Green TS Dissolve 50 mg of bromocresol green in 100 mL of ethanol (95). Filter if necessary.

Bromocresol Green TS (for cyclodextrin glucanotransferase activity test) Dissolve 70 mg of bromocresol green in 4 mL of ethanol (99.5), add 16 mL of water, and mix. Vibrate supersonically for 30 minutes, and filter through a 0.45 μm filter.

Bromocresol Purple $C_{21}H_{16}Br_2O_5S$ [K8841, Special Grade] [115-40-2]

Bromocresol Purple TS Dissolve 50 mg of bromocresol purple in 100 mL of ethanol (95). Filter if necessary.

Bromophenol Blue $C_{19}H_{10}Br_4O_5S$ [K8844] [115-39-9]

Bromophenol Blue–Sodium Hydroxide TS Dissolve 0.1 g of bromophenol blue in 3 mL of sodium hydroxide TS (0.05 mol/L) while shaking well, and add water to make 25 mL.

Bromophenol Blue TS Dissolve 0.1 g of bromophenol blue in 100 mL of 50% (vol)

ethanol. Filter if necessary.

Bromophenol Blue TS for Citric Acid To bromophenol blue TS, add an equal volume of ethanol (95), and adjust the pH to 7.0 with sodium hydroxide TS (0.01 mol/L).

Bromothymol Blue $C_{27}H_{28}Br_2O_5S$ [K8842, Special Grade] [76-59-5]

Bromothymol Blue TS Dissolve 0.1 g of bromothymol blue in 100 mL of 50% (vol) ethanol. Filter it if necessary.

Brucine *n*-Hydrate $C_{23}H_{26}N_2O_4 \cdot nH_2O$ [K8832, Special Grade] [357-57-3, anhydrous]

1,4-BTMSB-*d*$_4$ $C_{12}H_{18}D_4Si_2$ Deuterated 1,4-bis(trimethylsilyl)benzene whose traceability to the International System of Units is ensured.

Buffer for Bacillus Natto Gum (pH 3.3) Dissolve 6.19 g of trisodium citrate dihydrate, 5.66 g of sodium chloride, 19.80 g of citric acid monohydrate, 130.0 mL of ethanol (95), 5.0 mL of 2,2′-thiodiethanol, 4.0 mL of a solution of polyoxyethylene(23) lauryl ether (1 in 4), and 0.1 mL of octanoic acid in water to make exactly 1000 mL.

Buffer for the α-Amylase Activity Test Use an appropriate one.

1. Acetate Buffer (1 mol/L) at pH 4.5
2. Acetate 4 (1 mol/L) at pH 5.0
3. Acetate Buffer (1 mol/L) at pH 6.0
4. Phosphate Buffer (1/3 mol/L) at pH 7.0
5. Phosphate Buffer (containing sodium chloride)
6. Acetate Buffer (0.02 mol/L, pH6.0, containing calcium chloride and sodium chloride)
7. pH 7.0 Sodium Phosphate Buffer (0.5 mol/L)

Buffer for the β-Amylase Activity Test Use an appropriate one.

1. Acetate Buffer (1 mol/L) at pH 4.5
2. Acetate Buffer (1 mol/L) at pH 5.0
3. Acetate Buffer (1 mol/L) at pH 5.5
4. Acetate Buffer (1 mol/L) at pH 6.0
5. Phosphate Buffer (1/3 mol/L) at pH7.0
6. Phosphate Buffer (containing sodium chloride)

Buffer for the Phospholipase Activity Test Use either of the following buffers.

1. Tris-malate buffer at pH 5.5
2. Acetic Acid–Sodium Hydroxide Buffer (0.4 mol/L, pH 6.0, containing calcium chloride)

Buffer for the Polyphenol Oxidase Activity Test Use an appropriate one.

1. Acetate Buffer (1 mol/L) at pH 4.5
2. Acetate Buffer (1 mol/L) at pH 6.0
3. Potassium Phosphate Buffer (1 mol/L) at pH7.0

1-Butanol $CH_3(CH_2)_2CH_2OH$ [K8810, Special Grade] [71-36-3]

2-Butanol $CH_3CH_2CH(OH)CH_3$ [K8812, Special Grade] [78-92-2]

2-Butanone $CH_3COC_2H_5$ [K8900, Special Grade] [78-93-3]

3-Butenyl Isothiocyanate C_5H_7NS [3386-97-8] A colorless to yellow clear liquid.

Content Not less than 95.0%.

Assay Analyze 0.5 μL of 3-butenyl isothiocyanate by gas chromatography using the operating conditions given below. Determine the content of 3-butenyl isothiocyanate from the area of the 3-butenyl isothiocyanate peak and the sum of all peak areas.

Operating conditions

Detector: Flame-ionization detector.

Column: A fused silica tube (0.2–0.25 mm internal diameter and 50–60 m length) coated with a 0.2–0.4 μm thick layer of dimethyl polysiloxane for gas chromatography.

Column temperature: Upon injection at 80°C, raise the temperature at a rate of 4°C/minute to 250°C.

Detector temperature: 250°C.

Injection port temperature: 100°C.

Carrier gas: Helium.

Flow rate: Adjust the retention time of 3-butenyl isothiocyanate to 10–30 minutes.

Injection method: Split.

Split ratio: 1:50.

Measurement time: 42 minutes.

Butyl Acetate $CH_3COOCH_2CH_2CH_2CH_3$ [K8377, Special Grade] [123-86-4]

Butylated Hydroxytoluene $C_{15}H_{24}O$ [128-37-0] White to slightly yellow crystals, powder, or granuls.

Content Not less than 98.0%.

Identification Determine the infrared absorption spectrum of butylated hydroxytoluene as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2960 cm^{-1}, 1430 cm^{-1}, 1360 cm^{-1}, 1230 cm^{-1}, 1150 cm^{-1}, 1120 cm^{-1}, 1030 cm^{-1}, 880 cm^{-1}, 870 cm^{-1}, 770 cm^{-1}, and 580 cm^{-1}.

Melting point 69–72°C.

Clarity of solution Almost clear (1 g, ethanol (99.5), 20mL).

Assay Prepare a test solution by adding acetone to1 g of butylated hydroxytoluene to make 10 mL. Analyze 1-μl portions of the test solution by gas chromatography using operating conditions below. Continue the chromatograph for about three times the retention time of the main peak. Measure the areas of all peaks obtained to determine the amount from the ratio of the main peak area using the peak area percentage method. Separately, perform a blank test to make correction.

Operating conditions

Detector: Flame-ionization detector.

Column: A fused silica capillary (about 0.25 mm internal diameter and about 30 m length) coated with a 0.25-μm thick layer of dimethyl polysiloxane for gas chromatography.

Column temperature: 190°C.

Injection port temperature: 240°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 1.33 mL/minute.

Injection method: Split.

Split ratio: 1:100.

sec-Butyl Isothiocyanate C_5H_9NS [4426-79-3] A colorless to yellow-brown clear liquid.

Content Not less than 99.0%.

Assay Analyze 1 μL of *sec*-butyl isothiocyanate by gas chromatography using the operating conditions given below. Determine the content of *sec*-butyl isothiocyanate from the area of the *sec*-butyl isothiocyanate peak and the sum of all peak areas.

Operating conditions

Detector: Thermal conductivity detector.

Column: A glass or stainless tube (3 mm internal diameter and 2 m length).

Column packing material

Liquid phase: Methylphenylsilicone polymer (20% of the support).

Support: 180–250 μm diatomaceous earth for gas chromatography.

Column temperature: 120°C.

Detector temperature: 250°C.

Injection port temperature: 200°C.

Carrier gas: Helium.

Flow rate: 20 mL/minute.

Measurement time: 3 times the retention time of the main peak.

tert-Butyl Methyl Ether $C_5H_{12}O$ [1634-04-4] A colorless liquid.

Content Not less than 99.5% of *tert*-butyl methyl ether ($C_5H_{12}O$).

Specific gravity d_{20}^{20}: 0.738–0.744.

Water Not more than 0.08%.

Assay Analyze 0.2-μL portions of *tert*-butyl methyl ether by gas chromatography using the operating conditions given below. Measure the peak area of each peak to determine the percentage of the main peak by the peak area percentage method.

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.53 mm internal diameter and 15 m length) coated with a 5.0 μm thick layer of a mixture of 5% phenyl/95% methylpolysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 40°C for 10 minutes, raise at 20°C/minute to 260°C, and maintain the temperature at 260°C for 4 minutes.

Injection port temperature: 200°C.

Detector temperature: 260°C.

Carrier gas: Helium or Nitrogen.

Flow rate: A constant rate of about 4 mL/min.

Injection method: Split.

Split ratio: 1 : 50.

Caffeine Monohydrate $C_8H_{10}N_4O_2 \cdot H_2O$ [5743-12-4] Use caffeine hydrate specified in the Japanese Pharmacopoeia.

Calcium Acetate Monohydrate $Ca(CH_3COO)_2 \cdot H_2O$ [K8364, Special Grade] [62-54-4]

Calcium Acetate TS (0.2 mol/L) Dissolve 35.2 g of calcium acetate monohydrate in water to make 1000 mL.

Calcium Carbonate $CaCO_3$ [K8617, Special Grade] [471-34-1]

Calcium Chloride Dihydrate $CaCl_2 \cdot 2H_2O$ [K8122, Special Grade] [10035-04-8]

Calcium Chloride for Water Determination $CaCl_2$ [K8125] [10043-52-4]

Calcium Chloride TS (1 mol/L) Dissolve 147 g of calcium chloride dihydrate in water to make 1000 mL.

Calcium Chloride TS (0.32 mol/L) Dissolve 47.0 g of calcium chloride dihydrate in water to make 1000 mL.

Calcium Chloride TS (0.22 mol/L) Dissolve 32.3 g of calcium chloride dihydrate in water to make 1000 mL.

Calcium Chloride TS (0.1 mol/L) Dissolve 14.7 g of calcium chloride dihydrate in water to make 1000 mL.

Calcium 3-[*N*-Ethyl-*N*-(4-sulfophenyl)amino]methylbenzenesulfonate $C_{15}H_{15}CaNO_6S_2$ A white to slightly reddish yellow powder.

Purity (1) Clarity of solution Clear (10 mg, Ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Dissolve 10 mg of calcium 3-[*N*-ethyl-N- (4-sulfophenyl)amino]methylbenzenesulfonate in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Use this as the test solution. Analyze 20 μL each of the test solution and ammonium acetate TS (0.02 mol/L) by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 35 minutes of injection. The area percentage of the main peak of the test solution is not less than 60.0% of the total area of all the peaks, excluding those from the ammonium acetate TS in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 260 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: Ammonium acetate TS (0.02 mol/L).

B: Acetonitrile (for HPLC).

Concentration gradient (A/B): Run a linear gradient from 95/5 to 60/40 in 20 minutes, and maintain at 60/40 for 15 minutes.

Flow rate: 1.0 mL/minutes.

Water Not more than 15.0% (50 mg, Coulometric Titration).

Calcium Hydroxide $Ca(OH)_2$ [K8575, Special Grade] [1305-62-0]

Calcium Hydroxide for pH Determination Use a saturated solution that is obtained at 23–27ºC and has pH 12.45 at 25ºC.

Calcium Hydroxide TS Weigh 10 g of calcium oxide, add 40 mL of freshly boiled and cooled water, and allow to stand for a while. Add 1000 mL of freshly boiled and cooled water, stopper tightly, shake, and allow to stand. Discard the supernatant by decantation, and to the residue, add 1000 mL of freshly boiled and cooled water. Stopper tightly, and allow to stand for 1 hour with occasional shaking vigorously. Collect the supernatant by decantation or filtration before use.

Calcium Oxide CaO [K8410, Special Grade] [1305-78-8]

Calcium Sulfate Dihydrate $CaSO_4 \cdot 2H_2O$ [K8963, Special Grade] [10101-41-4]

Campesterol $C_{28}H_{48}O$ [474-62-4] A white crystalline powder.

Identification Prepare a test solution and a standard solution by dissolving 20 mg each of campesterol and stigmasterol in 5mL of acetone. Analyze 2 μL each of both solutions by gas chromatography using operating conditions given in the Assay for Vegetable Sterol (High Concentration of Free Sterol) in the Monographs. The relative retention time of the main peak of the test solution to the retention time of stigmasterol in the standard solution is about 0.95.

Melting point 157–160°C.

Purity Related substances Analyze 2-μL portions of the test solution prepared in Identification by gas chromatography using operating conditions given in the Assay for Vegetable Sterol (High Concentration of Free Sterol) in the Monographs. Continue the chromatography for two times the retention time of the main peak. Exclude the solvent peak from the measurement. Measure the area of each peak to determine the amount of main peak by the peak area percentage method. It is not less than 93.0%.

Carbazole $C_{12}H_9N$ [86-74-8] White leafy or plate crystals or powder.

Content Not less than 95.0%.

Assay Weigh accurately about 25 mg of carbazole, and dissolve it in acetone to make exactly 5 mL. Analyze 1 μL of this solution by gas chromatography using the operating conditions given below. Determine the carbazole content from the area of carbazole peak and the sum of the areas of all the peaks. Perform a blank test to make any necessary correction.

Operating conditions

Detector: Flame-ionization detector.

Column: A fused silica tube (0.53 mm internal diameter and 30 m length) coated with a 1.0 μm layer of 5% phenyl polysilphenylene-siloxane.

Column temperature: Upon injection at 120°C, maintain the temperature for 2 hours, raise at a rate of 10°C/minute to 200°C, and maintain at 200°C for 10 minute. Then raise the temperature at a rate of 10°C/minute to 300°C, and maintain at 300°C for 5 minutes.

Injection port temperature: 200°C.

Detector temperature: 300℃.

Carrier gas: Helium.

Flow rate: 6 mL/minutes.

Injection method: Split.

Split ratio: 1:5.

Measurement time: 35 minutes.

Carbazole–Ethanol TS Dissolve 1.0 g of carbazole in 800 mL of ethanol (99.5).

***N*-Carbobenzoxy-L-glutamyl-L-tyrosine** $C_{22}H_{24}N_2O_8$ Use a product suitable for the corresponding enzyme activity tests.

Carbon Dioxide CO_2 [124-38-9] "Carbon Dioxide"

Carbon Monoxide CO [630-08-0] A colorless gas. Carbon monoxide is produced by reacting formic acid with sulfuric acid and passing the produced gas through sodium hydroxide TS layer. Carbon Monoxide contained in a hermetic, pressure-resistant metal container may be used.

Carboxymethylcellulose $(C_8H_{16}O_8)_n$ Use a product suitable for the corresponding enzyme activity tests.

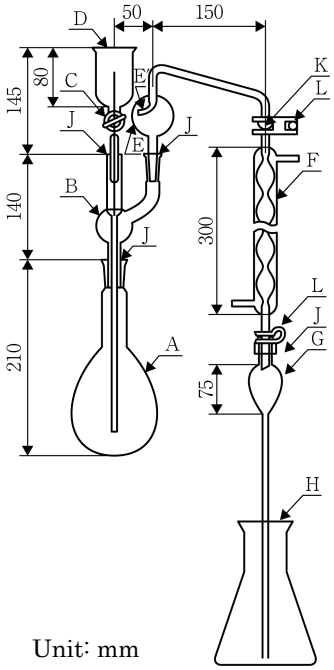
Carob Bean Gum [9000-40-2] "Carob Bean Gum"

Casein (milk-derived) [9000-71-9] A white to light yellow powder or small granules.

Identification Dissolve about 0.1 g of casein (milk-derived) in 5 mL of sodium hydroxide solution (1 in 10), and add 1 drop of 10% (w/v) copper(II) sulfate. A purple color is produced. When burnt, it emits a characteristic odor of protein.

Purity Nitrogen 13.0–16.0% (after drying).

Apparatus Use the apparatus as illustrated in the figure.



A: Kjeldahl flask (300 mL)
B: Connecting delivery tube
C: Ground-glass cock
D: Injection funnel
E: Kjeldahl trap bulb
(E′: a small hole)
F: Allihn condenser
G: Back-flow stopper (about 50 mL)
H: Receiver (300-mLErlenmeyer flask)
J: Interchangeable ground-glass joint
K: Ground-glass ball joint (taper)
L: Joint pin

Weigh 0.15 g of casein (milk-derived), dried at 105°C, into Kjeldahl flask A, and add 5.5 g of a mixture (that was prepared by mixing well 10 g of powdered potassium sulfate and 1 g of powdered copper(II) sulfate pentahydrate) and 20 mL of sulfuric acid. Heat gently with the flask tilted at about 45 degrees until the contents of the flask become light green, and continue heating for an additional three hours. Leave it to cool, and add 150 mL of water gradually. Add 2 to 3 boiling chips, and connect the flask to the distillation apparatus. Put the absorption solution (a mixture of exactly measured 20 mL of 0.05 mol/L sulfuric acid, 0.2 mL of bromocresol green–methyl red mixture TS, and 100 mL of water) into receiver H, and immerse the end of back-flow stopper G in the solution. Pour 100 mL of sodium hydroxide solution (3 in 10) through injection funnel D, wash the funnel with 10 mL of water, and close the ground-glass cock. Heat the Kjeldahl flask gradually, distill, and collect 100 mL of the initial distillate (stop distillation when the contents start bumping). Lower the receiver so that the end of the back-flow stopper is above the solution surface, wash the end with a small amount of water, and remove the received from the apparatus. Titrate the contents of the receiver with 0.1 mol/L sodium hydroxide. The endpoint is when the color of the solution changes from red to red-purple. Separately, perform a blank test to make any necessary correction.

Each mL of 0.05 mol/L sulfuric acid = 1.4007 mg of N

Loss on drying Not more than 14.0% (1 g, 105°C, 2 hours).

Casein TS (pH 2.0) Weigh accurately about 1 g of casein (milk-derived), dry at 105°C for 2 hours, and determine the loss on drying. Weigh exactly an amount of casein (milk-derived) equivalent to 1.2 g on the dry basis, and add 12 mL of lactic acid TS and 150 mL of water. Warm it in a water bath to dissolve, and cool with running water. Add hydrochloric acid TS (1 mol/L) to adjust to pH 2.0. Add water to make exactly 200 mL.

Prepare fresh before use.

Casein TS (pH 7.0) Weigh accurately about 1 g of casein (milk-derived), dry at 105°C for 2 hours, and determine the loss on drying. Weigh exactly an amount of casein (milk-derived) equivalent to 0.6 g of on the dry basis, and add 80 mL of disodium phosphate TS (0.05 mol/L). Warm it in a water bath for 20 minutes to dissolve. Cool with running water, and add hydrochloric acid TS (1 mol/L) to adjust the pH to 7.0. Add water to make exactly 100 mL. Prepare fresh before use.

Casein TS (pH 8.0) Weigh accurately about 1 g of casein (milk-derived), dry at 105°C for 2 hours, and determine the loss on drying. Weigh exactly an amount of casein (milk-derived) equivalent to 1.2 g on the dry basis, and add 160 mL of disodium phosphate TS (0.05 mol/L). Warm it in a water bath to dissolve. Cool with running water, and add sodium hydroxide TS (0.1 mol/L) to adjust to pH 8.0. Add water to make exactly 200 mL. Prepare fresh before use.

(+)-Catechin for Assay $C_{15}H_{14}O_6 \cdot nH_2O$ [154-23-4, anhydrous] A white to light brown or light yellow-green powder.

Identification (1) Dissolve 5 mg of (+)-catechin for assay in 5 mL of a 1:1 mixture of water/ethanol (95). To 1 mL of this solution, add 6 mL of valine solution (1 in 25) in methanol and 3 mL of hydrochloric acid, and shake. A light red or red color develops.

(2) Determine the absorption spectrum of (+)-catechin for assay as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at about 1690 cm^{-1}, 1610 cm^{-1}, 1520 cm^{-1}, 1450 cm^{-1}, 1350 cm^{-1}, 1240 cm^{-1}, 1150 cm^{-1}, 1100 cm^{-1}, 1040 cm^{-1}, 830 cm^{-1}, 770 cm^{-1}.

Purity (1) Clarity of solution Colorless to yellow and clear (50 mg, a 1:1 mixture of water/ethanol (95) 1 mL).

(2) Related substances Prepare a test solution by dissolving 20 mg of (+)-catechin for assay in 20 mL of a 500:500:1 mixture of water/ethanol (for HPLC)/ formic acid. Prepare a control solution by diluting 1 mL of the test solution to exactly 50 mL with a 500:500:1 mixture of water/ethanol (for HPLC)/formic acid. Analyze 10 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for 2 times the retention time of the main peak. The sum of the areas of all the peaks of the test solution, excluding the main peak of and the solvent peak, is not larger than the area of the main peak from the control solution. If necessary, determine 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 (for HPLC)/formic acid.

Concentration gradient (A/B): Run a linear gradient from 90/10 to 60/40 in 40 minutes.

Flow rate: Adjust the retention time of the main peak to about 15 minutes.

(–)-Catechin Gallate $C_{22}H_{18}O_{10}$ [130405-40-2] A white to light yellow or light red powder.

Identification Proceed as directed in Identification (1) for (+)-catechin for assay.

Purity Related substances Prepare a test solution by dissolving 20 mg of (–)-catechin gallate in 20 mL of a 500:500:1 mixture of water/ethanol (for HPLC)/ formic acid. Analyze 10-μL portions of the test solution by liquid chromatography using the operating conditions given in Purity for (+)-Catechin for Assay. Measure the area of each peak to determine the percentage of the main peak by the peak area percentage method. Continue the chromatography for the retention time of the main peak and exclude the solvent peak from the measurement. It is not less than 90.0%.

D(+)-Cellobiose $C_{12}H_{22}O_{11}$ 4-*O*-β-D-Glucopyranosyl-D-glucose Use a product suitable for the corresponding enzyme activity tests.

Centrifugal Ultrafiltration Unit A polypropyrene tube (about 3 cm diameter and 11–12 cm length) lined with a regenerated cellulose film with 3000 molecular weight cut off, or other units comparable to this in resolution capability.

Cerium(IV) Sulfate Tetrahydrate $Ce(SO_4)_2 \cdot 4H_2O$ [K8976, Special Grade] [10294-42-5]

CHES Buffer (0.5 mol/L) Dissolve 103 g of 2-(cyclohexylamino)ethanesulfonic acid in 600 mL of water, adjust the pH with sodium hydroxide TS (1 mol/L) to the value specified in the corresponding section of this publication, and add water to make 1000 mL.

CHES Buffer (0.1 mol/L) Dissolve 20.7 g of 2-(cyclohexylamino)ethanesulfonic acid in 900 mL of water, adjust the pH with sodium hydroxide TS (1 mol/L) to the value specified in the corresponding section of this publication, and add water to make 1000 mL.

Chitosan (Poly(1→4)-β-D-glucosamine) Use a product suitable for the corresponding enzyme activity tests.

Chloramphenicol $C_{11}H_{12}Cl_2N_2O_5$ [56-75-7] Use chloramphenicol specified in the Japanese Pharmacopoeia.

1-Chloro-2,4-dinitrobenzene $C_6H_3(NO_2)_2Cl$ [97-00-7] Light yellow crystals or crystalline powder. Freely soluble in diethyl ether and practically insoluble in water.

Content Not less than 99.0%.

Assay Prepare a test solution by dissolving 1 g of 1-chloro-2,4-dinitrobenzene in acetone to make exactly 10 mL. Analyze 1μL each of the test solution and acetone by gas chromatography using the operating conditions given below. Normalize the sum of the areas of all the peaks, excluding the peaks from acetone, that appear within the specified measurement time to 100. Determine the peak area percentage of 1-chloro-2,4-

dinitrobenzene.

Operating conditions

Detector: Flame-ionization detector.

Column: A fused silica tube (0.32 mm internal diameter and 30 m length) coated with a 5.0-μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Upon injection at 150°C, raise the temperature at a rate of 10°C/minute to 250°C, and maintain at 250°C for 10 minutes.

Injection port temperature: 280℃.

Detector temperature: 280°C.

Carrier gas: Helium.

Flow rate: 3 mL/minute.

Injection method: Split.

Split ratio: 1:45.

Measurement time: 20 minutes.

Chloroform $CHCl_3$ [K8322, Special Grade] [67-66-3]

Chloroform (Ethanol-free) Measure 20 mL of chloroform, add 20 mL of water, shake well and gently for 3 minutes, and separate the chloroform layer from the mixture. Repeat twice the above procedure with 20 mL of water each time. Filter the chloroform layer through a dry filter paper. To the filtrate, add 5 g of sodium sulfate, and shake well for 5 minutes. Allow to stand for 2 hours, and filter through a dry filter paper.

Chloroform for Water Determination To 1000 mL of chloroform, add 30 g of synthetic zeolite for desiccation, and stopper tightly. With occasional shaking, allow to stand for about 8 hours. After additional about 16 hours of standing, confirm that the chloroform layer is clear and separate it. Store protected from moisture. The water content should not be more than 0.1 mg in 1 mL of the sample.

Chlorogenic Acid Hemihydrate 5-Caffeoylquinic Acid Hemihydrate $2C_{16}H_{18}O_9 \cdot 1H_2O$ Use a product suitable for the corresponding enzyme activity tests.

5α-Cholestane $C_{27}H_{48}$ [481-21-0] A white to milky white powder.

Content Not less than 97.0%.

Identification Prepare a test solution and a standard solution by separately dissolving 0.1 g each of 5α-cholestane and stigmasterol in 100 mL of ethyl acetate. Analyze 2 μL each of the test solution and the standard solution by gas chromatography using operating conditions given in the Assay for Vegetable Cholesterol (High Concentration of Free Sterol) in the Monographs. The relative retention time of the main peak of the test solution to the retention time of stigmasterol is about 0.53.

Melting point 77–83°C.

Assay Analyze 2-μL portions of the test solution prepared in Identification by gas chromatography using operating conditions given in the Assay for Vegetable Cholesterol (High Concentration of Free Sterol) in the Monographs. Continue the chromatography for three times the retention time of the main peak, and exclude the solvent peak from

measurement. Measure the area of each peak, and determine the percentage of the main peak by the peak area percentage method.

Cholestanol $C_{27}H_{48}O$ 5α-Cholestan-3β-ol [80-97-7] A white powder.

Identification Proceed as directed in Identification for Campesterol. The relative retention time of the main peak of the test solution to the retention time of stigmasterol is 0.79.

Melting point 138–143°C.

Purity Proceed as directed in Purity for Campesterol.

Cholesterol See Cholesterol for Assay.

Cholesterol for Assay $C_{27}H_{46}O$ [57-88-5] White to slightly pale yellow crystals or powder.

Content Not less than 90.0%.

Identification Determine the absorption spectrum of cholesterol for assay as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3420 cm^{-1}, 2930 cm^{-1}, 1470 cm^{-1}, 1380 cm^{-1}, 1060 cm^{-1}, 1020 cm^{-1}, 960 cm^{-1}, 840 cm^{-1}, and 800 cm^{-1}.

Specific rotation $[\alpha]_D^{20}$: − 34 to − 39°. Weigh accurately about 0.5 g of cholesterol for assay, dried previously, and dissolve in 1,4-dioxane to make exactly 25 mL. Measure the optical rotation of the resulting solution.

Melting point 146−149°C.

Purity Acid To 1 g of cholesterol for assay, add 50 mL of a 1:1 mixture of ethanol (95) and diisopropyl ether and 3 drops of phenolphthalein TS, and then add 0.05 mol/L sodium hydroxide until the solution is light red. Immediately stopper and shake, and add 0.2 mL of 0.1 mol/L sodium hydroxide. The solution is light red to red.

Loss on drying Not more than 0.2% (1 g, 105°C, 2 hours).

Assay Weigh 0.1 g of cholesterol for assay, add 1 mL of pyridine, then promptly add 0.5 mL of *N*, *O*-bis(trimethylsilyl)trifluoroacetamide using a syringe, and heat in a water bath for 5 minutes. Use this solution as the test solution. Separately prepare a blank test solution in the same manner. Analyze 1 μL each of the test solution and the blank test solution by gas chromatography. Determine the content of cholesterol from the area of the cholesterol peak of the test solution and the sum of the peak areas.

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: 300°C.

Injection port temperature: 300°C.

Detector temperature: 300°C.

Carrier gas: Helium.

Flow rate: 1.33 mL/minute.

Injection method: Split.

Split ratio: 1:100.

Measurement time: 3 times the retention time of the main peak.

Choline Chloride $[(CH_3)_3NCH_2CH_2OH]Cl$ [67-48-1] White crystals or crystalline powder.

Content 95.0%. Weigh accurately about 0.2 g of choline chloride, previously dried 110°C for 3 hours, dissolve in 20 mL of acetic acid for nonaqueous titration, add 50 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid. To confirm the endpoint, use a potentiometer with a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes. Separately, perform a blank test to make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 13.962 mg of $[(CH_3)_2NCH_2CH_2OH]Cl$

Choline Chloride for Water Determination $[(CH_3)_3NCH_2CH_2OH]Cl$ [67-48-1] A white crystalline powder.

Melting point 303–305°C (decomposition).

Water Not more than 1 mg/g of sample.

Choline Oxidase It is obtained from *Alcaligenes* sp. One unit is the amount of enzyme required to produce 1 μmol of hydrogen peroxide from choline as a substrate in 1 minute at 37°C and pH 8.0. Use a product suitable for the corresponding enzyme activity tests.

Chromium(VI) Oxide CrO_3 [1333-82-0] Dark red-purple, deliquescent acicular or prismatic crystals or flakes. Freely soluble in water. Can ignite when it comes into contact with a flammable organic solvent.

Content Not less than 8.0%.

Assay Weigh accurately about 0.7 g of chromium(VI) oxide into a 100-mL volumetric flask, and add water to the volume. Use this solution as the test solution. Transfer exactly measured 10 mL (70 mg of the sample) of the test solution into a 300-mL ground-glass stoppered iodine flask, add 100 mL of water, 5 mL of hydrochloric acid, and 3 g of potassium iodide. Immediately stopper, allow to stand for 15 minutes in a dark place, and add 100 mL of water. Titrate the resulting solution with 0.1 mol/L sodium thiosulfate. Add 3 mL of starch TS as the indicator near the endpoint when the solution is light yellow. The endpoint is when the solution is green. Separately, perform a blank test with 110 mL of water to make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 3333 mg of CrO_3

Chromotropic Acid TS Weigh 0.5 g of disodium chromotropate dihydrate, add diluted sulfuric acid (2 in 3) to make 50 mL, and shake. Centrifuge the mixture, and use the supernatant as Chromotropic Acid TS. Prepare fresh before use.

Citrate Buffer (0.1 mol/L)

Solution 1 Dissolve 21.0 g of citric acid monohydrate in water to make 1000 mL.

Solution 2 Dissolve 29.4 g of trisodium citrate dihydrate in water to make 1000 mL.

Mix Solutions 1 and 2 to adjust the pH to the corresponding value specified in this

publication.

Citrate Buffer (0.05 mol/L)

Solution 1 Dissolve 10.5 g of citric acid monohydrate in water to make 1000 mL.

Solution 2 Dissolve 14.7 g of trisodium citrate dihydrate in water to make 1000 mL.

Mix Solutions 1 and 2 to adjust the pH to the corresponding value specified in this publication.

Citrate Buffer (pH 2.2) Mix 1.4 g of trisodium citrate dihydrate, 13 g of citric acid monohydrate, and 10.9 g of sodium chloride, and dissolve the mixture in water to make 1000 mL.

Citrate Buffer (pH 3.0)

Solution 1 Dissolve 21 g of citric acid monohydrate in water to make 1000 mL.

Solution 2 Dissolve 71.6 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Mix 159 volumes of Solution 1 and 41 volumes of Solution 2.

Citrate Buffer (pH 5.0)

Solution 1 Dissolve 21 g of citric acid monohydrate in water to make 1000 mL.

Solution 2 Dissolve 71.6 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Mix 97 volumes Solution 1 and 103 volumes Solution 2.

Citrate Buffer (pH 5.28) Dissolve 34.3 g of trisodium citrate dihydrate in 400 mL of water, add 7.5 mL of hydrochloric acid, 5 mL of benzyl alcohol, and water to make 1000 mL. Adjust the pH to 5.28 ± 0.03 with diluted hydrochloric acid (1 in 4) or sodium hydroxide solution (1 in 25).

Citrate Buffer (pH 6.0)

Solution 1 Dissolve 21 g of citric acid monohydrate in water to make 1000mL.

Solution 2 Dissolve 71.6 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Mix 72 volumes of Solution 1 and 128 volumes of Solution 2. If necessary, adjust the pH to 6.0 with either of Solution 1 or Solution 2.

Citrate Buffer (pH 7.0).

Solution 1 Dissolve 21 g of citric acid monohydrate in water to make 1000 mL.

Solution 2 Dissolve 71.6 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Mix 35 volumes of Solution 1 and 165 volumes of Solution 2, and adjust the pH to 7.0 with either solution.

Citric Acid Monohydrate $H_3C_6H_5O_7 \cdot H_2O$ [K8283, Special Grade] [5949-29-1]

Citric Acid–Hydrochloric Acid Buffer (0.1 mol/L)

Solution 1 Dilute 9 mL of hydrochloric acid to 1000 mL with water.

Solution 2 Dissolve 26.3 g of disodium hydrogen citrate sesquihydrate (2/3) in water to make 1000 mL.

Mix Solutions 1 and 2 to adjust the pH to the corresponding value specified in this publication.

Citric Acid–Phosphoric Acid Buffer (0.1 mol/L)

Solution 1 Dissolve 21.0 g of citric acid monohydrate in water to make 1,000 mL.

Solution 2 Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in water to make 1,000 mL.

Mix Solutions 1 and 2 to adjust the pH to the corresponding value specified in this publication.

Citric Acid–Sodium Hydroxide Buffer (0.2 mol/L) Dissolve 42 g of citric acid monohydrate in 800 mL of water, and adjust the pH with sodium hydroxide TS (1 mol/L) to the corresponding value specified in this publication. Add water to make 1000 mL.

Citric Acid–Sodium Hydroxide Buffer (0.1 mol/L) Dissolve 21 g of citric acid monohydrate in 500 mL of water, and adjust the pH with sodium hydroxide TS (2 mol/L) to the corresponding value specified in this publication. Add water to make 1000 mL.

Citric Acid–Sodium Hydroxide Buffer (0.05 mol/L, pH 5.0, containing cysteine) Dissolve 10.5 g of citric acid monohydrate, 0.23 g of a solution of polyoxyethylene(23) laury ether (3 in 100), and 3.0 g of L-cysteine together in about 900 mL of water. Adjust its pH with sodium hydroxide TS (4 mol/L) to 5.0, and add water to make 1000 mL.

Citric Acid–Sodium Hydroxide Buffer (0.02 mol/L) Dissolve 4.2 g of citric acid monohydrate in 500 mL of water, and adjust the pH with sodium hydroxide TS (2 mol/L) to the corresponding value specified in this publication. Add water to make 1000 mL.

Citrinin $C_{13}H_{14}O_3$ [518-75-2] Yellow, odorless crystals. Very soluble in water.

Identification Proceed as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at about 1634 cm^{-1}, 1492 cm^{-1}, 1266 cm^{-1}, 1018 cm^{-1} and 818 cm^{-1}.

Purity Related substances Prepare a test solution by dissolving about 10 mg citrinin, weighed accurately, in methanol to make 100 mL. Prepare a control solution by measuring exactly 1 mL of the test solution and diluting with methanol to exactly 100 mL. Analyze 5 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Measure the peak areas. The sum of the areas of all the peaks, other than the main peak and methanol peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Detector: Spectrophotofluorometer (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 materials: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 30°C.

Mobile phase: A 100:100:0.1 mixture of acetonitrile/water/trifluoroacetic acid.

Flow rate: 1.0 mL/min.

Cobalt(II) Chloride Hexahydrate $CoCl_2 \cdot 6H_2O$ [K8129, Special Grade] [7791-13-1]

Cobalt(II) Chloride TS (0.5 mmol/L) Dissolve 0.12 g of cobalt(II) chloride hexahydrate in water to make 1000 mL. Prepare fresh before use.

Cobalt(II) Chloride TS (0.1 mol/L) Dissolve 23.8 g of cobalt(II) chloride hexahydrate in water to make 1000 mL.

Cobalt(II) Nitrate Hexahydrate $Co(NO_3)_2 \cdot 6H_2O$ [K8552, Special Grade] [10026-22-9]

Color Fixing TS for D-Glucose Determination Dissolve 0.50 g of phenol, 130 units of mutarotase, 9000 units of glucose oxidase, 650 units of peroxidase, and 0.1 g of 4-aminoantipyrine in phosphate buffer (pH 7.1), and make exactly 1000 mL. Store at 2–10°C, and use within 1 month of preparation.

Copper(II) Acetate Monohydrate $Cu(CH_3COO)_2 \cdot H_2O$ [6046-93-1] Blue-green crystals or crystalline powder. Soluble in water.

Identification (1) Heat a solution of 1 g of copper(II) acetate monohydrate in 10 mL of diluted sulfuric acid (1 in 2). An odor of acetic acid is emitted.

(2) Add 5 mL of diluted ammonia solution (2 in 3) to a solution of 0.1 g of Copper(II) Acetate Monohydrate in 20 mL of water. The solution is deep blue.

Assay Dissolve 0.4 g of copper(II) acetate monohydrate in water to make exactly 250 mL. To 25 mL of this solution, exactly measured, add 75 mL of water and 5 mL of diluted ammonia solution (1 in 15), titrate with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 50 mg of murexide–sodium chloride indicator). The endpoint is when the color of the solution changes from yellow-green to red-purple.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate = 1.9965 mg of $(CH_3COO)_2Cu \cdot H_2O$

Copper(II) Acetate TS Dissolve 13.3 g of copper(II) acetate monohydrate by adding 5 mL of acetic acid and 195 mL of water.

Copper(II) Chloride Dihydrate $CuCl_2 \cdot 2H_2O$ [K8145, Special Grade] [10125-13-0]

Copper(II) Citrate TS (Alkaline) Weigh 173 g of trisodium citrate dihydrate and 117 g of sodium carbonate decahydrate, add 100 mL of water, and dissolve them while heating. Filter if necessary. Add the resulting solution gradually to a solution of 17.3 g of copper(II) sulfate pentahydrate in 700 mL of water, while stirring. Cool, and add water to make 1000 mL.

Copper(II) Sulfate $CuSO_4$ [K8984, First Grade] [7758-98-7]

Copper(II) Sulfate Pentahydrate $CuSO_4 \cdot 5H_2O$ [K8983, Special Grade] [7758-99-8]

10% (w/v) Copper(II) Sulfate TS Dissolve 15.6 g of cupper(II) sulfate pentahydrate in water to make 100 mL.

Copper TS (for xylanase/dextranase activity test) Dissolve 71 g of disodium hydrogenphosphate dodecahydrate and 40 g of potassium sodium (+)-tartrate

tetrahydrate in 650 mL of water. To this solution, add 100 mL of sodium hydroxide TS (1 mol/L), and then add gradually 80 mL of a solution (1 in 10) of copper(II) sulfate pentahydrate while stirring gently. To the resulting solution, add 180 g of sodium sulfate to dissolve it, and then add 25 mL of potassium iodide solution (9 in 250) and water to make 1000 mL. Allow it to stand for 2 days at 25–35°C, filter to remove the precipitate, and store the resulting at 25–35°C.

Copper TS (for maltotriohydrolase activity test)

Solution 1 Dissolve 25 g of sodium carbonate, 25 g of potassium sodium (+)-tartrate tetrahydrate, 20 g of sodium hydrogen carbonate, and 200 g of sodium sulfate in water to make 1000 mL.

Solution 2 Dissolve 30 g of copper(II) sulfate pentahydrate in 150 mL of water, and add 4 drops of sulfuric acid and water to make 200 mL.

Before use, mix 25 volumes of Solution 1 and 1 volume of Solution 2.

Creatine Monohydrate $C_4H_9N_3O_2 \cdot H_2O$ Use a product suitable for the corresponding enzyme activity tests.

Cresidine Azo Schaeffer's Salt $C_{18}H_{15}N_2NaO_5S$ Monosodium 6-hydroxy-5-(2-methoxy-5-methylphenylazo)-2- naphthalenesulfonate.

A red to red-brown powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength 498–504 nm): Not less than 440. Weigh accurately 10 mg of cresidine azo Schaeffer's salt, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits an absorption maximum at a wavelength of 498–504 nm. Measure the absorbance of this solution at the maximum between 498–504 nm against ammonium acetate TS (0.02 mol/L), and determine the specific absorbance.

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: To 5 mg of cresidine azo Schaeffer's salt, add ammonium acetate TS (0.02 mol/L) to make exactly 50 mL. Analyze 10 μL each of the test solution and ammonium acetate TS (0.02 mol/L) by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 30 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the ammonium acetate TS in the test solution.

Operating conditions

Detector: Visible spectrophotometer (wavelength: 510 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: Ammonium acetate TS (0.02 mol/L).

B: Acetonitrile (for HPLC).

Concentration gradient (A/B): Run a linear gradient from 80/20 to 20/80 in 20 minutes, and maintain 20/80 for 10 minutes.

Flow rate: 1.0 mL/min.

Water Not more than 10.0% (50 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

Cresidine Sulfonic Acid Azo β-Naphthol $C_{18}H_{15}N_2NaO_5S$ Monosodium 4-(2-hydroxy-1-naphthylazo)-5-methoxy-2-methylbenzenesulfonate.

A red to red-brown powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 497–503 nm): Not less than 530. Weigh accurately 10 mg of cresidine sulfonic acid azo β-naphthol, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits an absorption maximum at a wavelength of 497–503 nm. Measure the absorbance at the maximum between 497–503 nm against ammonium acetate TS (0.02 mol/L) to determine the specific absorbance.

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add ammonium acetate TS (0.02 mol/L) to 5 mg of cresidine sulfonic acid azo β-naphthol to make exactly 50 mL. Analyze 10 μL each of the test solution and the ammonium acetate TS (0.02 mol/L) by liquid chromatography using the operating conditions given in Purity (2) for cresidine azo Schaeffer's salt. Measure the area of each peak that appears within 30 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the ammonium acetate TS in the test solution.

Water Not more than 5.0% (50 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

Cresidine Sulfonic Acid Azo G Salt $C_{18}H_{13}N_2Na_3O_{11}S_3$ Trisodium 7-hydroxy-8-(2-methoxy-5-methyl-4-sulfophenylazo)-1,3-naphthalene-disulfonate.

An red to reddish yellow powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength 497–503 nm): Not less than 440. Weigh accurately 10 mg of cresidine sulfonic acid azo G salt, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L)

to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits an absorption maximum at a wavelength of 497–503 nm. Measure the absorbance at the maximum between 497–503 nm against ammonium acetate TS (0.02 mol/L) to determine the specific absorbance.

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add the mobile phase to 5 mg of cresidine sulfonic acid azo G salt to make exactly 25 mL. Analyze 10 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 20 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the mobile phase in the test solution.

Operating conditions

Detector: Visible spectrophotometer (wavelength: 505 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 3:2 mixture of ammonium acetate–tetra-*n*-butylammonium bromide TS/acetonitrile (for HPLC).

Flow rate: 1.0 mL/min.

Water Not more than 5.0% (50 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

Cresidine Sulfonic Acid Azo R Salt $C_{18}H_{13}N_2Na_3O_{11}S_3$ Trisodium 3-hydroxy-4-(2-methoxy-5-methyl-4-sulfophenylazo)-2,7-naphthalene- disulfonate.

A red-brown powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 512–518 nm): Not less than 420. Weigh accurately 10 mg of cresidine sulfonic acid azo R salt, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits an absorption maximum at a wavelength of 512–518 nm. Measure the absorbance of this solution at the maximum between 512–518 nm against ammonium acetate TS (0.02 mol/L), and determine the specific absorbance.

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add the mobile phase to 5

mg of cresidine sulfonic acid azo R salt to make exactly 25 mL. Analyze 10 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 20 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the mobile phase in the test solution.

Operating conditions

Detector: Visible spectrophotometer (wavelength: 515 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 3:2 mixture of ammonium acetate–tetra-*n*-butylammonium bromide TS/acetonitrile (for HPLC).

Flow rate: 1.0 mL/min.

Water Not more than 10.0% (50 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

p-Cresol $CH_3C_6H_4OH$ [K8306, Special Grade] [106-44-5]

Cresol Red $C_{21}H_{18}O_5S$ [K8308, Special Grade] [1733-12-6]

Cresol Red–Thymol Blue TS Mix 0.1 g of cresol red and 0.3 g of thymol blue, dissolve the mixture in 100 mL of ethanol (95), and add water to make 400 mL. Filter if necessary.

Crystalline Cellulose Use a product suitable for the corresponding enzyme activity tests.

Crystal Violet $C_{25}H_{30}ClN_3 \cdot 9H_2O$ [K8294, Special Grade] [548-62-9]

Crystal Violet–Acetic Acid TS Dissolve 50 mg of crystal violet in 100 mL of acetic acid.

Curdlan $(-C_6H_{10}O_5-)_n$ A insoluble polysaccharide having a linear β-1,3-glucan structure. It is produced by *Alcaligenes faecalis* var. *myxogenes*.

Cyanidin 3-Glucoside Chloride $C_{21}H_{21}ClO_{11}$ [7084-24-4]

Identification (1) Weigh 1 mg of cyanidin 3-glucoside chloride, and add citrate buffer (pH 3.0) to make 5 mL. A red to dark red-orange color develops.

(2) To the solution prepared in (1), add sodium hydroxide solution (1 in 25) to make the solution alkaline. The solution turns dark-green.

(3) A solution of cyanidin 3-glucoside chloride in citrate buffer (pH 3.0) exhibits an absorption maximum at a wavelength of 505–525 nm.

(4) Determine the absorption spectrum of cyanidin 3-glucoside chloride as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3378 cm^{-1}, 1640 cm^{-1}, 1332cm^{-1}, 1070 cm^{-1}, and 630 cm^{-1}.

Purity Related substances Use the solution prepared in Identification (1) as the test solution. Prepare Control Solution A by adding citrate buffer (pH 3.0) to 1 mL of the

test solution, exactly measured, to make exactly 100 mL. Analyze the test solution and Control Solution A by liquid chromatography using the operating conditions given below. Continue the chromatography for three times the retention time of the main peak, and measure the peak areas. Exclude the solvent peaks from measurement. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from Control Solution A.

Operating conditions

Use the conditions given in Identification (4) for Purple Corn Color in the Monographs.

Detection sensitivity

Adjust the sensitivity so that the automatic integration method can measure the peak area of the main peak from 10 μL of Control Solution B. Also, adjust so that the peak height of the main peak from 10 μL of Control Solution A is about 20% of the full scale. Prepare Control Solution B as follows: Measure exactly 1 mL of Control Solution A, and add citrate buffer (pH 3.0) to make exactly 20 mL.

Cycloartenol Ferulate $C_{40}H_{58}O_4$ [21238-33-5]

Description A white to light brown powder.

Identification (1) This test must be carried out, protected from light. Determine the absorption spectrum of a solution (1 in 50,000) of cycloartenol ferulate in heptane as directed under Ultraviolet-Visible Spectrophotometry. It exhibits absorption maxima in the ranges 229–233 nm, 289–293 nm, and 313–317 nm, respectively.

(2) Determine the absorption spectrum of cycloartenol ferulate as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2940 cm^{-1}, 1691 cm^{-1}, 1511 cm^{-1}, and 1270 cm^{-1}.

Purity (1) Clarity Almost clear (2 mg, acetone 2 mL).

(2) Related substances Prepare a test solution by dissolving 2.0 mg of cycloartenol ferulate in 2 mL of acetone. Prepare a control solution by making 1 mL of the test solution up to exactly 100 mL with methanol. Analyze 5 μL each of the test solution and control solution by thin-layer chromatography using a 5:2 mixture of hexane/acetone as the developing solution. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support. 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 it under ultraviolet light (main wavelength: 365 nm). All the spots, other than the main spot with about R_f 0.4, from the test solution are not more intense than the spot from the control solution.

(3) Prepare a test solution by dissolving 2 mg of cycloartenol ferulate in 2 mL of acetone. Analyze 5 μL of the test solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and exclude the solvent peak from the measurement. Measure the area of each peak to determine the percentage of the main peak by the peak area percentage

method. Separately, perform a blank test to make any necessary correction. The percentage of the main peak is not less than 98.0%.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 315).

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 40:7:3 mixture of acetonitrile/methanol/tetrahydrofuran.

Flow rate: 1.2 mL/minute.

Loss on drying Not more than 1.0% (105°C, 1 hour).

α-Cyclodextrin for Assay $C_{36}H_{60}O_{30}$ [10016-20-3] White, odorless crystals or crystalline powder having a slightly sweet taste.

Identification To 0.2 g of α-cyclodextrin for assay, add 2 mL of iodine TS, heat in a water bath to dissolve it, and cool in cold water. A dark red-purple precipitate is formed.

Specific rotation $[\alpha]_D^{20}$: +147 to +152° (after drying. 1 g, water, 100 mL).

Purity Related substances Prepare a test solution by dissolving about 1.5 g of α-cyclodextrin for assay in water to make exactly 100 mL. Prepare a control solution by adding water to 1 mL of the test solution, measured exactly, to make exactly 100 mL. Analyze 20–100 μL each of the test solution and the control solution by liquid chromatography using the conditions below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Use the conditions specified in the Assay for α-Cyclodextrin in the Monographs.

Loss on drying Not more than 14.0% (120°C, 2 hours).

β-Cyclodextrin for Assay $C_{42}H_{70}O_{35}$ [7585-39-9] White, odorless crystals or a crystalline powder. Odorless having a slightly sweet taste.

Identification To 0.2 g of β-cyclodextrin for assay, add 2 mL of iodine TS, heat in a water bath to dissolve, and cool in cold water. A red-brown precipitate is formed.

Specific rotation $[\alpha]_D^{20}$: +160 to +164° (after drying. 1 g, water, 100 mL).

Purity Related substances Prepare a test solution by dissolving about 1.5 g of β-cyclodextrin for assay in water to make 100 mL. Prepare a control solution by taking exactly 1 mL of the test solution and adding water to make 100 mL. Analyze 20–100 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak. Measure the peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Use the conditions specified in the Assay for β-Cyclodextrin in the Monographs.

Loss on drying Not more than 14.0% (120°C, 2 hours).

γ-Cyclodextrin for Assay $C_{48}H_{80}O_{40}$ [17465-86-0] White, odorless crystals or crystalline powder having a slightly sweet taste.

Identification To 0.2 g of γ-cyclodextrin for assay, add 2 mL of iodine TS, heat in a water bath to dissolve, and cool in cold water. A brown precipitate is formed.

Specific rotation $[\alpha]_D^{20}$: +172 to +178° (after drying, 1 g, water, 100 mL).

Purity Related substances Prepare a test solution by dissolving about 1.5 g of γ-cyclodextrin for assay in water to make 100 mL. Prepare a control solution by adding water to 1 mL of the test solution, measured exactly, to make exactly 100 mL. Analyze 20–100 μL each of the test solution and the control solution by liquid chromatography using the conditions below. Continue the chromatography for two times the retention time of main peak, and measure the peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Use the conditions specified in the Assay for γ-Cyclodextrin in the Monographs.

Loss on drying: Not more than 14.0% (120°C, 2 hours).

Cyclohexane C_6H_{12} [K8464, Special Grade] [110-82-7]

1,2-Cyclohexanediaminetetraacetic Acid Monohydrate $C_{14}H_{22}N_2O_8 \cdot H_2O$ [13291-61-7] A white powder.

Content Contains not less than 99.0% of *trans*-1,2-cyclohexanediaminetetraacetic acid monohydrate ($C_{14}H_{22}N_2O_8 \cdot H_2O$).

Identification Determine the absorption spectrum of 1,2-cyclohexanediaminetetraacetic acid monohydrate as directed in the Disk Method under Infrared Spectrometry. It exhibits absorptions at wavenumbers of about 3000 cm^{-1}, 1750 cm^{-1}, 1710 cm^{-1}, 1590 cm^{-1}, 1430 cm^{-1}, 1400 cm^{-1}, 1240 cm^{-1}, and 1220 cm^{-1}.

Purity Clarity of solution Almost clear. Prepare a test solution by dissolving 4.0 g of 1,2-cyclohexanediaminetetraacetic acid monohydrate in 25 mL of sodium hydroxide TS (1 mol/L) and diluting the solution to 100 mL with water.

Assay Weigh accurately about 0.4 g of 1,2-cyclohexanediaminetetraacetic acid monohydrate, and dissolve it in 11 mL of sodium hydroxide TS (1 mol/L). Add 2 mL of ammonia buffer (pH 10.7) and water to make exactly 100 mL. Titrate the resulting solution with 0.05 mol/L zinc chloride (indicator: 5 drops of eriochrome black TS).

Each mL of 0.05 mol/L zinc chloride = 18.22 mg of $C_{14}H_{22}N_2O_8 \cdot H_2O$

2-Cyclohexylaminoethanesulfonic Acid $C_8H_{17}NO_3S$ Use a product suitable for the corresponding enzyme activity tests.

L-Cysteine $C_3H_7NO_2S$ Use a product suitable for the corresponding enzyme activity tests.

L-Cysteine Hydrochloride Monohydrate $C_3H_7NO_2S \cdot HCl \cdot H_2O$ [K8470, Special Grade] [7048-04-6]

L-Cysteine Hydrochloride TS Dissolve 1 g of L-cysteine hydrochloride monohydrate in water to make 5 mL. Prepare fresh before use.

Cysteine–Sulfuric Acid TS Dissolve 0.30 g of L-cysteine hydrochloride monohydrate in 10 mL of water. To 0.5 mL of this solution, add 25 mL of 86% (vol) sulfuric acid, and mix. Prepare fresh before use.

Decane $CH_3(CH_2)_8CH_3$ [124-18-5] A colorless transparent liquid.

Content Not less than 99.5%. Analyze 1 μL of decane by gas chromatography using the operating conditions given below. Determine the content of decane from the area of the decane peak and the sum of the areas of all the peaks.

Operating conditions

Detector: Flame-ionization detector.

Column: A fused silica tube (0.32 mm internal diameter and 30 m length) coated with a 5-μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Upon injection at 50°C, raise the temperature at a rate of 10°C/minute to 150°C.

Injection port temperature: 200°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 3.4 mL/minute.

Injection method: Split.

Split ratio: 1:100.

Measurement time: 10 minutes.

Decanoic Acid $C_{10}H_{20}O_2$ [334-48-5] A colorless to light yellow, clear liquid or white to faint yellow crystals or lumps.

Content Not less than 99.0%.

Identification Determine the absorption spectrum of decanoic acid as directed in the Disc Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2676 cm^{-1}, 1700 cm^{-1}, 1299 cm^{-1}, 1268 cm^{-1}, 1232 cm^{-1}, 1200 cm^{-1}, 1075 cm^{-1}, 934 cm^{-1}, 825 cm^{-1}, and 686 cm^{-1}.

Purity Congealing point 29–33°C.

Assay Weigh accurately about 0.05 g of decanoic acid, add 1 mL of *N,O*-bis(trimethylsilyl)trifluoroacetamide, stopper tightly, and mix well. Heat the mixture on a water bath for 30 minutes, and cool to room temperature. Analyze appropriate portions of the resulting solution by gas chromatography using the operating conditions given below. Determine the area percentage of the main peak.

Operating conditions

Detector: Flame-ionization detector.

Column: A silicate glass capillary column (0.53 mm in internal diameter and 15 m

in length) coated with a 1.5-μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Raise the temperature at a rate of 10°C/minutes from 60°C to 280°C.

Injection port temperature: 280°C.

Detector temperature: 280°C.

Injection method: Split (20:1). Set conditions so that any component of the sample does not exceed the column acceptable range.

Carrier gas: Helium.

Flow rate: Adjust so that the peaks of components to be determined appear in 5–20 minutes of injection.

Deuterated Acetone CD_3COCD_3 [666-52-4] Use a product exclusively produced for NMR spectrum measurement.

Deuterated Acetonitrile CD_3CN [2206-26-0] Use a product produced exclusively for NMR spectral measurement.

Deuterated Chloroform $CDCl_3$ [865-49-6] Use a product produced exclusively for NMR spectrum measurement.

Deuterated Dimethyl Sulfoxide C_2D_6OS [2206-27-1] Use a product produced exclusively for NMR spectra measurement.

Deuterated Methanol CD_3OD [811-98-3] Use deuterated methanol produced exclusively for NMR spectral measurement.

Devarda's Alloy [K8653, Nitrogen Analysis Grade] [8049-11-4]**Dextran (molecular weight 70,000)** $(C_6H_{10}O_5)_n$ Derived from *Leuconostoc* spp. Use a product suitable for the corresponding enzyme activity tests.

Dextran (molecular weight: 150,000) $(C_6H_{10}O_5)_n$ Use a product suitable for the corresponding enzyme activity tests.

Dextran (molecular weight 2,000,000) $(C_6H_{10}O_5)_n$ Derived from *Leuconostoc* spp. Use a product suitable for the corresponding enzyme activity tests.

Dextrin Hydrate $(C_6H_{10}O_5)_n \cdot nH_2O$ [K8646, Special Grade] [9004-53-9]

Dextrin TS Dissolve 5.0 g of dextrin hydrate in Tris buffer (0.005 mol/L, pH7.0, containing calcium chloride) to make 200 mL.

Diacylglycerol TS Dissolve 3.0 mg of 1,2-dipalmitoyl-*rac*-glycerol in 1 mL of a 2:1 mixture of chloroform/methanol.

4,4′-Diaminodiphenylamine Sulfate $C_{12}H_{13}N_3 \cdot H_2SO_4$ [53760-27-3] A colorless to grayish blue, crystalline powder. Slightly soluble in water and soluble in dilute mineral acid.

Clarity Clear. Weigh 1.0 g of 4,4′-diaminodiphenylamine sulfate, add 20 mL of diluted sulfuric acid (1 in 16), and dissolve by heating. Use this as test solution.

Residue on ignition Not more than 0.1% (1 g). Without adding sulfuric acid, gradually heat the sample on a sand bath, and ignite after incineration.

4,4′-Diaminodiphenylamine TS Triturate well 4,4′-diaminodiphenylamine sulfate with a small quantity of ethanol (95), and add ethanol (95) again. Heat on a water bath under a reflux condenser to make a saturated solution.

2,3-Diaminonaphthalene $C_{10}H_{10}N_2$ Light yellow-brown crystals or powder. [771-97-1]

Melting point 193–198°C.

Sensitivity Measure exactly 1 mL Selenium Standard Solution, and add water to make exactly 100 mL. Measure exactly 10 mL of this solution, and add water to make exactly 50 mL. To exactly measured 1 mL of this solution, add 50 mL of diluted nitric acid (1 in 60), and use the resulting solution as Solution A. Place 40 mL each of Solution A and diluted nitric acid (1 in 60) into separate beakers, and 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, and wash the beakers with 10 mL each of water, and add the washings to the corresponding separating funnels. To each, add 0.2 g of hydroxylammonium chloride, and dissolve them while gently shaking. Add 5 mL of a solution that is prepared by dissolving 0.10 g of 2,3-diaminonaphthalene and 0.5 g of hydroxylammonium chloride in hydrochloric acid TS (0.1 mol/L), making up to 100 mL, and filtering. Shake them, and allow to stand for 100 minutes. To each, add 5.0 mL of cyclohexane, and shake well for 2 minutes to extract selenium. Separately, collect the cyclohexane layers, and centrifuge them at 3000 rpm for 10 minutes. Use the upper layers. Against the cyclohexane layer from the diluted nitric acid (1 in 60) as the reference solution, measure the absorbance of the cyclohexane layer from Solution A by ultraviolet-visible spectrophotometry. The absorbance is not less than 0.08 at a wavelength of 378 nm.

2,3-Diaminonaphthalene TS Dissolve 0.10 g of 2,3-diaminonaphthalene and 0.5 g of hydroxylammonium chloride in hydrochloric acid TS (0.1 mol/L) to make 100 mL, and filter if necessary. Prepare fresh before use.

2,4-Diaminophenol Dihydrochloride $C_6H_{10}C_{12}N_2O$ Use a product suitable for the corresponding activity enzyme activity tests.

Diammonium 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonate) $C_{18}H_{16}N_4O_6S_4$-$(NH_4)_2$ Use a product suitable for the corresponding enzyme activity tests.

Diammonium Cerium(IV) Nitrate $Ce(NH_4)_2(NO_3)_6$ [K8556, Special Grade] [16774-21-3]

Diammonium Hydrogen Citrate $C_6H_{14}N_2O_7$ [K8284, Special Grade] [3012-65-5]

Diarsenic Trioxide As_2O_3 [K8044, Special Grade] [1327-53-3]

2,6-Dibromo-*N*-chloro-*p*-benzoquinone Monoimine $C_6H_2Br_2ClNO$ [K8491, Special Grade] [537-45-1]

Dibutylamine $C_8H_{19}N$ [111-92-2] A colorless, clear liquid.

Content Not less than 99.0% of dibutylamine ($C_8H_{19}N$).

Specific gravity d_{20}^{20}: 0.756–0.764.

Water Not more than 0.3%.

Assay Analyze 0.2-μL portions of dibutylamine by gas chromatography using the operating conditions given below. Measure the peak area of each peak to determine the percentage of the main peak by the peak area percentage method.

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.32 mm internal diameter and 25 m length) coated with a 1.2 μm thick layer of polyethylene glycol for gas chromatography.

Column temperature: Maintain the temperature at 60°C for 2 minutes, raise at 5°C/minute to 100°C, and maintain the temperature at 100°C for 20 minutes.

Injection port temperature: A constant temperature of 150–170°C.

Detector temperature: 200°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the retention time of dibutylamine to about 20 minutes.

Injection method: Split.

Split ratio: 1 : 80.

Dibutylamine–Toluene TS (1 mol/L) Dissolve 129.3 g of dibutylamine in toluene to make 1000 mL. Prepare before use.

Dibutyl Ether $[CH_3(CH_2)_3]_2O$ [142-96-1] A colorless clear liquid.

Refractive index n_D^{20}: 1.398–1.400.

Specific gravity d_{20}^{20}: 0.764–0.770.

Boiling point 141–143°C.

2,6-Dichloroindophenol Sodium Salt Dihydrate $C_{12}H_6Cl_2NNaO_2 \cdot 2H_2O$ [620-45-1] A green to dark green crystalline powder with metallic luster. Store in a stoppered Cobacontainer, protected from light.

Content 2,6-dichlorolindophenol sodium salt dihydrate contains not less than 95.0% of 2,6-dichloroindophenol sodium salt ($C_{12}H_6Cl_2NNaO_2$ = 290.08) when calculated on the dried basis.

Identification Determine the absorption spectrum of 2,6-dichloroindophenol sodium salt dihydrate as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3470 cm^{-1}, 2940 cm^{-1}, 1700 cm^{-1}, 1450 cm^{-1}, 1370 cm^{-1}, 1240 cm^{-1}, 1170 cm^{-1}, 1080 cm^{-1}, 1030 cm^{-1}, 890 cm^{-1}.

Purity (1) Water insoluble substances Not more than 0.3 %. Weigh accurately a glass filter (G4), dried for 30 minutes at 105°C and then left to cool in a desiccator. Weigh 0.5 g of 2,6-dichloroindophenol sodium salt dihydrate, add 200 mL of water, and dissolve it by heating at a temperature of 100°C or lower. After cooling, filter the solution through the glass filter, and wash the filter with 30 mL of boiling water. Dry the filter at 105°C to constant weight, and weigh it.

(2) Ethanol insoluble substances Not more than 0.3 %. Weigh 0.5 g of 2,6-dichloroindophenol sodium salt dihydrate into a flask, add 120 mL of ethanol (95), heat

under a reflux condenser for 15 minutes, and cool. Filter the contents in the flask under suction through a crucible-shaped glass filter (G4), dried to constant weight at 105 ± 2°C. Wash the glass filter with ethanol (95), volatilize the ethanol, and dry the filter to constant weight at 105 ± 2°C. Weigh the residue.

(3) Interference color Weigh 50 mg of 2,6-dichloroindophenol sodium salt dihydrate, dissolve it by adding 4mL of sodium hydrogen carbonate solution (1 in 100) and 50 mL of water, and make up to exactly 200 mL with water. Filter the solution through a filter paper for quantitative analysis (5C). Discard the initial 20 mL, collect subsequent 15 mL, add 5 mL of L(+)-ascorbic acid TS, and allow to stand for 5 minutes at 20°C. Measure the absorbance of the resulting solution against water at a wavelength of 500 nm. It is not more than 0.05.

Loss on drying 10–14.5% (0.50 g, 120°C, 3 hours).

Assay Weigh accurately 0.3 g of 2,6-dichloroindophenol sodium salt dihydrate, dissolve it in 50 mL of acetic acid for acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid. To confirm the endpoint, use a potentiometer with a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes. Perform a blank test to make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 29.01 mg $C_{12}H_6Cl_2NNaO_2$

2,6-Dichloroindophenol Sodium Salt TS Weigh 0.1 g of 2,6-dichloroindophenol sodium salt dihydrate, and add 100 mL of water. Warm and filter it. Store in a brown bottle, and use within 3 days.

Dichloromethane CH_2Cl_2 [K8161, Special Grade] [75-09-2]

2,6-Dichloroquinonechloroimide $C_6H_2Cl_3NO$ [101-38-2]

Melting point 65–67°C.

Clarity of ethanolic solution Clear (0.10 g, ethanol (95) 10 mL).

Residue on ignition Not more than 0.2%.

Diethanolamine $C_4H_{11}NO_2$ [111-42-2] A colorless, viscous liquid.

Melting point 27–30°C.

Water Not more than 1 mg in 1 g of diethanolamine.

Diethylene Glycol Monoethyl Ether for Water Determination To 1000 mL of 2-(2-ethoxyethoxy)ethanol, add 30 g of synthesized zeolite for desiccation, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, and then leave it further for about 16 hours. Collect the 2-(2-ethoxyethoxy)ethanol that is clear, and store it protected from moisture. It shall not contain more than 0.3 mg of water per mL.

Diethylene Glycol Succinate Polyester Use diethylene glycol succinate polyester of high quality prepared for gas chromatography.

Diethyl Ether $C_2H_5OC_2H_5$ [K8103, Special Grade] [60-29-7]

Diethyl Ether for Vitamin A Determination Distill diethyl ether, and discard 10% of the initial distillate and 10% of the distillation residue. Determine the absorbance of the

distillate, using water as the reference. The absorbance is not more than 0.01 at 300–350 nm.

Peroxide Measure 5 mL of Diethyl Ether for Vitamin A Determination, and add 5 mL of iron(II) sulfate TS and 5 mL of ammonium thiocyanate solution (2 in 25). No red color develops.

_N,N_-Diethyl-_N′_-1-naphthylethylenediamine Oxalate $C_{18}H_{24}N_2O_4$ [29473-53-8] A white crystalline powder.

Content Not less than 98.0%.

Assay Weigh accurately about 0.5 g, add 100 mL of water, and heat in a water bath to dissolve. Titrate with 0.1 mol/L sodium hydroxide. To confirm the endpoint, use a potentiometer with a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes.

Each mL of 0.1 mol/L sodium hydroxide = 33.24 mg of $C_{18}H_{24}N_2O_4$

_N,N_-Diethyl-_p_-phenylenediamine Sulfate $(C_2H_5)_2NC_6H_4NH_2 \cdot H_2SO_4$ [6283-63-2] A white to slightly pale brown granules, or powder. Soluble in water.

Content Contains not less than 98.0% of *N,N*-diethyl-*p*-phenylenediamine sulfate ($(C_2H_5)_2NC_6H_4NH_2 \cdot H_2SO_4$).

Identification 5 mL of a 1 in 40 solution of *N,N*-diethyl-*p*-phenylenediamine sulfate produces a white precipitate when 1 mL of barium chloride solution (1 in 10) is added.

Purity (1) Clarity of solution Almost clear (0.5 g, water 20 mL).

(2) Absorbance Weigh exactly 0.02 g of *N,N*-diethyl-*p*-phenylenediamine sulfate, add 2.5 mL of phosphate buffer (pH 6.5, containing 1,2-cyclohexanediaminetetraacetic acid) and 0.48 g of sodium sulfate, dissolve the solids, and dilute to exactly 50 mL with water. Refer to this solution as Solution A. Measure the absorbance of the solution against water by ultraviolet-visible spectrophotometry. It is not more than 0.005 at a wavelength of 555 nm. Then, prepare a solution obtained by dissolving 0.3 g of potassium iodide in 30 mL of Solution A and leaving for 2 minutes and measure its absorbance against water in the same manner. It is not more than 0.005 at a wavelength of 555 nm. Separately, perform a blank test for each solution to make any necessary correction.

Assay Weigh accurately about 0.2 g of *N,N*-diethyl-*p*-phenylenediamine sulfate and dissolve it in 50 mL of water. Titrate the solution with 0.1 mol/L sodium hydroxide. Use a potentiometer to confirm the endpoint. The endpoint is when the titration curve shows a second inflection point. Make correction with the volume of titration consumed by the first inflection point.

Each mL of 0.1 mol/L sodium hydroxide = 26.23 mg of $(C_2H_5)_2NC_6H_4NH_2 \cdot H_2SO_4$

Difenoconazole for Assay $C_{19}H_{17}Cl_2N_3O_3$ [119446-68-3] A white crystalline powder or powder.

Content Not less than 97.0% of difenoconazole ($C_{19}H_{17}Cl_2N_3O_3$).

Identification Determine the absorption spectrum of difenoconazole for assay as

directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 1605 cm^{-1}, 1585 cm^{-1}, 1507 cm^{-1}, 1478 cm^{-1}, 1227 cm^{-1}, 1048 cm^{-1}, 848 cm^{-1}, and 679 cm^{-1}.

Assay Weigh accurately about 10 mg of difenoconazole for assay and about 1 mg of 1,4-BTMSB-d_4, and add 1 mL of deuterated acetone to dissolve them together. Transfer the resulting solution to an NMR tube of 5 mm in external diameter, and stopper tightly. Measure ^{1}HNMR spectra using a spectrometer with a proton resonance frequency of 400 MHz or more under the following operating conditions. Assuming the signal of 1,4-BTMSB-d_4 as δ 0 ppm, when the signal area intensities at around δ 7.33–7.35 ppm and δ 7.48–7.53 ppm are designated as A_1 (corresponding to 1 hydrogen atom) and A_2 (corresponding to 1 hydrogen atom), respectively, confirm that A_1/A_2 is 1.0. Then, assuming the signal area intensity of 1,4-BTMSB-d_4 as 18.00, when the sum of A_1 and A_2, the sum of the number of hydrogens, and the purity of 1,4-BTMSB-d_4 are designated as I, N, and P(%), respectively, determine the content of difenoconazole by the following formula. If the signal from difenoconazole is overlapped with the signal from a contaminant, do not use its signal area intensity and the number of hydrogens for the assay.

$$\text{Content (\%) of difenoconazole } (C_{19}H_{17}Cl_2N_3O_3) = \frac{\text{Weight (mg) of 1,4-BTMSB-}d_4 \times \text{I} \times \text{P}}{\text{Weight (mg) of the sample} \times \text{N}} \times 1.794$$

Operating conditions

Digital resolution: not more than 0.25.

Spinning: Off.

^{13}C decoupling: Present.

Acquisition time: Not less than 4 seconds.

Spectral range: At least 20 ppm including between -5 ppm and 15 ppm.

Flip angle: 90°.

Delay time: Not less than 64 seconds.

Dummy scans: Not less than 2.

Number of accumulation: Not less than 32.

Measurement temperature: A constant temperature at 20°C–30°C.

Digitonin $C_{56}H_{92}O_{29}$ [11024-24-1] White crystals or crystalline powder.

Identification Determine the absorption spectrum as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3400 cm^{-1}, 2930 cm^{-1}, 1640 cm^{-1}, 1370 cm^{-1}, 1070 cm^{-1}, 890 cm^{-1}.

Specific rotation $[\alpha]_D^{20}$: −47 to −50°. Weigh accurately about 2 g of digitonin, dried for 2 hours at 105°C, and dissolve it in diluted acetic acid (3 in 4) to make exactly 50 mL. Measure the rotation of the resulting solution.

Sensitivity Weigh 0.5 g of digitonin, add 20 mL of ethanol (95), dissolve it by warming,

and add ethanol (95) to make 50 mL. Use this solution as the test solution. Dissolve 20 mg of cholesterol in ethanol (95) to make 100 mL. To 10 mL of this solution, add 0.5 mL of the test solution, and cool the mixture to about 10°C. Allow to stand for 30 minutes with occasional shaking. A precipitate is formed.

2,3-Dihydro-2,3-dioxo-1*H*-indole-5-sulfonic Acid Sodium Salt Dihydrate $C_8H_4NNaO_5S \cdot 2H_2O$ [207399-16-4] Reddish yellow to red-brown crystals or powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 241–247 nm): 852–1040.

Weigh accurately about 10 mg of 2,3-dihydro-2,3-dioxo-1*H*-indole-5-sulfonic acid sodium salt dihydrate, dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to the resulting solution as Solution A. To exactly measured 5 mL of Solution A, add ammonium acetate TS (0.02 mol/L) to make exactly 50mL. This solution exhibits an absorption maximum at a wavelength of 241–247 nm. Measure the absorbance (A_B) of this solution at the maximum between 241–247 nm against ammonium acetate TS (0.02 mol/L) to determine the specific absorbance by the following formula:

$$E_{1\,cm}^{1\%} = A_B \times \frac{10}{\text{Weight (g) of the sample}} \times \frac{100}{100 - \text{Loss on drying (\%)}}$$

Purity (1) Clarity Weigh accurately about 10 mg of 2,3-dihydro-2,3-dioxo-1*H*-indole-5-sulfonic acid sodium salt dihydrate, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. The resulting solution is clear.

(2) Related substances Analyze 10 μL each of Solution A prepared for the specific absorbance measurement and ammonium acetate TS (0.02 mol/L) by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 40 minutes of injection. The area percentage of the main peak of Solution A is not less than 95.0% of the total area of all the peaks, excluding those from the ammonium acetate TS in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 245 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: An 85:15 mixture of ammonium acetate–tetra-*n*-butylammoniumbromide TS/acetonitrile (HPLC).

Flow rate: 1.0 mL/minute.

Loss on drying 9.8–14.8% (50 mg, 135°C, 6 hours).

2-(2,4-Dihydroxy-3,5-diiodobenzoyl)benzoic Acid $C_{14}H_8I_2O_5$ [3480-21-5] A slightly pale yellow to yellow-brown powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 348–354 nm): 426–520. Weigh accurately about 20mg of 2-(2,4-dihydroxy-3,5-diiodobenzoyl)benzoic acid, dissolve it in acetonitrile to make exactly 10 mL. To exactly measured 5 mL of this solution, add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to the resulting solution as Solution A. To exactly measured 5 mL of Solution A, add ammonium acetate TS (0.02 mol/L) to make exactly 50mL. This solution exhibits an absorption maximum at a wavelength of 348–354 nm. Measure the absorbance (A_B) of this solution at the maximum between 348–354 nm against the reference solution prepared as follows: Make 5 mL of acetonitrile up to 100 mL with ammonium acetate TS (0.02 mol/L), and then make 5 mL of the resulting solution up to 100 mL with ammonium acetate TS (0.02 mol/L). Determine the specific absorbance by the following formula:

$$E_{1cm}^{1\%} = A_B \times \frac{20}{\text{Weight (g) of the sample}} \times \frac{100}{100 - \text{Water (\%)}}$$

Purity (1) Clarity Clear (20 mg, acetonitrile 10 mL).

(2) Related substances Analyze 20 μL each of Solution A prepared for the specific absorbance measurement and a solution prepared by diluting 5 mL of acetonitrile up to 100 mL with ammonium acetate TS (0.02 mol/L) by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 30 minutes of injection. The area percentage of the main peak of Solution A is not less than 95.0% of the total area of all the peaks, excluding those from acetonitrile and the ammonium acetate TS in Solution A.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 350 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: An 85:15 mixture of ammonium acetate TS (0.02 mol/L)/acetonitrile (HPLC).

Flow rate: 1.0 mL/minute.

Water Not more than 1.0% (50 mg, Coulometric Titration).

1,3-Dihydroxynaphthalene $C_{10}H_6(OH)_2$ [132-86-5] Red-brown crystals or a gray to grayish brown powder. Freely soluble in water, in ethanol (95), and in diethyl ether.

Melting point 122–124°C (decomposition).

Sensitivity To 2 drops of L(+)-tartaric acid solution (1 in 1000), add 1 mL of a solution of 1,3-Dihydroxynaphthalene in diluted sulfuric acid (1 in 10,000), and heat at 90°C for 1 hour. A blue-green to green-blue color develops.

Diisopropyl Ether [K9528, Special Grade] [108-20-3]

Dimedone $C_8H_{12}O_2$ [126-81-8] A white to pale yellow crystalline powder.

Melting point 145–149°C.

Dimedone TS Dissolve 5 g of dimedone in ethanol (99.5) to make 100 mL. Prepare fresh before use.

1,2-Dimethoxyethane $C_4H_{10}O_2$ [110-71-4] A colorless, transparent liquid having a diethyl ether-like odor. Very soluble in water, in ethanol (95), and in hydrocarbon solvents.

Content Not less than 99.0% of 1,2-dimethoxyethane ($C_4H_{10}O_2$).

Boiling point 82–83°C.

Assay Analyze 1,2-dimethoxyethane by gas chromatography using the following conditions, and calculate the area percentage of the main peak.

Operating conditions

Detector: Flame-ionization detector.

Column: A glass or stainless steel tube (3–4 mm internal diameter and 2 m length).

Column packing material

Liquid phase: 10% Polyethylene glycol 20M of the amount of support.

Support: 177- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of 70–80°C.

Carrier gas: Helium.

Flow rate: A constant rate of 50 mL/min.

1,2-Dimethoxyethane TS (containing 5% methanol) To 5 mL of methanol, add 1,2-dimethoxyethane to make 100 mL. The solution is stable at least for 3 months in a refrigerator.

***p*-Dimethylaminobenzaldehyde** $(CH_3)_2NC_6H_4CHO$ [K8496, Special Grade] [100-10-7]

***p*-Dimethylaminobenzaldehyde TS** Dissolve 125 mg of *p*-dimethylaminobenzaldehyde in 100 mL of cooled diluted sulfuric acid (13 in 20), and add 50 μL of iron(III) chloride hexahydrate solution (1 in 10). Use within 7 days of preparation.

***p*-Dimethylaminocinnamaldehyde** $C_{11}H_{13}NO$ [6023-18-5] Orange crystals or crystalline powder having a characteristic odor.

Melting point 140–142°C.

Purity Clarity Dissolve 0.2 g of 4-dimethylaminocinnamaldehyde in 20 mL of ethanol (95). The solution is clear.

Loss on drying Not more than 0.5% (105°C, 2 hours).

Residue on ignition Not more than 0.1% (1 g).

Nitrogen content 7.8–8.1% (dry before the test at 105°C for 2 hours, Nitrogen Determination).

***p*-Dimethylaminocinnamaldehyde TS** Before use, add 1 mL of acetic acid to 10 mL of a solution (1 in 2000) of *p*-dimethylaminocinnamaldehyde in ethanol (95).

***N*-(3,3-Dimethylbutyl)-L-α-aspartyl-L-phenylalanine** $C_{19}H_{28}N_2O_5$ A white to off-white powder. It is principally produced by hydrolyzing neotame in an alkaline state.

Identification Measure the absorption spectrum of *N*-(3,3-dimethylbutyl)-L-α-

aspartyl-L-phenylalanine as directed in the Disk Method under the Infrared Spectrophotometry. It exhibits absorptions at about 3290 cm^{-1}, 3150 cm^{-1}, 2960 cm^{-1}, 1690 cm^{-1}, 1560 cm^{-1}, 750 cm^{-1}, and 700 cm^{-1}.

Purity Related substances Prepare a test solution by dissolving about 0.1 g of *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine in 100 mL of the mobile phase directed under the Assay for Neotame in the Monographs. Prepare a control solution by diluting 1 mL of the test solution, measured exactly, with the mobile phase solution to make exactly 100 mL. Analyze 25 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for 5 times the retention time of the main peak, and measure peak areas. Exclude the solvent peak from measurement. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Use the operating conditions given in the Assay for Neotame in the Monograph

Adjust the retention time of *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine to about 4 minutes.

Residue on ignition Not more than 0.2 %.

Dimethyl Carbonate $C_3H_6O_3$ [616-38-6] A colorless to slightly light yellow liquid.

Content Not less than 98.0% of dimethyl carbonate ($C_3H_6O_3$).

Refractive index n_D^{20}: 1.365–1.372.

Water Not more than 0.2%.

Assay Analyze 0.2-μL portions of dimethyl carbonate by gas chromatography using the operating conditions given below. Measure the peak area of each peak to determine the percentage of the main peak by the peak area percentage method.

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.32 mm internal diameter and 15 m length) coated with a 5.0 μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 50°C for 10 minutes, raise at 20°C/minute to 250°C, and maintain the temperature at 250°C for 5 minutes.

Injection port temperature: 200°C.

Detector temperature: 260°C.

Carrier gas: Helium.

Flow rate: A constant rate of about 1.5 mL/min.

Injection method: Split.

Split ratio: 1 : 200.

***N,N*-Dimethyl Casein** Milk-derived Dimethyl Casein Use a product suitable for the corresponding enzyme activity tests.

***N,N*-Dimethylformamide** $HCON(CH_3)_2$ [K8500, Special Grade] [68-12-2]

Dimethylglyoxime $(CH_3)_2C_2(NOH)_2$ [K8498, Special Grade] [95-45-4]

Dimethyl Sulfoxide $(CH_3)_2SO$ [K9702, Special Grade] [67-68-5]

Dimethyl Sulfoxide for Ultraviolet Absorption Spectrum Measurement A colorless, clear liquid.

Identification Determine the absorption spectrum as directed in the Liquid Film Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2990 cm^{-1}, 2910 cm^{-1}, 1440 cm^{-1},1310 cm^{-1}, 1050 cm^{-1}, 950 cm^{-1}, 700 cm^{-1}, 670 cm^{-1}.

Density 1.098–1.103 g/mL (20°C).

Absorbance Not more than 0.20. Measure the absorbance against water at a wavelength of 280 nm.

Purity Clarity Clear (2 mL, water 20 mL).

Water Not more than 0.05% (10 g, Volumetric Titration, Direct Titration).

Dimethyl Sulfoxide TS Place 300 mL of dimethyl sulfoxide for ultraviolet absorption spectrum measurement in a 1-L separating funnel, and add 75 mL of phosphoric acid. Shake the mixture, and allow to stand for 10 minutes. Add 150 mL of 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement, shake, and allow to stand for 10 minutes. Separate the lower layer, and store in a tightly stoppered glass bottle.

3,5-Dinitrobenzoyl Chloride $(NO_2)_2C_6H_3COCl$ [99-33-2] A slightly yellowish crystalline powder. Soluble in diethyl ether.

2,4-Dinitrophenylhydrazine $C_6H_6N_4O_4$ [K8480, Special Grade] [119-26-6]

2,4-Dinitrophenylhydrazine Hydrochloride TS Transfer 10 mL of hydrochloric acid into a 100 mL Erlenmeyer flask, add 5 g of 2,4-dinitrophenylhydrazine, and shake gently until free base (red color) converts to hydrochloride (yellow color). Add 100 mL of ethanol (95), and heat on a water bath to dissolve. Cool, crystallize at room temperature, and filter. Wash with diethyl ether, and dry at room temperature, and store in a desiccator. Use it as the reagent 2,4-dinitrophenylhydrazine hydrochloride. During storage, it gradually converts from hydrochloride to free base, but the free base is removed by rinsing with 1,2-dimethoxyethane. Prepare 2,4-Dinitrophenylhydrazine Hydrochloride TS by dissolving 0.5 g of 2,4-dinitrophenylhydrazine hydrochloride in 15 mL of 1,2-dimethoxyethane TS (containing 5% methanol). Store in a refrigerator.

3,5-Dinitrosalicylic Acid $(NO_2)_2C_6H_2(OH)COOH$ Use a product suitable for the corresponding enzyme activity tests.

3,5-Dinitrosalicylic Acid–Lactose TS Dissolve 1.20 g of lactose monohydrate in water to make 100 mL. To 1 mL of this solution, add water to make 100 mL. Mix 50 mL of the resulting solution with 150 mL of 3,5-dinitrosalicylic acid TS. Prepare fresh before use.

3,5-Dinitrosalicylic Acid–Phenol TS

Solution 1 Dissolve 44.0 g of 3,5-dinitrosalicylic acid in water to make 4.4 L. To this solution, add 1275 g of potassium sodium (+)-tartrate tetrahydrate to dissolve it. Add

1500 mL of sodium hydroxide solution (9 in 200), and mix.

Solution 2 Dissolve 45 g of phenol in 110 mL of sodium hydroxide solution (1 in 10), and add water to make 500 mL.

To Solution 1, add 345 mL of Solution 2 and 34.5 g of sodium hydroxide, leave for 2 days in a dark place, and filter. Store in a tightly stoppered brown bottle in a dark place at room temperature. Use within 1 year.

3,5-Dinitrosalicylic Acid–Phenol TS (for agarase activity test) Dissolve 10.6 g of 3,5-dinitrosalicylic acid and 19.8 g of sodium hydroxide in 1416 mL of water. Dissolve 306 g of potassium sodium (+)-tartrate tetrahydrate and 8.3 g of sodium pyrosulfite in this solution. Then to the resulting solution, add 7.6 g of phenol to dissolve. Filter the solution through a filter paper, and leave it for 1 day protected from light. Filter through a filter paper before use when a precipitate is formed.

3,5-Dinitrosalicylic Acid–Phenol TS (for cellulase activity test) Dissolve 31.8 g of 3,5-dinitrosalicylic acid in 4 L of water while stirring, and then dissolve 59.4 g of sodium hydroxide in this solution. To this solution, add 918 g of potassium sodium (+)-tartrate tetrahydrate, 22.8 mL of phenol, and 24.9 g of sodium pyrosulfite, and dissolve them. Make up to 5 L with water, and filter. Use after left to stand for 1 day or more.

3,5-Dinitrosalicylic Acid–Potassium Sodium Tartrate TS Dissolve 0.1 g of 3,5-dinitrosalicylic acid and 6.0 g of potassium sodium (+)-tartrate tetrahydrate by adding 20 mL of sodium hydroxide TS (2 mol/L) and 10 mL of water.

3,5-Dinitrosalicylic Acid TS Weigh 10.0 g of 3,5-dinitrosalicylic acid, add 400 mL of water, and suspend it by warming while stirring. Add gradually 150 mL of sodium hydroxide solution (8 in 75), and dissolve it by warming below 50°C while stirring. Then add gradually 300 g of potassium sodium (+)-tartrate tetrahydrate, dissolve it, and make up to 950 mL with water. Completely dissolve by warming below 50°C while stirring. Cool this solution to room temperature, and make up to 1000 mL with water. Filter through a glass filter. Store in a tightly stoppered brown glass bottle in a dark place at room temperature, and use within 6 months.

3,5-Dinitrosalicylic Acid TS (for pectinase activity test) Dissolve 1.6 g of sodium hydroxide in 50 mL of water. To this solution, add gradually 1.0 g of 3,5-dinitrosalicylic acid to dissolve it, and make up to 100 mL with water.

1,4-Dioxane $C_4H_8O_2$ [K8461, Special Grade] [123-91-1]

1,2-Dipalmitoyl-*rac*-glycerol $C_{35}H_{68}O_5$ Use a product suitable for the corresponding enzyme activity tests.

L-α-Dipalmitoylphosphatidylcholine $C_{40}H_{80}NO_8P$
1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine Use a product suitable for the corresponding enzyme activity tests.

Diphenylamine $(C_6H_5)_2NH$ [K8487, Special Grade] [122-39-4]

Diphenyl Ether $C_{12}H_{10}O$ [101-84-8] Colorless crystals having a characteristic odor.
Boiling point 254–259°C.

Melting point 25–28°C.

Purity Related substances Prepare a test solution by dissolving 1.0 g of diphenyl ether in 100 mL of ethyl acetate. Prepare a control solution by adding ethyl acetate to 1 mL of the test solution, exactly measured, to make exactly 100 mL. Analyze 0.5 μL each of the test solution and the control solution by gas chromatography using the operating conditions given below, and measure the peak areas. Continue the chromatography for two times the retention time of the main peak. Exclude the solvent peaks from measurement. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Detector: Flame-ionization detector.

Column: A fused silica tube (0.53 mm internal diameter and 12 m length) coated with a 1.0-μm thick layer of dimethylpolysiloxane.

Column temperature: Raise the temperature from 100°C to 300°C at a rate of 10°C/minute.

Inlet temperature: 300°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the peak of diphenyl ether appears about 3 minutes after injection.

Injection method: Split.

Split ratio: 1:10.

Dipotassium Hydrogenphosphate K_2HPO_4 [K9017, Special Grade] [7758-11-4]

1,3-Di(4-pyridyl)propane $C_{13}H_{14}N_2$ [17252-51-6] A light yellow powder.

Melting point 61–62°C.

Water Not more than 1 mg/g.

Disodium Chromotropate Dihydrate $C_{10}H_6Na_2O_8S_2 \cdot 2H_2O$ [K8316, Special Grade] [5808-22-0]

Disodium 4,4′-(Diazoamino)dibenzenesulfonate $C_{12}H_9N_3Na_2O_6S_2$ [56120-28-6] A white to reddish yellow powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 356–362 nm): Not less than 640. Weigh accurately about 10 mg of disodium 4,4′-(diazoamino)dibenzenesulfonate, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate solution (0.02 mol/L) to make exactly 100 mL. This solution exhibits absorption maxima in the ranges 238–244 nm and 356–362 nm, respectively. Measure the absorbance of this solution at the maximum between 356–362 nm against ammonium acetate TS (0.02 mol/L), and determine the specific absorbance.

Purity (1) Clarity of solution Clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add the mobile phase to 5 mg of disodium 4,4′-(diazoamino)dibenzenesulfonate to make exactly 50 mL. Analyze 10 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 20 minutes of injection. The area percentage of the main peak in the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the mobile phase in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 360 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 19:1 mixture of ammonium acetate (0.02 mol/L)/acetonitrile (for HPLC).

Flow rate: 1.0 mL/min.

Water Not more than 10.0% (50 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

Disodium Dihydrogen Ethylenediaminetetraacetate Dihydrate $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ [K8107] [6381-92-6]

Disodium Dihydrogen Ethylenediaminetetraacetate–Hydrochloric Acid TS (0.001 mol/L) Dissolve 0.37 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate in 100 mL of hydrochloric acid (0.01 mol/L), and add water to make 1000 mL.

Disodium Dihydrogen Ethylenediaminetetraacetate–Sodium Hydroxide TS Dissolve 1 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate and 1.2 g of sodium hydroxide in water to make 1000 mL.

Disodium Dihydrogen Ethylenediaminetetraacetate–Tris TS Place 18.6 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate and 6.05 g of 2-amino-2-hydroxymethyl-1,3-propanediol into a 250-mL beaker, and add 200 mL of hot water, and stir. Adjust the pH to 7.5–7.6 with sodium hydroxide solution (1 in 5). After cooling, adjust the pH to 8.0 with sodium hydroxide solution (1 in 5), transfer the solution to a 250-mL volumetric flask, and add water to the volume of 250 mL. Mix well, and store in a plastic bottle.

Disodium Dihydrogen Ethylenediaminetetraacetate TS (0.2 mol/L) Dissolve 74.4 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate in water to make 1000 mL.

Disodium Dihydrogen Ethylenediaminetetraacetate TS (0.005 mol/L) Dissolve 1.86 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate in water to make 1000 mL.

Disodium 5′-Guanylate *n*-Hydrate $C_{10}H_{12}N_5Na_2O_8P \cdot nH_2O$ [5550-12-9] “Disodium

5′-Guanylate”

Disodium Hydrogen Citrate Sesquihydrate $2NaOCOCH_2C(OH)(COOH)CH_2COONa \cdot 3H_2O$ Use a product suitable for the corresponding enzyme activity tests.

Disodium Hydrogenphosphate Na_2HPO_4 [K9020, Special Grade] [7558-79-4]

Disodium Hydrogenphosphate Dodecahydrate $Na_2HPO_4 \cdot 12H_2O$ [K9019, Special Grade] [10039-32-4]

Disodium Hydrogenphosphate for pH Determination Na_2HPO_4 [K9020, pH Standard Solution Grade] [7558-79-4]

Disodium Hydrogenphosphate TS (0.2mol/L, containing albumin) Dissolve 28.4 g of disodium hydrogenphosphate and 0.5 g of bovine serum albumin in water to make 1000 mL.

Disodium Hydrogenphosphate TS (0.05 mol/L) Dissolve 7.098 g of disodium hydrogenphosphate in water to make 1000 mL.

Disodium Hydrogenphosphate TS (0.01 mol/L) Dissolve 1.42 g of disodium hydrogenphosphate in water to make 1000 mL.

Disodium Hydrogenphosphate TS (0.01mol/L, containing albumin) Dissolve 1.4 g of disodium hydrogenphosphate and 0.5 g of bovine serum albumin in water to make 1000 mL.

Disodium 3-Hydroxy-2,7-naphthalenedisulfonate $C_{10}H_6Na_2O_7S_2$ [135-51-3] A white to gray-yellowish green powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength of 278–284 nm): Not less than 110. Weigh accurately about 10 mg of disodium 3-hydroxy-2,7-naphthalenedisulfonate, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Exactly measure 10 mL of Solution A, add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits absorption maxima in the ranges 233–239 nm, 270–276 nm, 278–284 nm, and 337–343 nm, respectively. Measure the absorbance of this solution at the maximum between 278–284 nm against ammonium acetate TS (0.02 mol/L), and determine the specific absorbance.

Purity (1) Clarity of solution Clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add ammonium acetate TS (0.02 mol/L) to 5 mg of disodium 3-hydroxy-2,7-naphthalenedisulfonate to make exactly 50 mL. Analyze 5 μL each of the test solution and ammonium acetate TS (0.02 mol/L) by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 55 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the ammonium acetate TS in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 235 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: Ammonium acetate (0.02 mol/L).

B: Acetonitrile (for HPLC).

Concentration gradient (A/B): Maintain 100/0 for 5 minutes, and run a linear gradient from 100/0 to 70/30 in 50 minutes.

Flow rate: 1.0 mL/min.

Water Not more than 10.0% (50 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

Disodium 3-Hydroxy-2,7-naphthalenedisulfonate (for unsulfonated primary aromatic amine determination) $C_{10}H_6Na_2O_7S_2$ [135-51-3] A white to grayish yellow green powder.

Identification Weigh accurately about 10 mg of disodium 3-hydroxy-2,7-naphthalenedisulfonate, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits absorption maxima in the ranges 233–239 nm, 270–276 nm, 278–284 nm, and 337–343 nm, respectively.

Purity Related substances Measure exactly 10 mL of Solution A, add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Analyze 20 μL of this solution by liquid chromatography, using the operating conditions given below. Measure the area of each peak. The area percentage of the main peak of the solution is not less than 85.0% of the total area of all the peaks that appear within 35 minutes of injection.

Operating conditions

Detector: Visible spectrophotometer or photodiode array (wavelength: 254 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 near 40°C.

Mobile phase

A: Ammonium acetate (0.02 mol/L).

B: A 7:3 mixture of acetonitrile/water.

Concentration gradient (A/B): Maintain 100/0 for 10 minutes, and run a linear gradient from 100/0 to 50/50 in 20 minutes, and maintain 50/50 for 5 minutes.

Flow rate: 1.0 mL/min.

Disodium 7-Hydroxy-1,3-naphthalenedisulfonate $C_{10}H_6Na_2O_7S_2$ [842-19-3] A white

to yellow-green powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 285–291 nm): Not less than 130. Weigh accurately about 10 mg of disodium 7-hydroxy-1,3-naphthalenedisulfonate, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits absorption maxima in the ranges 234–240 nm, 285–291 nm, and 333–339 nm, respectively. Measure the absorbance of this solution at the maximum between 285–291 nm against ammonium acetate TS (0.02 mol/L), and determine the specific absorbance.

Purity (1) Clarity of solution Clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add the mobile phase to 5 mg of disodium 7-yhdroxy-1,3-naphthalenedisulfonate to make exactly 50 mL. Analyze 10 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 20 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the mobile phase in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 235 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 13:7 mixture of ammonium acetate–tetra-*n*-butylammonium bromide TS/acetonitrile (for HPLC).

Flow rate: 1.0 mL/min.

Water Not more than 15.0% (50 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

Disodium 3-Hydroxy-2,7-naphthalenedisulfonate TS (0.05 mol/L) Dissolve 1.74 g of disodium 3-hydroxy-2,7-naphthalenedisulfonate (for unsulfonated primary aromatic amine determination) in water to make 100 mL.

Disodium 5′-Inosinate *n*-Hydrate $C_{10}H_{11}N_4Na_2O_8P \cdot nH_2O$ [4691-65-0] "Disodium 5′-Inosinate"

Disodium 2-Ketoglutarate *n*-Hydrate $C_5H_4Na_2O_5 \cdot nH_2O$ [305-72-6, anhydrous] A white powder. Soluble in water.

Disodium Molybdate(VI) Dihydrate $Na_2MoO_4 \cdot 2H_2O$ [K8906, Special Grade] [10102-40-6]

Disodium *p*-Nitrophenyl Phosphate Hexahydrate $O_2NC_6H_4OPO(ONa)_2 \cdot 6H_2O$ Use a product suitable for the corresponding enzyme activity tests.

Disodium 1-Nitroso-2-naphthol-3,6-disulfonate $C_{10}H_5NNa_2O_8S_2$ [525-05-3] Yellow crystals or powder.

Identification Determine the spectrum of disodium 1-nitroso-2-naphthol-3,6-disulfonate as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3400 cm^{-1}, 1639 cm^{-1}, 1451 cm^{-1}, 1270 cm^{-1}, 1231 cm^{-1}, 1173 cm^{-1}, 1049 cm^{-1}, 848 cm^{-1}, and 662 cm^{-1}.

Sensitivity Weigh 0.2 g of disodium 1-nitroso-2-naphthol-3,6-disulfonate into a 100-mL volumetric flask, dissolve it in water, and make up to 100 mL with water. Use this solution as the test solution. To 5 mL of Cobalt Standard Solution, add 0.5 g of sodium acetate and 0.2 mL of diluted acetic acid (1 in 3), and then add 1.0 mL of the test solution. The color of solution becomes red.

Storage standard Store in a tightly stoppered container protected from light.

Disodium 6,6′-Oxybis(2-naphthalenesulfonate) $C_{20}H_{12}Na_2O_7S_2$ [61551-82-4] A whitish powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 240 nm): Not less than 2020. Weigh accurately 10 mg of disodium 6,6′-oxybis(2-naphthalenesulfonate), previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits absorption maxima at wavelengths of around 220 nm and around 240 nm.

Purity Other aromatic compounds Measure exactly 1.0 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Analyze 20 μL of this solution by liquid chromatography using the operating conditions specified in Purity (7) for Food Red No. 40 in the Monographs, JSFA-IX. Only one peak of disodium 6,6′-oxybis(2-naphthalenesulfonate) is observed.

Dithiothreitol $C_4H_{10}O_2S_2$ [27565-41-9] Crystals.

Melting point 42–43°C.

Dodecylbenzene $C_{18}H_{30}$ [123-01-3] A colorless liquid.

Specific gravity d_4^{20}: 0.855–0.859.

***n*-Dodecylbenzene Sulfonic Acid** $C_{18}H_{30}O_3S$ [27176-87-0] White lumps or powder.

Identification (1) Ignite 1 g of *n*-dodecylbenzene sulfonic acid, add 20 mL of water to the residue to dissolve it. Refer to this solution as Solution A. To 10 mL of this solution, add 1 mL of diluted hydrochloric acid (2 in 3) and 1 mL of barium chloride dihydrate solution (1 in 10). A white precipitate is formed.

(2) Determine the spectrum of *n*-dodecylbenzene sulfonic acid as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2920 cm^{-1}, 1180 cm^{-1}, 1130 cm^{-1}, 1040 cm^{-1}, and 1010 cm^{-1}.

Purity (1) Dodecylbenzene Not more than 0.1% as $C_{12}H_{25}C_6H_5$. To 0.5 g of *n*-dodecylbenzene sulfonic acid, add10 mL of water, 10 mL of ethanol (99.5), and 5 mL of hexanes (for the determination of the pesticide residue PCB), shake the mixture vigorously for 1 minute, and allow to stand for 5 minutes. Refer to the hexane layer as Solution B. Dissolve 0.1 g of dodecylbenzene in hexane (for pesticide residue and polychlorinated biphenyl analysis) to make 100 mL, and refer to the resulting solution as Solution C. Analyze 5 μL each of Solutions B and C by gas chromatography using the operating conditions given below. The height of the peak of dodecylbenzene in Solution B is not more than 1/10 of that of dodecylbenzene in Solution B.

Operating conditions

Detector: Flame-ionization detector.

Column: A glass or stainless steel tube (3 mm internal diameter and 2 m length).

Column packing material

Liquid phase: 0.5% Phosphoric acid and 10% diethylene glycol succinate to the support.

Support: 180–250 μm diatomaceous earth for gas chromatography.

Column temperature: 150°C.

Injection port temperature: 200°C.

Carrier gas: Helium.

Flow rate: 45 mL/minute.

(2) Prepare a test solution by dissolving 20 mg of *n*-dodecylbenzene sulfonic acid in 100 mL of a 50:50 mixture of water/acetonitrile (for HPLC). Analyze 20 μL of the test solution by liquid chromatography using the operating conditions given below. Four main peaks are observed. The area of the impurity whose peak is the largest of the impurity peaks, other than the solvent peak, is not more than 10% of the area of the smallest one of the four main peaks. Separately, perform a blank test to make any necessary correction.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 222 nm).

Column: A stainless steel (4.6 mm internal diameter and 15 cm length).

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

Column temperature: 25°C.

Mobile phase: A solution of 1 g of tetramethyl ammonium bromide in 500 mL of a 50:50 mixture of water/acetonitrile (for HPLC).

Flow rate: 1.0 mL/minute.

DPD–EDTA TS Pulverize 1.1 g of *N,N*-diethyl-*p*-phenylenediamine sulfate in an agate mortar. Add 0.2 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate and a small amount of water to dissolve them if necessary by heating with stirring. Add 8 mL of 25% (w/v) of diluted sulfuric acid, and mix. Dilute the resulting solution to 1000 mL with water.

Dragendorff Reagent

Solution 1 Dissolve 0.85 g of basic bismuth nitrate by adding 10 mL of acetic acid and 40 mL of water.

Solution 2 Dissolve 8 g of potassium iodide by adding 20 mL of water.

Prepare the reagent by mixing 5 mL of Solution 1, 5 mL of Solution 2, 20 mL of acetic acid, and 100 mL of water before use.

Dried Bacterial Cells Inoculate *Bacillus subtilis* 168 into 50 mL of Luria-Bertani medium in a 500-mL baffled Erlenmeyer flask, and incubate it in a rotator at 37°C at 160 rpm for about 18 hours. Inoculate 10 mL of the resulting culture into 500 mL of LB medium in 3-L Erlenmeyer flask, and incubate it at 37°C at 80 rpm for 4–5hours. Confirm that the absorbance at 660 nm is about 1.8. Centrifuge this culture at 8000 rpm for 15 minutes at 10°C) to recover the cells. Wash the cells with 50 mL of water, and centrifuge at 10°C at 8000 rpm for 15 minutes to recover them. Disperse the cells uniformly in 50 mL of acetone, and centrifuge at 10°C at 8000 rpm for 15 minutes to recover them. Repeat the dispersion in acetone once more. Vacuum-dry the acetone-treated cells for 16–24 hours at room temperature.

Luria-Bertani medium

Tripton: 10 g

Yeast extract: 5 g

Sodium chloride: 10 g

Water: 1000 mL

Mix all the ingredients, and autoclave at 121°C for 20 minutes.

Dried Yeast (for the Glucanase Activity Test) Incubate *Cndida utilis* NBRC 0396, and collect the proliferated cells by centrifugation. Wash the cells with water, and freeze-dry. Grind them to align the size.

DSS-d_6 **$C_6H_9D_6NaO_3SSi$** [284664-85-3] Sodium 3-(trimethylsilyl)-1-propane-1,1,2,2,3,3-d_6-sulfonate whose traceability to the International System of Units is ensured.

Dulcoside A $C_{38}H_{60}O_{17}$ [64432-06-0] A white powder.

Identification (1) Determine the spectrum of dulcoside A as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3400 cm^{-1}, 2920 cm^{-1}, 1730 cm^{-1}, 1640 cm^{-1}, 1450 cm^{-1}, 1340 cm^{-1}, 1230 cm^{-1},1080 cm^{-1}, 900 cm^{-1}, 810 cm^{-1}, and 640 cm^{-1}.

(2) Dissolve 10 mg of dulcoside A by adding 0.5 mL of methanol, 0.5 mL of chloroform, and 0.1 mL of water. Using 5 μL of the resulting solution, proceed as directed in Identification (2) for steviolbioside. The main spot is observed at an R_f value of about 0.7.

Purity Related substances Prepare a test solution by dissolving 5 mg of dulcoside A in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC). Analyze 10 μL of the test solution by liquid chromatography using operating conditions specified in the Assay for Stevia Extract in the Monographs. Continue the chromatography for 30 minutes and

exclude the solvent peak from measurement. Measure the areas of all the peaks to determine the percentage of the main peak by the peak area percentage method. It is not less than 95.0%.

Egg Yellow Use a product suitable for the corresponding enzyme activity tests.

Egg White Use fresh egg white.

Egg White TS Weigh 10 g of egg white, add 40 mL of water, and shake.

Ehrlich's TS Dissolve 0.8 g of *p*-dimethylaminobenzaldehyde in 30 mL of ethanol (99.5), add 30 mL of hydrochloric acid, and cool. Prepare fresh before use.

Eosine Y $C_{20}H_6Br_4Na_2O_5$ [17372-87-1] A red to red-brown powder.

Identification Dissolve 0.10 g of eosine Y in water to make 100 mL. To 1 mL of this solution, exactly measured, add water to make exactly 200 mL. The resulting solution exhibits an absorption maximum at a wavelength of 514–518 nm.

Absorbance Measure the absorbance of the test solution for the identification test by ultraviolet-visible spectrophotometry against water. The absorbance at a wavelength of 515 nm is 0.50–0.80.

(–)-Epicatechin $C_{15}H_{14}O_6$ [490-46-0] A white to light yellow powder.

Identification Proceed as directed in Identification (1) for (+)-catechin for assay.

Purity Dissolve 20 mg of (–)-epicatechin in 20 mL of a 500:500:1 mixture of water/methanol (for HPLC)/formic acid. Analyze 10-μL portions of the resulting solution by liquid chromatography using the operating conditions specified in Purity (2) for (+)-catechin for assay. Continue the chromatography for two times the retention time of the main peak and exclude the solvent peak from measurement. Measure the area of each peak to determine the percentage of the main peak by the peak area percentage method. It is not less than 90.0%.

(–)-Epicatechin Gallate $C_{22}H_{18}O_{10}$ [1257-08-5] A grayish white powder.

Identification Proceed as directed in Identification (1) for (+)-catechin for assay.

Purity Dissolve 20 mg of (–)-epicatechin gallate in 20 mL of a 500:500:1 mixture of water/methanol (for HPLC)/formic acid. Analyze 10-μL portions of the resulting solution by liquid chromatography using the operating conditions specified in Purity (2) for (+)-catechin for assay. Continue the chromatography for two times the retention time of the main peak and exclude the solvent peak from measurement. Measure the area of each peak to determine the percentage of the main peak by the peak area percentage method. It is not less than 90.0%.

Eriochrome Black T $C_{20}H_{12}N_3NaO_7S$ [K8736, Special Grade] [1787-61-7]

Eriochrome Black T TS Dissolve 0.5 g of eriochrome black T and 4.5 g of hydroxylammonium chloride in 100 mL of ethanol (95). Store in a light-resistant container.

Eriochrome Black T–Sodium Chloride Indicator Mix 0.1 g of eriochrome black T and 10 g of sodium chloride, and triturate thoroughly to be homogeneous.

***meso*-Erythritol** $C_4H_{10}O_4$ [149-32-6] White crystals or crystalline powder.

Clarity of solution Clear (1.0 g, water 20 mL).

Melting point 118–120°C.

Water Not more than 0.5% (1 g, Volumetric Titration, Direct Titration).

Residue on ignition Not more than 0.1% (2 g).

Esterized Pectin Use a product suitable for the corresponding enzyme activity tests.

Ethanol (95) C_2H_5OH [K8102, Special Grade, First Grade] [64-17-5]

Ethanol (99.5) C_2H_5OH [K8101, Special Grade] [64-17-5]

Ethanol (Aldehyde-free) [K8001, Ethanol (Aldehyde and Ketone Tests Grade)] To 500 mL of ethanol (99.5), add 10 g of 2,4-dinitrophenylhydrazine and 0.2 mL of hydrochloric acid, reflux under a condenser for 2 hours, and distill. Discard the first 100 mL distillate, and use subsequent 300 mL. The distillate must be colorless (CH_3COCH_3: not more than 1 ppm by mass fraction).

Ethanol (Neutralized) Measure a suitable quantity of ethanol (95), add several drops of phenolphthalein TS, and add sodium hydroxide solution (1 in 1250) until a light pink color develops. Prepare fresh before use.

2-(2-Ethoxyethoxy)ethanol $C_2H_5(OCH_2CH_2)_2OH$ [111-90-0] A colorless, clear liquid. Miscible with water. The boiling point is about 203°C.

Refractive index n_D^{20}: 1.425–1.429.

Specific gravity d_{20}^{20}: 0.990–0.995.

Acid (as CH_3COOH) Not more than 0.01%.

Ethyl Acetate $CH_3COOC_2H_5$ [K8361, Special Grade] [141-78-6]

Ethyl 2-Acetoxy-2-methylacetoacetate $C_9H_{14}O_5$ Use a product suitable for the corresponding enzyme activity tests.

Ethylene Glycol $HOCH_2CH_2OH$ [K8105, Special Grade] [107-21-1]

Ethylene Glycol for Water Determination Distill ethylene glycol, and collect the fraction at 195–198°C. The water content in 1 mL of it is not more than 1.0 mg.

Ethylene Glycol Chitin Use a product suitable for the corresponding enzyme activity tests.

Ethylene Oxide–Tetrahydrofuran TS for Polysorbate A colorless, transparent liquid. Use promptly after opening because it is highly volatile.

Content About 44.05 g of ethylene oxide (C_2H_4O) in 1000 mL (1 mol/L).

Assay Cool Ethylene Oxide–Tetrahydrofuran TS for Polysorbate in methanol with dry ice, and use as the test solution. Place the test solution in a glass tube (2 mm external diameter), and seal the tube with a fluorine resin tape. Place deuterated chloroform, cooled in methanol with dry ice, in an NMR sample tube (5 mm external diameter), and also place the glass tube containing the test solution, and seal tightly. Measure the ^{1}HNMR spectrum at once. Designate the signal area intensity (at approximate 3.95 ppm) of tetrahydrofuran as A when the signal area intensity (approximate 2.85 ppm) of Ethylene Oxide–Tetrahydrofuran TS is assumed as 1. Determine the content of ethylene oxide by the following formula:

Content (g/L) of ethylene oxide (C_2H_4O) = (11.01/(12.24 + 20.26 × A)) × 1000

Ethyl Formate $HCOOC_2H_5$ [109-94-4] A colorless, transparent liquid having a characteristic odor.

Content Not less than 97.0% of ethyl formate ($HCOOC_2H_5$ = 74.08).

Refractive index n_D^{20}: 1.3595–1.3601.

Specific gravity d_4^{20}: 0.915–0.924.

Boiling point 53–54°C.

Assay Weigh accurately about 5.0 g of ethyl formate, and proceed as directed under Ester Value and Acid Value in the Flavoring Substances Tests. Calculate the content by the following formula:

$$\text{Content (\%) of ethyl formate } (HCOOC_2H_5) = \frac{\text{Saponification value} - \text{Acid value}}{561.1} \times 74.08$$

***N*-Ethylmaleimide** $C_4H_2O_2NC_2H_5$ [128-53-0] White crystals. Very soluble in ethanol (95) and in diethyl ether. A solution of *N*-ethylmaleimide (1 in 10,000) exhibits an absorption maximum at 298–302 nm.

Melting point 44.0–46.0°C.

***N*-Ethyl-*N*-(1-methylethyl)propane-2-amine** $C_8H_{19}N$ [7087-68-5] A colorless or very slightly yellow, clear liquid.

Content Not less than 95.0%.

Density 0.750–0.760 g/mL (20°C).

Assay Analyze 1-µl portions of *N*-ethyl-*N*-(1-methylethyl)propane-2-amine by gas chromatography using operating conditions below. Measure the areas of all peaks obtained to determine the amount from the ratio of the main peak area using the peak area percentage method.

Operating conditions

Detector: Thermal conductivity detector.

Column: A fused silica capillary (about 0.53 mm internal diameter and about 15 m length) coated with a 1.5-µm thick layer of dimethyl polysiloxane for gas chromatography.

Column temperature: Inject at 50°C, and raise to 150°C at a rate of 10°C/minute.

Injection port temperature: 200°C.

Detector temperature: 250°C.

Injection method: Split.

Split ratio: 1:120.

Carrier gas: Helium.

Flow rate: 5 mL/minute.

Measurement time: 15 minutes.

Fehling's TS

Copper Solution Dissolve 34.66 g of fine crystals of copper(II) sulfate pentahydrate in water to make 500 mL. Store in an almost-filled, grass-stoppered bottle.

Alkaline Tartrate Solution Weigh 173 g of potassium sodium (+)-tartrate tetrahydratee and 50 g of sodium hydroxide, and mix them. Dissolve the mixture in water to make 500 mL. Store in a rubber-stoppered container.

Mix equal volumes of both solutions before use.

Ferroin TS To 0.70 g of iron(II) sulfate heptahydrate, add 70 mL of water and 1.78 g of 1,10-phenanthroline chloride monohydrate to dissolve the solid, and make up with water to 100 mL.

Ferulic Acid for Assay $C_{10}H_{10}O_4$ [1135-24-6] White to light yellow crystals or powder.

Identification Determine the absorption spectrum of a solution (1 in 200,000) of ferulic acid for assay in methanol by ultraviolet-visible spectrophotometry. It exhibits absorption maxima in the ranges 215–219 nm, 231–235 nm, and 318–322 nm, respectively.

Purity (1) Clarity Clear (10 mg, methanol 10 mL).

(2) Related substances Prepare a test solution by dissolving 1 mg of ferulic acid for assay in 1 mL of methanol. Analyze 2 μL of the test solution by thin-layer chromatography using a 20:12:3 mixture of ethyl acetate/acetone/water as the developing solution. Any control solution is not used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support. 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 uniformly with sulfuric acid. Dry the plate at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm). Only one spot is observed at an R_f value of about 0.6.

(3) Prepare a test solution by dissolving 5 mg of ferulic acid for assay in 10 mL of a 1:1 mixture of water/methanol (for HPLC). Prepare a control solution by diluting exactly measured 1 mL of the test solution to exactly 100 mL with a 1:1 mixture of water/methanol (for HPLC). Analyze 10 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Measure the area of each peak to determine the percentage of the main peak by the peak area percentage method. The sum of the areas of all the peaks, excluding the main peak of the test solution, is not larger than the area of the main peak of the control solution. Preparation of the test and control solutions must be carried out, protected from light.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 240 nm).

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

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

Column temperature: 40°C.

Mobile phase: To a solution of 7.8 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, add 2 mL of phosphoric acid. To 850 mL of this solution, add

150 mL of acetonitrile (for HPLC).

Flow rate: 1.0 mL/minute.

Fludioxonil for Assay $C_{12}H_6F_2N_2O_2$ [131341-86-1] Colorless to white crystals or a white crystalline powder.

Content Not less than 99.0% of fludioxonil ($C_{12}H_6F_2N_2O_2$).

Identification Measure the absorption spectrum of fludioxonil for assay as directed in the Paste Method or Disk Method under Infrared Spectrophotometry. It exhibits absorptions at about 3289 cm^{-1}, 2223 cm^{-1}, 1652 cm^{-1}, 1530 cm^{-1}, and 1236 cm^{-1}.

Melting point 200–201°C.

Assay Weigh accurately about 20 mg of fludioxonil for assay and about 4 mg of DSS-d_6, and dissolve them together in 2 mL of deuterated dimethyl sulfoxide. Transfer the resulting solution to an NMR tube of 5 mm in external diameter, stopper tightly, and measure ^{1}HNMR spectra using an NMR spectrometer with a proton resonance frequency of 400 MHz or more. Assuming the signal of DSS-d_6 as δ 0 ppm, when the signal area intensities at around δ 7.31–7.40 ppm, δ 7.56 ppm, and δ 7.85 ppm are designated as A_1 (corresponding to 3 hydrogens), A_2 (corresponding to 1 hydrogen), and A_3 (corresponding to 1 hydrogen), respectively, confirm that each of $(A_1/3)/A_2$, $(A_1/3)/A_3$, and A_2/A_3 is 1.0. Assuming the signal area intensity of DSS-d_6 as 9.000, when the sum of A_1, A_2, and A_3, the sum of the number of hydrogens, and the purity of DSS-d_6 are designated as I, N, and P(%), respectively, determine the content by the following formula. If the signal from Fludioxonil is overlapped with the signal from a contaminant, do not use its signal area intensity for the assay.

$$\text{Content (\%) of fludioxonil } (C_{12}H_6F_2N_2O_2) = \frac{\text{Weight (mg) of DSS-}d_6 \times \text{I} \times \text{P}}{\text{Weight (mg) of the sample} \times \text{N}} \times 1.106$$

Operating conditions

Spinning: Off.

^{13}C decoupling: Present.

Acquisition time: 4 seconds.

Spectral range: At least 20 ppm including between −5 ppm and 15 ppm.

Flip angle: 90°.

Delay time: 60 seconds.

Dummy scans: 1 or more.

Number of accumulation: Not less than 8.

Fluorescein $C_{20}H_{12}O_5$ [2321-07-5] A yellow-red to red-brown powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 487–493 nm): 2173–2655. Weigh accurately about 20 mg of fluorescein, and dissolve it in diluted ammonia solution(28) (1 in 25) to make 10 mL. Refer to this solution as Solution A. To

exactly 5 mL of Solution A, add ammonium acetate TS (0.02 mol/L) to make 100 mL. A solution prepared by making 5 mL of the resulting solution up to 200 mL with ammonium acetate TS (0.02 mol/L) exhibits an absorption maximum at a wavelength of 487–493 nm. Measure the absorbance (A_B) of this solution at the maximum between 487–493 nm against an solution prepared as follows: To 5 mL of ammonium solution (28) (1 in 25), add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL, and then make 5 mL of this solution up to exactly 200 mL with ammonium acetate TS (0.02 mol/L). Determine specific absorbance by the following formula:

$$E_{1cm}^{1\%} = A_B \times \frac{10}{\text{Weight (g)of the sample}} \times \frac{100}{100 - \text{loss on drying (\%)}}$$

Purity (1) Clarity Weigh accurately about 20 mg of fluorescein, previously dried, and dissolve it in diluted ammonia solution (28) (1 in 25) to 10 mL. This solution is clear.

(2) Related substances Prepare a test solution by measuring exactly 1 mL of Solution A prepared for the specific absorbance measurement and making up to 50 mL with ammonium acetate TS (0.02 mol/L). Analyze 20 μL each of the test solution and a solution prepared by diluting 1 mL of diluted ammonia solution (28) (1 in 25) to exactly 50 mL with ammonium acetate TS (0.02 mol/L) by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 25 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the ammonia solution and the ammonium acetate TS in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 230 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

A: Ammonium acetate (0.02 mol/L).

B: Acetonitrile (for HPLC).

Concentration gradient (A/B): Run a linear gradient from 95/5 to 30/70 in 15 minutes, and maintain at 30/70 for 10 minutes.

Flow rate: 1.0 mL/minute.

Loss on drying Not more than 10.0% (50 mg, 135°C, 6 hours).

Folin's TS Weigh 20 g of sodium tungstate(VI) dihydrate and 5 g of disodium molybdate(VI) dihydrate, transfer into a 300-mL flask, and add about 140 mL of water, 10 mL of diluted phosphoric acid (17 in 20), and 20 mL of hydrochloric acid. Equip the flask with a reflux condenser with a ground-glass joint, and boil gradually for 10 hours. Add 30 g of lithium sulfate monohydrate and 10 mL of water, and add a very small

amount of bromine until the dark green color of the solution changes to yellow. Boil gradually for 15 minutes without a condenser to expel the excess bromine. Cool, add water to make 200 mL, and filter through a filter paper for qualitative analysis (No. 2). Store in a tightly stoppered bottle.

Formaldehyde Solution HCHO [K8872, Special Grade] [50-00-0]

Formaldehyde Solution–Sulfuric Acid TS Measure 0.2 mL of formaldehyde solution, and mix with 10 mL sulfuric acid. Prepare fresh before use.

Formic Acid HCOOH [K8264, Special Grade] [64-18-6]

Formic Acid TS (15 mol/L) Dissolve 705 g of formic acid in water to make 1000 mL.

α-D-Fructofuranose β-D-fructofuranose1,2′:2,3′-dianhydride $C_{12}H_{20}O_{10}$ Use a product suitable for the corresponding enzyme activity tests.

Fructose (for enzyme) $C_6H_{12}O_6$ Use a product suitable for the corresponding enzyme activity tests.

D(–)-Fructose $C_6H_{12}O_6$ [57-48-7] Use fructose specified in the Japanese Pharmacopoeia.

D(–)-Fructose for Enzyme Activity Determination $C_6H_{12}O_6$ [57-48-7] Colorless to white crystals or powder.

Specific rotation $[\alpha]_D^{20}$: −90 to −94° Weigh accurately about 4 g of D(–)-fructose for enzyme activity determination, add 0.2 mL of ammonia TS and 80 mL of water to dissolve it, allow to stand for 30 minutes, and add water to make exactly 100 mL. Measure the optical rotation of the resulting solution.

Purity (1) Clarity of solution Clear (1.0 g, water 20 mL).

(2) Loss of drying Not more than 2.0% (reduced pressure, 18 hours).

(3) Related substances Prepare a test solution by dissolving 20 mg of D(–)-fructose for enzyme activity determination in 2 mL of water. Prepare a control solution by diluting exactly measured 1 mL of the test solution with water to exactly 50 mL. Analyze 10 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for three times the retention time of the main peak, and measure the peak areas. The sum of the areas of all peaks, other than the main peak and the solvent peak, from the test solution is not greater than the area of main peak from the control solution.

Operating conditions

Detector: Differential refractometer.

Column: A stainless steel tube (3–8 mm internal diameter and 15–30 cm length).

Column packing material: 5–10 μm aminopropyl-bonded silica gel for liquid chromatography.

Column temperature: A constant temperature of 35–40°C.

Mobile phase: A 7 : 3 mixture of acetonitrile/water.

Flow rate: Adjust the retention time of D(–)-fructose to 4–7 minutes.

Fuchsin $C_{20}H_{20}ClN_3$ [632-99-5] Green, lustrous lumps or crystalline powder.

Sparingly soluble in water and in ethanol(95).

Loss on drying 17.5–20.0% (1 g, 105°C, 4 hours).

Residue on ignition Not more than 0.1% (1 g).

Fuchsin–Sodium Hydrogen Sulfite TS Dissolve 0.2 g of fuchsin in 120 mL of hot water. After cooling, add 2 g of sodium hydrogen sulfite, 2 mL of hydrochloric acid, and water to make 200 mL. Before use, leave for at least 1 hour. Store in a brown bottle in a cold place.

Fumonisin B_1 $C_{34}H_{59}NO_{15}$ [116355-83-0] A white to yellowish white powder.

Identification Determine the absorption spectrum of fumonisin B_1 as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3450 cm^{-1}, 2934 cm^{-1}, 1730 cm^{-1}, and 1632 cm^{-1}.

Purity Prepare a test solution by dissolving 10 mg of fumonisin B_1 in 10 mL of a 1:1 mixture of water/acetonitrile. Analyze 10 μL of the test solution by thin-layer chromatography using a 7:3 mixture of methanol/water as the developing solvent. Control solution is not used. Use a thin-layer plate coated with octadecylsilanized silica gel as the solid support. 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 prepared by dissolving 1 g of vanillin in 100 mL of a 4:1 mixture of sulfuric acid/ethanol (95). Only one spot is observed in natural light.

Galactan A polysaccharide that consists mainly of galactose (not less than 80%). Use a product suitable for the corresponding enzyme activity tests.

Galactitol $C_6H_{14}O_6$ [608-66-2] White crystals or crystalline powder.

Clarity of solution Clear (1.0 g, water 30 mL).

Melting point 188–189°C.

Water Not more than 0.5% (1 g, Volumetric Titration , Direct Titration).

Residue on ignition Not more than 0.1% (2 g).

D-Galacturonic Acid for Assay $C_6H_{10}O_7 \cdot H_2O$ [685-73-4] A white to pale-brown powder.

Content Not less than 98.0%.

Assay Weigh accurately about 0.3 g of galacturonic acid for assay, dissolve it in 50 mL of water, and titrate with 0.1 mol/L sodium hydroxide. To confirm the endpoint, use a potentiometer with a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes.

Each mL of 0.1 mol/L sodium hydroxide = 21.215 mg of $C_6H_{10}O_7 \cdot H_2O$ mL

Gallic Acid Monohydrate $C_7H_6O_5 \cdot H_2O$ [149-91-7]

Content 98.0–103.0%.

Description White to pale brown crystals or powder.

Identification To 5 mL of a solution of gallic acid monohydrate (1 in 1000), add 3 drop of iron(III) chloride solution (1 in 500). A dark blue color is formed.

Purity (1) Clarity Slightly turbid. Prepare a test solution by adding 20 mL of water to 1.0 g of gallic acid monohydrate and boiling.

(2) Sulfate Not more than 0.02 % as SO_4.

Test Solution: To 1.0 g of gallic acid monohydrate, add 45 mL of hot water, leave to cool white stirring, make up to 50 mL with water. Filter this solution, discard the initial 10 mL of the filtrate. To the subsequent 25 mL of filtrate, add 0.3 mL of diluted hydrochloric acid solution (2 in 3), 3 mL of ethanol (95), and 2 mL of a solution of barium chloride dihydrate (1 in 10), and allow to stand for 30 minutes.

Control Solution : Weigh 1.48 g of sodium sulfate decahydrate, dried at 110ºC for 2 hours, dissolve it in water to make exactly 1000 mL. Or, to 10 mL of Standard Solution [1000 mg/L of sulfate ion (SO_4^{2-})] specified in the Measurement Ac, add 0.3 mL of diluted hydrochloric acid (2 in 3), 15 mL of water, 3 mL of ethanol (95), and 2 mL of a solution of barium chloride dihydrate (1 in 10), and allow to stand for 30 minutes.

The white turbidity formed in the test solution is not darker than that formed in the control solution.

(3) Tannic acid To 1.0 g of gallic acid monohydrate, add 20 mL of water, shake well, and filter. When 5–6 drops of a warmed gelatin solution (1 in 100) is added to the filtrate, the solution is slightly turbid.

Loss on drying 8.0−11.0% (1 g, 105ºC, 2 hours).

Residue on ignition Not more than 0.1% (1 g). Weigh 1 g of gallic acid monohydrate into a platinum crucible, add 0.2 mL of sulfuric acid, and heat gradually until the sample is carbonized. Heat strongly by a gas burner until it is incinerated, and weigh the residue.

Assay Weigh accurately 0.3 g of gallic acid monohydrate, and dissolve it by adding 50 mL of ethanol (neutralized) and 50 mL of water. Titrate this solution with 0.1 mol/L sodium hydroxide. To confirm the endpoint, use a potentiometer with a glass indicator electrode and a silver-silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes.

Each mL of 0.1 mol/L sodium hydroxide = 18.813 mg of $C_6H_2(OH)_3COOH \cdot H_2O$

Gelatin [9000-70-8] Light yellow to yellow-brown crystals or crystalline powder or lumps.

Purity (1) Clarity Slightly turbid. To 1.0 g of gelatin, add 40 mL of water, dissolve it by heating in a water bath. Examine the turbidity of the resulting solution.

(2) Heavy metals Not more than 50 μg/g as Pb.

Test Solution: Weigh 0.5 g of gelatin in a ceramic crucible, heat gradually to carbonize the sample, and allow to cool. Add 2 mL of nitric acid and 0.5 mL of sulfuric acid, heat carefully until white fumes are no longer evolved, ignite to incineration, and allow to cool. To the ash, add 3 drops of hydrochloric acid and 10 mL of water, heat the crucible for 2 minutes in a water bath, and make a 30 mL solution with water. Filter it if necessary. To the resulting solution, add 1 drop of phenolphthalein TS, and then add ammonia solution until the solution is light red. Add 2 mL of sodium acetate solution (1

in 5) and 1 drop of sodium sulfide TS, and leave the mixture for 5 minutes.

Control Solution: Transfer 2 mL of nitric acid into a ceramic crucible, add 0.5 mL of sulfuric acid, heat to evaporate, and allow to cool. Add 3 drops of hydrochloric acid and 10 mL of water, then add 2.5 mL of Lead Standard Solution (for heavy metals limit test), and make a 30 mL solution with water. To the resulting solution, add 1 drop of phenolphthalein TS, and then add ammonia solution until the solution is light red. Add 2 mL of sodium acetate solution (1 in 5) and 1 drop of sodium sulfide TS, and leave the mixture for 5 minutes.

The test solution is not darker in color than the control solution.

(3) Arsenic Not more than 1 μg/g as As.

Test Solution: Heat 15 g of gelatin with 60 mL of diluted hydrochloric acid (1 in 5) to dissolve it, add 15 mL of bromine TS, and heat to remove the excess bromine. Neutralize the solution with ammonia TS, add 1.5 g of disodium hydrogenphosphate dodecahydrate, and allow to cool. Add 30 mL of magnesia TS, and allow to stand for 1 hour. Collect the precipitate by filtration, and wash it five times with 10 mL of diluted ammonia solution (1 in 4) each time. Dissolve the precipitate in 3 mL of diluted hydrochloric acid (1 in 4) while shaking, and make up to 50 mL with water. Use 5 mL of this solution as the test solution.

Procedure: Proceed as directed in the Method using Apparatus B. Prepare the standard color by the following: To 30 mL of Arsenic Standard Solution, add 60 mL of diluted hydrochloric acid (1 in 5) and 15 mL of bromine TS, and heat to remove the eccess bromine. Neutralize the solution with diluted ammonia solution (2 in 5), add 1.5 g of disodium hydrogenphosphate dodecahydrate, and allow to cool. Add 30 mL of magnesia TS, and allow to stand for 1 hour. Collect the precipitate by filtration, and wash it five times with 10 mL of diluted ammonia solution (1 in 4) each time. Dissolve the precipitate in 3 mL of diluted hydrochloric acid (1 in 4) while shaking, and make up to 50 mL with water. Use 5 mL of this solution.

Loss on drying Not more than 15.0%. Weigh accurately 10 g of quartz sand, dried for 3 hours at 110°C, add 1 g of gelatin, then weigh accurately the mixture. Add 20 mL of water, and leave it for 30 minutes with occasional shaking. With occasional shaking, evaporate the solution to dryness in a water bath, and dry for 3 hours at 110°C.

Gelatin TS Dissolve gently 1 g of gelatin in 50 mL of water while heating, and filter if necessary. Prepare fresh before use.

Geniposide $C_{17}H_{24}O_{10}$ [24512-63-8] White, odorless crystals or crystalline powder.

Identification Weigh accurately about 5 mg of geniposide, dissolve it in methanol to make exactly 10 mL. Measure exactly 1 mL of this solution, and add methanol to make 10 mL. This solution exhibits an absorption maximum at a wavelength of about 238 nm.

Specific absorbance $E_{1cm}^{1\%}$(maximum absorption wavelength near 240 nm): 249–269.

Measure the absorbance of the following solution at the maximum at about 240 nm: Dissolve about 10 mg of geniposide, weighed accurately, in methanol (1 in 2) to make

exactly 500 mL.

Purity Related substances Prepare a test solution by dissolving 10 mg of geniposide, weighed accurately, in a 17:3 mixture of water/acetonitrile, and making exactly 100 mL. Prepare a control solution by diluting 2 mL of the test solution, measured exactly, with a 17:3 mixture of water/acetonitrile to exactly 100 mL. Analyze 20 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for about two times the retention time of main peak, and measure the peak areas. Exclude the solvent peaks from measurement. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

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.

Mobil phase: A 17:3 mixture of water/acetonitrile.

Flow rate: Adjust the retention time of geniposide to about 15 minutes.

β-Glucan (barley-derived) $(C_6H_{12}O_6)_n$ It is obtained from barley. Use a product suitable for the corresponding enzyme activity tests.

Glucoamylase A white to brown powder or a light yellow to dark brown liquid. Is odorless or has a characteristic odor. It is obtained from *Aspergillus niger*. One unit is the amount of enzyme required to produce 1 mg of D-glucose from starch as a substrate in 60 minutes at 40°C and pH 4.5.

D(+)-Glucose $C_6H_{12}O_6$ [K8824] [50-99-7] Use glucose specified in the Japanese Pharmacopoeia.

α-D-Glucose 1,6-Diphospate Potassium Salt *n*-Hydrate $C_6H_{10}K_4O_{12}P_2 \cdot nH_2O$ Use a product suitable for the corresponding enzyme activity tests.

Glucose Oxidase A white powder. It is obtained from *Penicillium* fungi. One unit is the amount of enzyme required to produce 1 μmol of D-glucono-1,5-lactone from D-glucose as a substrate in 1 minute at 25°C and pH 7.0.

Glucose Oxidase (*Aspergillus*-derived) It is obtained from *Aspergillus* fungi. One unit is the amount of enzyme required to oxidize 1 μmol of D(+)-glucose as a substrate in 1 minute at 37°C and pH 7.0. Use a product suitable for the corresponding enzyme activity tests.

Glucose Oxidase (*Aspergillus niger*-derived) It is obtained from *Aspergillus niger*. One unit is the amount of enzyme required to oxidize D(+)-glucose as a substrate into 1 μmol of D-gluconolacotone and hydrogen peroxide in 1 minute at 35°C and pH 5.1. Use a product suitable for the corresponding enzyme activity tests.

Glucose Oxidase–Peroxidase TS Weigh 9000–15,000 unit of glucose oxidase (*Aspergillus niger*-derived), 1000–3000 unit of peroxidase (horseradish-derived, pyrogallol substrate), and 1.00 g of diammonium-2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate). Dissolve them in pH 7.0 potassium phosphate buffer (0.1 mol/L) to make 1000 mL.

Glucose-6-phosphate Dehydrogenase It is obtained from *Leuconostoc mesenteroides.* One unit is the amount of enzyme required to oxidize 1 μmol of glucose-6-phosphate in 1 minute at 25°C and pH 7.8 when glucose-6-phosphate and nicotinamide adenine dinucleotide (oxidized form) are used as substrate Use a product suitable for the corresponding enzyme activity tests. It has 1 unit of activity per μL and the specific activity is 550 unit per mg. It contains 3.2 mol/L ammonium sulfate.

L-Glutamic Acid Dehydrogenase (bovine liver-derived) Obtained from bovine liver. Has an enzyme activity. One unit of enzyme activity is equivalent to the amount of the enzyme required to liberate 1 μmol of L-glutamic acid per minute at pH 7.3 and at 25°C when 2-ketoglutaric acid as the substrate is used.

L-Glutamic Acid for Assay $C_5H_9NO_4$ L-Glutamic Acid [K9047, Special Grade] [56-86-0]

L(+)-Glutamine $C_5H_{10}N_2O_3$ Use a product suitable for the corresponding enzyme activity tests.

L-Glutamyl-L-tyrosyl-L-glutamic Acid $C_{19}H_{25}N_3O_9$ Use a product suitable for the corresponding enzyme activity tests.

Glutamyl-valyl-glycine for Assay $C_{12}H_{21}N_3O_6$ A white to light red powder.

Content Not less than 99.0% of glutamyl–valyl–glycine ($C_{12}H_{21}N_3O_6$) when calculated on the dried basis.

Identification Determine the infrared absorption spectrum of glutamyl-valyl-glycine for assay 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 Related substances Not more than 0.50%. Prepare a test solution by dissolving 25 mg of glutamyl-valyl-glycine for assay in water and making 25 mL. Prepare a standard solution as follows: Dilute exactly measured 5 mL of the test solution with water to make 50 mL, and dilute again exactly measured 5 mL of the resulting solution with water to make 50 mL. Analyze a 20-μL portion each of the test solution and the standard solution by liquid chromatography using operating conditions below. Measure the sum of the areas of all peaks, other than the main peak, for the test solution and the main peak area for the standard solution. Calculate the amount of the related substances by the formula:

Amount (%) of related substances

$$= \frac{\text{Sum of the areas of all peaks, other than main peak, for the test solution}}{\text{Main peak area for the standard solution}}$$

Operating conditions

Use the conditions given in the Assay for Glutamyl-valyl-glycine in the Monographs.

Loss on drying Not more than 1.0% (105°C, 1 hour).

Assay To about 0.4 g of glutamyl–valyl–glycine for assay, accurately weighed, add 3 mL of formic acid to dissolve, and add 50 mL of acetic acid. Titrate with 0.1 mol/L perchloric acid. To confirm the endpoint, use a potentiometer with a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes.

Perform a blank test to make any necessary correction. Then, calculate the content on the dried basis.

Each mL of 0.1 mol/L perchloric acid = 30.33 mg of $C_{12}H_{21}N_3O_6$

Glutaric Acid $HOOC(CH_2)_3COOH$ [110-94-1] A white crystalline powder. Soluble in water.

Melting point 95–99°C.

Glutathione, Reduced Form $C_{10}H_{17}N_3O_6S$ Use a product suitable for the corresponding enzyme activity tests.

Description White crystals or crystalline powder.

Specific rotation $[\alpha]_n^{20}$: −16 to −19° (1 g, water, 100 mL)

Loss on drying Not more than 0.5% (1.0 g, reduced pressure, desiccant phosphorus(V) oxide, room temperature, 4 hours).

Residue on ignition Not more than 0.2%.

Glycerol $CH_2(OH)CH(OH)CH_2OH$ [K8295, Special Grade] [56-81-5]

Glycine H_2NCH_2COOH [K8291, Special Grade] [56-40-6]

Glycine–Sodium Hydroxide Buffer (0.25 mol/L, pH10.0, containing sodium chloride) Dissolve 18.8 g of glycine and 14.6 g of sodium chloride in 700 mL of water, and adjust the pH to 10.0 with sodium hydroxide TS (2 mol/L). Add water to make 1000 mL.

Glycine–Sodium Hydroxide Buffer (0.025 mol/L, pH10.0, containing sodium chloride) Dissolve 1.88 g of glycine and 1.46 g of sodium chloride in 700 mL of water, and adjust the pH to 10.0 with sodium hydroxide TS (0.2 mol/L). Add water to make 1000 mL.

Glycyrrhetinic Acid 3-*O*-Glucuronide for Assay $C_{36}H_{54}O_{10}$ [34096-83-8] A white powder.

Purity (1) Dissolve 1 mg of glycyrrhetinic acid 3-glucuronide for assay in 4 mL of ethanol (95) (1 in 2). Analyze 2 μL of this solution by thin-layer chromatography using a 7:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent without using any control solution. Use a thin-layer plate coated with silica gel for thin-layer chromatography (containing fluorescent indicator) and dried at 110°C for 1 hour. When the solvent front has ascended to a point about 10 cm above the starting line, stop the development, air-dry the plate, and examine in ultraviolet lay (main wavelength: 254 nm). Only one spot is observed.

(2) Dissolve 1 mg of glycyrrhetinic acid 3-glucuronide for assay in 0.2 mL of the mobile phase. Analyze 2 μL of this solution by liquid chromatography using the operating condition given below. Continue the chromatography for three times the retention time of the main peak, and exclude the solvent peak from measurement. Determine the content of glycyrrhetinic acid 3-glucuronide from the area of the main peak and the sum of the areas of all the peaks. It is not less than 99.0%.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 254 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: A 54:45:1 mixture of water/acetonitrile (HPLC)/acetic acid.

Flow rate: 1.0 mL/min.

Loss on drying Not more than 1% (reduced pressure in a desiccator, 2 hours).

Glycyrrhizic Acid for Thin-Layer Chromatography $C_{42}H_{62}O_{16}\cdot nH_2O$

Description A white crystalline powder having a characteristic sweet taste. Soluble in boiling water and in ethanol (95), and practically insoluble in diethyl ether.

Melting point 213–218°C (decomposition).

Purity Related substances Prepare a test solution by dissolving 10 mg of glycyrrhizic acid for thin-layer chromatography in 5 mL of a 1:1 mixture of water/ethanol (95). Prepare a control solution by diluting 1 mL of the test solution, measured exactly, with a 1:1 mixture of water/ethanol (95) to exactly 100 mL. Analyze 10 μl each of the test solution and control solution by thin-layer chromatography as directed under Identification for Crude Licorice Extract. Spots, other than the main spot with about R_f 0.3, from the test solution are not more intense than the spot from the reference solution.

Graphite Carbon Cartridge (500 mg) Use a polyethylene column with the internal diameter of 10–15 mm packed with 0.5 g graphite carbon or its equivalent in separation capability.

Guanosine 2′(3′)-Monophosphate Disodium Salt Use a product suitable for the corresponding enzyme activity tests.

Helium He [7440-59-7] Use helium containing not less than 99.995% (vol) of He.

Hemoglobin (bovine-derived) Use hemoglobin that us derived from bovine and is suitable for the corresponding enzyme activity tests.

HEPES Buffer (0.05 mol/L) Dissolve 11.9 g of 2-[4-(2-hydroxyethyl)-1-piperadinyl] ethansulfonic acid in 600 mL of water. Adjust the pH with sodium hydroxide TS (0.05 mol/L) to the value specified in the corresponding section of this publication, and add water to make 1000 mL.

Heptane C_7H_{16} [K9701, Special Grade] [142-82-5]

1-Heptanesulfonic Acid Sodium Salt $C_7H_{15}NaO_3S$ [22767-50-6] White crystals or

crystalline powder.

Content Not less than 98.0%.

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

Loss on drying Not more than 3.0% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.4 g of 1-heptanesulfonic acid sodium, previously dried, dissolve it in 50 mL of water. Transfer in a chromatography column (9 mm internal diameter and 160 mm height) packed with 10 mL of strongly acidic ion exchange resin for column chromatography (425–600 μm, H type), run through at a flow rate of about 4 mL/minute, and collect the effluent. Wash the chromatography column with 150 mL of water at a flow rate of about 4 mL/minute. Combine the washings with the effluent, and titrate with 0.1 mol/L sodium hydroxide (indicator: 10 drops of bromothymol blue). The end point is when the color of the solution changes from yellow to blue.

Each mL of 0.1 mol/L sodium hydroxide = 20.23 mg of $C_7H_{15}NaO_3S$

Hesperidin $C_{28}H_{34}O_{15}$ Use a product for suitable for the corresponding enzyme activity tests.

Hexaammonium Heptamolybdate Tetrahydrate $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ [K8905, Special Grade] [12054-85-2]

Hexaammonium Heptamolybdate TS for Modified Starch Dissolve 50 g of hexaammonium heptamolybdate tetrahydrate in 900 mL of warm water, cool the solution to room temperature, and add water to make 1000 mL.

Hexachlorobenzene C_6Cl_6 [118-74-1] Contains not less than 98% of hexachlorobenzene.

Melting point 226°C.

Hexadecane for Ultraviolet Absorption Spectrum Measurement $CH_3(CH_2)_{14}CH_3$ [544-76-3] Prepare a test solution by adding 2,4,4-trimethylpentane for ultraviolet absorption spectrum measurement to 1 mL of hexadecane for ultraviolet absorption spectrum measurement and making exactly 25 mL. Measure the absorbance of the test solution in a 5-cm path length cell using 2,4,4-trimethylpentane for ultraviolet absorption spectrum measurement as the reference solution. It is not more than 0.00 (absorbance/cm light pass length) at 280–400 nm. If necessary, purify the test solution by filtration through a column packed with active silica gel or by distillation.

1,1,1,3,3,3-Hexamethyldisilazane $(CH_3)_3SiNHSi(CH_3)_3$ [999-97-3] A colorless or almost colorless liquid. Store in a tightly stoppered container protected from light.

Content Not less than 95.0%.

Identification Determine the absorption spectrum of 1,1,1,3,3,3-hexamethyldisilazane as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3370 cm^{-1}, 2940 cm^{-1}, 1700 cm^{-1}, 1450 cm^{-1}, 1370 cm^{-1}, 1240 cm^{-1}, 1170 cm^{-1}, 1080 cm^{-1}, 1030 cm^{-1}, and 890 cm^{-1}.

Density 0.772−0.776 g/mL (20°C).

Assay Analyze 1 μL of 1,1,1,3,3,3-hexamethyldisilazane by gas chromatography using the operating conditions given below. Determine the purity of the 1,1,1,3,3,3-hexamethyldisilazane from the peak area of 1,1,1,3,3,3-hexamethyldisilazane and the sum of the areas of all the peaks.

Operating conditions

Detector: Flame-ionization detector.

Column: A fused silica tube (0.32 mm internal diameter and 30 m length) coated with a 5 μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Upon injection at 50°C, raise the temperature at a rate of 10°C/minute to 200°C, and maintain at 200°C for 5 minutes.

Injection port temperature: 200°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 3.0 mL/minute.

Injection method: Split.

Split ratio: 1:45.

Measurement time: 20 minutes.

Hexamethylenetetramine $C_6H_{12}N_4$ [K8847, Special Grade] [100-97-0]

Hexane C_6H_{14} [K8848, Special Grade] [110-54-3]

Hexane (for HPLC) C_6H_{14} [K 8848] [110-54-3] A colorless, clear, volatile liquid.

Identification Determine the absorption spectrum of hexane (for HPLC) as directed in the Liquid Film Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2960 cm^{-1},1470 cm^{-1}, 1380 cm^{-1}, and 730 cm^{-1}.

Density 0.658–0.662 g/mL (Specific Gravity Method 4, 20°C).

Water Not more than 0.01% (20 g, Volumetric Titration , Direct Titration).

Absorbance Not more than 0.25 at 210 nm, not more than 0.04 at 230 nm, not more than 0.02 at 240 nm.

Determine the absorbance at each wavelength against water as the reference.

Hexane (for pesticide residue and polychlorinated biphenyl analysis) C_6H_{14} [K 8825] [110-54-3]

Hexane for Ultraviolet Absorption Spectrum Measurement When determined, using water as the reference, the absorbance is not more than 0.10 at a wavelength of 220 nm and not more than 0.02 at 260 nm. No characteristic absorbance is observed at 260–350 nm.

1-Hexanol $CH_3(CH_2)_5OH$ [111-27-3] A colorless, clear liquid.

Specific gravity d_4^{20}: 0.818–0.819.

Boiling point 157°C.

L-Histidine $C_6H_9N_3O_2$ [71-00-1] White crystals or powder.

Content Not less than 98.0% of L-histidine.

Specific Rotation $[\alpha]_D^{20}$: +12.0 to +13.0° (1 g, hydrochloric acid, 10 mL).

Assay Dissolve about 0.15 g of L-histidine, accurately weighed, in 2 mL of formic acid, add 50 mL of acetic acid, and titrate with 0.1 mol/L perchloric acid. To confirm the endpoint, use a potentiometer with a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes. Perform a blank test to make correction.

Each mL of 0.1 mol/L perchloric acid = 15.52 mg of $C_6H_9N_3O_2$

Hydrazine Monohydrate $H_2NNH_2 \cdot H_2O$ [7803-57-8] A colorless, hygroscopic liquid having a characteristic odor. Very soluble in water but not miscible with diethyl ether.

Content Not less than 98% of hydrazine monohydrate ($H_2NNH_2 \cdot H_2O$).

Assay Weigh accurately about 1 g of hydrazine monohydrate, and dissolve it in water to make exactly 200 mL. Transfer exactly 10 mL of this solution into a 300-mL stoppered Erlenmeyer flask, add 20 mL of water and 30 mL of hydrochloric acid, and cool. Titrate with 0.05 mol/L potassium iodate. Add 5 mL of chloroform just before the endpoint, and stir constantly. The endpoint is when pink color of chloroform disappears.

Each mL of 0.05 mol/L potassium iodate = 2.503 mg of $H_2NNH_2 \cdot H_2O$

Hydrazinium Sulfate $N_2H_6SO_4$ [K8992, Special Grade] [10034-93-2]

4-Hydrazinobenzenesulfonic Acid $C_6H_8N_2O_3S$ [98-71-5] A white to pale brown powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 250–256 nm): Not less than 730. Weigh accurately about 10 mg of 4-hydrazinobenzenesulfonic acid, previously dried for 24 hours in a vacuum desiccator, add ammonium acetate TS (0.02 mol/L), and dissolve it to make exactly 100 mL. Refer to this solution as Solution A. Exactly measure 10 mL of Solution A, add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits an absorption maximum at a wavelength of 250–256 nm. Measure the absorbance of this solution at the maximum between 250–256 nm against ammonium acetate TS (0.02 mol/L) to determine the specific absorbance.

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add the mobile phase to 5 mg of 4-hydrazinobenzenesulfonic acid to make exactly 25 mL. Analyze 10 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 40 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the mobile phase in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 254 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: Dissolve 1.54 g of ammonium acetate and 3.22 g of tetra-*n*-butylammonium bromide in 900 mL of water. Adjust the pH to 6 with a 10:1 mixture of water/acetic acid, and make up with water to 1000 mL. To 850 mL of his solution, add 150 mL of acetonitrile (for HPLC).

A 4:1 mixture of ammonium acetate–TS/acetonitrile (for HPLC).

Flow rate: 1.0 mL/min.

Loss on drying 3.6–5.4% (50 mg, 105°C, 2 hours).

Hydriodic Acid HI [K8917, Special Grade] [10034-85-2]

Hydrochloric Acid HCl [K8180, Special Grade, Arsenic Analysis Grade] [7647-01-0]

Hydrochloric Acid (Arsenic-free) HCl [K8180, Arsenic Analysis Grade] [7647-01-0]

10% Hydrochloric Acid TS Measure 23.6 mL of hydrochloric acid, and add water to make 100 mL.

Hydrochloric Acid (Purified) HCl Measure 1000 mL of diluted hydrochloric acid (1 in 2), add 0.3 g of potassium permanganate, and distill. Discard 250 mL of the initial distillate, and collect the subsequent 500 mL of distillate.

Hydrochloric Acid–Sodium Acetate Buffer (0.1 mol/L)

Solution 1 Add water to 9 mL of hydrochloric acid to make 1000 mL.

Solution 2 Dissolve 13.6 g of sodium acetate trihydrate in water to make 1000 mL.

Mix Solution 1 and Solution 2, and adjust the pH to the value specified in the corresponding monographs.

Hydrochloric Acid TS (6 mol/L) Add water to 540 mL of hydrochloric acid to make 1000 mL.

Hydrochloric Acid TS (4 mol/L) Add water to 360 mL of hydrochloric acid to make 1000 mL.

Hydrochloric Acid TS (3 mol/L) Add water to 270 mL of hydrochloric acid to make 1000 mL.

Hydrochloric Acid TS (2 mol/L) Add water to 180 mL of hydrochloric acid to make 1000 mL.

Hydrochloric Acid TS (1 mol/L) Add water to 90 mL of hydrochloric acid to make 1000 mL.

Hydrochloric Acid TS (0.5 mol/L) Add water to 45 mL of hydrochloric acid to make 1000 mL.

Hydrochloric Acid TS (0.3 mol/L) Add water to 27 mL of hydrochloric acid to make 1000 mL.

Hydrochloric Acid TS (0.2 mol/L) Add water to 18 mL of hydrochloric acid to make 1000 mL.

Hydrochloric Acid TS (0.1 mol/L) Add water to 9 mL of hydrochloric acid to make 1000 mL.

Hydrochloric Acid TS (0.05 mol/L) Add water to 4.5 mL of hydrochloric acid to make

1000 mL.

Hydrochloric Acid TS (0.02 mol/L) Add water to 100 mL of hydrochloric acid TS (0.2 mol/L) to make 1000 mL.

Hydrochloric Acid TS (0.01 mol/L) Add water to 100 mL of hydrochloric acid TS (0.1 mol/L) to make 1000 mL.

Hydrochloric Acid TS (0.025 mol/L) Add water to 250 mL of hydrochloric acid TS (0.1 mol/L) to make 1000 mL.

Hydrochloric Acid TS (0.004 mol/L) Dilute hydrochloric acid TS (0.1 mol/L) to 25 times its original volute with water.

Hydrochloric Acid TS (0.001 mol/L) Add water to 10 mL of hydrochloric acid TS (0.1 mol/L) to make 1000 mL.

Hydrofluoric Acid HF [K8819, Special Grade] [7664-39-3]

Hydrogen H_2 [K0512] [1333-74-0] Use hydrogen containing not less than 99.99% (vol) of H_2.

Hydrogen Peroxide H_2O_2 [Hydrogen Peroxide Solution (30%), K8230, Special Grade] [7722-84-1]

Hydrogen Peroxide TS Use oxydol specified in the Japanese Pharmacopoeia.

Hydrogen Sulfide H_2S [7783-06-4] A colorless gas having a characteristic odor. Heavier than air, and soluble in water. It is prepared by reacting iron(II) sulfide with diluted sulfuric acid (1 in 20) or diluted hydrochloric acid (1 in 4).

Hydrogen Sulfide TS Use a saturated solution of hydrogen sulfide. Store in a small, almost filled, light-resistant bottle, and if possible in a cold place. It has a strong odor of hydrogen sulfide.

***p*-Hydroxybenzoic Acid Hydrazide** $HOC_6H_4CONHNH_2$ Use a product suitable for 4-droxybenzoic acid hydrazide enzyme activity tests.

***p*-Hydroxybenzoic Acid Hydrazide TS** Dissolve 0.14 g of bithmus(III) acetate, 0.5 g of *p*-hydroxybenzoic acid hydrazide, and 1.25 g of potassium sodium (+)-tartrate tetrahydrate in sodium hydroxide TS (0.5 mol/L) to make 25 mL.

***p*-Hydroxybenzoic Acid Propyl** $HOC_6H_4COOCH_2CH_2CH_3$ [94-13-3] White crystals or crystalline powder.

Content Not less than 95.0%.

Assay Prepare a test solution by dissolving about 1.0 g of *p*-hydroxybenzoic acid propyl, weighed accurately, in acetone to make exactly 10 mL. Analyze 1 μL of the test solution by gas chromatography using the operating conditions given below. Determine the content from the area of the *p*-hydroxybenzoic acid propyl peak and the sum of the areas of all the peaks. Separately perform a blank test to make any necessary correction.

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/minute to 250°C.

Detector temperature: 250°C.

Injection port temperature: 250°C.

Carrier gas: Helium.

Flow rate: 1.33 mL/minute.

Injection method: Split.

Split ratio: 1:100.

Measurement time: 15 minutes.

2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic Acid $C_8H_{18}N_2O_4S$ [K9804]

2-Hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic Acid $C_{21}H_{14}N_2O_7S$ [K8776, Special Grade] [3737-95-9]

Hydroxylamine TS Dissolve 20 g of hydroxylammonium chloride in 40 mL of water, and add 400 mL of ethanol (95), 300 mL of 0.5 mol/L ethanol potassium hydroxide, and 2.5 mL of bromophenol blue–sodium hydroxide TS. Allow to stand for 30 minutes, and filter. Prepare fresh before use.

Hydroxylammonium Chloride $HONH_3Cl$ [K8201, Special Grade] [5470-11-1]

5-Hydroxy-1-(4-sulfophenyl)-3-pyrazolecarboxylic Acid $C_{10}H_8N_2O_6S$ [21951-33-7] A white to pale yellow powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 256–266 nm): Not less than 494. Weigh accurately about 10 mg of 5-hydroxy-1-(4-sulfophenyl)-3-pyrazolecarboxylic acid, previously dried for 24 hours in a vacuum desiccator, dissolve it in ammonium acetate TS (0.02 mol/L), and make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits an absorption maximum at a wavelength of 256–266 nm. Measure the absorbance of this solution at the maximum between 256–266 nm against ammonium acetate TS (0.02 mol/L).

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add the mobile phase to 5 mg of 5-hydroxy-1-(4-sulfophenyl)-3-pyrazolecarboxylic acid to make exactly 50 mL. Analyze 10 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given below. Measure the areas of the peaks that appear within 20 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the mobile phase in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 260 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 13:7 mixture of ammonium acetate–tetra-*n*-butylammonium bromide TS/acetonitrile (for HPLC).

Flow rate: 1.0 mL/min.

Water Not more than 10.0% (50 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

Imidazole for Water Determination $C_3H_4N_2$ [288-32-4] A white crystalline powder. Very soluble in water and in methanol. Shall not contain more than 1 mg of water per mL.

Melting point 89–92°.

Specific Absorbance $E_{1cm}^{1\%}$ (313 nm): Not more than 0.031 (8 g, water, 100 mL).

2,2′-Iminodiethanol Hydrochloride $C_4H_{11}NO_2 \cdot HCl$ [14426-21-2] A light yellow liquid.

Refractive index n_D^{20}: 1.515–1.519.

Specific gravity d_{20}^{20}: 1.259–1.263.

Water Not more than 1 mg/g sample.

Indigo Carmine $C_{16}H_8N_2Na_2O_8S_2$ [K8092, Special Grade] [860-22-0]

Indigo Carmine TS Weigh an amount of the sample equivalent to 0.18 g of indigo carmine ($C_{16}H_8N_2Na_2O_8S_2$), and dissolve it in water to make 100 mL. Use within 2 months of preparation.

***myo*-Inositol for Assay** $C_6H_{12}O_6$ [87-89-8]

Description White, odorless crystals or crystalline powder having a sweet taste.

Identification Determine the absorption spectrum of *myo*-inositol for assay, previously dried at 105°C for 4 hours, as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at about 3380 cm^{-1}, 3220 cm^{-1}, 1446 cm^{-1}, 1147 cm^{-1}, 1114 cm^{-1}, and 1049 cm^{-1}.

Purity Related substances Prepare a test solution by dissolving 0.2 g of *myo*-inositol for assay in 20 mL of water. Prepare a control solution by diluting 1 mL of the test solution, exactly measured, with water to exactly 100 mL. Analyze 10 μL each of the test solution and the control solution by liquid chromatography using the operating conditions below. Continue the chromatography for two times the retention time of the main peak and measure the peak areas of both solutions. Exclude the solvent main peak from the measurement. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Use the conditions specified in the Assay for *myo*-Inositol in the Monographs.

Inulin (chicory-derived) $(C_6H_{10}O_5)_n$ Use a product suitable for the corresponding

enzyme activity tests.

Inulin (dahlia-derived) $(C_6H_{10}O_5)_n$ Use a product suitable for the corresponding enzyme activity tests.

Iodine I_2 [K8920, Special Grade] [7553-56-2]

Iodine–Potassium Iodide TS Dissolve 0.5 g of iodine and 1.5 g of potassium iodide in 25 mL of water.

Iodine–Potassium Iodide TS (0.4 mmol/L) Dilute iodine–potassium iodide TS (0.08 mol/L) with water to 200 times the original volume.

Iodine–Potassium Iodide TS (0.2 mmol/L) Dilute iodine–potassium iodide TS (0.04 mol/L) with water to 200 times the original volume.

Iodine–Potassium Iodide TS (0.08 mol/L) Dissolve 10.0 g of potassium iodide and 1.0 g of iodine in water to make 100 mL. Store protected from light.

Iodine–Potassium Iodide TS (0.04 mol/L) Dissolve 5.0 g of potassium iodide and 1.0 g of iodine in water to make 100 mL.

Iodine–Potassium Iodide TS (for α-amylase activity test) Dissolve 11 g of potassium iodide and 5.5 g of iodine together in water to make 250 mL. Store protected from light. Mix 1 mL of this solution and 200 mL of potassium iodide solution (1 in 20), and add water to make 250 mL.

Iodine Trichloride ICl_3 [K8403, Special Grade] [865-44-1]

Iodine TS Dissolve 14 g of iodine in 100 mL of potassium iodide solution (2 in 5), and add 1 mL of diluted hydrochloric acid (1 in 4) and water to make 1000 mL. Store protected from light.

Iodine TS (2.75 mmol/L) Dissolve 20.0 g of potassium iodide and 7.0 g of iodine in 50 mL of water. Add 0.5 mL of 10% hydrochloric acid TS and water to make 500 mL. Dilute this solution with water to 20 times the original volume.

Iodine TS (0.005 mol/L) Dilute 0.05 mol/L Iodine with water to 10 times the original volume.

Iodine TS (for α-glucosyltransferase activity test) Dissolve 26 g of potassium iodide in water. To this solution, add 2.6 g of iodine to dissolve, and add water to make 100 mL. Mix 0.5 mL of the resulting solution and 2 mL of hydrochloric acid TS (1 mol/L), add water to make 260 mL.

Iodine TS (for isoamylase activity test) Dissolve 8.30 g of potassium iodide and 0.635 g of iodine together in water to make 100 mL. Mix this solution and diluted hydrochloric acid (1 in 120) in a volume ratio of 2:8. Store protected from light.

Iodomethane for Assay CH_3I [K8919, Special Grade] [74-88-4] A clear, colorless liquid. Upon exposure to light, turns brown releasing iodine. Miscible with ethanol (95) and with diethyl ether, and slightly soluble in water.

For the tests, use the fraction obtained by distillation at 42.2–42.6°C.

Content Not less than 98.0% of methyl iodide (CH_3I).

Specific gravity d_{25}^{25}: 2.27–2.28.

Purity Analyze 1 μL of iodomethane for assay by gas chromatography using the conditions directed in the Assay for Hydroxypropyl Methylcellulose in the Monographs. Measure the peak area of each peak recorded in the chromatogram, and obtain the content of methyl iodide by the peak area percentage method. The content is not less than 99.8%. Adjust the detection sensitivity, so that the peak height of methyl iodide obtained from 1 μL of iodomethane for assay is about 80% of the full scale.

Assay Perform the test in the same manner as for Isopropyl Iodide for Assay.

Each mL of 0.1 mol/L silver nitrate solution = 14.19 mg of CH_3I

Iron(III) Chloride Hexahydrate $FeCl_3 \cdot 6H_2O$ [K8142, Special Grade, Phosphoric Acid Analysis Grade] [10025-77-1]

Iron(III) Chloride–Hydrochloric Acid TS Dissolve 5 g of iron(III) chloride hexahydrate by adding 5 mL of hydrochloric acid and water to make 100 mL.

10% (w/v) Iron(III) Chloride–Hydrochloric Acid TS Dissolve16.7 g of iron(III) chloride hexahydrate by adding 9 mL of diluted hydrochloric acid (2 in 3) and water to make 100 mL.

Iron(III) Chloride TS Dissolve 9 g of iron(III) chloride hexahydrate in water to make 100 mL.

Iron(III) Chloride TS (for transglutaminase activity test) Dissolve 5.0 g of iron(III) chloride hexahydrate in hydrochloric acid TS (0.1 mol/L) to make 100 mL. Mix equal volumes of this solution, diluted hydrochloric acid (57 in 200), and trichloroacetate solution (3 in 25).

0.2% (w/v) Iron(III) Chloride TS To 2 mL of iron(III) chloride, add water to make 100 mL. Prepare fresh before use.

Iron Fragment Fe Not less than 97.7% Fe. Use iron in fragmentary form. It is attracted by a magnet.

Iron(II) Sulfate Heptahydrate $FeSO_4 \cdot 7H_2O$ [K8978, Special Grade] [7782-63-0]

Iron(III) Sulfate *n*-Hydrate $Fe_2(SO_4)_3 \cdot nH_2O$ [K8981, Special Grade] [15244-10-7]

Iron(II) Sulfate TS Dissolve 8 g of iron(II) sulfate heptahydrate in 100 mL of freshly boiled and cooled water. Prepare fresh before use.

Iron(III) Sulfate TS Weigh 50 g of iron(III) sulfate *n*-hydrate, add about 500 mL of water, and shake well. Add 200 mL of sulfuric acid, dissolve it by shaking well, and add water to make 1000 mL.

Iron(II) Sulfide FeS [K8948, for hydrogen sulfide generation] [1317-37-9]

Iso-*α*-bitter Acids for Assay An international calibration standard (DCHA-Iso) with a known concentration. It is a mixture of isohumulone, isoadhumulone, and isocohumulone and their isomers. Use the content (%) of the total iso-*α*-bitter acids as the content (%) of iso-*α*-bitter acids.

Isomaltose $C_{12}H_{22}O_{11}$ Use a product suitable for the corresponding enzyme activity tests.

Isomaltulose $C_{12}H_{22}O_{11} \cdot H_2O$ 6-0-α-D-glucopyranosyl-D-fructorse Use a product

suitable for the corresponding enzyme activity tests.

Isopropyl Iodide for Assay C_3H_7I [75-30-9] A clear, colorless liquid. Upon exposure to light, turns brown releasing iodine. Miscible with ethanol (95), with diethyl ether, and with petroleum benzine. Not miscible with water. Use the distillate obtained at 89.0–89.5°C for the following tests.

Content Not less than 98.0% of isopropyl iodide (C_3H_7I).

Specific gravity d_4^{20}: 1.700–1.710.

Purity Analyze 1 μL of isopropyl iodide for assay by gas chromatography using the conditions directed in the Assay for Hydroxypropyl Methylcellulose in the Monographs. Using the automatic integration method, determine the peak area of each peak recorded in the chromatogram, and obtain the content of isopropyl iodide by the peak area percentage method. The content is not less than 99.8%. Adjust the detection sensitivity, so that the peak height of isopropyl iodide obtained from 1 μL of Isopropyl Iodide for Assay is about 80% of the full scale.

Assay Place 10 mL of ethanol (95) in a 100-mL brown volumetric flask, and weigh accurately the flask with the ethanol. To the flask, add 1 mL of isopropyl iodide for assay, and weigh the flask accurately. To the mixture, add ethanol (95) to make exactly 100 mL. Measure exactly 20 mL of this solution into another volumetric flask, add exactly 50 mL of 0.1 mol/L silver nitrate solution and 2 mL of nitric acid, and stopper. Allow to stand in a dark place for 2 hours with occasional shaking. Then leave to stand in a dark place for night, and shake occasionally for additional 2 hours. Add water to make exactly 100 mL, and filter through a dry filter paper. Remove the initial 20 mL of the filtrate, and collect exactly the following 50 mL. Titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate solution. Use 2 mL of iron(III) ammonium sulfate–sufuric acid TS as an indicator. Separately perform a blank test.

Each mL of 0.1 mol/L silver nitrate solution = 17.00 mg of C_3H_7I

Isoquercitrin $C_{21}H_{20}O_{12}$ [482-35-9] A light yellow to yellow powder.

Identification Dissolve 10 mg each of isoquercitrin and rutin for assay separately in a small amount of methanol, and add an 80:20:0.1 mixture of water/acetonitrile/phosphoric acid to make 10 mL each. Use them as the test solution and as the standard solution, respectively. Analyze 10-μL portions of each solution by liquid chromatography using operating conditions specified in the Assay for Enzymatically Modified Rutin (Extract) in the Monographs. Use a photodiode array detector instead of an ultraviolet spectrophotometer. When determined at 254 nm, the retention time of the main peak of the test solution is longer than that of the rutin peak of the standard solution. Compare the absorption spectrum of the main peak at 200–400 nm with that of the rutin peak from the standard solution. Both spectra exhibit an absorption maximum at the same wavelength.

Purity Related substances Analyze 10-μL portions of the test solution prepared for the identification test by liquid chromatography using the operating conditions specified

in the Assay for Enzymatically Modified Rutin (Extract) in the Monographs. Continue the chromatography for two times the retention time of the main peak, and exclude the solvent peak from measurement. Measure the area of each peak to determine the percentage of the main peak by the peak area percentage method. It is not less than 75.0%.

Isotonic Sodium Chloride Solution Use isotonic sodium chloride solution specified in the Japanese Pharmacopoeia.

1-Kestose $C_{18}H_{32}O_{16}$ Use a product suitable for the corresponding enzyme activity tests.

Lactic Acid $CH_3CH(OH)COOH$ [K8726, Special Grade] [598-82-3]

Lactic Acid TS Dissolve 12.0 g of lactic acid in water to make 100 mL.

Lactoferrin for Assay A light red-yellow to yellow-red powder or crystalline powder. Obtained from the milk from mammals and consists mainly of lactoferrin.

Specific absorbance $E_{1cm}^{1\%}$ (280 nm): 12.0–13.5 (on the dried basis). Prepare a test solution by dissolving accurately weighed 0.1 g of lactoferrin for assay in water to make 200 mL and filtering the resulting solution through a membrane filter with a pore size of 0.45 μm. Measure the absorbance of the test solution at a wavelength of 280 nm, and calculate on the dried basis.

Purity (1) Iron 0.005–0.05% as Fe. Weigh 1.0 g of lactoferrin for assay in ceramic crucible, add 0.2 mL of sulfuric acid, and carbonize the sample by heating gradually. Heat it strongly using gas burner to incinerate, and allow to cool. Add 5 mL of hydrochloric acid (2 in 3), and dissolve it by heating. Add water to make 50 mL, and filter. To 2 mL of the filtrate, add water to make 10 mL. Use this solution as the test solution. Separately, prepare two standard solutions with different concentrations by placing exactly 2 mL of Iron Standard Solution into two separate test volumetric flasks, adding 0.2 mL of hydrochloric acid (2 in 3) and water to each to make 10 mL and 100 mL of solutions, respectively. Measure the atomic absorbance of the test solution and the standard solutions using the following operating conditions. Determine the iron concentration in the test solution using a calibration curve prepared and the atomic absorbance of the test solution. Calculate the iron content (%) in the sample.

Operating conditions

Light source: Iron hollow cathode lamp.

Wavelength: 248.3 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(2) Related substances Prepare a test solution by dissolving 0.1 g of lactoferrin for assay in sodium chloride solution (3 in 100) to make exactly 50 mL. Analyze 25 μL of the test solution by liquid chromatography using the operating conditions for the assay of Lactoferrin Concentrate in the Monographs. Determine the content of lactoferrin from the peak area of the lactoferrin and the total peak area. It is not less than 95.0%.

Separately, perform a blank test to make any necessary correction.

Loss on drying Not more than 6.0% (105°C, 5 hours).

Lactose Monohydrate $C_{12}H_{22}O_{11}\cdot H_2O$ [64044-51-5, a mixture of α- and β-lactose monohydrate] Use lactose hydrate specified in the Japanese Pharmacopoeia.

Lanthanum(III) Oxide La_2O_3 [1312-81-8] White crystals.

Loss on ignition Not more than 0.5% (1 g, 1000°C, 1 hour).

Lanthanum Oxide TS Place 5.86 g of lanthanum(III) oxide into a 100-mL volumetric flask, moisten with 2 to 3 mL of water, and slowly add 25 mL of hydrochloric acid. Shake to dissolve it completely, and make up to the volume with water.

Lead(II) Acetate Trihydrate $Pb(CH_3COO)_2\cdot 3H_2O$ [K8374, Special Grade] [6080-56-4]

Lead(II) Acetate TS Dissolve 11.8 g of lead(II) acetate trihydrate in water to make 100 mL, and add 2 drops of diluted acetic acid (1 in 4). Store in a tightly-stoppered container.

Lead(II) Acetate TS (Basic) Weigh 3 g of lead(II) acetate trihydrate and 1 g of lead(II) monoxide, add 0.5 mL of water, and triturate them. Transfer the resultant yellowish mixture into a beaker, and heat on a water bath with a watch glass covering it. When the contents have become uniformly white to reddish white, add 9.5 mL of boiling water in small portions, and allow to stand with a watch glass covering it. Collect the supernatant by decantation, and add water to adjust the specific gravity (d_{25}^{25}) to between 1.23 and 1.24. Store in a tight-stoppered container.

Lead(II) Nitrate $Pb(NO_3)_2$ [K8563, Special Grade] [10099-74-8]

Lead(II) Oxide PbO [K8090, Special Grade] [1317-36-8]

L-α-Lecithin (soybean-derived) L-α- phosphatidylcholine Use a product suitable for the corresponding enzyme activity tests.

L-Leucyl-glycyl-glycine $C_{10}H_{19}N_3O_4$ Use a product suitable for the corresponding enzyme activity tests.

L-Leucyl-*p*-nitroanilide Hydrochloride $C_{12}H_{17}N_3O_3\cdot HCl$ Use a product suitable for the corresponding enzyme activity tests.

Light Green SF Yellow $C_{37}H_{34}N_2Na_2O_9S_3$ [5141-20-8] Disodium 4-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}methyliumyl)benzenesulfo-nate. Darkish green granules or powder.

Identification Add 1 mL of sodium hydroxide solution (1 in 10) to 5 mL of a solution of light green SF yellow (1 in 1000). The solution turns light green.

Specific absorbance $E_{1cm}^{1\%}$: Not less than 606 (maximum absorption wavelength near 633 nm). Weigh accurately 10 mg of light green SF yellow, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Measure exactly 10 mL of this solution, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits an absorption maximum at a wavelength of 631–635 nm.

Linoleic acid $C_{18}H_{32}O_2$ Use a product suitable for the corresponding enzyme activity tests.

Liquid Paraffin [8042-47-5] A colorless, clear liquid.

Identification Determine the absorption spectrum of liquid paraffin as directed in the Liquid Film Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2923 cm^{-1}, 2854 cm^{-1}, 1461 cm^{-1}, 1376 cm^{-1}, and 725 cm^{-1}.

Density 0.825–0.850 g/mL (20°C).

Purity (1) Polynuclear aromatic hydrocarbons All glass instruments used in the test should be washed by hexane.

Test Solution: Transfer 25 mL of liquid paraffin into a 100-mL separating funnel, add 25 mL of hexane (for HPLC), and shake the funnel vigorously. Add 5mL of dimethyl sulfoxide for ultraviolet absorption 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 stoppered centrifuge tube, and centrifuge at 2500−3000 rpm for about 10 minutes.

Control Solution: Proceed as directed for the test solution using a mixture of 25 mL of hexane for ultraviolet absorption spectrum measurement and 5 mL of dimethyl sulfoxide for ultraviolet absorption spectrum measurement. Use the supernatant obtained as the control solution.

Procedure: Measure the absorbance in a 10-mm cell of the test solution against the control solution at a wavelength of 260–350 nm. It is not more than 0.10.

(2) Readily carbonizable substances Place 10 g of liquid paraffin into a Nessler tube, washed with 85% sulfuric acid TS, immerse the tube in a water bath so that the solution surface in the tube is below the water surface in the bath, and heat for 10 minutes while shaking vigorously 2–3 times. Remove the test tube from the bath, and examine the solution. The color of sulfuric acid layer is not darker than Matching Fluid D.

Lithium Acetate Dihydrate $CH_3COOLi \cdot 2H_2O$ [6108-17-4] Colorless to white crystals. Freely soluble in water.

Melting point 70°C.

Clarity of solution Colorless and almost clear (0.5 g, water 10 mL).

Lithium Chloride LiCl [7447-41-8] White, deliquescent crystals or small lumps.

Content Not less than 99.0% of lithium chloride (LiCl) on the dried basis.

Identification To 5 mL of a solution of lithium chloride (1 in 100), add 1 mL of silver nitrate solution (1 in 50). A white precipitate is formed. The precipitate dissolves with the addition of 10 mL of diluted ammonia solution (28) (2 in 5).

Loss on drying Not more than 2.0% (130°C, 42 hours).

Assay Prepare a test solution as follows: Weigh accurately about 0.5 g of lithium chloride, previously dried at 130°C for 4 hours, add water to make up to exactly 100 mL, and to exactly 20 mL of this solution, add 50 mL of water. To the test solution, gradually add exactly 40 mL of 0.1 mol/L silver nitrate while stirring. Then add 9 mL of diluted nitric acid (1 in 3) and 3 mL of nitrobenzene. Titrate with 0.1 mol/L ammonium

thiocyanate (indicator: 3 mL of ammonium iron(III) sulfate–nitric acid TS). Separately, perform a blank test.

Each mL of 0.1 mol/L silver nitrate = 4.239 mg of LiCl

Lithium Lactate $LiC_3H_5O_3$ [867-55-0] Odorless, white crystals or powder.

pH 6.0–7.5 (1.0 g, water 20 mL).

Residue on ignition 56.5– 58.0% (use after drying at 105°C for 4 hours).

Lithium Sulfate Monohydrate $Li_2SO_4 \cdot H_2O$ [K8994, Special Grade] [10102-25-7]

Litmus [1393-92-6] A blue to purplish blue powder or lumps. Soluble in water and in ethanol (95), and its solution is blue to purplish blue.

Identification Dissolve 0.5 g of litmus in 50 mL of warm water, add 10% sulfuric acid TS dropwise until the solution produces a red color, and boil for 10 minutes. If the color changes to blue during boiling, add 10% sulfuric acid TS until the color changes to red. Then add a saturated solution of barium hydroxide until it produces a purple color, and filter. Refer to the resulting solution as Solution A. To 100 mL of freshly boiled and cooled water, add 0.5 mL of Solution A and 0.05 mL of diluted hydrochloric acid (1 in 120). A red color is produced. To 100 mL of freshly boiled and cooled water, add 0.5 mL of Solution A and 50 μL of sodium hydroxide solution (1 in 250). A blue color is produced.

Litmus Milk Dissolve 10 g of powdered skim milk, 50 mg of litmus, and 50 mg of sodium sulfate in 100 mL of water, and mix well. Prepare fresh before use.

Litmus Paper (Blue) [Litmus Paper, K9071, Blue Litmus Paper]

Litmus Paper (Red) [Litmus Paper, K9071, Red Litmus Paper]

Locust Bean Gum (for enzyme) Use a product suitable for the corresponding enzyme activity tests.

L-Lysine Monohydrochloride $H_2N(CH_2)_4CH(NH_2)COOH \cdot HCl$ [657-27-2] White crystals or crystalline powder. Freely soluble in water but practically insoluble in diethyl ether.

Content Not less than 99.0% of L-lysine monohydrochloride ($H_2N(CH_2)_4CH(NH_2)COOH \cdot HCl$) when dried.

Purity Other amino acids Prepare a test solution by dissolving 0.20 g of L-lysine monohydrochloride in water to make exactly 50 mL. Apply 5 μL of the test solution, using a mycrosylinge or micropipette in 2–6 mm spots, on the starting line 20 mm from the bottom of the thin-layer plate, with 10-mm apart from the sides of the plate, at 10-mm or more intervals. Dry the plate. Wind a filter paper on the inside wall, moister the filter with an appropriate developing solvent, and fill the developing chamber with the solvent up to 10 mm deep. Tightly seal the chamber, allow it to stand for about 1 hour at room temperature to saturate the chamber with the vapor of the developing solvent. Use a 10:5:2:10 mixture of acetone/ammonia solution/water/1-butanole as the development 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. Place the plate into the chamber with care without touching the inside wall, and seal tightly, and allow

to stand at room temperature to develop the chromatogram. Remove the plate from the chamber when the solvent front has ascended to a point about 10 cm above the starting line, and mark the location of the solvent front quickly. Air-dry the plate at 100°C for 30 minutes, and leave it to cool. Spray the plate with a solution (1 in 50) of ninhydrin in acetone, and heat at 80°C for 10 minutes to form color. More than 1 spot is not detected.

Assay Weigh accurately about 0.1 g of L-lysine monohydrochloride, dried at 105°C for 3 hours, into a titration beaker, add 3 mL of formic acid and exactly 20 mL of 0.1 mol/L perchloric acid, and cover the beaker with a watch dish, and heat it to dissolve. Leave it cool, and make 60 mL with acetic acid for nonaqueous titration. Titrate the resulting solution with 0.1 mol/L sodium acetate. To confirm the endpoint, use a potentiometer with a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes. Separately, perform a blank test to make necessary correction.

Each mL of 0.1 mol/L perchloric acid = 9.132 mg of $H_2N(CH_2)_4CH(NH_2)COOH \cdot HCl$

L-α-Lysophosphatidylcholine Use a product suitable for 1-acyl-sn-glycerol 3-phosphocholine enzyme activity test.

Magnesia TS Mix 5.5 g of magnesium chloride hexahydrate and 7 g of ammonium chloride, and dissolve the mixture in 65 mL of water. Add 35 mL of ammonia TS, allow to stand for several days in a tightly stoppered bottle, and filter. If the solution is not clear, filter before use.

Magnesia TS (for red phosphorus determination) To 50 g of magnesium chloride hexahydrate, add 100 g of ammonium chloride and 800 mL of water to dissolve the solids, add 3 drops of phenolphthalein TS. To this solution, add ammonia solution (2 in 5) until the color of the solution becomes dark red, leave it for 2 days, and filter. To the filtrate, add water to make 1000 mL. Adjust the pH to 6–7 with diluted hydrochloric acid (1 in 11).

Magnesium Acetate Tetrahydrate $(CH_3COO)_2Mg \cdot 4H_2O$ [16674-78-5] Colorless to white crystals or powder. Deliquescent and soluble in water.

Content 99.0%.

Identification Magnesium acetate tetrahydrate responds to all the tests for Acetate Salt and for Magnesium Salt.

Assay Weigh accurately about 0.5 g of magnesium acetate tetrahydrate, and dissolve it in 100 mL of water. Add 2 mL of ammonium buffer (pH 10.7), and titrate with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate. Use two drops of eriochrome black T TS as the indicator. The endpoint is when the solution turns from red to blue.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate solution = 21.47 mg of $Mg(CH_3COO)_2 \cdot 4H_2O$

Magnesium Chloride Hexahydrate $MgCl_2 \cdot 6H_2O$ [K8159, Special Grade] [7791-18-6]

Magnesium Chloride TS (1 mol/L) Dissolve 203 g of magnesium chloride hexahydrate

in water to make 1000 mL.

Magnesium Chloride TS (0.1 mol/L) Dissolve 20.3 g of magnesium chloride hexahydrate in water to make 1000 mL.

Magnesium Nitrate Hexahydrate $Mg(NO_3)_2 \cdot 6H_2O$ [K8567, Special Grade] [13446-18-9]

Magnesium Oxide MgO [K8432, Special Grade] [1309-48-4]

Magnesium Powder Mg [K8876, Special Grade] [7439-95-4]

Magnesium Sulfate Heptahydrate $MgSO_4 \cdot 7H_2O$ [K8995, Special Grade] [10034-99-8]

Magnesium Sulfate TS (0.5 mol/L) Dissolve 11 g of magnesium sulfate heptahydrate in 50 mL of water to make 100 mL (0.5 mol/L).

Magnesium Sulfate TS (0.1 mol/L) Dissolve 24.6 g of magnesium sulfate heptahydrate in water to make 1000 mL.

Malachite Green Oxalate $C_{52}H_{54}N_4O_{12}$ [Malachite Green (Oxalate), K8878, Special Grade] [2437-29-8]

Maleic Acid HOOCCH:CHCOOH Use a product suitable for the corresponding enzyme activity tests.

Maleic Acid–Magnesium Sulfate–Cobalt Chloride TS Dissolve 23.2 g of maleic acid in 800 mL of water. To this solution, add 4.9 g of magnesium sulfate and 10 mL of cobalt(II) chloride TS (0.1 mol/L) to dissolve them, adjust the pH of the solution to 6.9 with sodium hydroxide (8 in 25). Then make up to 1000 mL.

Maleic Acid TS (0.05 mol/L, pH 5.6) Dissolve 6.7 g of maleic acid, 2.92 g sodium chloride, and 0.29 g calcium chloride dihydrate in water. Adjust the pH to 5.6, and make up to 1000 mL.

Maltopentaose $C_{30}H_{52}O_{26}$ Use a product suitable for the corresponding enzyme activity tests.

D(+)-Maltose Monohydrate $C_{12}H_{22}O_{11} \cdot H_2O$ Use a product suitable for the corresponding enzyme activity tests.

Maltotetraose $C_{24}H_{42}O_{21}$ Use a product suitable for the corresponding enzyme activity tests.

Maltotriose $C_{18}H_{32}O_{16}$ Use a product suitable for the corresponding enzyme activity tests.

Manganese(II) Chloride Tetrahydrate $MnCl_2 \cdot 4H_2O$ [K8160, Special Grade] [13446-34-9]

Manganese(II) Sulfate Pentahydrate $MnSO_4 \cdot 5H_2O$ [K8997, Special Grade] [15244-36-7]

D(–)-Mannitol $C_6H_{14}O_6$ [K8882, Special Grade] [69-65-8]

D-Mannitol for Assay Weigh 40 g of "D-Mannitol" into a 300-mL flask, and add 100 mL of water. Dissolve it by warming in a water bath, and cool to 40°C. Transfer the solution into a 300-mL beaker, add 20 mg of "D-Mannitol," mix, and allow to stand for 24 hours. Filter the formed crystals by suction, and wash with 10 mL of cold water. Dry the

resultant recrystallized product under reduced pressure at 105°C for 4 hours.

McIlvaine Buffer

Solution 1 Dissolve 21.0 g of citric acid monohydrate in water to make 1000 mL.

Solution 2 Dissolve 28.4 g of disodium hydrogenphosphate in water to make 1000 mL.

Mix solutions 1 and 2, and adjust the pH to the value specified in the corresponding sections in this publication.

McIlvaine Buffer (0.1 mol/L)

Solution 1 Dissolve 35.8 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Solution 2 Dissolve 21.0 g of citric acid monohydrate in water to make 1000 mL.

Mix solutions 1 and 2, and adjust the pH to the value specified in the corresponding sections in this publication.

McIlvaine Buffer (0.02 mol/L)

Solution 1 Dissolve 4.2 g of citric acid monohydrate in water to make 1000 mL.

Solution 2 Dissolve 5.7 g of disodium hydrogenphosphate in water to make 1000 mL.

Mix solutions 1 and 2, and adjust the pH to the value specified in the corresponding sections in this publication.

2-Melcaptoethanol $HSCH_2CH_2OH$ [60-24-2] A colorless, clear liquid.

Specific gravity d_4^{20}: 1.112–1.117.

Melibiose $C_{12}H_{22}O_{11}$ 6-*O*-α-D-Galactopyranosyl-D-glucose.

Use a product suitable for the corresponding enzyme activity tests.

Menaquinone-4 for Assay $C_{31}H_{40}O_2$ [863-61-6] A yellow powder or crystalline powder.

Melting point 36.0–38.0°C.

Purity (1) Clarity Yellow, clear (0.10 g, hexane 1 mL).

(2) Related substances The following operation should be protected from direct sunlight, and the equipment used should be light-resistant.

Prepare a test solution as follows. Dissolve 0.1 g of menaquinone-4 for assay in 50 mL of 2-propanol, and add ethanol (99.5) to make exactly 100 mL. Measure exactly 10 mL of this solution, and add ethanol (99.5) to make 100 mL. Measure exactly 2 mL of the obtained solution, and add 4 mL of 2-propanol. Use the prepared solution as the test solution. Prepare a control solution by diluting 2 mL of the test solution, measured exactly, to exactly 100 mL with a 2:1 mixture of 2-propanol/ethanol (95). Analyze 20 μL each of the test solution and the control solution by liquid chromatography using the operating conditions below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the main peak area from the control solution.

Operating conditions

Use the conditions specified in the Assay for Menaquinone (Extract) in the Monographs.

Mercury(II) Chloride $HgCl_2$ [K8139, Special Grade] [7487-94-7]

MES Buffer (0.05 mol/L, pH 6.0, containing sodium chloride) Dissolve 9.8 g of 2-(*N*-morpholino)ethanesulfonic acid *n*-hydrate and 17.5 g of sodium chloride in 900 mL of water. Adjust the pH with 1.5 mL of polyoxyethylene(23) lauryl ether (3 in 20) to 6.0, and add water to make 1000 mL.

Metaphosphoric Acid HPO_3 [37267-86-0]

Content Not less than 32.0% as metaphosphoric acid.

Description White deliquescent lumps.

Identification Prepare a test solution by dissolving 0.5 g of metaphosphoric acid in 50 mL of water. Neutralize 10 mL of the test solution with diluted ammonia solution (2 in 5), add 5 mL of silver nitrate solution (1 in 50). A white precipitate is formed. When 10 mL of albumin TS is added to 10 mL of the test solution, a white gluey precipitate is formed.

Purity Potassium permanganate reducing substances Weigh 2.0 g of metaphosphoric acid into ground-glass stoppered flat-bottom test tube, add 10 mL of water, 5 mL of diluted sulfuric acid (1 in 16), and 0.1 mL of 0.02 mol/L potassium manganite, shake the mixture, and heat for 5 minutes on a hot plate or a water bath. Examine the resulting solution from the sides and from the top with a white background. The solution remains red (not more than about 0.02% as H_3PO_3).

Assay Weigh accurately about 6 g of metaphosphoric acid, and dissolve it in 75 mL of water. Titrate the resulting solution with 1 mol/L sodium hydroxide. A potentiometer is generally used to confirm the endpoint. Use a glass electrode as the supporting electrode and silver–silver chloride electrode as the reference electrode. Integrated type can be used for the reference and supporting electrodes.

Each mL of 1 mol/L sodium hydroxide = 79.98 mg of HPO_3

Methanesulfonic Acid CH_4O_3S [75-75-2] A colorless to light yellow-brown, clear liquid.

Content Not less than 98.0% of methanesulfonic acid.

Assay Mix about 2 g of methanesulfonic acid, accurately weighed, with 40 mL of water. Titrate with 1 mol/L sodium hydroxide using 2 drops of bromothymol blue TS as an indicator. Perform a blank test to make correcction.

Each mL of 1 mol/L sodium hydroxide = 96.11 mg of CH_4O_3S

Methanol CH_3OH [K8891, Special Grade] [67-56-1]

Methanol (for HPLC) A colorless, clear, volatile liquid.

Measure the absorption spectrum of methanol (for HPLC) as directed in the Liquid Film Method under Infrared Spectrophotometry. It exhibits absorptions at about 2950 cm^{-1}, 2830 cm^{-1}, 1450 cm^{-1}, 1030 cm^{-1}, and 660 cm^{-1}.

Density 0.789–0.792/mL (Specific Gravity Method 4, 20°C).

Water Not more than 0.05% (10 g, Coulometric Titration).

Absorbance Not more than 0.06 at 240 nm, and not more that 0.01 at 260–400nm. Measure the absorbance of methanol in 10-mm cell at each wavelength against water.

Methanol for Water Determination To 1000 mL of methanol, add 30 g of synthetic zeolite for desiccation, stopper tightly, and allow to stand for about 8 hours with occasional gentle shaking. Then leave it for an additional 16 hours, and collect clear methanol, and store protected from moisture. Water in 1 mL of methanol for water determination is not more than 0.1 mg. Methanol containing components (such as sulfure dioxide and pyridine) of water determination TS is usable.

4-Methoxybenzaldehyde $C_8H_8O_2$ [123-11-5] A colorless to light-yellow, clear liquids. Miscible with ethanol (95) and with diethyl ether, but practically insoluble in water.

Content Not less than 97.0%.

Specific gravity d_4^{20}: 1.123–1.129.

Assay Weigh accurately about 0.8 g of 4-methoxybenzaldehyde, add exactly 7.5 mL of hydroxylamine TS, shake well, and allow to stand for 30 minutes. Titrate with 0.5 mol/L hydrochloric acid (indicator: 3 drops of bromophenol blue TS) until the color of the solution changes from blue to yellow-green through green. Separately, perform a blank test.

Each mL of 0.5 mol/L hydrochloric acid = 68.08 mg of $C_8H_8O_2$

0.5% 4-Methoxybenzaldehyde–Ethyl Acetate TS Mix 0.5 mL of 4-methoxybenzaldehyde and 99.5 mL of ethyl acetate.

4-Methoxybenzaldehyde–Sulfuric Acid TS To 9 mL of ethanol (95), add 0.5 mL of 4-methoxybenzaldehyde and 0.5 mL of sulfuric acid, and mix well.

2-Methoxyethanol $CH_3OCH_2CH_2OH$ [K8895, Special Grade] [109-86-4]

2-Methoxy-5-methylaniline $C_8H_{11}NO$ [120-71-8] A white to gray crystalline powder. Sparingly soluble in water, and soluble in methanol and in ethanol (95).

Identification (1) Dissolve 2-methoxy-5-methylaniline in a 1:1 mixture of methanol/ammonium acetate TS (0.01 mol/L). It exhibits an absorption maximum at about 290 nm.

(2) Determine the absorption spectrum as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3410 cm^{-1}, 2950 cm^{-1}, 1630 cm^{-1}, 1520 cm^{-1}, 1230 cm^{-1}, 1030 cm^{-1}, and 780 cm^{-1}.

Melting point 47–54°C.

1-Methoxy-2-propanol $C_5H_{12}O_2$ [107-98-2] A colorless, clear liquid.

Specific gravity d_{20}^{20}: 0.920–0.925.

Refractive index 1.402–1.405.

Water Not more than 0.5% (0.1 g, Coulometric Titration).

2-(Methylamino)pyridine $C_6H_8N_2$ [4597-87-9] A light yellow liquid.

Specific gravity d_{20}^{20}: 1.050–1.065.

Boiling point 200–202°C.

Water Not more than 1 mg/g.

2-(Methylamino)pyridine for Water Determination Distill 2-(methylamino)pyridine protected from moisture, and store protected from moisture. It shall not contain more than 1 mg of water per mL.

Methyl Behenate See Methyl Docosanoate.

Methyl Benzoate $C_6H_5COOCH_3$ [93-58-3] A colorless, clear liquid.

Refractive index n_D^{20}: 1.515–1.520.

Specific gravity d_{20}^{20}: 1.087–1.095.

Purity Dissolve 0.1 mL of methyl benzoate in the mobile phase directed under the Assay for Thiamine Hydrochloride in the Monographs, and make 50 mL. Analyze 10 μL of this solution by liquid chromatography using the operating conditions directed under the Assay for Thiamine Hydrochloride. Continue the chromatography for twice the retention time of the main peak, and measure the peak area of each peak. Calculate the content of methyl benzoate. The content is not less than 99.0%.

3-Methyl-1-butanol $(CH_3)_2CHCH_2CH_2OH$ [K8051, Special Grade] [123-51-3]

3-Methylbutyl Acetate $CH_3COO(CH_2)_2CH(CH_3)_2$ [123-92-2]

Content Not less than 98.0%.

Description A colorless, clear volatile liquid.

Identification Determine the absorption spectrum of 3-methylbutyl acetate as directed in the Liquid Film Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2958 cm^{-1}, 1743 cm^{-1}, 1465 cm^{-1}, 1309 cm^{-1}, 1245 cm^{-1}, 1056 cm^{-1}, and 605 cm^{-1}.

Density 0.868–0.879 g/mL (Specific Gravity Method 4, 20°C).

Assay Analyze 1 μL of 3-methylbutyl acetate by gas chromatography using the operating conditions given below. Normalize the sum of the areas of all the peaks appearing within the measurement time to 100, and obtain the peak area percentage of 3-methylbutyl acetate to determine the content.

Operating conditions

Detector: Flame-ionization detector.

Column: A fused silica gel tube (0.53 mm internal diameter and 15 m length) coated with a 1.5-μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Upon injection at 50°C, raise the temperature at a rate of 10°C/minute to 150°C.

Injection port temperature: 200°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 5 mL/minute.

Injection method: Split.

Split ratio: 1:20.

Measurement time: 10 minutes.

Methyl Caprate See Methyl Decanoate.

Methyl Caprylate See Methyl Octanate.

Methyl Decanoate $C_{11}H_{22}O_2$ [110-42-9] A colorless, clear liquid.

Refractive index n_D^{20}: 1.424–1.427.

Specific gravity d_{20}^{20}: 0.872–0.876.

Methyl Docosanoate $C_{23}H_{46}O_2$ [929-77-1] A colorless crystalline powder.

Melting point 53–56°C.

Methylene Blue $C_{16}H_{18}N_3S \cdot Cl \cdot 3H_2O$ [K8897, Special Grade] [7220-79-3]

Methylene Blue TS Dissolve 0.1 g of methylene blue in 100 mL of ethanol (95). Filter if necessary.

0.001% (w/v) Methylene Blue TS Measure 1 mL of methylene blue TS, and add water to make 100 mL.

α-Methyl-D(+)-Glucoside $C_7H_{14}O_6$ Use a product suitable for the corresponding enzyme activity tests.

2-Methylimidazole $C_4H_6N_2$ [693-98-1] White to light-yellow, hygroscopic crystals or crystalline powder having a slight, characteristic odor. Soluble in water, in ethanol (95), in ethyl acetate, and in acetone.

Content Not less than 98% of 2-methylimidazole ($C_4H_6N_2$).

Boiling point 267–268°C.

Melting point 142–145°C.

Assay Weigh accurately about 0.2 g of 2-methylimidazole, and dissolve it in 50 mL of acetic acid for nonaqueous titration. Titrate with 0.1 mol/L perchloric acid. Use a potentiometer to determine the endpoint. Perform a blank test in the same manner, and make necessary correction.

Each mL of 0.1 mol/L perchloric acid = 8.211 mg of $C_4H_6N_2$

4-Methylimidazole $C_4H_6N_2$ [822-36-6] Light-yellow, hygroscopic crystals or crystalline powder having a slight, characteristic odor. Soluble in water, in ethanol (95), in acetone, and in chloroform.

Content Not less than 97% of 4-methylimidazole ($C_4H_6N_2$).

Boiling point 262–264°C.

Melting point 46–48°C.

Assay Weigh accurately about 0.2 g of 4-methylimidazole, and dissolve it in 50 mL of acetic acid for nonaqueous titration. Titrate with 0.1 mol/L perchloric acid. Use a potentiometer to determine the endpoint. Perform a blank test in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid = 8.211 mg of $C_4H_6N_2$

Methyl Laurate $C_{13}H_{26}O_2$ [111-82-0] A colorless or yellow liquid.

Refractive index n_D^{20}: 1.431.

Specific gravity d_{20}^{20}: 0.87.

Melting point About 5°C.

Methyl Myristate See Methyl Tetradecanoate.

Methyl Octanate $C_9H_{18}O_2$ [111-11-5] A colorless, clear liquid.

Refractive index n_D^{20}: 1.415–1.420.

Density 0.874–0.880 g/mL (20°C).

Methyl Oleate $C_{19}H_{36}O_2$ [112-62-9] A colorless to pale yellow liquid.

Refractive index n_D^{20}: 1.452.

Specific gravity d_{20}^{20}: 0.88.

Methyl Orange $C_{14}H_{14}N_3NaO_3S$ [K8893, Special Grade] [547-58-0]

Methyl Orange–Indigo Carmine TS Mix 0.1 g of methyl orange and 0.25 g of indigo carmine, and add water to make 100 mL. Store protected from light, and use it within 15 days after preparation.

Methyl Orange TS Dissolve 0.1 g of methyl orange in 100 mL of water. Filter if necessary.

Methyl Orange–Xylene Cyanol FF TS Mix 1.0 g of methyl orange and 1.4 g of xylene cyanol FF, and dissolve the mixture in 500 mL of 50% (vol) ethanol.

Methyl Palmitate $C_{17}H_{34}O_2$ [112-39-0] White or yellow crystalline lumps.

Refractive index n_D^{20}: 1.451.

Melting point About 30°C.

4-Methyl-2-pentanone $CH_3COCH_2CH(CH_3)_2$ [K8903, Special Grade] [108-10-1]

3-Methyl-1-phenyl-5-pyrazolone $C_{10}H_{10}N_2O$ [K9548, Special Grade] [89-25-8]

2-Methyl-1-propanol $(CH_3)_2CHCH_2OH$ [K8811, Special Grade] [78-83-1]

2-Methyl-2-propanol $(CH_3)_3COH$ [75-65-0] White lumps, and a colorless, transparent liquid having a characteristic odor when melted. Very soluble in water and in diethyl ether.

Content Not less than 99.0%.

Assay Analyze 0.5 μL of 2-methyl-2-propanol by gas chromatography using the operating conditions given below. Determine the content of 2-methyl-2-propanol from the 2-methyl-2-propanol peak and the sum of the areas of all the peaks.

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 polyethylene glycol for gas chromatography.

Column temperature: 80°C.

Injection port temperature: 130°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 1.33 mL/minute.

Injection method: Split.

Split ratio: 1:100.

Measurement time: 30 minutes.

Methyl Red $C_{15}H_{15}N_3O_2$ [K8896, Special Grade] [493-52-7]

Methyl Red–Methylene Blue Mixture TS Mix equal volumes of methyl red TS and methylene blue TS.

Methyl Red TS Dissolve 0.1 g of methyl red in 100 mL of ethanol (95). Filter if necessary.

Methyl Salicylate $HOC_6H_4COOCH_3$ [119-36-8] A colorless or slightly pale-yellow, oily substance having a characteristic odor. Sparingly soluble in water and miscible well with diethyl ether.

Content Not less than 98.0%.

Assay Analyze 1 μg of methyl salicylate by gas chromatography using the operating conditions given below. Determine the content of methyl salicylate from the area of the methyl salicylate peak and the sum of the areas of all the peaks.

Operating conditions

Detector: Thermal conductivity detector.

Column: A fused silica tube (0.53 mm internal diameter and 15 m length) coated with a 1.5-μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Upon injection at 100°C, raise the temperature at a rate of 10°C/minute to 250°C.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 5 mL/minute.

Injection method: Split.

Split ratio: 1:20.

Measurement time: 15 minutes.

Methyl Stearate $C_{19}H_{38}O_2$ [112–61-8] White to yellow crystalline lumps.

Melting point About 38°C.

Methyl Tetradecanoate $C_{15}H_{30}O_2$ [124-10-7] A colorless, transparent liquid.

Refractive index n_D^{20}: 1.434–1.438.

Specific gravity d_{20}^{20}: 0.853–0.873.

Methyl Yellow $C_{14}H_{15}N_3$ [K8494, Special Grade] [60-11-7]

Methyl Yellow TS Dissolve 0.10 g of methyl yellow in 200 mL of ethanol (95).

Mixture of Four Steviol Glycosides Dissolve four glycosides (stevioside, rebaudioside A, rebaudioside C, and dulcoside A) in a 7:3 mixture of water/acetonitrile (for HPLC) to prepare it so that the concentration of each glycoside is 0.1 mg/mL.

Mixture of Nine Steviol Glycosides Dissolve nine glycosides (stevioside, rebaudioside A, rebausioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside, steviolbioside) in a 7:3 mixture of water/acetonitrile (for HPLC) to prepare it so that the concentration of each glycoside is 0.1 mg/mL.

Mogroside V for Assay ($C_{60}H_{102}O_{29}$) [88901-36-4] A white to light yellow powder having a sweet taste.

Identification Determine the absorption spectrum of mogroside V for assay, previously dried at 105°C for 2 hours, as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3430 cm^{-1}, 2930 cm^{-1}, 1634 cm^{-1}, 1383 cm^{-1}, 1170 cm^{-1}, 1075 cm^{-1}, and 1038 cm^{-1}.

Purity Related substances Prepare a test solution by dissolving 5 mg of mogroside V for assay in 1 mL of a 74:26 mixture of acetonitrile/water. Prepare a control solution by diluting 0.5 mL of the test solution, measured exactly, with a 74:26 mixture of acetonitrile/water to make exactly 10 mL. Analyze 5 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. Exclude the solvent peaks from the measurement. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Follow the conditions specified in the Assay for Luohanguo Extract in the Monographs.

Molybdenum(VI) Oxide MoO_3 [1313-27-5] A white to yellowish green powder. Sparingly soluble in water.

Content Not less than 99.0%.

Purity Phosphate (PO_4) Not more than 0.0005%.

Test Solution: Weigh 1.5 g of molybdenum(VI) oxide into a 200-mL polyethylene beaker, dissolve it in 10 mL of sodium hydroxide solution (1 in 10), add 30 mL of water, and adjust the pH to 4–5 with diluted hydrochloric acid (1 in 10) using a pH test paper. Then add 2 mL of bromine TS, adjust the pH to 1.7–1.9 with diluted hydrochloric acid (1 in 10) using a pH meter, and transfer the solution in the polyethylene beaker to a 200 mL of glass beaker. Heat the solution until it starts boiling, cool to about 20°C, and add water to make 90 mL. Transfer the resulting solution to a 200-mL separating funnel, add 10 mL of hydrochloric acid and 20 mL of diethyl ether, agitate for 3 minutes, and allow to stand. Collect the diethyl ether layer, wash it 4 times with 10 mL of diluted hydrochloric acid (1 in 10) each time, add 0.2 mL of tin(II) chloride dihydrate (1 in 50) to the diethyl ether, shake well for 30 seconds, and allow to stand. Dilute the collected diethyl ether to 25 mL with diethyl ether.

Standard Solution: Weigh 0.5 g of molybdenum(VI) oxide into a 200-mL polyethylene beaker, dissolve in 10 mL of sodium hydroxide solution (1 in 10), add 0.5 mL of Phosphate Standard Solution and 30 mL of water, and adjust the pH to 4–5 with diluted hydrochloric acid (1 in 10) using a pH test paper. Then add 2 mL of bromine TS, adjust the pH to 1.7–1.9 with diluted hydrochloric acid (1 in 10) using a pH meter, and transfer the solution in the polyethyle beaker to a 200 mL of glass beaker. Heat the solution until it starts boiling, cool to about 20°C, and add water to make 90 mL. Transfer the resulting solution to a 200-mL separating funnel, add 10 mL of hydrochloric acid and 20 mL of

diethyl ether, agitate for 3 minutes, and allow to stand. Collect the diethyl ether layer, wash it 4 times with 10 mL of diluted hydrochloric acid (1 in 10) each time, add 0.2 mL of tin(II) chloride dihydrate (1 in 50) to the diethyl ether, shake well for 30 seconds, and allow to stand. Dilute the collected diethyl ether to 25 mL with diethyl ether. The blue color of the test solution is not darker than that of the standard solution.

Assay Weigh accurately about 0.15 g of molybdenum(VI) oxide, dissolve it in 2 mL of sodium hydroxide solution (1 in 10), and add 5 mL of hexamethylenetetramine solution (1 in 10). Adjust the pH to 5–6 with diluted nitric acid (1 in 11), warm the solution to 50–70°C, add 4-(2-pyridylazo) resorcinol TS, and titrate with 0.05 mol/L lead(II) nitrate. The endpoint is when the color of the solution changes from yellow to yellowish red.

Each mL of 0.05 mol/L lead(II) nitrate = 7.198 mg MoO_3

Monoglucosyl Hesperidin for Assay $C_{34}H_{44}O_{20}$ A light yellow to yellow-brown crystalline powder having a slight characteristic odor.

Identification (1) Dissolve 5 mg of monoglucosyl hesperidin for assay 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 10 mg of monoglucosyl hesperidin for assay in 500 mL of water. The solution exhibits an absorption maximum at a wavelength of 280–286 nm.

Loss on drying Not more than 6.0% (Not more than 2.7 kPa, 120°C, 2 hours).

Purity Related substances Prepare a test solution by dissolving about 0.1 g of monoglucosyl hesperidin for assay, weighed accurately, in an 80:20:0.01 mixture of water/acetonitrile/acetic acid to make exactly 200 mL. Prepare a control solution by diluting 1 mL of the test solution, measured exactly, to exactly 50 mL with an 80:20:0.01 mixture of water/acetonitrile/acetic acid. Analyze 10 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Use the conditions specified in the Assay for Enzymatically Modified Hesperidin in the Monographs.

Monoglucosyl Rutin A yellow to yellow-brown powder.

Identification Prepare a test solution by dissolving about 10 mg of monoglucosyl rutin in an 80:20:0.1 mixture of water/acetonitrile/phosphoric acid to make 10 mL. Prepare a standard solution by dissolving about 10 mg of rutine for assay in a small amout of methanol and making up to 10 mL with an 80:20:0.1 mixture of water/acetonitrile/phosphoric acid. Analyze 10 μL each of the test solution and the standard solution by liquid chromatography using the operating conditions given in the Assay for Enzymatically Modified Rutin (Extract) in the Monographs. Use a photodiode array detector for the detector. When analyzing them at a wavelength of 254 nm, the

retention time of the main peak from the test solution is shorter than that of rutin peak from the standard solution. Compare the absorption spectra of at wavelengths of 200−400 nm between both peaks. The both spectra exhibit absorption maxima at the same wavelengths.

Purity Related substances Analyze a 10-μL portion of the test solution prepared in Identification by liquid chromatography using the operating conditions given in the Assay for Enzymatically Modified Rutin (Extract) in the Monographs. Continue the chromatography for two times the retention time of the main peak, and exclude the solvent peak from measurement. Measure the area of each peak and determine the percentage of the main peak by the peak area percentage method. It is not less than 65.0%.

L-(+)-Monosodium L-Aspartate Monohydrate $C_4H_6NNaO_4 \cdot H_2O$ [3792-50-5] "Monosodium L-Aspartate"

Monosodium L-Glutamate Monohydrate $C_5H_8NNaO_4 \cdot H_2O$ [6106-04-3] "Monosodium L-Glutamate"

Monosodium 6-Hydroxy-2-naphthalenesulfonate $C_{10}H_7NaO_4S$ [135-76-2] A white to pale brown powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 277–283 nm): Not less than 190. Weigh accurately about 10 mg of monosodium 6-hydroxy-2-naphthalenesulfonate, dried previously for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits absorption maxima in the ranges 277–283 nm and 327–333 nm, respectively. Measure the absorbance at the maximum between 277–283 nm against ammonium acetate TS (0.02 mol/L) to determine the specific absorbance.

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add ammonium acetate TS (0.02 mol/L) to 10 mg of monosodium 6-hydroxy-2-naphthalenesulfonate to make exactly 25 mL. Analyze 10 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given below. Measure the areas of the peaks that appear within 50 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the ammonium acetate TS in the test solution.

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: 30°C.

Mobile phase

A: Ammonium acetate TS (0.02 mol/L).

B: Methanol (for HPLC).

Concentration gradient (A/B): Run a linear gradient from 100/0 to 0/100 in 50 minutes.

Flow rate: 1.0 mL/min.

Water Not more than 20.0% (50 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

MOPS Buffer (0.1 mol/L, pH 7.0) Dissolve 21 g of 3-(*N*-morpholino)propanesulfonic acid in 900 mL of water. Adjust the pH to 7.0 with sodium hydroxide solution of an appropriate concentration, and dilute with water to exactly 1000 mL.

MOPS Buffer (0.04 mol/L) Dissolve 8.4 g of 3-(*N*-morpholino)propanesulfonic acid in 900 mL of water. Adjust the pH with sodium hydroxide TS (4 mol/L) to the value specified in the corresponding section, and add water to make 1000 mL.

MOPS Buffer (0.04 mol/L, pH 7.0, containing magnesium sulfate and sodium chloride) Weigh 62.3 g of magnesium sulfate heptahydrate and 25.3 g of sodium chloride, add 200 mL of MOPS buffer (0.04 mol/L) at pH 7.0, and dissolve them slowly while warming. Adjust the pH to 7.0 with sodium hydroxide TS (2 mol/L) or hydrochloric acid TS (2 mol/L). Add MOPS buffer at pH 7.0 to make 250 mL.

MOPS Buffer (0.04 mol/L, containing magnesium sulfate, sodium chloride, and cobalt chloride) Mix 0.1 mL of a solution of cobalt(II) chloride hexahydrate (1 in 10) with MOPS buffer (0.04 mol/L, pH 7.0, containing magnesium sulfate and sodium chloride) to make 10 mL.

MOPS Buffer (0.02 mol/L, pH 7.0, containing magnesium sulfate) Dissolve 123 g of magnesium sulfate heptahydrate and 21.0 g of 3-(*N*-morpholino)propanesulfonic acid, in 4.8 L of water. Then, dissolve 50 g of polyoxyethylene(10) octylphenyl ether in the resulting solution. Adjust the pH to 7.0 with sodium hydroxide TS (4 mol/L), and add water to make 5L.

Morpholine C_4H_9NO [110-91-8] A basic, colorless liquid having an ammonia-like odor. Soluble in water.

Refractive index n_D^{20}: 1.452–1.457.

Specific gravity d_{20}^{20}: 0.998–1.005.

2-(*N*-Morpholino)ethanesulfonic Acid *n*-Hydrate $C_6H_{13}NO_4S \cdot nH_2O$ Use a product suitable for the corresponding enzyme activity tests.

3-(*N*-Morpholino)propanesulfonic Acid $C_7H_{15}NO_4S$ [1132-61-2] A white crystalline powder. Freely soluble in water and practically insoluble in ethanol (99.5).

Melting point 275–280°C.

Murexide $C_8H_8N_6O_6$ [3051-09-0] A red-purple powder. Practically insoluble in

water, in ethanol (95), and in diethyl ether.

Absorbance Dissolve 10 mg of murexide in water to make 100 mL. Dilute 5 mL of this solution with water to exactly 50 mL. Measure the absorbance of the resulting solution against water as directed under Ultraviolet-Visible Spectrophotometry. The solution exhibits an absorption maximum at a wavelength of about 522 nm. The absorbance is 0.35.

Loss on drying Not more than 2.0% (105°C, constant temperature).

Murexide–Sodium Chloride Indicator Mix 0.1 g of murexide and 10 g of sodium chloride, and grind the mixture homogeneously. Store protected from light.

Mutarotase [9031-76-9] A white, 50% glycerol suspension. It is obtained from the kidney of swine. One unit is equivalent to the amount of enzyme required to produce 1 μmol of β-D-glucose from α-D-glucose as a substrate in 1 minute at 25°C and pH 7.2.

Myricitrin for Assay $C_{21}H_{20}O_{12}$ [17912-87-7] A light grayish yellow to light yellow, almost odorless powder.

Identification Determine the absorption spectrum of myricitrin for assay as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 1660 cm^{-1}, 1605 cm^{-1}, 1345 cm^{-1}, 1200 cm^{-1}, and 970 cm^{-1}.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength at about 354 nm): Not less than 340. Weigh accurately about 50 mg of myricitrin for assay, dried in a desiccator for 24 hours, and dissolve it in methanol to make exactly 100 mL. Measure exactly 2 mL of the obtained solution, and add methanol to make exactly 100 mL. Measure the absorbance of this solution as directed under Ultraviolet–Visible Spectrophotometry.

Purity Related substances Dissolve 50 mg of myricitrin for assay in 25 mL of methanol. Measure exactly 5 mL of this solution, and add an 800:200:1 mixture of water/acetonitrile/phosphoric acid to make exactly 50 mL. Use this solution as the test solution. Measure exactly 1 mL of the test solution, add 5 mL of methanol, and add an 800:200:1 mixture of water/acetonitrile/phosphoric acid to make exactly 50 mL. Use this solution as the control solution. Analyze 20 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. Remove solvent peaks from measurement. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Use the operating conditions given in the Assay for Chinese Bayberry Extract in the Monographs.

Naphthalene $C_{10}H_8$ [91-20-3] Colorless, foliaceous or lustrous rod-like crystals having a characteristic odor. Sublimes gradually at ordinary temperature. Upon ignition, burns with sooty flames. Practically insoluble in water.

Content Not less than 99.0%.

Assay Prepare a test solution by dissolving 1.0 g of naphthalene in acetone to make exactly 10 mL. Analyze 1 μL each of the test solution and acetone by gas chromatography using the operating conditions. Normalize the sum of the areas of all the peaks, except for acetone peak, appearing within the measurement time to 100, calculate the peak percentage of naphthalene, and determine the naphthalene content.

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.25 internal diameter and 30 m length) coated with a 0.25-μm thick of polyethylene glycol for gas chromatography.

Column temperature: 200°C.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Injection method: Split.

Flow rate: 1.33 mL/minute.

Split ratio: 1:100.

Measurement time: Three times the retention time of the main peaks.

1-Naphthol $C_{10}H_7OH$ [K8698, Special Grade] [90-15-3] Store protected from light.

***p*-Naphtholbenzein** $C_{27}H_{18}O_2$ [K8693, Special Grade] [145-50-6]

***p*-Naphtholbenzein TS** Dissolve 1 g of *p*-naphtholbenzein in acetic acid for nonaqueous titration to make 100 mL.

Naphthol-Creatine TS Dissolve 5 g of 1-naphthol and 0.5 g of creatine monohydrate in 500 mL of sodium hydroxide TS (1 mol/L). Prepare before use. Protect from light.

1-Naphthylamine $C_{10}H_9N$ [K8692, Special Grade] [134-32-7]

***N*-1-Naphthylethylenediamine Dihydrochloride** $C_{12}H_{14}N_2 \cdot 2HCl$ [K8197, Special Grade] [1465-25-4] Prepare fresh before use.

Naringin *n*-Hydrate $C_{27}H_{32}O_{14} \cdot nH_2O$ Naringenin 7-Rhamnoglucoside Hydrate. Use a product suitable for the corresponding enzyme activity tests.

Nelson's TS A reagent for saccharide determination that contains hexaammonium heptamolybdate tetrahydrate and disodium arsenate. Use a product suitable for the corresponding enzyme activity tests.

Neotame for Assay $C_{20}H_{30}N_2O_5$ [165450-17-9] A white to off-white powder. It is produced by a single step reaction of aspartame and 3,3-dimethylbutyl aldehyde.

Identification Measure the absorption spectrum of neotame for assay as directed in the Disk Method under the Infrared Spectrophotometry. It exhibits absorption at about 3320 cm^{-1}, 2960 cm^{-1}, 1730 cm^{-1}, 1690 cm^{-1}, 1590 cm^{-1}, 1210 cm^{-1}, 760 cm^{-1}, and 700 cm^{-1}.

Purity Related substances Prepare a test solution by dissolving about 0.1 g of neotame for assay in 100 mL of the mobile phase directed under Assay for Neotame.

Prepare a control solution by diluting 1 mL of the test solution, measured exactly, with the mobile phase to make exactly 100 mL. Analyze 25 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for 1.5 times the retention time of the main peak, and measure peak areas. Exclude the solvent peak from measurement. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Use the operating conditions given in the Assay for Neotame in the Monographs.

Neutral Red $C_{15}H_{17}ClN_4$ [553-24-2] A dark green powder or small lumps having a metallic luster. Freely soluble in water and practically insoluble in diethyl ether.

Absorbance Not less than 0.50 (on the dried basis). Weigh accurately about 0.1 g of neutral red into a beaker, add 80 mL of water, heat in a water bath, and cool. Transfer the solution to a 100-mL volumetric flask, wash the beaker with 15 mL of water, add the washings to the flask, and make up to the volume with water. Transfer exactly 10 mL of this solution into a 100-mL volumetric flask, and make up to the volume with phosphate buffer (pH 6.4), and leave for about 5 minutes. Measure the absorbance of the resulting solution as directed under Ultraviolet-Visible Spectrophotometry at a wavelength of 525 nm against phosphate buffer (pH 6.4).

Loss on drying Not more than 10.0% (105°C, 4 hours).

Nickel(II) Chloride Hexahydrate $NiCl_2 \cdot 6H_2O$ [K8152, Special Grade] [7791-20-0]

β-Nicotinamide Adenine Dinucleotide $C_{21}H_{27}N_7O_{14}P_2$ [β-NAD^+, K9802] [53-84-9]

β-Nicotinamide Adenine Dinucleotide (oxidized form) $C_{21}H_{27}N_7O_{14}P_2$ Use a product suitable for the corresponding enzyme activity tests.

β-Nicotinamide Adenine Dinucleotide Disodium Salt *n*-Hydrate (reduced form) $C_{21}H_{27}N_7Na_2O_{14}P_2 \cdot nH_2O$ [606-68-8] A white to pale yellow powder. Soluble in water.

β-Nicotinamide Adenine Dinucleotide TS Dissolve 40 mg of β-nicotinamide adenine dinucleotide in 10 mL of water. Prepare fresh before use.

Ninhydrin $C_9H_6O_4$ [K8870] [485-47-2]

Ninhydrin–Acetic Acid TS Dissolve 8.2 g of sodium acetate trihydrate in water, and add 2.5 mL of acetic acid. To this solution, add 2.0 g of ninhydrin, and then add water to make 100 mL.

Ninhydrin–2-Methoxyethanol–Citric Acid Buffer Weigh 1.0 g of ninhydrin, dissolve in 25 mL of 2-methoxyethanol, add 25 mL of citric acid–sodium hydroxide buffer (0.2 mol/L) at pH 5.0, and mix.

Ninhydrin–2-Methoxyethanol TS To 750 mL of 2-methoxyethanol, and add 250 mL of acetate buffer. To this solution, add 20 g of ninhydrin first, then 0.38 g of tin(II) chloride dihydrate while passing through nitrogen, and dissolve them. Allow to stand in a cold, dark place for 24 hours. Store protected from light.

Ninhydrin TS Dissolve 1 g of ninhydrin in water to make 1000 mL.

Ninhydrin TS for Bacillus Natto Gum Assay

Solution 1 Dissolve 39 g of ninhydrin and 81 mg of sodium tetrahydroborate for amino acid analysis in 979 mL of 1-methoxy-2-propanol, and mix under nitrogen gas.

Solution 2 Dissolve 204 g of lithium acetate dihydrate, 123 mL of acetic acid, and 401 mL of 1-methoxy-2-propanol in water to make 1000 mL, and mix under nitrogen gas.

Mix equal volumes of Solutions 1 and 2.

Ninhydrin TS for Modified Starch Dissolve 3.0 g of ninhydrin in sodium hydrogen sulfite solution (1 in 20) to make 100 mL.

Nitric Acid HNO_3 [K8541, Special Grade] [7697-37-2]

Nitric Acid (for trace metal determination) HNO_3 [K8541, for trace metal determination] [7697-37-2] Use a reagent with a nitric acid concentration of 69–70%, unless otherwise specified.

10% Nitric Acid TS Measure 10.5 mL of nitric acid, and add water to make 100 mL.

Nitric Acid TS (1 mol/L) Measure an appropriate amount of nitric acid depending on the concentration: 6.4 mL for a concentration of 69–70%, 6.9 mL for a concentration of 65–66%, or 7.6 mL for a concentration of 60–61%. Add water to make 100 mL.

2,2′,2″-Nitrilotriethanol $(CH_2CH_2OH)_3N$ [K8663, Special Grade] [102-71-6]

Nitrobenzene $C_6H_5NO_2$ [K8723, Special Grade] [98-95-3]

Nitrogen N_2 [7727-37-9] Use nitrogen specified in the Japanese Pharmacopoeia.

Nitromethane CH_3NO_2 [K9523, Special Grade] [75-52-5]

***p*-Nitrophenyl 2-Acetamido-2-deoxy-β-D-glucopyranoside** $C_{14}H_{18}N_2O_8$

p-Nitrophenyl-*N*-acetyl-β-D-glucosaminide Use a product suitable for the corresponding enzyme activity tests.

***p*-Nitrophenyl Di-*N*-acetyl-β-chitobioside** Use a product suitable for the corresponding enzyme activity tests.

***p*-Nitrophenyl Butyrate** $NO_2C_6H_4OCO(CH_2)_2CH_3$ Use a product suitable for the corresponding enzyme activity tests.

***p*-Nitrophenyl α-D-Galactopyranoside** $C_{12}H_{15}NO_8$ Use a product suitable for the corresponding enzyme activity tests.

***o*-Nitrophenyl β-D-Galactopyranoside** $C_{12}H_{15}NO_8$ Use a product suitable for the corresponding enzyme activity tests.

***p*-Nitrophenyl α-D-Glucopyranoside** $C_{12}H_{15}NO_8$ Use a product suitable for the corresponding enzyme activity tests.

***p*-Nitrophenyl β-D-Glucopyranoside** $C_{12}H_{15}NO_8$ Use a product suitable for the corresponding enzyme activity tests.

***p*-Nitrophenyl Palmitate** $C_{22}H_{35}NO_4$ Use a product suitable for the corresponding enzyme activity tests.

5-Nitroso-8-hydroxyquinoline $C_9H_6N_2O_2$ [3565-26-2] A dark greenish gray crystalline powder. Practically insoluble in water.

Sensitivity Prepare a test solution by dissolving 0.1 g of 5-nitroso-8-

hydroxyquinoline in 100 mL of sulfuric acid. Place 0.05 mL of a 1 in 1000 solution of resorcinol in ethanol (99.5) into a test tube, and evaporate to dryness on a water bath. To the residue, add 0.05 mL of the test solution, and warm. The color of the solution becomes red-purple.

Nitrous Oxide N_2O [10024-97-2] A colorless, odorless gas. Use gas filled in a hermetic, pressure-resistant metal container.

NN Indicator Mix 0.5 g of 2-hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3- naphthoic acid and 50 g of potassium sulfate, and triturate thoroughly until it is homogeneous.

Non-reducing-end Blocked *p*-Nitrophenyl α-D-Maltoheptoside-Enzyme A reagent for the α-amylase activity test containing 54.5 mg of non-reducing-end blocked *p*-nitrophenyl α-D-maltoheptoside and 125 units of α-glucosidase (pH 6.0). Use a product suitable for the corresponding enzyme activity tests.

Norbixin $C_{24}H_{28}O_4$ [542-40-5]

Content Not less than 70%.

Description A strongly yellowish red powder.

Identification Dissolve 5.0 mg of norbixin in potassium hydroxide solution (1 in 200) to make exactly 25 mL. Refer to this solution as Solution A. A solution prepared by making 1 mL of Solution A up to 50 mL with potassium hydroxide solution (1 in 200) exhibits absorption maxima in the ranges 448–456 nm and 476–484 nm, respectively.

Assay Analyze 10 μL of Solution A by liquid chromatography using the operating conditions given below. Continue the chromatograph for two times the retention time of the main peak. Determine the ratio of the area of the main peak to the sum of the areas of all the peaks on the chromatogram.

Operating conditions

Detector: Visible absorption photometer (wavelength 460 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 250 mm length).

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

Column temperature: 40°C.

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

Flow rate: Adjust the retention time of the main peak to about 10 minute.

Octacosane $C_{28}H_{58}$ [630-02-4] White crystals or crystalline powder.

Melting point 60.0–63.0°C.

Octadecylsilanized Silica Gel Minicolumn (500 mg) Use a polyethylene column (10–25 mm internal diameter) packed with 0.5 g of octadecylsilanized silica gel or a column comparable in separation property to the former one.

Octane C_8H_{18} [111-65-9]

Specific gravity d_4^{20}: 0.700–0.705.

Purity Analyze 2 μL of octane by gas chromatography using the conditions directed in the Assay for Hydroxypropyl Methylcellulose in the Monographs. Determine the peak

area of each peak in the chromatogram, and obtain the content of octane by the peak area percentage method. The content is not less than 99.0%.

Octanoic Acid $CH_3(CH_2)_6COOH$ [124-07-2] Use a product that is produced for amino acid analysis.

Description A colorless to light yellow, clear liquid.

Congealing point 15–17°C.

Octanoic Acid for Assay $C_8H_{16}O_2$ [124-07-2] A colorless to light yellow, clear liquid.

Content Not less than 98.0% of octanoic acid ($C_8H_{16}O_2$).

Identification Determine the absorption spectrum of octanoic acid for assay as directed in the Liquid Film Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2930 cm^{-1}, 2860 cm^{-1}, 1710 cm^{-1}, 1460 cm^{-1}, 1420 cm^{-1}, 1280 cm^{-1}, 1230 cm^{-1}, 1200 cm^{-1}, 1110 cm^{-1}, 940 cm^{-1}, and 720 cm^{-1}.

Congealing point 15–17°C.

Refractive index n_D^{20}: 1.425–1.431.

Specific gravity d_{20}^{20}: 0.909–0.915.

Assay Weigh accurately about 0.05 g of octanoic acid for assay, add 1 mL of *N,O*-bis(trimethylsilyl)trifluoroacetamide, stopper tightly, and mix well. Heat the mixture on a water bath for 30 minutes, and allow to cool. Analyze appropriate portions of the resulting solution by gas chromatography using the operating conditions given below. Determine the area percentage of the main peak.

Operating conditions

Detector: Flame-ionization detector.

Column: A fused silica capillary column (0.53 mm internal diameter and 15 m length) coated with a 1.5-µm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Raise the temperature at a rate of 10°C/minutes from 50°C to 280°C, and then maintain at 280°C for 2 minutes.

Injection port temperature: 280°C.

Detector temperature: 280°C.

Injection method: Split (20:1). Set conditions so that any component of the sample does not exceed the column acceptable range).

Carrier gas: Helium.

Flow rate: Adjust so that the peak of the component to be determined appears in 5–20 minutes of injection.

Octenyl Succinic Anhydride $C_{12}H_{18}O_3$ [26680-54-6] A mixture of the *cis* and *trans* forms of octenyl succinic anhydride. A colorless or pale yellow liquid.

Content Not less than 95.0% of octenyl succinic anhydride ($C_{12}H_{18}O_3$).

Refractive index n_D^{20}: 1.468–1.470.

Specific gravity d_4^{20}: 1.025–1.028.

Assay Weigh accurately about 1.5 g of octenyl succinnic anhydride into a 200-mL

stoppered Erlenmeyer flask, dissolve it by adding exactly 25 mL of 0.5 mol/L methanolic morpholine, and allow to stand for 1 hour. Titrate the excess morpholine with 0.5 mol/L methanolic hydrochloric acid. Record the volume of the methanolic hydrochloric acid consumed as S (mL). Use BANASS–brilliant yellow TS as the indicator. The endpoint is when the color of the solution changes from red to blue-purple. Perform a blank test, and record the volume of the 0.5 mol/L methanolic hydrochloric acid consumed as B (mL). Determine the content by the following formula.

$$\text{Content (\%) of octenylsuccinic anhydride } (C_{12}H_{18}O_3) = \frac{(B - S) \times 0.1051}{\text{Weight (g) of the sample}} \times 100$$

Olive Oil Use a product suitable for the corresponding enzyme test.

Orcinol Monohydrate $CH_3C_6H_3(OH)_2 \cdot H_2O$ [6153-39-5] Colorless crystals. Turns red by oxidation in air. Soluble in water, in ethanol (95), and in diethyl ether. A solution of orcinol in ethanol (95) should be prepare fresh before use.

Melting point 107–108°C.

Orcinol–Ethanol TS Dissolve 0.1 g of orcinol monohydrate in 1 mL of ethanol (95). Prepare fesh before use.

Oxalic Acid Dihydrate $HOOCCOOH \cdot 2H_2O$ [K8519, Special Grade] [6153-56-6]

Palladium Nitrate $Pd(NO_3)_2$ [10102-05-3] Blackish brown deliquescent crystals. Soluble in water with turbidity.

Content 97.0–102.0%.

Assay Weigh accurately about 0.2 g of palladium nitrate, add 2 mL of diluted hydrochloric acid (2 in 3) and 50 mL of water, and dissolve it by heating in a water bath. After cooling, transfer the solution to a 200-mL volumetric flask, and make up to the volume with water. To exactly measured 40 mL of the resulting solution, add exactly 40 mL of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate and 50 mL of water, and adjust the pH to 5 with sodium acetate solution (1 in 5). Boil the solution for 5 minutes, and add 80 mL of water. Add xylenol orange as the indicator, titrate with 0.01 mol/L zinc acetate while maintaining it at pH 5. The endpoint of when the color of the solution changes from yellow to reddish yellow. Separately perform a blank test to make any necessary correction.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate = 2.3043 mg of $Pd(NO_3)_2$

Palladium Nitrate TS To 0.108 g of palladium nitrate, add 10 mL of diluted nitric acid (1 in 2), dilute with water to make exactly 500 mL. Measure exactly 20 mL of this solution, and add water to make exactly 200 mL.

Palmitic Acid $C_{16}H_{32}O_2$ [K8756, Special Grade] [57-10-3]

Panose $C_{18}H_{32}O_{16}$ Use a product suitable for the corresponding enzyme activity tests.

Pararosaniline–Formaldehyde TS Dissolve 40 mg of pararosaniline hydrochloride in 20 mL of hydrochloric acid, and add water to make 100 mL. Mix this solution with an

equal volume of formaldehyde solution (3 in 500) prepared fresh before use.

Pararosaniline Hydrochloride $C_{19}H_{17}N_3 \cdot HCl$ [569-61-9]

Melting point 268–270°C.

Partially Hydrolyzed Saponin for Assay White crystals having slightly odor.

Identification Proceed as directed in the Disk Method under Infrared Spectrophotometry. The spectrum exhibits absorptions at about 3240 cm^{-1}, 2920 cm^{-1}, 1640 cm^{-1}, 1150 cm^{-1}, 1080 cm^{-1}, and 1,020 cm^{-1}.

Purity Related substances Prepare a test solution by dissolving 10 mg of partially hydrolyzed saponin for assay in 20 mL of a 65:35 mixture of 0.1% phosphoric acid/acetonitrile. Prepare a control solution by diluting 4 mL of the test solution, exactly measured, to exactly 100 mL with a 65:35 mixture of 0.1% phosphoric acid/acetonitrile. Analyze 20 μL each of the test solution and the standard solution by liquid chromatography using the conditions given below. Continue the chromatography for 30 minutes, and measure the each peak area. Remove solvent peaks from measurement. The sum the areas of all the peaks of the test solution, other than the main peak, is not greater than the area of the main peak from the control solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 210 nm).

Column: A stainless steel tube of 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 65:35 mixture of 0.1% phosphoric acid/acetonitrile.

Flow rate: Adjust the retention time of partial hydrolyzed saponin to about 10 minutes.

Loss on drying Not more than 2.0% (105°C, 3 hours).

Pectate Lyase [9015-75-2] A water-soluble enzyme obtained from *Aspergillus* sp. It contains glycerol as an enzyme stabilizer. One unit is equivalent to the amount of enzyme required to release 1 μmol of uronic acid polymers with 4-deoxy-α-D-galact-4-enuronic acid residues at the non-reducing terminal from polygalacturonic acid as a substrate in 1 minute at 40°C and pH 8.0.

Pectate Lyase Solution for Pectin Determination Dissolve 1400 units pectate lyase in Tris buffer (pH7.0) for pectin determination to make 100 mL.

Pectin (citrus-derived) Use pectin that is derived from citrus and suitable for the corresponding enzyme activity tests.

Pectin (apple-derived) Use pectin that is derived from apples and suitable for the corresponding enzyme activity tests.

Pectic Acid (citrus-derived) $(C_6H_8O_6)n$ Use pectic acid that is derived from citrus and suitable for the corresponding enzyme activity tests.

Pentaerythritol $C_5H_{12}O_4$ [115-77-5]

Content 47–51%.

Description White crystals, crystalline powder, or granules.

Assay Weigh accurately about 0.4 g of pentaerythritol, add 20 mL of a 9:1 mixture of pyridine/acetic anhydride, and heat in a water bath for 1 hour. After cooling, add 1 mL of water, heat the solution in a water bath for 10 minutes, leave to cool, and add 5 mL of ethanol (95). Titrate the resulting solution with 1 mol/L sodium hydroxide (indicator: 3 drops of phenolphthalein). Separately, perform a blank test to make any necessary correction.

Each mL of 1 mol/L sodium hydroxide = 0.017007 g of $C(CH_2OH)_4$

3-Pentanone $C_5H_{10}O$ [96-22-0] A colorless to light yellow liquid.

Content Not less than 98.0% of 3-pentanone ($C_5H_{10}O$).

Refractive index n_D^{20}: 1.390 –1.396.

Water Not more than 0.2%.

Assay Analyze 0.2-μL portions of 3-pentanone by gas chromatography using the operating conditions given below. Measure the peak area of each peak to determine the percentage of the main peak by the peak area percentage method.

Operating conditions

Detector: Flame ionization detector.

Column: A fused silica tube (0.32 mm internal diameter and 15 m length) coated with a 5.0 μm thick layer of a mixture of 5% phenyl/95% methylpolysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 70°C for 10 minutes, raise at 20°C/minute to 250°C, and maintain the temperature at 250°C for 6 minutes.

Injection port temperature: 250°C.

Detector temperature: 260°C.

Carrier gas: Helium.

Flow rate: A constant rate of about 1.5 mL/min.

Injection method: Split.

Split ratio: 1 : 300.

Perchloric Acid $HClO_4$ [K8223, Special Grade] [7601-90-3]

***o*-Periodic Acid** $I(OH)_5O$ [10450-60-9] White, deliquescent crystals or crystalline powder. Soluble in water and slightly soluble in diethyl ether.

Content Not less than 99.0%.

Identification (1) Prepare a test solution by dissolving 2 g of *o*-periodic acid in 20 mL of water. To 10 mL of the test solution, add 0.1 g of sodium hydrogen carbonate, and then add 0.1 mL of silver nitrate solution (1 in 50). A black precipitate is produced.

(2) To 10 mL of the test solution of (1), add 0.1 mL of hydroxylammonium chloride (1 in 10). A yellow–brown color develops.

Assay Weigh accurately about 1 g of *o*-periodic acid, dissolve it in water to make 250 mL. Place 25 mL of this solution into a 200-mL iodine flask, and add 30 mL of water, 3 g

of potassium iodide, and 5 mL of diluted sulfuric acid (1 in 6). Immediately stopper tightly, gently shake, and allow to stand in a dark place for 10 minutes. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 3 mL of starch TS). Add the indicator near the endpoint, when the solution is a light yellow. The endpoint is when the blue color of the solution disappears. Separately, perform a blank test to make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 2.8493 mg of $I(OH)_5O$

Peroxidase [9003-99-0] A red-brown powder. It is obtained from horse radish. One unit is equivalent to the amount of enzyme required to produce 1 μmol of water from hydrogen peroxide as a substrate in 1 minute at 25°C and pH 7.0.

Peroxidase (horse radish-derived, guaiacol substrate) It is obtained from horse radish. One unit is equivalent to the amount of enzyme required to oxidize 1 μmol of guaiacol as a substrate in 1 minute at 25°C and pH 7.0. Use a product suitable for the corresponding enzyme activity tests.

Peroxidase (horse radish-derived, pyrogallol substrate) It is obtained from horse radish. One unit is equivalent to the amount of enzyme required to produce 1 mg of purpurogallin from pyrogallol as a substrate in 20 seconds at 20°C and pH 6.0. Use a product suitable for the corresponding enzyme activity tests.

Peroxidase TS (25 units/mL) Dissolve peroxidase (horse radish-derived, pyrogallol substrate) in water to make a reagent with an activity of 25 units per 1 mL.

Petroleum Benzine [K8594, Special Grade] [8030-30-6]

Petroleum Ether [K8593, Special Grade] [8032-32-4]

1,10-Phenanthroline Monohydrate $C_{12}H_8N_2 \cdot H_2O$ [K8789, Special Grade] [3829-86-5]

1,10-Phenanthroline TS Dissolve 0.15 g of 1,10-phenanthroline monohydrate in 10 mL of a freshly prepared solution of iron(II) sulfate heptahydrate (37 in 2500). Prepare fresh before use.

1,10-Phenanthrolinium Chloride Monohydrate $C_{12}H_9ClN_2 \cdot H_2O$ [K8202, Special Grade] [3829-86-5]

Phenol C_6H_5OH [K8798, Special Grade] [108-95-2]

Phenol–Nitroprusside TS (Basic) To 8 to 10 mL of sodium hydroxide solution (13 in 50), add 0.1 mL of sodium nitroprusside solution (1 in 100), stir, and add 10 mL of phenol solution in ethanol (5 in 8) and water to make 50 mL. Prepare fresh before use.

Phenolphthalein $C_{20}H_{14}O_4$ [K8799, Special Grade] [77-09-8]

Phenolphthalein–Sodium Carbonate TS Mix 0.5 mL of 2% (w/v) phenolphthalein TS and 0.5 mL of sodium carbonate TS (0.5 mol/L) and add water to the mixture to make 100 mL. Prepare fresh before use.

Phenolphthalein TS Dissolve 1 g of phenolphthalein in 100 mL of ethanol (95).

2% (w/v) Phenolphthalein TS Dissolve 2.0 g of phenolphthalein in 100 mL of ethanol (99.5).

Phenol Red $C_{19}H_{14}O_5S$ [K8800, Special Grade] [143-74-8]

Phenol Red TS Dissolve 0.1 g of phenol red in 100 mL of ethanol (95). Filter if necessary.

Phenol Red TS (pH 4.7)

Solution 1 To 33 mg of phenol red, add 1.5 mL of sodium hydroxide solution (2 in 25) and water to make 100 mL.

Solution 2 Dissolve 25 mg of ammonium sulfate in 235 mL of water, add 105 mL of sodium hydroxide solution (2 in 25) and 135 mL of diluted acetic acid (3 in 25), and mix.

Mix 1 volume of Solution 1 and 19 volumes of Solution 2. If necessary, adjust its pH to 4.7 with the sodium hydroxide solution or acetic acid.

Phenol–Sodium Pentacyanonitrosylferrate(III) TS Dissolve 5 g of phenol and 25 mg of sodium pentacyanonitrosylferrate(III) dihydrate in water to make 500 mL. Store in a cold, dark place.

Phenol TS (0.25 mol/L) Dissolve 23.5 g of phenol in water to make 1000 mL. Store in a glass container protected from light at 30°C. Before use, leave for 24 hours.

L-Phenylalanine $C_9H_{11}NO_2$ [63-91-2] "L-Phenylalanine"

1-Phenylazo-2-naphthalenol $C_{16}H_{12}N_2O$ Sudan I (alias) [842-07-9] Yellowish red lumps or powder.

Content Not less than 98.0%.

Identification Add ethanol (95) to about 0.1 g of 1-phenylazo-2-naphthalenol, accurately weighed, dissolve it ultrasonically, and make exactly 100 mL. A solution prepared by diluting 1 mL of this solution to 100 mL with ethanol (95) exhibits an absorption maximum at a wavelength of 477–483 nm.

Purity (1) Clarity Dissolve 0.10 g of 1-phenylazo-2-naphthalenol ultrasonically in ethanol (95) to make exactly 100 mL. The resulting solution is almost clear.

(2) Related substances Prepare a test solution by dissolving 5 mg of 1-phenylazo-2-naphthalenol in acetonitrile (for HPLC) to make 100 mL. Analyze 10 μL each of the test solution and acetonitrile (for HPLC) by liquid chromatography using operating conditions given below. Measure the area of each peak that appeares within 30 minutes of injection. The area percentage of the main peak of the test solution is not less than 98.0% of the total area of all the peaks, excluding those from the acetonitrile in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 230 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: 40°C.

Mobile phase: A 9:1 mixture of acetonitrile (for HPLC)/water.

Flow rate: 1.0 mL/minute.

Loss on drying Not more than 2.0% (0.5 g, 105°C, 4 hours).

***p*-Phenylenediamine Dihydrochloride** $C_6H_4(NH_2)_2 \cdot 2HCl$ [624-18-0] A white to light

yellow or white to light red crystalline powder. Freely soluble in water.

Clarity of solution Clear (1.0 g, water 10 mL).

Molecular absorption coefficient Dissolve 60 mg of *p*-phenylenediamine dihydrochloride in 100 mL of water. Measure 1.0 mL of the solution, and add phosphate buffer (pH 7) to make 50 mL. Measure the absorbance of this solution at a wavelength of 237–241 nm against phosphate buffer (pH 7). The molecular absorption coefficient is not less than 8000.

Phenylhydrazine $C_6H_5NHNH_2$ [100-63-0] A colorless to light yellow, transparent liquid having a faint aroma. Soluble in diethy ether and slightly soluble in water.

Content Not less than 99.0%.

Assay Analyze 1 μL of phenylhydrazine by gas chromatography using the operating conditions given below. Determine the content from the area of the phenylhydrazine peak and the sum of the areas of all the peaks.

Operating conditions

Detector: Flame-ionization detector or thermal conductivity detector.

Column: A fuse silica tube (0.53 mm internal diameter and 15 m length) coated with a 1.5 μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Upon injection at 100°C, raise the temperature at a rate of 10°C/minute to 250°C.

Injection port temperature: 250°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 5.0 mL/minute.

Injection method: Split.

Split ratio: 1:20.

Measurement time: 15 minutes.

Phenylhydrazinium Chloride $C_6H_5NHNH_2 \cdot HCl$ [K8203, Special Grade] [59-88-1]

Phenylhydrazinium Chloride–Sodium Acetate TS Dissolve 0.5 g of phenylhydrazinium chloride in 10 mL of a solution of sodium acetate trihydrate (2 in 15). Filter if necessary. Prepare fresh before use.

***p*-Phenylphenol** $C_6H_5C_6H_4OH$ [92-69-3] White crystals having a sublimation property. Soluble in ethanol (95) and in diethyl ether. Slightly soluble in petroleum ether.

Melting point 163–167°C.

Water Not more than 0.2%.

Residue on ignition Not more than 0.2%.

***p*-Phenylphenol TS** Dissolve 0.75 g of *p*-phenylphenol in 50 mL of sodium hydroxide solution (1 in 25). Filter if necessary. Prepare fresh before use.

Phosphate Buffer (containing disodium dihydrogen ethylenediaminetetraacetate) Dissolve 24.0 g of disodium hydrogenphosphate, 46.0 g of potassium dihydrogen phosphate, and 0.8 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate in

water to make 1000 mL.

Phosphate Buffer (containing sodium chloride) Dissolve 33.0 g of disodium hydrogenphosphate, 14.0 g of potassium dihydrogen phosphate, and 3.3 g of sodium chloride in water to make 1000 mL.

Phosphate Buffer (0.5 mol/L)

Solution 1 Dissolve 71.0 g of disodium hydrogenphosphate in water to make 1000 mL.

Solution 2 Dissolve 68.0 g of potassium dihydrogen phosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Phosphate Buffer (0.4 mol/L)

Solution 1 Dissolve 54.4 g of potassium dihydrogen phosphate in water to make 1000 mL.

Solution 2 Dissolve 143 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Phosphate Buffer (1/3 mol/L)

Solution 1 Dissolve 47.3 g of disodium hydrogenphosphate in water to make 1000 mL.

Solution 2 Dissolve 45.4 g of potassium dihydrogen phosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Phosphate Buffer (0.2 mol/L)

Solution 1 Dissolve 28.4 g of disodium hydrogenphosphate in water to make 1000 mL.

Solution 2 Dissolve 27.2 g of potassium dihydrogen phosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Phosphate Buffer (0.1 mol/L)

Solution 1 Dissolve 14.2 g of disodium hydrogenphosphate in water to make 1000 mL.

Solution 2 Dissolve 13.6 g of potassium dihydrogen phosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Phosphate Buffer (1/15 mol/L)

Solution 1 Dissolve 9.1 g of potassium dihydrogen phosphate in water to make 1000 mL.

Solution 2 Dissolve 9.5 g of disodium hydrogenphosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Phosphate Buffer (0.05 mol/L)

Solution 1 Dissolve 6.8 g of potassium dihydrogen phosphate in water to make 1000 mL.

Solution 2 Dissolve 17.9 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Phosphate Buffer (0.02 mol/L)

Solution 1 Dissolve 2.84 g of disodium hydrogenphosphate in water to make 1000 mL.

Solution 2 Dissolve 2.72 g of potassium dihydrogen phosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Phosphate Buffer (0.01 mol/L)

Solution 1 Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL.

Solution 2 Dissolve 3.58 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Phosphate Buffer (0.01 mol/L, pH 2.6)

Solution 1 Dissolve 1.56 g of sodium dihydrogenphosphate dihydrate in water to make 1000 mL.

Solution 2 Dissolve 1.15 g of phosphoric acid in water to make 1000 mL.

Mix both solutions, and adjust the pH to 2.6 with both solutions.

Phosphate Buffer (0.01 mol/L, pH 7.0, containing albumin) Dissolve 0.1 g of bovine serum albumin by adding 10 mL of phosphate buffer (0.1 mol/L) at pH 7.0 and water to make 100 mL. Mix 10 mL of this solution and 100 mL of phosphate buffer (0.1 mol/L) at pH 7.0, and add water to make 1000 mL.

Phosphate Buffer (0.005 mol/L)

Solution 1 Dissolve 0.68 g of potassium dihydrogen phosphate in water to make 1000 mL.

Solution 2 Dissolve 1.79 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Phosphate Buffer (pH3.3) Dissolve 12 g of sodium dihydrogenphosphate dihydrate in water to make 1000 mL of a solution. Add phosphoric acid to adjust the pH to 3.3.

Phosphate Buffer (pH 6.2)

Solution 1 Dissolve 9.08 g of potassium dihydrogen phosphate in water to make 1000 mL.

Solution 2 Dissolve 9.46 g of disodium hydrogenphosphate in water to make 1000 mL.

Mix 800 mL of Solution 1 and 200 mL of Solution 2, and adjust the pH to 6.2 with either solution if necessary.

Phosphate Buffer (pH 6.4)

Solution 1 Dissolve 6.80 g of potassium dihydrogen phosphate in water (carbon dioxide-removed) to make exactly 500 mL.

Solution 2 0.2 mol/L sodium hydroxide: Transfer 30 mL of water to a 100-mL polyethylene container, add 36 g of sodium hydroxide in small amounts, stopper, and allow to stand for 4–5 days. Place 10 mL of the supernatant of this solution into a 1000-mL polyethylene container, and add 1000 mL of water. Refer to this solution as Solution A.

Dry amidosulfuric acid (reference material) as directed in its certificate. Weigh accurately 0.4–0.5 g of it into a 100-mL conical beaker, and dissolve it in 25 mL of water. Add a few drops of bromothymol blue as the indicator, and titrate with Solution A. The endpoint is when the color of the solution changes from yellow to bluish green. Calculate the factor of Solution A by the formula:

$f = m/(0.019419 \times V) \times A/100$

f = factor of 0.2 mol/L sodium hydroxide,
m = weight (g) of amidosulfuric acid (reference material) collected,
A = content (%) of amidosulfuric acid (reference material),
V = amount (mL) of 0.2 mol/L sodium hydroxide consumed.

Place exactly 50 mL of Solution 1 and exactly 6.3 mL of Solution 2 into a 100-mL volumetric flask, and make up to the volume with water (carbon dioxide-removed). When the factor of Solution 2 is not 1.000, correct the volme of Solution 2 to be added based on the actual factor.

Phosphate Buffer (pH 6.5) Dissolve 10.5 g of disodium hydrogenphosphate dodecahydrate and 5.8 g of potassium dihydrogen phosphate in 750 mL of water. Adjust the pH to 6.5 with sodium hydroxide TS (1 mol/L), and to add water to make 1000 mL.

Phosphate Buffer (pH 6.5, containing 1,2-cyclohexanediaminetetraacetic acid) Dissolve 2.7 g of potassium dihydrogen phosphate in water to make exactly 100 mL. Adjust the pH to 6.5 with sodium hydroxide TS (0.2 mol/L). Add 0.13 g of 1,2-cyclohexanediaminetetraacetic acid in the resulting solution to dissolve.

Phosphate Buffer (pH 6.8) Mix 3.40 g of potassium dihydrogen phosphate and 3.55 g of disodium hydrogenphosphate, and dissolve the mixture in water to make 1000 mL.

Phosphate Buffer (pH 7)

Solution 1 Dissolve 27.218 g of potassium dihydrogen phosphate for pH determination in water to make 1000 mL.

Solution 2 Use sodium hydroxide TS (0.2 mol.L).

Mix 50.0 mL of Solution 1 and 29.54 mL of Solution 2, and add water to make 200 mL. If necessary, adjust the pH to 7.0 adding either solution.

Phosphate Buffer (pH 7.1)

Solution 1 Dissolve 21.2 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Solution 2 Dissolve 8.2 g of potassium dihydrogen phosphate in water to make 1000 mL.

Mix 2 volumes of Solution 1 and 1 volume of Solution 2, and adjust the pH to 7.1 using either solution.

Phosphate Buffer (pH 7.3) Dissolve 138 g of sodium dihydrogen phosphate dihydrate in 800 mL of water. Adjust the pH of the solution to 7.3 with sodium hydroxide solution (1 in 2), and add water to make 1000 mL.

Phosphate Buffer (pH 7.5)

Solution 1 Dissolve 53.7 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Solution 2 Dissolve 20.4 g of potassium dihydrogen phosphate in water to make 1000 mL.

Mix 21 volumes of Solution 1 and 4 volumes of Solution 2, and adjust the pH to 7.5, using either Solution 1 or 2.

Phosphate Buffer (pH 7.6)

Solution 1 Dissolve 4.54 g of potassium dihydrogen phosphate in water in water to make 500 mL.

Solution 2 Dissolve 4.73 g of disodium hydrogenphosphate in water to make 500 mL.

Mix 13 volumes of Solution 1 and 87 volumes of Solution 2, and adjust the pH to 7.6, using either Solution 1 or 2.

Phosphate Buffer (pH 8)

Solution 1 Dissolve 23.88 g of disodium hydrogenphosphate in water to make 1000 mL.

Solution 2 Dissolve 9.07 g of potassium dihydrogen phosphate in water to make 1000 mL.

Mix 50 volumes of Solution 1 and 7 volumes of Solution 2, and adjust the pH to 8 using both solutions.

L-α-Phosphatidylinositol Sodium Salt Use a product suitable for the corresponding enzyme activity tests.

Phosphinic Acid H_3PO_2 [6303-21-5] A colorless to slightly pale yellow, viscous liquid. The density is 1.13 g/mL

Content 30.0–32.0%.

Assay Weigh accurately about 1.0 g of phosphinic acid, dissolve it in water to make exactly 250 mL. Transfer exactly 25 mL of this solution into a 300-mL ground glass stoppered iodine flask, and add exactly 40 mL of 0.05 mol/L bromine, 100 mL of water, and 10 mL of diluted sulfuric acid (1 in 6). Shake the mixture gently, and allow to stand for 3 hours. Add 20 mL of potassium iodide solution (1 in 10), and titrate with 0.1 mol/L sodium thiosulfate. Add 3 mL of starch TS near the endpoint when the solution is light yellow. The endpoint is when the blue color of the solution disappears. Separately, perform a blank test.

Each mL of 0.1 mol/L sodium thiosulfate = 1.6499 mg of H_3PO_2

Phosphoglucomutase Use a product suitable for the corresponding enzyme activity tests. Derived from the muscles of rabbits. One unit of phosphoglucomutase is equivalent to the amount of the enzyme required to transform α-D-glucose-1-phosphoric acid as the substrate to 1 μmol of α-D-glucose-6-phosphoric aicd per 1 minute at pH 7.4 and 30°C. Phosphoglucomutase contains 2.0–15.0 mg of protein per mL and has not less than 100 units of activity per 1 mg of protein. This substance contains 0.01% (w/v) disodium dihydrogen ethylenediaminetetraacetate and 3.2 mol/L ammonium sulfate.

Phosphomolybdic Acid *n*-Hydrate $H_3(PMo_{12}O_{40})\cdot nH_2O$ [51429-74-4] Yellow crystals or crystalline powder. Freely soluble in water and in diethyl ether.

Identification (1) A 10 mL solution (1 in 10) of phosphomolybdic acid *n*-hydrate produces a yellow precipitate when 2 mL of ammonia TS is added, but the precipitate dissolves when 2 mL of ammonia TS is added. The solution produces a yellow precipitate again when 5 mL of diluted nitric acid (1 in 2) is added.

(2) A 5 mL solution (1 in 10) of phosphomolybdic acid *n*-hydrate produces a white precipitate when 1 mL of ammonia TS and 1 mL of magnesia TS are added.

Phosphoric Acid H_3PO_4 [K9005, Special Grade] [7664-38-2]

Phosphoric Acid TS (0.1 mol/L) Add water to 11.5 g of phosphoric acid to make 1000 mL.

Phosphoric Acid–Tetra-*n*-butylammonium Bromide TS Dissolve 1 mL of phosphoric acid and 3.22 g of tetra-*n*-butylammonium bromide in water to make 1000 mL.

Phosphorous(V) Oxide P_2O_5 [K8342, Special Grade] [1314-56-3]

***o*-Phthalaldehyde** $C_6H_4(CHO)_2$ [643-79-8] Light yellow to yellow crystals.

Purity Related substances Prepare a test solution by dissolving 1 g of *o*-phthalaldehyde in 10 mL of ethanol (95). Prepare a control solution by diluting exactly 1 mL of the test solution to exactly 100 mL with ethanol (95). Analyze 10 μL each of the test solution and the control solution by gas chromatography using the operating conditions given below. Continue the chromatography for seven times the retention time of the main peak, and measure the peak areas. Exclude the solvent peaks from

measurement. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from control solution.

Operating conditions

Detector: Thermal conductivity detector.

Column: A glass tube of 3 mm internal diameter and 2 m length.

Column packing material

Liquid phase: 10% methyl silicone polymer of the amount of solid support.

Solid phase: 177- to 250-μm diatomaceous earth for gas chromatography, treated with acid and silane.

Column temperature: A constant temperature of about 180°C.

Carrier gas: Helium.

Flow rate: Aadjust the retention time of *o*-phthalaldehyde to about 3–4 minutes at a constant rate about 50 mL/minute.

Phthalaldehyde TS Dissolve 40 mg of *o*-phthalaldehyde in 1 mL of methanol. To this solution, add 1 mL of sodium tetraborate decahydrate solution (1 in 50) and 50 μL of 2-melcaptoethanol, and mix. Store in a tightly stoppered, light-resistant bottle. Use within 1 week of the preparation.

***o*-Phthalaldehyde TS (for peptidase activity test)** Dissolve 40 mg of *o*-phthalaldehyde in 1 mL of ethanol (99.5). To this solution, add 25 mL of sodium tetraborate TS (0.1 mol/L), 2.5 mL of sodium lauryl sulfate solution (1 in 5), 0.1 mL of 2-melcaptoethanol, and water to make 50 mL.

Phthalic Acid $C_8H_6O_4$ [88-99-3] A white crystalline powder. Freely soluble in methanol but slightly soluble in water and in diethyl ether.

Content Not less than 99.0% of phthalic acid ($C_8H_6O_4$).

Purity Other Aromatic Compounds Weigh accurately 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. To 10.0 mL of this solution, add a 7:3 mixture of diluted acetic acid (1 in 100)/methanol to make exactly 100 mL. Analyze the resulting solution by liquid chromatography using the operating conditions specified in Purity (5) for Benzoic Acid in the Monographs. Only one peak of phthalic acid is observed.

Assay Weigh accurately about 2 g of phthalic acid, dissolve it in 50 mL of ethanol (neutralized), and titrate with 0.1 mol/L sodium hydroxide (indicator: a few drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide = 8.307 mg of $C_8H_6O_4$

Phthalic Anhydride $C_6H_4(CO)_2O$ [85-44-9]

Content Not less than 99.5%.

Description White crystals or crystalline powder or flakes.

Identification Determine the infrared absorption spectrum of phthalic anhydride as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 1860 cm^{-1}, 1770 cm^{-1}, 1610 cm^{-1}, 1480 cm^{-1}, 1370 cm^{-1}, 1260

cm^{-1}, 1120 cm^{-1}, 910 cm^{-1}, and 720 cm^{-1}.

Melting point 131−133°C.

Assay Weigh accurately about 2.0 g, dissolve it by adding exactly 50 mL of 1 mol/L sodium hydroxide, titrate this solution with 1 mol/L hydrochloric acid (indicator: 3 drops of phenolphthalein). The end point is when the color of the solution disappear.

Each mL of 1 mol/L sodium hydroxide = 74.06 mg of $C_6H_4(CO)_2O$

Phytonadione $C_{31}H_{46}O_2$ [84-80-0] Use phytonadione specified in the Japanese Pharmacopoeia.

Polyethylene Glycol 600 [25322-68-3] A product with the average molecular weight of 560–640.

Description A colorless to pale yellow, clear liquid or white lumps.

Identification Dissolve 50 mg of polyethylene glycol 600 in 5 mL of 10% hydrochloric acid TS, add 1 mL of a solution of barium chloride dihydrate (3 in 25), and mix. Filter the mixture if necessary. Add 1 mL of phosphomolybdic acid *n*-hydrate solution (1 in 10). A yellow-green precipitate is produced.

pH 4.0–7.0 (5 g, water 100 mL, 25°C).

Viscosity 100–150 mPa・s (25°C). Measure the viscosity with 200 mL of the sample using a rotational viscometer.

Congealing point 15–25°C.

Purity Acid Not more than 0.1% as CH_3COOH. Dissolve 10 g of polyethylene glycol 600 in 50 mL of water (carbon dioxide-removed), and add 3 drops of phenolphthalein solution. Titrate with 0.1 mol/L sodium hydroxide. One mL of 0.1 mol/L sodium hydroxide is equivalent to 6.005 mg of CH_3COOH.

Water Not more than 0.3% (2 g, Volumetric Titration, Direct Titration).

Average molecular weight 560–640. Place 42 g of phthalic anhydride into a 1-L light resistant stoppered bottle containing 300 mL of newly distilled pyridine, mix vigorously to dissolve, and allow to stand at least 16 hours. Measure exactly 25 mL of the obtained solution into a 200-mL pressure-resistant bottle with a stopper, add about 2.4 g of polyethylene glycol 600, accurately weighed, and stopper tightly. Wrap the bottle with strong cloth, and warm in a 98 ± 2°C water bath for 30 minutes. Maintain the bottle so that its contents are under the water. Take it out of the bath, and cool to room temperature in air. Add exactly 50 mL of 0.5 mol/L sodium hydroxide, then add 5 drops of phenolphthalein solution (1 in 100) in pyridine, and titrate with 0.5 mol/L sodium hydroxide. The endpoint is when the solution remains light red for 15 seconds. Separately, perform a blank test.

Average molecular weight = Weight (g) of the sample × 4000/(a − b)

a: the volume (mL) of 0.5 mol/L sodium hydroxide consumed in the blank test,

b: the volume (mL) of 0.5 mol/L sodium hydroxide consumed in the test.

Polyethylene Glycol 8000 $H(OCH_2CH_2)_nOH$ Use a product suitable for the corresponding enzyme activity tests.

ε-Polylysine Hydrochloride for Assay [26124-78-7] A white to light yellow powder.

Identification Add 1 mL of methyl orange TS to a solution of 0.1 g of ε-polylysine hydrochloride for assay in 100 mL of phosphate buffer (pH 6.8). A red-brown precipitate is formed.

Purity Related substances Prepare a test solution by dissolving 15 mg of ε-polylysine hydrochloride for assay in 100 mL of the same mobile phase used in the Assay for ε-Polylysine in the Monographs. Prepare a control solution by diluting 2 mL of the test solution, measured exactly, to exactly 100 mL with the mobile phase. Analyze 100 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for about two times the retention time of the main peak, and measure the peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Use the conditions specified in the Assay for ε-Polylysine in the Monographs.

Polyoxyethylene(23) Lauryl Ether [9002-92-0] Use lauromacrogol specified in the Japanese Pharmacopoeia.

Polyoxyethylene(23) Lauryl Ether TS Add water to 15 g of polyoxyethylene(23) lauryl ether to make 100 mL.

Polyoxyethylene(10) Octylphenyl Ether $(C_2H_4O)_nC_{14}H_{22}O$ 4-(1,1,3,3-tetramethylbutyl)phenyl polyethylene glycol (alias) Use a product suitable for the corresponding enzyme activity tests.

Polyoxyethylene(10) Octylphenyl Ether TS Dissolve 10 g of polyoxyethylene(10) octylphenyl ether in potassium phosphate buffer (0.2 mol/L) at pH 7.0 to make 100 mL.

Polysorbate 20 [9005-64-5] Polysorbate 20 is mainly obtained by addition polymerization of ethylene oxide to sorbitan monolaurate. A pale yellow to yellow liquid having a slight characteristic odor.

Identification (1) To 0.5 g of polysorbate 20, add 10 mL of water and 10 mL of sodium hydroxide TS (1 mol/L), and boil for 5 minutes. Acidify with 10% hydrochloric acid TS, and oily materials are separated.

(2) Weigh 5 g of polysorbate 20, and saponify it as directed under the Fats and Related Substances Tests, and evaporate the ethanol completely. Add 50 mL of water, and dissolve the residue. Acidify with hydrochloric acid (indicator: methyl orange), extract twice with 30-mL portions of diethyl ether. Combine the diethyl ether layers, and wash repeatedly with 20-mL portions of water until the washing becomes neutral. Evaporate diethyl ether on the water bath. The acid value of the residue is 275–285. For saponification, use 50 mL of 3.5% (w/v) potassium hydroxide–ethanol TS.

Acid value Not more than 4.0.

Saponification value 43–55 (Fats and Related Substances Tests).

Loss on drying Not more than 3.0% (5 g, 105°C, 1 hour).

Residue on ignition Not more than 1.0%. Weigh accurately about 3 g of polysorbate 20, heat gently at first, then gradually ignite (800–1,200°C), and incinerate completely. If carbonized material remains, add hot water and leach. Filter through a filter paper for quantitative analysis (No. 5C). Ignite the residue together with the filter paper. To the residue, add filtrate, and evaporate to dryness. Ignite carefully until the carbonized material disappears. When carbonized material still remains, add 15 mL of ethanol (95), crush the carbonized materials with a glass rod, burn the ethanol, and then re-ignite carefully. Allow to cool in a desiccator containing silica gel, and weigh accurately.

50% Polysorbate 20 TS Mix polysorbate 20 and water in the same weight ratio, and autocrave at 121°C for 15 minutes.

Polysorbate 80 [9005-65-6] Use polysorbate 80 specified in the Japanese Pharmacopoeia.

Polyvinyl Alcohol I $(-CH_2CHOH-)$ Use a product suitable for the corresponding enzyme activity tests.

Description Colorless to white or pale yellow granules or powder, and odorless or has a little of acetic acid. Practically insoluble in ethanol (95) and in diethyl ether. It is hygroscopic. When heated with water, it is a clear viscous liquid.

Viscosity 25.0–31.0 mm^2/s. Weigh 4.00 g of polyvinyl alcohol I, previously dried, add 95 mL of water, and allow to stand for 30 minutes. Dissolve it by heating on a water bath under a reflux condenser for 2 hours while stirring. After cooling, make 100.0 g with water, and mix. Leave it until the bubbles disappear. Perform the test at a temperature of 20°C as directed in Method 1 of Viscosity Measurement.

pH 5.0–8.0 (1.0 g, water 25 mL).

Degree of Saponification value 98.0–99.0 (mol%). Weigh accurately about 3.0 g of polyvinyl alcohol I, dried, into a stoppered Erlenmeyer flask, add 100 mL of water, dissolve it by heating on a water bath. After cooling, add 25 mL of sodium hydroxide TS (0.1 mol/L), stopper the flask, and allow to stand for 2 hours. Add 30 mL of sulfuric acid TS (0.05 mol/L), shake the flask well, and titrate with sodium hydroxide TS (0.1 mol/L) (3 drops of phenolphthalein TS). Separately, perform a blank test to make any necessary correction. If the amount of sodium hydroxide TS (0.1 mol/L) consumed is 25 mL of more, about 2.0 g of the sampe should be used.

$$\text{Degree of saponification (mol/\%)} = 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A = \frac{0.6005 \times (a - b) \times f}{\text{Weight (g) of the sample}}$$

a = amount (mL) of sodium hydroxide TS (0.1 mol/L) consumed,
b = amount (mL) of sodium hydroxide TS (0.1 mol/L) consumed in the blank test,
f = factor of sodium hydroxide TS (0.1 mol/L).

Purity Clarity Add 1.0 g of polyvinyl alcohol I to 20 mL of water, disperse it by shaking well, warm at 60–80°C for 2 hours, and cool. The liquid obtained is colorless and clear.

Polyvinyl Alcohol I–Polyvinyl Alcohol II TS Weigh 18 g of polyvinyl alcohol I and 2 g of polyvinyl alcohol II, add 800 mL of water, dissolve them by heating at 75–80°C for about 1 hour while stirring, and cool. Filter if necessary. Make up to 1000 mL with water.

Polyvinyl Alcohol I TS To 20 g of polyvinyl alcohol, add 800 mL of water, dissolve it by heating at 75–80°C for about 1 hour while stirring, and cool. Filter if necessary. Make up to 1000 mL with water.

Polyvinyl Alcohol II $(-CH_2CHOH-)$ Use a product suitable for the corresponding enzyme activity tests.

Description Colorless to white or pale yellow granules or powder, and odorless or has a little of acetic acid. Practically insoluble in ethanol (95) and in diethyl ether. It is hygroscopic. When warmed with water, it is a clear viscous liquid.

Viscosity 4.6–5.4 mm^2/s. Weigh 4.00 g of polyvinyl alcohol II, previously dried, add 95 mL of water, and allow to stand for 30 minutes. Dissolve it by heating at 60–80°C for 2 hours while stirring. After cooling, make 100.0 g with water, and mix. Leave it until the bubbles disappear. Perform the test at a temperature of 20°C as directed in Method 1 of Viscosity Measurement.

pH 5.0–8.0 (1.0 g, water 25 mL).

Degree of Saponification 86.5–89.5 (mol%). Weigh accurately about 2.0 g of polyvinyl alcohol II, previously dried, into a stoppered Erlenmeyer flask, add 100 mL of water, dissolve it by heating while stirring. After cooling, add 25 mL of sodium hydroxide TS (0.5 mol/L), stopper the flask, and allow to stand for 2 hours. Add 30 mL of sulfuric acid TS (0.25 mol/L), shake the flask well, and titrate with sodium hydroxide TS (0.5 mol/L) (3 drops of phenolphthalein TS). Separately, perform a blank test to make any necessary correction.

$$\text{Degree of saponification (mol/\%)} = 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A = \frac{3.0025 \times (a - b) \times f}{\text{Weight (g) of the sample}}$$

a = amount (mL) of sodium hydroxide TS (0.5 mol/L) consumed,

b = amount (mL) of sodium hydroxide TS (0.5 mol/L) consumed in the blank test,

f = factor of sodium hydroxide TS (0.5 mol/L).

Purity Clarity Add 1.0 g of polyvinyl alcohol II to 20 mL of water, disperse it by shaking well, warm on a water bath for 2 hours, and cool. The liquid obtained is colorless and clear.

Potassium Acetate CH_3COOK [K8363, Special Grade] [127-08-2]

Potassium Antimonyl Tartrate TS Dissolve 1.37 g of bis[(+)-tartrato]diantimonate(III)dipotassium trihydrate by adding 350 mL of water gradually, and make up with water to 500 mL.

Potassium Bromate $KBrO_3$ [K8530, Special Grade] [7758-01-2]

Potassium Bromate–Potassium Bromide TS Mix 1.4 g of potassium bromate and 8.1 g of potassium bromide, dissolve the mixture in water to make 100 mL.

Potassium Bromide KBr [K8506, Special Grade] [7758-02-3]

Potassium Bromide for Infrared Absorption Spectrophotometry A powder prepared by crushing potassium bromide single crystals or potassium bromide, passing it through a 74-μm standard sieve, and drying the at 120°C for 10 hours or at 500°C for 5 hours. The infrared spectrum of a disk formed with this powder shows no characteristic absorption.

Potassium Carbonate K_2CO_3 [K8615, Special Grade] [584-08-7]

Potassium Chlorate $KClO_3$ [K8207, Special Grade] [3811-04-9]

Potassium Chloride KCl [K8121, Special Grade, Reagent for Electrical Conductivity Determination] [7447-40-7]

Potassium Chloride–Hydrochloric Acid TS Weigh 14.9 g of potassium chloride, and add water to make 1000 mL. Confirm that its pH range is 5.2–7.2.

Potassium Chloride TS (0.2 mol/L) Weigh 250 g of potassium chloride, and add 8.5 mL of hydrochloric acid and 750 mL of water to dissolve it.

Potassium Chromate K_2CrO_4 [K8312, Special Grade] [7789-00-6]

Potassium Cyanide KCN [K8443, Special Grade] [151-50-8]

Potassium Dichromate $K_2Cr_2O_7$ [K8517, Special Grade] [7778-50-9]

Potassium Dichromate (Reference Material) $K_2Cr_2O_7$ [Reference Material for Volumetric Analysis, K8005] [7778-50-9]

In addition to Reference Material for Volumetric Analysis specified in JIS K8005, a certified reference material capable of being used for volumetric analysis is usable.

Potassium Dihydrogen Phosphate KH_2PO_4 [K9007, Special Grade] [7778-77-0]

Potassium Dihydrogen Phosphate for pH Determination KH_2PO_4 [K9007, pH Standard Solution Grade] [7778-77-0]

Potassium Dihydrogen Phosphate TS (0.2 mol/L, containing disodium dihydrogen ethylenediaminetetraacetate dihydrate) Dissolve 5.4 g of potassium dihydrogen and 74 mg of disodium dihydrogen ethylenediaminetetraacetate dihydrate in water to make 200 mL.

Potassium Dihydrogen Phosphate TS (0.02 mol/L) Dissolve 2.72 g of potassium dihydrogen in water to make 1000 mL.

Potassium Hexacyanoferrate(II) Trihydrate $K_4Fe(CN)_6 \cdot 3H_2O$ [K8802, Special Grade] [14459-95-1]

Potassium Hexacyanoferrate(III) $K_3[Fe(CN)_6]$ [K8801, Special Grade] [13746-66-2]

Potassium Hexacyanoferrate(III) TS (0.05 mol/L) Dissolve 16.5 g of potassium hexacyanoferrate(III) and 22 g of sodium carbonate in water to make 1000 mL.

Potassium Hexacyanoferrate(III) TS (0.025 mol/L) Dissolve 1.65 g of potassium hexacyanoferrate(III) and 2.12 g of sodium carbonate in water to make 200 mL. Before use, leave it in dark place for 2–3 days.

Potassium Hexahydroxoantimonate(V) $K[Sb(OH)_6]$ [12208-13-8] White granules or crystalline powder. Slightly soluble in water.

Sensitivity Add water to 1.0 g of potassium hexahydroxoantimonate(V) to make 100 mL, and dissolve it by heating in a water bath. Add 0.2 mL of sodium chloride solution (1 in 10) to 20 mL of the resulting solution while keeping it at 20°C, and leave for 10 minutes. Crystals are formed.

Potassium Hexahydroxoantimonate(V) TS Weigh 2 g of potassium hexahydroxoantimonate, add 100 mL of water, boil for about 5 minutes, and cool quickly. Add 10 mL of potassium hydroxide solution (3 in 20), allow to stand for 24 hours, and filter.

Potassium Hydrogen Phthalate (Reference Material) $C_6H_4(COOK)(COOH)$ [Reference Material for Volumetric Analysis, K8005] [877-24-7] In addition to Reference Material for Volumetric Analysis specified in JIS K8005, a certified reference material capable of being used for volumetric analysis is usable.

Potassium Hydrogen Phthalate for pH Determination $C_6H_4(COOK)(COOH)$ [K8809, pH Standard Solution Grade] [877-24-7]

Potassium Hydrogen Sulfate $KHSO_4$ [K8972, Special Grade] [7646-93-7]

Potassium Hydroxide KOH [K8574, Special Grade] [1310-58-3]

10% (w/v) Potassium Hydroxide–Ethanol TS Dissolve 10 g of potassium hydroxide in ethanol (95) to make 100 mL. Prepare fresh before use.

3.5% (w/v) Potassium Hydroxide–Ethanol TS Dissolve 35 g of potassium hydroxide in 20 mL of water, and add ethanol (95) to make 1000 mL. Stopper tightly, and store.

Potassium Hydroxide Solution (Highly Purified) KOH [1310-58-3]

Content 40.0–50.0%.

Assay Weigh accurately about 2 g of potassium hydroxide solution (highly purified) into a 200-mL ground-glass stoppered Erlenmeyer flask, and dissolve it in 50-mL of water (carbon dioxide-removed). Stopper the flask, and leave it for 5 minutes. Titrate the resulting solution with 1 mol/L hydrochloric acid. To confirm the endpoint, use a potentiometer or an indicator (3 drops of phenolphthalein TS). For a potentiometer, use a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes. If the indicator is used, the endpoint is when the faint red color of the solution persists for 30 seconds.

Each mL of 1 mol/L hydrtochloric acid = 56.11 mg of KOH

Potassium Hydroxide Solution (for Semiconductor) KOH [1310-58-3]

Content 40.0–50.0%.

Assay Weigh accurately about 2 g of potassium hydroxide solution (for

semiconductor) into a 200-mL ground-glass stoppered Erlenmeyer flask, and dissolve it in 50-mL of water (carbon dioxide-removed). Stopper the flask, and leave it for 5 minutes. Titrate the resulting solution with 1 mol/L hydrochloric acid. To confirm the endpoint, use a potentiometer or an indicator (3 drops of phenolphthalein TS). A potentiometer is used, use a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes. If the indicator is used, the endpoint is when the faint red color of the solution persists for 30 seconds.

Each mL of 1 mol/L hydrochloric acid = 56.11 mg of KOH

Potassium Hydroxide TS (0.01 mol/L) Dilute 1 mol/L potassium hydroxide to 100 times its original volume with water (carbon dioxide-removed). Store in a tightly stoppered container made of resin, like polyethylene.

Potassium Iodate KIO_3 [K8922, Special Grade] [7758-05-6]

Potassium Iodate (Reference Material) KIO_3 [Reference Material for Volumetric Analysis, K8005] [7758-05-6]

Potassium Iodate TS Dissolve 7.1 g of potassium iodate (reference material) in water to make 1000 mL. Store protected from light.

Potassium Iodate TS (0.05 mol/L) Dissolve 1.07 g of potassium iodate in water to make 100 mL. Store protected from light.

Potassium Iodide KI [K8913, Special Grade] [7681-11-0] In addition to Reference Material for Volumetric Analysis specified in JIS K8005, a certified reference material capable of being used for volumetric analysis is usable.

Potassium Iodide–Starch Paper Immerse a piece of filter paper in potassium iodide–starch TS, freshly prepared, and dry the filter paper in a clean room. Store in a glass-stoppered bottle, protected from light and moisture.

Potassium Iodide–Starch TS Dissolve 0.5 g of starch (soluble) in 50 to 60 mL of water by heating. Add 0.5 g of potassium iodide and water to dissolve it, and dilute with water to make 100 mL.

Potassium Iodide TS Dissolve 16.5 g of potassium iodide in water to make 100 mL. Store protected from light.

Potassium Iodide TS (for β-amylase/invertase activity test) Dissolve 30 g of potassium iodide in 70 mL of water. Prepare fresh before use.

50% (w/v) Potassium Iodide TS Dissolve 50 g of potassium iodide in water to make 100 mL, and add 2 drops of sodium hydroxide solution (1 in 2).

Potassium Nitrate KNO_3 [K8548, Special Grade] [7757-79-1]

Potassium Periodate KIO_4 [K8249, Special Grade] [7790-21-8]

Potassium Permanganate $KMnO_4$ [K8247, Special Grade] [7722-64-7]

Potassium Phosphate Buffer (1 mol/L)

Solution 1 Dissolve 174 g of dipotassium hydrogenphosphate in water to make 1000 mL.

Solution 2　Dissolve 136 g of potassium dihydrogen phosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Potassium Phosphate Buffer (0.4 mol/L)

Solution 1　Dissolve 54.4 g of potassium dihydrogen phosphate in water to make 1000 mL.

Solution 2　Dissolve 69.7 g of dipotassium hydrogenphosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Potassium Phosphate Buffer (0.2 mol/L)

Solution 1　Dissolve 27.2 g of potassium dihydrogen phosphate in water to make 1000 mL.

Solution 2　Dissolve 34.8 g of dipotassium hydrogenphosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Potassium Phosphate Buffer (0.1 mol/L)　Dissolve 5.3 g of potassium dihydrogen phosphate and 10.6 g of dipotassium hydrogenphosphate in 950 mL of water. Adjust the pH with sodium hydroxide TS (2 mol/L) or hydrochloric acid TS (2 mol/L) to the corresponding value specified in this publication.

Potassium Phosphate Buffer (0.05 mol/L)

Solution 1　Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL.

Solution 2　Dissolve 8.71 g of dipotassium hydrogenphosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Potassium Phosphate Buffer (0.02 mol/L)

Solution 1　Dissolve 3.5 g of dipotassium hydrogenphosphate in water to make 1000 mL.

Solution 2　Dissolve 2.7 g of potassium dihydrogen phosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Potassium Phosphate Buffer (0.005 mol/L)

Solution 1　Dissolve 0.68 g of potassium dihydrogen phosphate in water to make 1000 mL.

Solution 2　Dissolve 0.87 g of dipotassium hydrogenphosphate in water to make 1000

mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Potassium Phosphate Buffer (0.005 mol/L, pH 7.0, containing zinc sulfate) To 1 mL of zinc sulfate heptahydrate solution (18 in 3125), add potassium phosphate buffer (0.005 mol/L) at pH 7.0 to make 100 mL.

Potassium Phosphate Buffer (pH 6.5, containing magnesium sulfate and disodium dihydrogen ethylenediaminetetraacetate) Dissolve 8.8 g of potassium dihydrogen phosphate and 6.1 g of dipotassium hydrogenphosphate in 900 mL of water. Add 10 mL of magnesium sulfate TS (0.1 mol/L), 10 mL of disodium dihydrogen ethylenediaminetetraacetate (0.005 mol/L), and water to make 1000 mL. Confirm that the pH is 6.50 ± 0.05.

Potassium Phosphate–Phosphoric Acid Buffer (1 mol/L) Dissolve 136 g of potassium dihydrogen phosphate in 800 mL of water, and adjust the pH with diluted phosphoric acid (67 in 1000) or sodium hydroxide TS (1 mol/L) to the corresponding value specified in this publication. Add water to make 1000 mL.

Potassium Phosphate–Sodium Hydroxide Buffer (0.2 mol/L) Dissolve 27.2 g of potassium dihydrogen phosphate in 800 mL of water, and adjust the pH with sodium hydroxide TS (2 mol/L) to the corresponding value specified in this publication. Add water to make 1000 mL.

Potassium Phosphate–Sodium Hydroxide Buffer (0.1 mol/L) Dissolve 13.6 g of potassium dihydrogen phosphate in 800 mL of water, and adjust the pH with sodium hydroxide TS (1 mol/L) to the corresponding value specified in this publication. Add water to make 1000 mL.

Potassium Phosphate–Sodium Hydroxide Buffer (0.1 mol/L, pH 7.0, containing phenol) Dissolve 1.36 g of potassium dihydrogen phosphate in 80 mL of water, add 3 mL of phenol solution (1 in 20) and 3 mL of polyoxyethylene(10) octyl phenyl ether solution (1 in 20). Adjust the pH to 7.0 with sodium hydroxide TS (1 mol/L), and add water to make 1000 mL.

Potassium Pyrophosphate $K_4O_7P_2$ [7320-34-5] A white crystalline powder. Very soluble in water.

Melting point 1109°C.

Potassium Pyrophosphate–Hydrochloric Acid Buffer (0.05 mol/L, pH9.0) Dissolve 0.83 g of potassium pyrophosphate in 40 mL of water. To this solution, add 1 mol/L hydrochloric acid to adjust its pH to 9.0, and add water to make 50 mL. Adjust the temperature to 22 ± 2°C before use.

Potassium Sodium (+)-Tartrate Tetrahydrate $NaOOCCH(OH)CH(OH)COOK \cdot 4H_2O$ [K8536, Special Grade] [6381-59-5]

Potassium Sulfate K_2SO_4 [K8962, Special Grade] [7778-80-5]

Potassium Thiocyanate KSCN [K9001, Special Grade] [333-20-0]

Potassium Trihydrogen Dioxalate Dihydrate for pH Determination $KH_3(C_2O_4)_2 \cdot 2H_2O$ [K8474, pH Standard Solution Grade] [6100-20-5]

Potate Starch Use a product suitable for the corresponding enzyme activity tests.

L-Proline *p*-Nitroanilide Trifluoroacetate Salt $C_{11}H_{13}N_3O_3 \cdot C_2HF_3O_2$ Use a product suitable for the corresponding enzyme activity tests.

1-Propanol $CH_3CH_2CH_2OH$ [K8838, Special Grade] [71-23-8]

2-Propanol $(CH_3)_2CHOH$ [K8839] [67-63-0]

2-Propanol for Vitamin A Determination Determine the absorbance of 2-propanol for vitamin A determination, using water as the reference. It is not more than 0.01 at 320–350 nm and not more than 0.05 at 300 nm.

Propiconazole for Assay $C_{15}H_{17}Cl_2N_3O_2$ [60207-90-1] A transparent viscous liquid or a colorless to yellow semi-gelatinous substance.

Content Not less than 97.0% of propiconazole ($C_{15}H_{17}Cl_2N_3O_2$).

Identification Measure the absorption spectrum of propiconazole for assay as directed in the Liquid Film Method under Infrared Spectrophotometry. It exhibits absorptions at about 2960 cm-1, 2870 cm-1, 1587 cm-1, 1506 cm-1, and 1466 cm-1, 1273 cm-1, 1138 cm-1, and 1028 cm-1. Use optical plates made from sodium chloride.

Specific gravity d_{20}^{20}: 1.288–1.290.

Assay Weigh accurately about 40 mg of propiconazole for assay and about 4 mg of 1,4-BTMSB-d_4, and dissolve them together in 4 mL of deuterated acetone. Transfer the resulting solution to an NMR tube of 5 mm in external diameter, stopper tightly, and measure ^{1}HNMR spectra using an NMR spectrometer with a proton resonance frequency of 400 MHz or more. Assuming the signal of 1,4-BTMSB-d_4 as δ0.00 ppm, determine the signal area intensity (A) (corresponding to 1 hydrogen) at around δ 7.05–7.13 ppm. Assuming the signal area intensity of 1,4-BTMSB-d_4 as 18.00, when the conversion value of A and the purity of 1,4-BTMSB-d_4 are designated as I and P(%), respectively, determine the content of propiconazole by the following formula. Confirm that the signal from propiconazole around δ 7.05–7.13 ppm is not overlapped with that from a contaminant.

$$\text{Content (\%) of propiconazole } (C_{15}H_{17}Cl_2N_3O_2) = \frac{\text{Weight (mg) of 1,4 BTMSB}-d_4 \times \text{I} \times \text{P}}{\text{Weight (mg) of the sample}} \times 1.511$$

Operating conditions

Digital resolution: Not more than 0.25.

Spinning: Off.

^{13}C decoupling: Present.

Acquisition time: Not less than 4 seconds.

Spectral width: Not less than 20 ppm including between −5 ppm and 15 ppm.

Flip angle: 90°.

Relaxation delay time: 64 seconds.

Dummy scans: 2 or more.

Number of accumulation: Not less than 8.

Temperature at measurement: A constant temperature of 20–30°C.

Propionic Acid C_2H_5COOH [79-09-4] "Propionic Acid"

Propylene Carbonate $C_4H_6O_3$ [108-32-7] A colorless liquid.

Boiling point 240–242°C.

Water Not more than 1 mg/g.

Propylene Carbonate for Water Determination To 1000 mL of propylene carbonate, add 30 g of synthetic zeolite for desiccation, stopper, and allow to stand for about 8 hours with occasional gentle shaking. Leave it for about additional 16 hours, and collect clear propylene carbonate. Store, protected from moisture. It shall not contain more than 0.3 mg of water per mL.

Propylene Chlorohydrin $CH_3CH(OH)CH_2Cl$ [127-00-4] A colorless to pale yellow liquid. Soluble in water, in ethanol (95), and in diethyl ether.

Content 70% of propylene chlorohydrin and 30% of 2-chloro-1-propanol.

Refractive index n_D^{20}: 1.4390–1.4410.

Specific gravity d_4^{20}: 1.111–1.115.

Boiling point 126–127°C.

Assay Determine the content as directed in operating condition (2) in Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests.

Propylene Glycol $CH_3CH(OH)CH_2OH$ [K8837, Special Grade] [57-55-6]

Protease Sample Diluent Use an appropriate one of the following diluents.

1. Phosphate Buffer (0.02 mol/L) at pH 8.0

2. Dissolve 0.35 g of calcium acetate monohydrate and 0.58 g of sodium chloride in water, adjust the pH to 6.0 with hydrochloric acid (1 mol/L) or sodium hydroxide (1 mol/L), and make up to 1000 mL with water.

3. Sodium Sulfite Solution (1 in 50)

4. Dilute hydrochloric acid TS (0.1mol/L) to 50 times the original volume with water, and cool with ice.

5. Dissolve 0.29 g calcium chloride dihydrate in water to make 1000 mL.

6. Dissolve 0.34 g of calcium sulfate dihydrate and 0.59 g of sodium chloride in water, add acetate buffer (1 mol/L) at pH 6.0, 0.5 mL of polyoxyethylene(10) octylphenyl ether solution (1 in 10), and water to make 1000 mL.

7. Dissolve 112 g of potassium chloride and 30.9 g of boric acid in 700 mL of water. In this solution, dissolve 8.6 g of sodium hydroxide, and make up to 1000 mL with water. To this solution, 1000 mL of sodium sulfite solution (1 in 5), 7.5 mL of a solution of polyoxyethylene(23) lauryl ether (3 in 10), and water to make 10 L. adjust the pH to 9.0 with hydrochloric acid (1 mol/L) or sodium hydroxide (1 mol/L).

8. Hydrochloric Acid–Sodium Acetate Buffer (0.1 mol/L) at pH 2.6.

D(+)-Psicose $C_6H_{12}O_6$ [551-68-8] A white to slightly pale yellow crystalized powder or powder.

Specific rotation $[\alpha]_D^{20}$: +2.0 to +6.0° (0.1 g, water, 10 mL).

Purity Related substances Prepare a test solution by dissolving 20 mg of D(+)-psicose in 2 mL of water. Prepare a control solution by diluting exactly measured 1 mL of the test solution with water to exactly 50 mL. Analyze 10 μL each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for three times the retention time of the main peak, and measure the peak areas. The sum of the areas of all peaks, other than the main peak and the solvent peak, from the test solution is not greater than the area of main peak from the control solution.

Operating conditions

Detector: Differential refractometer.

Column: A stainless steel tube (3–8 mm internal diameter and 15–30 cm length).

Column packing material: 5–10 μm aminopropyl-bonded silica gel for liquid chromatography.

Column temperature: A constant temperature of 35–40°C.

Mobile phase: A 7 : 3 mixture of acetonitrile/water.

Flow rate: Adjust the retention time of D(+)-psicose to 6–9 minutes.

Pullulan $[(C_6H_{10}O_5)n]m$ Use a product suitable for the corresponding enzyme activity tests.

Pullulan (red) A product obtained by coloring partially hydrolyzed pullulan at a rate of one molecular–(3-(phenylazo)-4-hydroxy-5-(4,6-dichloro-1,3,5-triazine-2-yl amino) naphthalene-2,7-bis(sulfonic acid sodium)– per 30 sugar moieties. It is red. Use a product suitable for the corresponding enzyme activity tests.

Pullulan (reduced) A product treated by a reducing agent to decrease the effects on the reducing sugar production in the pullulanase activity test. Use a product suitable for the corresponding enzyme sctivity tests.

Pullulanase [9075-68-7] An enzyme (pullulan 6-glucanohydrolase, EC 3.2.1.41). It decomposes pullulan, which is obtained from the culture of bacteria (*Bacillus, Klebsiella, Sulfolobus solfataricus*). It hydrolyzes α-1,6-glucosidic linkages, producing maltotrioses.

Active Unit One unit of Pullulanase is equivalent to the amount of the enzyme required to liberate 1 μmol of maltotriose from pullulan in 1 minute at pH 5.0 and 30°C.

Pullulanase TS Dissolve pullulanase in water so that its activity is 10 units per mL.

Pullulanase TS (100 units/mL) Dissolve pullulanase in water so that its activity is 100 units per mL. One unit is the amount of the enzyme that is required to produce reducing sugar equivalent to 1 μmol of glucose per 1 minute at pH 6.0 and 40°C from pullulan as the substrate.

Purified Water Use purified water specified in the Japanese Pharmacopoeia.

Purified Water for Ion Chromatography Distilled purified water with electric

conductivity not more than 1 μS/cm. Use a product suitable for ion chromatography.

Pyrazole $C_3H_4N_2$ [288-13-1] White to pale yellow crystals or crystalline powder.

Melting point 67–71°C.

Pyridine C_5H_5N [K 8777, Special Grade] [110-86-1]

Pyridine (Dehydrated) Measure 100 mL of pyridine, add 10 g of potassium hydroxide, and allow to stand for 24 hours. Collect the supernatant by decantation, and distill.

Pyridine for Water Determination To pyridine, add potassium hydroxide or barium oxide. Stopper tightly, and allow to stand for a couple of days. Distill the mixture, protected from exposure to moisture. Store protected from moisture. It shall not contain more than 1 mg per mL.

Pyridine–Pyrazolone TS Weigh 0.20 g of 3-methyl-1-phenyl-5-pyrazolone, add 100 mL of water of about 75°C, dissolve by shaking, and cool to room temperature (it does not need to be dissolved completely). Mix the resulting solution with a solution of 20 mg of bis(3-methyl-1-phenyl-5-pyrazolone) in 20 mL of pyridine.

Pyridine–Sodium Hydroxide TS Dissolve 1.2 g of sodium hydroxide in 200 mL of water, add 100 mL of pyridine, and mix.

4-(2-Pyridylazo)resorcinol Monosodium Salt Monohydrate $C_{11}H_8N_3NaO_2 \cdot H_2O$ [16593-81-0] An orage powdery solid.

Clarity Almost clear. Prepare a test solution by dissolving 0.1 g of 4-(2-pyridylazo)resorcinol monosodium salt monohydrate in water to make 100 mL.

Sensitivity To 10.0 mL of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate, add water to make 100 mL. Adjust the pH to 4.0 with diluted nitric acid (3 in 25), then adjust it to 5–6 with a saturated solution of hexamethylenetetramine, and add 0.2 mL of the test solution prepared in Clarity. Heat the resulting solution to 60°C, and titrate with 0.1 mol/L lead nitrate. The color of solution changes from yellow to light red. When 0.05 mL of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate is added, the color changes to yellow.

4-(2-Pyridylazo)resorcinol TS Dissolve 0.10 g of 4-(2-pyridylazo)resorcinol monosodium salt monohydrate in water to make 100 mL.

Pyrimethanil for Assay $C_{12}H_{13}N_3$ [53112-28-0] A white crystalline powder.

Content Not less than 99.0% of pyrimetahnil ($C_{12}H_{13}N_3$).

Identification Determine the infrared absorption spectrum of pyrimethanil as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3263 cm^{-1}, 1588 cm^{-1}, 1496 cm^{-1}, 1251 cm^{-1}, 757 cm^{-1}, 715 cm^{-1}.

Melting point 96–98°C.

Assay Weigh accurately about 20 mg of pyrimethanil for assay and about 4 mg of 1,4-BTMSB-d_4, and add 2 mL of deuterated methanol to dissolve them. Transfer the resulting solution to an NMR tube of 5 mm in external diameter, stopper tightly, and measure ^{1}HNMR spectra using an NMR spectrometer with a proton resonance frequency of 400 MHz or more. Assuming the signal of 1,4-BTMSB-d_4 as δ 0.23 ppm, when the

signal area intensities at around δ 2.32 ppm, and δ 6.56, δ6.80–7.40, and δ 7.66 ppm are designated as A_1 (corresponding to 6 hydrogens), A_2 (corresponding to 1 hydrogen), A_3 (corresponding to 3 hydrogens), and A_4 (corresponding to 2 hydrogens), respectively, confirm that each of $(A_1/6)/A_2$, $(A_1/6)/(A_3/3)$, $(A_1/6)/(A_4/2)$, $A_2/(A_3/3)$, $A_2/(A_4/3)$, and $(A_3/3)/(A_4/2)$ is 1.0. Then, assuming the signal area intensity of 1,4-BTMSB-d_4 as 18.00, when the sum of A_1, A_2, A_3, and A_4 is designated as 1, when the sum of the number of hydrogens and the purity of 1,4-BTMSB-d_4 are designated as N and P(%), respectively, determine the content of pyrimethanil by the following formula. If the signal from Pyrimethanil for Assay is overlapped with the signal from a contaminant, do not use its signal area intensity for the assay.

$$\text{Content (\%) of pyrimethanil } (C_{12}H_{13}N_3) = \frac{\text{Weight (mg) of 1,4-TMSB-}d_4 \times \text{I} \times \text{P}}{\text{Weight (mg) of the samp N}} \times 0.8797$$

Operating conditions

Spinning: Off.

^{13}C decoupling: Present.

Acquisition time: 4 seconds.

Spectral range: At least 20 ppm including between −5 ppm and 15 ppm.

Flip angle: 90°.

Delay time: Not less than 60 seconds.

Dummy scans: Not less than 1.

Number of accumulation: Not less than 8.

Pyrogallol $C_6H_3(OH)_3$ [K8780, Special Grade] [87-66-1]

Pyrogallol–Sodium Hydroxide TS Dissolve 10 g of pyrogallol in 80 mL of sodium hydroxide solution (3 in 10), and add sodium hydroxide solution (3 in 10) to make 100 mL. Prepare fresh before use.

Pyrogallol TS (Alkaline) Transfer 4.5 g of pyrogallol in a gas washing bottle, and purge air by blowing nitrogen into the bottle for 2 or 3 minutes. Add a solution prepared by dissolving 65 g of potassium hydroxide in 85 mL of water into the bottle. Purge the air completely from the bottle with nitrogen in the same manner.

Pyrophosphate Buffer (pH9.0) Dissolve 3.3 g of potassium pyrophosphate, 15 mg of dithiothreitol, and 40 mg of disodium dihydrogen ethylenediaminetetraacetate dihydrate in water to make 70 mL. Add citric acid monohydrate (21 in 100) to adjust its pH to 9.0, and add water to make exactly 100 mL. Prepare fresh before use.

Pyrrole C_4H_4NH [109-97-7] A colorless, transparent liquid having a characteristic odor. Insoluble in water and soluble in diether ether.

Content Not less than 99.0%.

Assay Analyze 1 μL of pyrrole by gas chromatography using the operating conditions

given below. Determine the content of pyrrole from the area of the pyrrole peak and the sum of the areas of all the peaks.

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 polyethylene glycol for gas chromatography.

Column temperature: Upon injection at 50°C, raise the temperature at a rate of 10°C/minute to 230°C.

Injection port temperature: 150°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 0.5 mL/minute.

Injection method: Split.

Split ratio: 1:100.

Measurement time: 18 minutes.

DL-2-Pyrrolidone-5-Carboxylic Acid $C_5H_7NO_3$ [149-87-1] Odorless white crystals or crystalline powder.

Content Not less than 97.0% of 2-pyrrolidone 5-carboxylic acid ($C_5H_7NO_3$), when dried.

Identification Determine the absorption spectrum of DL-2-pyrrolidone-5- carboxylic acid as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3400 cm^{-1}, 1720 cm^{-1}, 1655 cm^{-1}, 1420 cm^{-1}, and 1230 cm^{-1}.

Loss on drying Not more than 1.5% (105°C, 3 hours).

Assay Weigh accurately about 0.2 g of pyrrolidone carboxylic acid, previously dried, and analyze by the Kjeldahl method under Nitrogen Determination.

Each mL of 0.05 mol/L sulfuric acid = 12.91 mg of $C_5H_7NO_3$

Quartz Sand SiO_2 [14808-60-7] While granules.

Identificatio33n (1) Transfer 0.5 g of triturated quartz sand to a platinum dish, add 20 mL of hydrofluoric acid, and evaporate on a water bath to dryness. It almost volatilizes.

(2) To 0.1 g of quartz sand, add 10 mL of sodium hydroxide solution (1 in 10), and heat. To this solution, add 1 mL of a solution of hexaammonium heptamolybdate tetrahydrate (11 in 100) and 4 mL of diluted hydrochloric acid (2 in 3). A yellow precipitate is produced.

Purity Granular size The amount passed through 600-μm mesh: not more than 50%, the amount not passed through 600-μm mesh and passed through 850-μm mesh: not less than 50%, and the amount not passed through 850-μm mesh: not more than 10%.

Place 850-μm and 600-μm sieves in a stack on a sieve receiver. Transfer 10 g of quartz sand to the upper 850-μm sieve, lid the sieve, and put the sieve stack on a sifting device. Shake it for 10 minutes, and weigh the sand remaining on each sieve and the sand passed

through the 600-μm sieve.

Residue on ignition Not more than 2.0%.

Weigh 1 g of quartz sand in a platinum crucible, add 0.2 mL of sulfuric acid, heat gradually to carbonize, and ignite with a gas burner to incineration. Weigh the residue.

Quinaldine Red $C_{21}H_{23}IN_2$ [117-92-0] A crystalline powder, and soluble in ethanol (95). Its solution in methanol (0.005 in 1000) exhibits an absorption maximum at a wavelength of about 526 nm. The absorbance is not less than 0.5 at the maximum absorption wavelength.

Quinaldine Red TS Dissolve 0.1 g of quinaldine red in 100 mL of acetic acid. Prepare fresh before use.

Quinoline C_9H_7O [K8279, Special Grade] [91-22-5]

Rebaudioside A $C_{44}H_{70}O_{23}$ [58543-16-1] White crystals or powder.

Identification (1) Determine the absorption spectrum of rebaudioside A as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3350 cm^{-1}, 2920 cm^{-1}, 1730 cm^{-1}, 1450 cm^{-1}, 1210 cm^{-1}, 1030 cm^{-1}, and 890 cm^{-1}.

(2) Dissolve 10 mg of rebaudioside A in 1 mL of water. Analyze 5 μL of this solution, as directed in Identification (2) for steviolbioside. The main spot is observed at an R_f value of about 0.5.

Purity Related substances Prepare a test solution by dissolving 5 mg of rebaudioside A in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC). Analyze 10 μL of the tes solution by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in the Monographs. Continue the chromatograph for 30 minute of the sample injection, and exclude the solvent peak from measurement. Measure the area of each peak to determine the percentage of the main peak by the peak area percentage method. It is not less than 95.0%.

Rebaudioside A for Assay $C_{44}H_{70}O_{23}$ [58543-16-1] White crystals or powder.

Identification Proceed as directed in Identification (1) and (2) for rebaudioside A.

Purity Related substances Prepare a test solution by dissolving 5 mg of rebaudioside A for assay in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC). Analyze 10 μL of the test solution by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in the Monographs. Continue the chromatograph for 30 minute of the sample injection, and exclude the solvent peak from measurement. Measure the area of each peak to determine the percentage of the main peak by the peak area percentage method. It is not less than 99.0%.

Loss on drying Not more than 5.0% (50 mg, 105°C, 2 hours).

Rebaudioside B $C_{38}H_{60}O_{18}$ [58543-17-2] A white powder.

Identification (1) Determine the absorption spectrum of rebaudioside B as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3400 cm^{-1}, 1700 cm^{-1}, 1370 cm^{-1}, 1240 cm^{-1}, 1080 cm^{-1}, 1040 cm^{-1},

and 890 cm^{-1}.

(2) Dissolve 10 mg of rebaudioside B in 1 mL of methanol. Analyze 5 μL of this solution as directed in Identification (2) for steviolbioside. The main spot is observed at an R_f value of about 0.7.

Purity Related substances Prepare a test solution by dissolving 5 mg of rebaudioside B in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC). Analyze 10 μL of the test solution by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in the Monographs. Continue the chromatograph for 40 minute of the sample injection, and exclude the solvent peak from measurement. Measure the area of each peak to determine the percentage of the main peak by the Peak Area Percentage Method. It is not less than 95.0%.

Rebaudioside C $C_{44}H_{70}O_{22}$ [63550-99-2] A white to light brown crystals or powder.

Identification (1) Determine the absorption spectrum of rebaudioside C as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2920 cm^{-1}, 1730 cm^{-1}, 1640 cm^{-1}, 1450 cm^{-1}, 1370 cm^{-1}, 1230 cm^{-1}, and 1210 cm^{-1}, 1080 cm^{-1}, 900 cm^{-1}, and 580 cm^{-1}.

(2) Prepare a test solution by dissolving 5 mg of rebaudioside C in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC) to make 5 mL. Analyze 10 μL of the test solution by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in the Monographs. The retention time of the main peak corresponds to that of rebaudioside C for identification.

Purity Related substances Analyze 10 μL of the solution prepared in Identification (2) by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in the Monographs. Continue the chromatograph for 30 minute of the sample injection, and exclude the solvent peak from measurement. Measure the area of each peak to determine the percentage of the main peak by the Peak Area Percentage Method. It is not less than 90.0%.

Rebaudioside C for Identification $C_{44}H_{70}O_{22}$ [63550-99-2] White to light brown crystals or powder.

Identification (1) Proceed as directed in Identification (1) for rebaudioside C.

(2) Prepare a test solution by dissolving 5 mg of rebaudioside C for identification in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC) to make 5 mL. Analyze 1 μL of the test solution by liquid chromatography using the operating conditions given below. The signal (m/z 949) of deprotonated molecular $[M\text{-}H]^-$ is observed in the mass spectrum of the main peak.

Operating conditions

Detector: Mass spectrometer (electrospray ionization spectrometer). Adjust the required parameters such as voltage to optimize the device.

Scanning mass range: m/z100–1500 (negative ion).

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: 40°C.

Mobile phase: A 17:8 mixture of formic acid (0.02 mol/L)/acetonitrile (for HPLC).

Flow rate: 0.5 mL/minute.

Purity Analyze 10 μL of the solution prepared in Identification (2) by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in the Monographs. Continue the chromatograph for 30 minute of the sample injection, and exclude the solvent peak from measurement. Measure the area of each peak to determine the percentage of the main peak by the Peak Area Percentage Method. It is not less than 90.0%.

Rebaudioside D $C_{50}H_{80}O_{28}$ [63279-13-0] A white to light brown crystals or powder.

Identification (1) Determine the absorption spectrum of rebaudioside D as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3400 cm^{-1}, 2920 cm^{-1}, 1730 cm^{-1}, 1660 cm^{-1}, 1450 cm^{-1}, 1370 cm^{-1}, and 1230 cm^{-1}, 1080 cm^{-1}, and 890 cm^{-1}.

(2) Prepare a test solution by dissolving 5 mg of rebaudioside D in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC) to make 5 mL. Analyze 10 μL of the test solution by liquid chromatography using the operating conditions given below. The retention time of the main peak corresponds to that of rebaudioside D for identification.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 210 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: 40°C.

Mobile phase

A: Phosphate buffer (0.01 mol/L, pH2.6).

B: Acetonitrile (for HPLC).

Concentration gradient (A/B): Maintain at 75/25 for 12 minutes, run a linear gradient from 75/25 to 50/50 in 13 minutes, and then maintain at 50/50 for 15 minutes.

Flow rate: 1.0 mL/minute.

Purity Analyze 10 μL of the solution prepared in Identification (2) by liquid chromatography using the operating conditions given in Identification (2). Continue the chromatograph for 40 minute of the sample injection, and exclude the solvent peak from measurement. Measure the area of each peak to determine the percentage of the main peak by the Peak Area Percentage Method. It is not less than 70%.

Rebaudioside D for Identification $C_{50}H_{80}O_{28}$ [63279-13-0] A white to light brown crystals or powder.

Identification (1) Proceed as directed in Identification (1) for rebaudioside D.

(2) Prepare a test solution by dissolving 5 mg of rebaudioside D for identification in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC) to make 5 mL. Analyze 1 μL of the test solution by liquid chromatography using the operating conditions specified in Identification (2) for rebaudioside C for identification. The signal (m/z 1128) of deprotonated molecular $[M-H]^-$ is observed in the mass spectrum of the main peak.

Purity Analyze 10 μL of the solution prepared in Identification (2) by liquid chromatography using the operating conditions given in Identification (2) for rebaudioside D. Continue the chromatograph for 40 minute of the sample injection, and exclude the solvent peak from measurement. Measure the area of each peak to determine the percentage of the main peak by the Peak Area Percentage Method. It is not less than 70%.

Rebaudioside F $C_{43}H_{68}O_{22}$ [438045-89-7] A white to light brown crystals or powder.

Identification (1) Determine the absorption spectrum of rebaudioside F as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2920 cm^{-1}, 1730 cm^{-1}, 1640 cm^{-1}, 1450 cm^{-1}, 1370 cm^{-1}, 1230 cm^{-1}, and 1210 cm^{-1}, 1080 cm^{-1}, 900 cm^{-1}, and 580 cm^{-1}.

(2) Prepare a test solution by dissolving 5 mg of rebaudioside F in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC) to make 5 mL. Analyze 10 μL of the test solution by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in the Monographs. The retention time of the main peak corresponds to that of rebaudioside F for identification.

Purity Analyze 10 μL of the solution prepared in Identification (2) by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in the Monographs. Continue the chromatograph for 30 minute of the sample injection, and exclude the solvent peak from measurement. Measure the area of each peak to determine the percentage of the main peak by the Peak Area Percentage Method. It is not less than 70.0%.

Rebaudioside F for Identification $C_{43}H_{68}O_{22}$ [438045-89-7] A white to light brown crystals or powder.

Identification (1) Proceed as directed in Identification (1) for rebaudioside F.

(2) Prepare a test solution by dissolving 5 mg of rebaudioside F for identification in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC) to make 5 mL. Analyze 1 μL of the test solution by liquid chromatography using the operating conditions specified in Identification (2) for rebaudioside C. The signal (m/z 936) of deprotonated molecular $[M-H]^-$ is observed in the mass spectrum of the main peak.

Purity Analyze 10 μL of the solution prepared in Identification (2) by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in the Monographs. Continue the chromatograph for 30 minute of the sample injection, and exclude the solvent peak from measurement. Measure the area of each peak to

determine the percentage of the main peak by the peak area percentage method. It is not less than 70.0%.

Red Phosphorus P [7723-14-0] An odorless dark red powder. Insoluble in water.

Content Not less than 98.0%.

Assay (1) Free phosphoric acid Weigh 5.0 g of red phosphorus, add 10 mL of sodium chloride solution (1 in 5), stir, then add 50 mL of sodium chloride solution (1 in 2), leave the mixture at room temperature, and filter. Wash the residue three times with 10 mL of sodium chloride solution (1 in 5) each time, and combine the washings with the filtrate. Titrate the resulting solution with 0.1 mol/L sodium hydroxide using thymol blue TS as the indicator.

Each mL of 0.1 mol/L sodium hydroxide = 4.900 mg of H_3PO_4

(2) Yellow Phosphorus Weigh 10.0 g of red phosphorus, add 50 mL of benzene, heat on a water bath under a reflux condenser for 3 hours. After cooling, filter the solution into a separating funnel. Wash the residue three times with 10 mL of benzene each time, add the washings to the funnel, add 0.5 mL of bromine, and shake. Then add 20 mL of water, shake, leave the mixture, and collect the lower layer (water layer). Wash the benzene layer three times with 20 mL of water each time, add the washings to the collected water layer, and add 10 mL of nitric acid saturated with bromine. Evaporate the solution on a water bath to about 10 mL, add 20 mL of water and 10 mL of ammonia solution, and neutralize it with nitric acid. Add 1 mL of nitric acid, and warm the mixture to about 60°C. Then while shaking, add 15 mL of a solution of hexaammonium heptamolybdate tetrahydrate (11 in 100), previously warmed to about 60°C, warm the mixture for 1 hour on a water bath at about 60°C, and filter. Wash well the precipitate and filter with ammonium nitrate (1 in 10). Transfer the precipitate with the filter into a 200-mL Erlenmeyer flask, add 50 mL of water, crush the filter, add an excessive amount of 0.1 mol/L sodium hydroxide to dissolve the precipitate. Titrate the resulting solution with 0.1 mol/L nitric acid (indicator: phenolphthalein TS). Separately perform a blank test to make any necessary correction to determine the content yellow phosphorus.

Each mL of 0.1 mol/L nitric acid = 0.13467 mg of P (yellow phosphorus)

(3) Magnesium pyrophosphate (total phosphorus) To about 0.5 g of red phosphorus, weighed accurately, add 30 mL of nitric acid saturated with bromine, allow to stand for 1 hour, heat on a water bath until the red color of bromine disappears, and cool. Add 1 g of potassium chlorate and 30 mL of hydrochloric acid, leave it for 10 minutes. Perform above procedure in an exhauster or a draft.

Heat the resulting solution gradually on a water bath and evaporate it to about 5 mL, add 200 mL of water, and heat 10 minutes. After cooling filter. Wash the precipitate and filter paper with water, and combine the washing with the filtrate, transfer the solution into a 500-mL volumetric flask, and make up to the volume with water. To exactly measured 25 mL of this solution, add 0.5 g of citric acid monohydrate, add 3 drops of bromothymol blue TS, and neutralize with diluted ammonia solution (28) (2 in 5). Then

add gradually 10 mL of magnesia TS (for red phosphorus assay) while stirring, add diluted ammonia solution (28) (1 in 10) dropwise to make a precipitate form completely. Add an amount of diluted ammonia solution (28) (2 in 5) equal to one-fifth of the resulting solution, leave it for 3 hours, and filter. Wash the precipitate with diluted ammonia solution (28) (1 in 10) until the washings are free of chloride ions. Transfer the filter paper with the precipitate to a ceramic crucible, previously dried at 105°C to constant weight, dry at 105°C, gradually heat to incineration, and ignite. Cool the crucible in a desiccator, and weigh it to determine the content magnesium pyrophosphate.

(4) Red phosphorus Determine the content of red phosphorus by the following formula. The conversion factors between magnesium pyrophosphate ($Mg_2P_2O_7$) and red phosphorus and between free phosphoric acid and red phosphorus are 0.2783 and 0.3161, respectively.

Content (%) of red phosphorus = $(A \times 0.2783) - (B \times 0.3161 + C)$

A = content (%) of magnesium pyrophosphate,

B = content (%) of free phosphoric acid,

C = content (%) of yellow phosphorus.

Resorcinol $C_6H_4(OH)_2$ [K9032, Special Grade] [108-46-3]

L-Rhamnose for Assay $C_6H_{12}O_5 \cdot H_2O$ [6014-42-2] A white crystals or powder.

Purity Related substances Prepare a test solution by dissolving 50 mg of L-rhamnose for assay in a 2:8 mixture of water/acetonitrile (HPLC) to make 10 mL. Analyze 20 μL of the test solution by liquid chromatography using the operating conditions given below. Determine the content of L-rhamnose from the area of the main peak and the sum of the areas of all the peaks. It contains not less than 98.0% of L-rhamnose. Separately, perform a blank test to make any necessary correction.

Operating conditions

Detector: Differential refractometer.

Column: A stainless steel (6-mm internal diameter and 15-cm length).

Column Packing material: 5-μm amino-bonded silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 8:2 mixture of acetonitrile (for HPLC)/water.

Flow rate: 1.0 mL/minute.

D-Ribose for Assay $C_5H_{10}O_5$ [50-69-1] White crystals or crystalline powder.

Identification To 5 mL of boiling Fehling's TS, add 2–3 drops of a solution of D-ribose for assay (1 in 20). A red precipitation is produced.

Specific rotation $[\alpha]_D^{20}$: −18 to −22°. Weigh accurately about 1 g of D-ribose for assay, and add 0.2 mL of ammonia TS and water to dissolve it, and make exact 50 mL. Measure the angular rotation of this solution, and calculate on the anhydrous basis.

Purity Related substances Prepare a test solution by dissolving about 0.5 g of D-ribose for assay in 25 mL of water. Prepare a control solution by diluting 1 mL of test solution, exactly measured, to exactly 100 mL with water. Analyze 10 μL each of the test

solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Follow the operating conditions for the Assay of D-Ribose in the Monographs.

Water Not more than 1.0% (1 g, Volumetric Titration, Direct Titration).

Rubusoside $C_{32}H_{50}O_{13}$ [64849-39-4] A white powder.

Identification (1) Determine the absorption spectrum of rubusoside as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 2940 cm^{-1}, 1720 cm^{-1}, 1660 cm^{-1}, 1450 cm^{-1}, 1240 cm^{-1}, 1210 cm^{-1}, 1170 cm^{-1}, 1070 cm^{-1}, and 890 cm^{-1}.

(2) Analyze 5 μL of a solution of 10 mg of rubusoside in 1 mL of methanol by thin-layer chromatography using the operating conditions specified in Identification (2) for steviolbioside. A major spot is observed at an Rf value of about 0.7.

Purity Related substances Prepare a test solution by dissolving 5 mg of rubusoside in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC). Analyze 10 μL of the test solution by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in the Monographs. Continue the chromatography for 30 minutes and exclude the solvent peak from the measurement. Measure the areas of all the peaks to determine the percentage of the main peak by the peak area percentage method. It is not less than 95.0%.

Rutin for Assay $C_{27}H_{30}O_{16}\cdot 3H_2O$ [250249-75-3] A light yellow to light yellow-green crystalline powder.

Identification Determine the absorption spectrum of rutin for assay as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 1655 cm^{-1}, 1605 cm^{-1}, 1505 cm^{-1}, 1360 cm^{-1}, 1300 cm^{-1}, 1200 cm^{-1}, and 810 cm^{-1}.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 350 nm): Not less than 290. Weigh accurately about 50 mg of rutin for assay, previous dried at 135°C for 2 hours, and dissolve it in methanol to make 100 mL. Measure exactly 2 mL of this solution, and add methanol to make exactly 100 mL. Measure the absorbance of the resultant solution as directed under Ultraviolet-Visible Spectrophotometry.

Purity Related substances Prepare a test solution. Dissolve about 50 mg of rutin for assay in 25 mL of methanol, then measure exactly 5 mL of the resultant solution, and add an 800:200:1 mixture of water/acetonitrile/phosphoric acid to make exactly 50 mL. Then prepare a control solution. Add 5 mL of methanol to 1 mL of the test solution, measured exactly, and then add an 800:200:1 mixture of water/acetonitrile/phosphoric acid to make exactly 50 mL. Analyze 20 μL each of the test solution and the control

solution by liquid chromatography, using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The sum of the areas of all the peaks, other than the main peak and the solvent peak, from the test solution is not greater than the area of the main peak from the control solution.

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 rutin to about 8–12 minutes.

D(–)-Salicin $C_6H_{11}O_5OC_6H_4CH_2OH$ Use a product suitable for the corresponding enzyme activity tests.

Salicylaldazine $C_{14}H_{12}N_2O_2$ [959-36-4]

Melting point 213–219°C.

Purity Dissolve 90 mg of salicylaldazine in toluene to make 100 mL. To exactly measured 1 mL of this solution, add toluene to make exactly 100 mL. When the resulting solution is analyzed as directed under Purity (5) for Polyvinylpyrrolidone, any spot other than the main spot does not appear.

Salicylaldehyde HOC_6H_4CHO [K8390, Special Grade] [90-02-8]

Salicylic Acid HOC_6H_4COOH [K8392, Special Grade] [69-72-7]

Salicylic Acid–Methanol TS Dissolve 10 g of salicylic acid in 100 mL of methanol for water determination. Prepare fresh before use.

Salts TS Dissolve 0.18 g of calcium acetate monohydrate, 2.72 g of sodium acetate trihydrate, and 5.84 g of sodium chloride in water to make 1000 mL. Mix this solution with 10 mL of diluted acetic acid (1 in 10).

Sarsasapogenin for Assay $C_{27}H_{44}O_3$ [126-19-2] A white, odorless crystalline powder.

Identification Dissolve 5 mg of sarsasapogenin for assay in 5 mL of ethyl acetate. Analyze 2 μL of this 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 form 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 with4-methoxybenzaldehyde–sulfuric acid TS. After heating at 110°C for 10 minutes, examine it. A major yellow-green to blue-green spot is observed at an R_f value of about 0.55.

Purity Related substances Prepare a test solution by dissolving 0.10 g of sarsasapogenin for assay in 10 mL of ethyl acetate. Prepare a control solution by adding ethyl acetate to 1 mL of the test solution, exactly measured, to make exactly 50 mL.

Analyze 5 μL each of the test solution and the standard solution by thin-layer chromatography as directed under Identification above. Spots, other than the main spot, from the test solution are not more intense than the main spot from the control solution.

Water Not more than 8.0% (0.1 g, Volumetric Titration, Direct Titration).

Sea Sand A mixture of granules with plural colors including white, gray, brown, and black.

Loss on ignition Not more than 0.4%.

Assay Weigh accurately about 1.0 g of sea sand into a constant-weight crucible or evaporating dish, and dry at 100°C for 1 hour. Put the crucible or evaporating dish into an electric furnace set at 600–700°C, and increase the temperature gradually to ignite it. After 2-hour ignition, promptly transfer the crucible or dish from the furnace to a desiccator, and leave to cool. Remove it from the desiccator, and weigh accurately. Ignite for about 1 hour, and weigh. Repeat this procedure to constant weight.

Selenium Dioxide SeO_2 [7446-08-4] White crystals. Freely soluble in water. Sublimates when heated.

Silica Gel SiO_2 [Z0701] [7631-86-9] Use JIS Silica Gel Desiccant A for Packaging that has been dried for about 2 hours at 170–190°C

Silica Gel Minicolumn (500 mg) A polyethylene column with an internal diameter of 10–25 mm that has been packed with 0.5 g of silica gel or any other column equivalent in separation capability to the specified one.

Silicon Dioxide SiO_2 [K8885, Special Grade] [7631-86-9]

Silicone Oil A clear, colorless, odorless liquid.

Kinematic viscosity 50–100 mm^2/s.

Silicone Resin A light-gray, translucent, almost odorless, viscous liquid or pasty substance.

Refractive index and viscosity Reciprocally shake 20 g of silicone resin with 100 mL of hexane for 3 hours at about 200 times/minute, then centrifuge it for 30 minutes at 10000 rpm, and collect the supernatant. Add 50 mL of hexane to the residue, and shake well to disperse in hexane, and centrifuge. Combine the supernatants, remove the hexane in the liquid obtained in a water bath of 50–60°C under reduced pressure. Dry it for 1 hour at 105°C. Kinematic viscosity of this resulting liquid is 100–1100 mm^2/s (at 25°C), and refractive index is 1.400–1.410 (at 25°C).

Specific gravity d_{20}^{20}: 0.98–1.02.

Loss on drying 0.45–2.25 g (100°C, 1 hour).

Perform the test on the extraction residue obtained in the refractive index and viscosity tests.

Silver *N,N*-Diethyldithiocarbamate $C_5H_{10}AgNS_2$ [K9512, Special Grade] [1470-61-7]

Silver Diethyldithiocarbamate–Quinoline TS Dissolve 50 mg of silver nitrate, ground into a fine powder, in 100 mL of quinoline, and add 0.2 g of silver *N,N*-diethyldithiocarbamate. Prepare fresh before use.

Silver Nitrate $AgNO_3$ [K8550, Special Grade] [7761-88-8]

Silver Nitrate–Ammonia TS Dissolve 1 g of silver nitrate in 20 mL of water. Add dropwise ammonia TS while stirring until the precipitate almost dissolves, and filter. Store it in a tightly-stoppered, light-resistant container.

Silver Nitrate–Ethanol TS Dissolve 15 of silver nitrate in 50 mL of water, add 400 mL of ethanol (95), mix, and add a few drops of nitric acid. Store in a brown bottle.

Silylation TS To 3 mL of *N,O*-bis(trimethylsilyl)acetamide, add 2 mL of *N,N*-dimethylformamide to dissolve it. Prepare fresh before use.

Sitostanol $C_{29}H_{52}O$ [83-45-4] A white crystalline powder.

Identification Proceed as directed in Identification for Campesterol. The relative retention time of the main peak of the test solution to the retention time of stigmasterol in the standard solution is about 1.13.

Melting point 144–145°C.

Purity Proceed as directed in Purity for Campesterol.

β-Sitosterol $C_{29}H_{52}O$ [83-46-5] A white crystalline powder.

Identification Proceed as directed in Identification for Campesterol. The relative retention time of the main peak of the test solution to the retention time of stigmasterol in the standard solution is about 1.12.

Melting point 136–146°C.

Purity Proceed as directed in Purity for Campesterol.

Skimmed Milk A powder produced by almost completely dehydrating raw milk or cow's milk from which milk fat is previously removed.

Soda Lime [for carbon dioxide absorption, Element Analysis Grade, K8603] [8006-28-8]

Sodium Acetate CH_3COONa [K8372, Special Grade] [127-09-3]

Sodium Acetate Trihydrate $CH_3COONa \cdot 3H_2O$ [K8371, Special Grade] [6131-90-4]

Sodium Acetate TS (1 mol/L) Dissolve 82.0 g of sodium acetate in water to make 1000 mL.

Sodium Acetate TS (0.5 mol/L) Dissolve 41.0 g of sodium acetate in water to make 1000 mL.

Sodium Alginate $(C_6H_7O_6Na)n$ Use a product suitable for the corresponding enzyme activity tests.

Sodium 4-Amino-1-naphthalenesulfonate Tetrahydrate $C_{10}H_8NNaO_3S \cdot 4H_2O$ [130-13-2] A white to light red powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 316–322 nm): Not less than 280. Weigh accurately about 10 mg of sodium 4-amino-1-naphthalenesulfonate tetrahydrate, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to it as Solution A. Measure exactly 10 mL of Solution A, add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits absorption maxima in the ranges 234–240 nm and

316–322 nm, respectively. Measure the absorbance of the resulting solution at the maximum between 316–322 nm against ammonium acetate TS (0.02 mol/L) to determine the specific absorbance.

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add the mobile phase to 5 mg of sodium 4-amino-1-naphthalenesulfonate tetrahydrate to make exactly 50 mL. Analyze 10 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 20 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the mobile phase in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 238 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 19:1 mixture of ammonium acetate TS (0.02 mol/L)/acetonitrile (for HPLC).

Flow rate: 1.0 mL/min.

Water 20.5–24.4% (50 mg, Coulometric Titration) The node solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

Sodium Azide NaN_3 [K9501, Special Grade] [26628-22-8] A while, odorless crystalline powder.

Melting point 275°C (decomposition).

Sodium Borate–Hydrochloric Acid Buffer (0.1 mol/L) Dissolve 38.1 g of sodium tetraborate decahydrate in 600 mL of water, adjust the pH to the value specified in the corresponding section of this publication with hydrochloric acid TS (1 mol/L), and add water to make 1000 mL.

Sodium Borate–Hydrochloric Acid Buffer (0.01 mol/L, pH 8.5, containing polysorbate) Dissolve 3.81 g of sodium tetraborate decahydrate in 800 mL of water. To this solution, add 50 μL of polysorbate 80, adjust the pH to 8.5 with hydrochloric acid TS (0.5 mol/L), and add water to make 1000 mL.

Sodium Bromide NaBr [K8514, Special Grade] [7647-15-6]

Sodium Carbonate Na_2CO_3 [K8625, Special Grade] [497-19-8]

Sodium Carbonate (Reference Material) Na_2CO_3 [Reference Material for Volumetric Analysis, K8005] [497-19-8] In addition to Reference Material for Volumetric Analysis specified in JIS K8005, a certified reference material capable of being used for volumetric

analysis is usable.

Sodium Carbonate Decahydrate $Na_2CO_3 \cdot 10H_2O$ [K8624, Special Grade] [6132-02-1]

Sodium Carbonate–Disodium Dihydrogen Ethylenediaminetetraacetate TS Dissolve 50 g of sodium carbonate and 37.2 of disodium dihydrogen ethylenediaminetetraacetate dihydrate in water to make 1000 mL.

Sodium Carbonate for pH Determination Na_2CO_3 [K8625, pH Standard Solution Grade] [497-19-8]

Sodium Carbonate TS Dissolve 10.6 g of sodium carbonate in water to make 100 mL.

Sodium Carbonate TS (1 mol/L) Dissolve 106 g of sodium carbonate in water to make 1000 mL.

Sodium Carbonate TS (0.55 mol/L) Dissolve 58.3 g of sodium carbonate in water to make 1000 mL.

Sodium Carbonate TS (0.5 mol/L) Dissolve 53 g of sodium carbonate in water to make 1000 mL.

Sodium Carbonate TS (0.25 mol/L) Dissolve 26.5 g of sodium carbonate in water to make 1000 mL.

Sodium Carbonate TS (0.2 mol/L) Dissolve 21.2 g of sodium carbonate in water to make 1000 mL.

Sodium Carboxymethylcellulose $[C_6H_7O_2(OH)_3 \cdot x(OCH_2COONa)_x]_n$

x: the degree of substitution (etherification),

n: degree of polymerization.

Use a product suitable for the corresponding enzyme activity tests.

Sodium Chloride NaCl [K8150, Special Grade] [7647-14-5]

Sodium Chloride (Reference Material) NaCl [Reference Material for Volumetric Analysis, K8005] [7647-14-5] In addition to Reference Material for Volumetric Analysis specified in JIS K8005, a certified reference material capable of being used for volumetric analysis is usable.

Sodium Chloride TS (2 mol/L) Dissolve 116.9 g of sodium chloride in water to make 1000 mL.

Sodium Chloride TS (0.5 mol/L) Dissolve 29.2 g of sodium chloride in water to make 1000 mL.

Sodium Deoxycholate $C_{24}H_{39}NaO_4$ [302-95-4] A white, odorless crystalline powder.

Identification Determine the absorption spectrum of sodium deoxycholate, previously dried, as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3400 cm^{-1}, 2940 cm^{-1}, 1562 cm^{-1}, and 1408 cm^{-1}.

Purity Related substances Prepare a sample solution by dissolving 0.10 g of sodium deoxycholate in 10 mL of methanol. Prepare a control solution by diluting 1 mL of the sample solution, measured exactly, with methanol to make exactly 100 mL. With the sample solution and the control solution, proceed as directed under Thin-Layer

Chromatography. Apply 10 μL each of these solutions on a thin-layer plate prepared with silica-gel for thin-layer chromatography. Develop until the solvent front ascends to a point about 10 cm above the starting line, using an 80:40:1 mixture of 1-butanol/methanol/ acetic acid as the developing solvent. Air-dry the thin-layer plate, spray uniformly with sulfuric acid, and heat at 105°C for 10 minutes. The spots other than the main spot from the sample solution are not more intense than the spot from the control solution.

Sodium Deoxycholate TS (3.3.mmol/L) Dissolve 1.38 g of sodium deoxycholate in water to make 1000 mL.

Sodium Deoxycholate TS (0.016 mol/L) Dissolve 6.7 g of sodium deoxycholate in water to make 1000 mL.

Sodium *N,N*-Diethyldithiocarbamate Trihydrate $(C_2H_5)_2NCS_2Na \cdot 3H_2O$ [K8454, Special Grade] [20624-25-3]

Sodium Dihydrogenphosphate Dihydrate $NaH_2PO_4 \cdot 2H_2O$ [K9009, Special Grade] [13472-35-0]

Sodium Dithionite $Na_2S_2O_4$ [7775-14-6] A white to grayish white crystalline powder having a strong pungent odor of sulfur dioxide.

Content Not less than 85.0%.

Assay To a mixture of 10 mL of formaldehyde solution and 10 mL of water (dissolved oxygen-removed), add 3 drops of phenolphthalein TS as the indicator, and neutralize with 0.1 mol/L sodium hydroxide. To this solution, add about 1.5 g of sodium dithionite, weighed accurately, stopper tightly, and allow to stand for 30 minute with occasional stirring. Dilute to exactly 250 mL with water. Measure exactly 25 mL of the resulting solution, add 4 mL of hydrochloric acid TS (1 mol/L), titrate with 0.05 mol/L iodine. Add starch TS as the indicator near the endpoint of the titration, when the solution is pale yellow. The endpoint is when its color is blue. Separately, perform a blank test.

Each mL of 0.05 mol/L iodine = 4.353 mg of $Na_2S_2O_4$

Sodium Dodecyl Sulfate (for enzyme) $C_{12}H_{25}NaO_4S$ Use a product suitable for the corresponding enzyme activity tests.

Sodium Dodecyl Sulfate–Bovine Serum Albumin TS Dissolve 1 g of sodium of dodecyl sulfate (for enzyme) and 1 g of bovine serum albumin TS in water while stirring to make 1000 mL. Be careful not to form bubbles. Prepare fresh before use.

Sodium Fluoride NaF [K8821, Special Grade] [7681-49-4]

Sodium Formate HCOONa [K8267, Special Grade] [141-53-7]

Sodium 2-Formyl-benzenesulfonate $C_7H_5O_4SNa$ [1008-72-6] A white to light brown crystals, powder, or lumps.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength at 249–255 nm): Not less than 396–484 nm. Dissolve about 10 mg of sodium 2-formyl-benzenesulfonate, weighed accurately, in ammonium acetate TS (0.02 mol/L), and make exactly 100 mL. Refer to this solution as Solution A. A solution prepared by diluting exactly measured 5

mL of solution to exactly 50 mL with ammonium acetate TS (0.02 mol/L) exhibits an absorption maximum at 249–255 mn. Measure the absorbance (A_B) of Solution A at the maximum at 249–255 mn against ammonium acetate TS (0.02 mol/L) to determine the specific absorbance by the formula:

$$E_{1cm}^{1\%} = A_B \times \frac{10}{\text{Weight (g) of the sample}} \times \frac{100}{100 - \text{Loss on drying (\%)}}$$

Purity (1) Clarity Clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution by dissolving 5 mg of sodium 2-formyl-benzenesulfonate in the mobile phase to make 50 mL. Analyze 20 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 25 minutes of injection. The peak area percentage of the main peak of the test soltion is not less than 95.0% of the total area of all the peaks, excluding those from the mobile phase in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 252 nm).

Column: A stainless steel tube (4.6 mm internal diameter and 15 cm length).

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

Column temperature: 40°C.

Mobile phase: A 75:25 mixture of phosphoric acid–tetra-n-butylammonium bromide TS/acetonitrile (for HPLC).

Flow rate: 1.0 mL/minute.

Loss on drying Not more than 2.0% (50 mL, 135°C, 6 hours).

Sodium 2-Formyl-5-hydroxybenzenesulfonate $C_7H_5O_5SNa$ [119557-97-0] A white to light brown powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength at 335–341 nm): Not less than 286. Dissolve about 10 mg of sodium 2-formyl-5-hydroxybenzenesulfonate, weighed accurately, in ammonium acetate TS (0.02 mol/L), and make exactly 100 mL. Refer to this solution as Solution A. A solution prepared by diluting exactly measured 10 mL of Solution A to exactly 50 mL with ammonium acetate TS (0.02 mol/L) exhibits absorption maxima in the ranges 226–231 nm, 288–294 nm, and 335–341 nm, respectively. Measure the absorbance (A_B) of Solution A at the maximum at 335–341 nm against ammonium acetate TS (0.02 mol/L) to determine the specific absorbance by the formula:

$$E_{1cm}^{1\%} = A_B \times \frac{5}{\text{Weight (g) of the sample}} \times \frac{100}{100 - \text{Water (\%)}}$$

Purity (1) Clarity Clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution by dissolving 5 mg of sodium 2-formyl-5-hydroxybenzenesulfonate in ammonium acetate TS (0.02 mol/L) to make 50 mL. Analyze 10 μL each of the test solution and ammonium acetate TS (0.02 mol/L) by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 30 minutes of injection. The peak area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the ammonium acetate TS in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 285 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: Phosphoric acid–tetra-*n*-butylammonium bromide TS.

B: Acetonitrile (for HPLC).

Concentration gradient (A/B): Run a linear gradient from 70/30 to 40/60 in 20 minutes, and maintain at 40/60 for 10 minutes.

Flow rate: 1.0 mL/minute.

Water Not more than 10.0% (50 mg, Coulometric Titration).

Sodium Hexacyanoferrate(II) Decahydrate $Na_4[Fe(CN)_6]\cdot 10H_2O$ [14434-22-1] Slightly pale yellow to yellow crystals or crystalline powder.

Content Not less than 95.0%.

Clarity Slightly turbid (1 g, 20 mL).

Assay Dissolve 1 g of sodium hexacyanoferrate(II) decahydrate in 210 mL of diluted sulfuric acid (1 in 21), and titrate with 0.02 mol/L potassium permanganate. The endpoint is when the faint red color persists for 15 seconds. Perform a blank test to make any necessary correction.

Each mL of 0.02 mol/L potassium permanganate = 48.41 mg of $Na_4[Fe(CN)_6]\cdot 10H_2O$

Sodium Hexanitrocobaltate(III) $Na_3[Co(NO_2)_6]$ [13600-98-1] A yellow-brown powder. Very soluble in water.

Sensitivity Add 20 mL of water to 1.0 g of sodium hexanitrocobaltate(III). To 4 mL of this solution, add 1 mL of potassium standard solution and water to make 10 mL. Then add 10 mL of ethanol (95), shake, and allow to stand for 30 minutes at 15°C or less. A turbidity is formed.

Sodium Hexanitrocobaltate(III) TS Dissolve 30 g of sodium hexanitrocobaltate(III) in water to make 100 mL. Prepare fresh before use.

Sodium Hydrogen Carbonate $NaHCO_3$ [K8622, Special Grade] [144-55-8]

Sodium Hydrogen Carbonate for pH Determination $NaHCO_3$ [K8622, pH Standard Solution Grade] [144-55-8]

Sodium Hydrogen Sulfite $NaHSO_3$ [K8059, Special Grade] [7631-90-5]

Sodium Hydrogen (+)-Tartrate Monohydrate $HOOCCH(OH)CH(OH)COONa \cdot H_2O$ [526-94-3] Colorless crystals or a white crystalline powder. Soluble in water and practically insoluble in diethyl ether.

Content Not less than 99.0%.

Assay Weigh accurately about 4.0 g of sodium hydrogen (+)-tartrate monohydrate, and dissolve it in 200 mL of carbon dioxide-free water by heating. After cooling, add 3 drops of phenolphthalein TS as the indicator, and titrate with 1 mol/L sodium hydroxide. The endpoint is when the faint red color persists for 30 seconds.

Each mL of 1 mol/L sodium hydroxide = 190.08 mg of $HOOCCH(OH)CH(OH)COONa \cdot H_2O$

Sodium Hydroxide NaOH [K8576, Special Grade] [1310-73-2]

Sodium Hydroxide Solution (Highly Purified) NaOH [Highly Purified Sodium Hydroxide Solution, K9906] [1310-73-2]

Sodium Hydroxide Solution (for semiconductor) NaOH [1310-73-2]

Content 40.0–50.0%.

Assay Weigh accurately about 2 g of sodium hydroxide solution (for semiconductor) into a 200-mL ground-glass stoppered Erlenmeyer flask, and dissolve it in 50 mL of water (carbon dioxide-removed). Stopper the flask, and leave it for 5 minutes. Titrate the resulting solution with 1 mol/L hydrochloric acid. To confirm the endpoint, use a potentiometer or an indicator (3 drops of phenolphthalein TS). When a potentiometer is used, use a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes. If the indicator is used, the endpoint is when the faint red color of the solution persists for 30 seconds.

Each mL of 1 mol/L hydrtochloric acid = 40.00 mg of NaOH

Sodium Hydroxide TS (10 mol/L) Dissolve 400 g of sodium hydroxide in water to make 1000 mL.

Sodium Hydroxide TS (5 mol/L) Dissolve 200 g of sodium hydroxide in water to make 1000 mL.

Sodium Hydroxide TS (4 mol/L) Dissolve 160 g of sodium hydroxide in water to make 1000 mL.

Sodium Hydroxide TS (3 mol/L) Dissolve 126 g of sodium hydroxide in water to make 1000 mL.

Sodium Hydroxide TS (2 mol/L) Dissolve 80 g of sodium hydroxide in water to make 1000 mL.

Sodium Hydroxide TS (1 mol/L) Dissolve 4.3 g of sodium hydroxide in water to make 100 mL. Store in a polyethylene bottle.

Sodium Hydroxide TS (0.5 mol/L) Dissolve 22 g of sodium hydroxide in water to make 1000 mL. Store in a polyethylene bottle.

Sodium Hydroxide TS (0.2 mol/L) Dissolve 8.0 g of sodium hydroxide in water to make 1000 mL. Store in a polyethylene bottle.

Sodium Hydroxide TS (0.12 mol/L) Dissolve 4.8 g of sodium hydroxide in water to make 1000 mL. Store in a polyethylene bottle.

Sodium Hydroxide TS (0.1 mol/L) Dissolve 4.3 g of sodium hydroxide in freshly boiled and cooled water to make 1000 mL. Prepare fresh before use.

Sodium Hydroxide TS (0.05 mol/L) Dilute 10 mL of Sodium hydroxide (0.5 mol/L) to 100 mL with water.

Sodium Hydroxide TS (0.04 mol/L) Weigh 1.6 g of sodium hydroxide, and dissolve in water to 1000 mL.

Sodium Hydroxide TS (0.02 mol/L) Dilute 200 mL of Sodium hydroxide (0.1 mol/L) to 1000 mL with water.

Sodium Hydroxide TS (0.01 mol/L) Dilute 10 mL of Sodium hydroxide (1 mol/L) to 1000 mL with water.

Sodium Hypochlorite NaClO [7681-52-9] "Sodium Hypochlorite" Use a product containing not less than 5% of an available chlorine.

Sodium Hypochlorite–Sodium Hydroxide TS To a volume of sodium hypochlorite TS equivalent to 1.05 g of sodium hypochlorite (NaClO = 74.44), add 15 g of sodium hydroxide and water to make 1000 mL. Prepare fresh before use.

Sodium Hypochlorite–Sodium Hydroxide TS (for the urease activity test) Dissolve 10 g sodium hydroxide and 15 mL of sodium hypochlorite in water to make 1000 mL. Prepare fresh before use.

Sodium Hypochlorite–Sodium Hydroxide TS for *A. niger*-derived Asparaginase Activity Determination To 2.5 mL of sodium hypochlorite TS, add water to make 10 mL. Standardize the resulting solution using 3 mL of it, as directed in Assay for Sodium Hypochlorite in the Monographs, and adjust it to make a solution of 0.32 to 0.38 mol/L sodium hypochlorite. Adjust its pH to 12.5 with sodium hydroxide solution with an appropriate concentration. To 3 mL of this solution, add 85 mL of water, and adjust its pH to 12.5 with sodium hypochlorite solution with an appropriate concentration. Add water to make 100 mL. Store in a cool, dark place.

Sodium Hypochlorite TS Use a solution containing 5% of available chlorine.

Sodium Iodide NaI [7681-82-5] A white, deliquescent crystalline powder.

Content Not less than 99.5% of sodium iodide (NaI) when dried.

Identification When burned in a colorless flame, a solution of sodium iodide (1 in 200) gives a yellow flame.

Loss on drying Not more than 0.5% (110°C, 2 hours).

Assay Weigh accurately about 0.5 g of sodium iodide, previously dried, into a stoppered 300-mL flask, dissolve it in 25 mL of water, and cool below 5°C. Add 35 mL of hydrochloric acid and 5 mL of chloroform, each previously cooled below 5°C. Titrate with 0.05 mol/L potassium iodide with constant shaking until the color of iodine in the water

layer disappears. Stopper tightly, and shake well. Then, shake vigorously after each drop of 0.05 mol/L potassium iodide is added. The end point is when the violet color of the chloroform phase completely disappears.

Each mL of 0.05 mol/L potassium iodide = 14.99 mg of NaI

Sodium Lauryl Sulfate $C_{12}H_{25}NaO_4S$ [151-21-3] Use sodium lauryl sulfate specified in the Japanese Pharmacopoeia.

Sodium Lauryl Sulfate–Propylene Glycol TS Dissolve 1 g of sodium lauryl sulfate in 80 mL of water, and mix it with 20 mL of propylene glycol.

Sodium Nitrite $NaNO_2$ [K8019, Special Grade] [7632-00-0]

Sodium 1-Octanesulfonate $C_8H_{17}NaO_3S$ [5324-84-5] A white powder.

Clarity of solution Clear (1.1 g, 50 mL).

Content Not less than 98.0%. Weigh accurately about 0.4 g of sodium 1-octanesulfonate, dried at 105°C for 2 hours, dissolve it in 25 mL of water, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2–3 drops of phenolphthalein TS). The endpoint is when the faint red color persists for 15 seconds.

Each mL of 0.1 mol/L sodium hydroxide = 21.672 mg of $CH_3(CH_2)_7SO_3Na$

Sodium Oxalate (Reference Material) NaOCOCOONa [Reference Material for Volumetric Analysis, K8005] [62-76-0] In addition to Reference Material for Volumetric Analysis specified in JIS K8005, a certified reference material capable of being used for volumetric analysis is usable.

Sodium Pentacyanonitrosylferrate(III) Dihydrate $Na_2[Fe(CN)_5NO]\cdot 2H_2O$ [K8722, Special Grade] [13755-38-9]

Sodium Pentacyanonitrosylferrate(III) TS Dissolve 1.0 g of sodium pentacyanonitrosylferrate(III) dihydrate in water to make 20 mL. Prepare fresh before use.

Sodium Periodate $NaIO_4$ [K8256, Special Grade] [7790-28-5]

Sodium Periodate TS Dissolve 1.25 g of sodium periodate in water to make 100 mL.

Sodium Periodate TS for Glycerol Weigh 6 g of sodium periodate, and dissolve it in a solution that was prepared by adding 12 mL of diluted sulfuric acid (3 in 1000) to 38 mL of freshly boiled and cooled water. Add freshly boiled and cooled water to make 100 mL. Filter if necessary.

Sodium Phosphate Buffer (0.5 mol/L)

Solution 1 Dissolve 78 g of sodium dihydrogenphosphate dihydrate in water to make 1000 mL.

Solution 2 Dissolve 179 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Sodium Phosphate Buffer (0.2 mol/L)

Solution 1 Dissolve 31.2 g of sodium dihydrogenphosphate dihydrate in water to

make 1000 mL.

Solution 2 Dissolve 71.6 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Sodium Phosphate Buffer (0.1 mol/L)

Solution 1 Dissolve 15.6 g of sodium dihydrogenphosphate dihydrate in water to make 1000 mL.

Solution 2 Dissolve 14.2 g of disodium hydrogenphosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Sodium Phosphate Buffer (0.05 mol/L)

Solution 1 Dissolve 7.8 g of sodium dihydrogenphosphate dihydrate in water to make 1000 mL.

Solution 2 Dissolve 7.1 g of disodium hydrogenphosphate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Sodium Phosphate Buffer (0.01 mol/L, pH 7.0, containing ethylene glycol) Mix 50 mL of sodium phosphate buffer (0.2 mol/L) at pH 7.0 and 100 mL of ethylene glycol, and add water to make 1000 mL.

Sodium Phosphate Buffer (0.004 mol/L)

Solution 1 Dissolve 0.62 g of sodium dihydrogenphosphate dihydrate in water to make 1000 mL.

Solution 2 Dissolve 1.43 g of disodium hydrogenphosphate dodecahydrate in water to make 1000 mL.

Mix both solutions, and adjust the pH to the corresponding value specified in this publication.

Sodium Phytate Hydrate $C_6H_{18}O_{24}P_6 \cdot m Na^+ \cdot n H2O$ Use a product suitable for the corresponding enzyme activity tests.

Sodium Polygalacturonate Use a citrus-derived product that is suitable for the corresponding enzyme activity tests.

Sodium Pyrosulfite $Na_2S_2O_5$ Use a product suitable for the corresponding enzyme activity tests.

Sodium Selenite Na_2SeO_3 [10102-18-8] A white crystalline powder.

Content Not less than 97.0%.

Purity (1) Clarity of solution Clear (2.0 g, water 20 mL).

(2) Selenium salts and sulfates Measure exactly 5 mL of the test solution prepared in Purity (1), add 10 mL of water, and adjust the pH to 6.0 with diluted hydrochloric acid (1 in 3). Add 1 mL of diluted hydrochloric acid (2 in 3), and add water to make exactly 25

mL. To the resulting solution, add 2 mL of barium chloride dihydrate solution (1 in 10), and allow to stand for 30 minutes. The solution is not turbid (not more than about 0.3% as SeO_4 or not more than about 0.05% as SO_4).

Assay Weigh accurately about 1 g of sodium selenium, and dissolve it in water to make exactly 200 mL. Transfer exactly measured 20 mL of this solution to an iodine flask, add 80 mL of water, 3 g of potassium iodide, and 5 mL of diluted hydrochloride acid (2 in 3). Immediately stopper, allow to stand for 5 minutes in a dark place. Titrate the free iodine with 0.1 mol/L sodium thiosulfate (indicator: 0.5 mL of starch TS). Starch TS should be added near the endpoint, when the color of the solution becomes light yellow. The endpoint is when the blue color of solution disappears. Separately, perform a blank test to make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 4.324 mg of Na_2SeO_3

Sodium Sulfate Na_2SO_4 [K8987, Special Grade] [7757-82-6]

Sodium Sulfate Decahydrate $Na_2SO_4 \cdot 10H_2O$ [K8986, Special Grade] [7727-73-3]

Sodium Sulfide Nonahydrate $Na_2S \cdot 9H_2O$ [K8949, Special Grade] [1313-84-4]

Sodium Sulfide TS Dissolve 5 g of sodium sulfide nonahydrate in a solution of 30 mL of glycerol in 10 mL of water. Allow to stand, and use the supernatant. Use within 3 months of preparation.

Sodium Sulfite Na_2SO_3 [K8061] [7757-83-7]

Sodium (+)-Tartrate Dihydrate $NaOOCCH(OH)CH(OH)COONa \cdot 2H_2O$ [K8540, Special Grade] [6106-24-7]

Sodium Tetraborate Decahydrate $Na_2B_4O_7 \cdot 10H_2O$ [K8866, Special Grade, pH Standard Solution Grade] [1303-96-4]

Sodium Tetraborate Decahydrate for pH Determination $Na_2B_4O_7 \cdot 10H_2O$ [K8866, pH Standard Solution Grade] [1303-96-4]

Sodium Tetraborate–Sulfuric Acid TS Dissolve 0.95 g of sodium tetraborate decahydrate in 100 mL of sulfuric acid.

Sodium Tetraborate TS (0.1 mol/L) Dissolve 38.1 g of sodium tetraborate decahydrate in water to make 1000 mL.

Sodium Tetrahydroborate $NaBH_4$ (for atomic absorption spectrometry) [16940-66-2]

Sodium Tetrahydroborate for Amino Acid Analysis $NaBH_4$ [16940-66-2] Use sodium tetrahydroborate produced for the amino acid analysis.

Description A white crystalline powder.

Sodium Tetrahydroborate TS Dissolve 5 g of sodium tetrahydroborate in 500 mL of sodium hydroxide TS (0.1 mol/L).

Sodium Thiosulfate Pentahydrate $Na_2S_2O_3 \cdot 5H_2O$ [K8637, Special Grade] [10102-17-7]

Sodium Thiosulfate TS (0.1 mol/L) Dissolve 26 g of sodium thiosulfate pentahydrate and 0.2 g of sodium carbonate in freshly boiled and cooled water to make 1000 mL.

Sodium Thiosulfate TS (0.05 mol/L) Dilute 0.1 mol/L sodium thiosulfate to twice the

original volume with freshly boiled and cooled water.

Sodium Thiosulfate TS (0.02 mol/L) Dilute 0.1 mol/L sodium thiosulfate to five times the original volume with freshly boiled and cooled water.

Sodium *p*-Toluenesulfonchloramide Trihydrate $C_7H_7ClNNaO_2S \cdot 3H_2O$ [K8318] [7080-50-4]

Sodium *p*-Toluenesulfonchloramide TS Dissolve 1.25 g of sodium *p*-toluenesulfonchloramide trihydrate in water to make 100 mL. Prepare fresh before use.

Sodium Tungstate(VI) Dihydrate $Na_2WO_4 \cdot 2H_2O$ [K8612, Special Grade] [10213-10-2]

Soluble Starch Use a product suitable for the corresponding enzyme activity tests.

pH 4.5–7.5 (2% solution).

Residue on ignition Not more than 0.6%.

Loss on drying Not more than 15% (105°C, 2 hours).

Soluble Starch TS Mix well 1.0 g of soluble starch with 10 mL of cold water. Add it gradually into 90 mL of hot water while stirring. Boil it gently for 3 minutes, and cool. Prepare fresh before use.

Somogyi's Copper TS Weigh 71 g of disodium hydrogenphosphate dodecahydrate and 40 g of (+)-potassium sodium tartrate tetrahydrate, and dissolve them in 650 mL of water. To this solution, add 100 mL of sodium hydroxide TS (1 mol/L). Then add 80 mL of copper(II) sulfate solution (1 in 10) while stirring, and warm. Dissolve 180 g of sodium sulfate in the resulting solution, and make up to 1000 mL with water. Leave the solution for 2 days at room temperature, and filter it twice through a filter papers (No. 2). Store in a tightly stoppered container, protected from light.

Somogyi TS (I) Weigh 4.0 g of copper(II) sulfate pentahydrate, 24 g of sodium carbonate, 16 g of sodium hydrogen carbonate, 180 g of sodium sulfate, 12g of (+)-potassium sodium tartrate tetrahydrate, and dissolve them in water to make 900 mL. Boil this solution for 10 minutes, and make up to 1000 mL with water. Stopper tightly, leave it for 1 week, and filter through a glass filter. Store, protected from light.

Somogyi TS (II) Weigh 25 g of sodium carbonate and 25 g of (+)-potassium sodium tartrate tetrahydrate, and dissolve them in water to make 150 mL. To this solution, add 40 mL of sodium hydroxide TS (1 mol/L), 60 mL of copper(II) sulfate pentahydrate (1 in 10), and 25 mL of potassium iodide solution (1 in 5), and mix them. Then add 500 mL of sodium sulfate solution (9 in 25), 50 mL of potassium iodide TS (0.05 mol/L), and water to make 1000 mL. Leave it for 2 days at room temperature, and filter through a filter paper.

Somogyi TS (III) Weigh 4.0 g of copper(II) sulfate pentahydrate, 24 g of sodium carbonate, 16 g of sodium hydrogen carbonate, 18 g of sodium sulfate, 12 g of (+)-potassium sodium tartrate tetrahydrate, dissolve them in water to make 1000 mL. Boil this solution for 10 minutes, stopper tightly, and leave for 1 week, protected from light. Filter twice the solution through two filter papers (No. 2) stacked. Store in a tightly stoppered container, protected from light.

D-Sorbitol $C_6H_{14}O_6$ [50-70-4] "D-Sorbitol"

D-Sorbitol for Assay $C_6H_{14}O_6$ Weigh 80 g of D-sorbitol into a 500-mL flask, add 220 mL of 90% methanol, and dissolve it while warming on a water bath under a reflux condenser. Cool, transfer into a 500-mL beaker, add 40 mg of "D-Sorbitol" as seed crystals, mix, and allow to stand for 72 hours. Filter the formed crystals by suction, and wash with 50 mL of methanol. Weigh 40 g of the recrystallized product, add 110 mL of 90% methanol, and repeat the process above to obtain a twice-recrystallized product, using a recrystallized product dried under reduced pressure at 80°C for 5 hours as the seed crystals. Dry the twice-recrystallized product under reduced pressure at 80°C for 5 hours.

Starch [K8658, Special Grade] [9005-84-9]

Starch (soluble) [K8659, Special Grade or First Grade] [9005-84-9]

Starch TS Weigh 1 g of starch (soluble), and mix well with 10 mL of cold water. Add the mixture gradually into 200 mL of hot water while stirring, and boil until the liquid becomes translucent. Allow to cool and stand, and use the supernatant as starch TS. Prepare fresh before use.

Stearic Acid $C_{18}H_{36}O_2$ [K8585, Special Grade] [57-11-4]

Steviolbioside $C_{32}H_{50}O_{13}$ [41093-60-1] A white to light brown powder.

Identification (1) Determine the spectrum of steviolbioside as directed in the Disk Method under Infrared Spectrophotometry. The spectrum exhibits absorptions at wavenumbers of about 3370 cm^{-1}, 2940 cm^{-1}, 1700 cm^{-1}, 1450 cm^{-1}, 1370 cm^{-1}, 1240 cm^{-1}, 1170 cm^{-1}, 1080 cm^{-1}, 1030 cm^{-1}, and 890 cm^{-1}.

(2) Dissolve 10 mg of steviolbioside in 1 mL of 1,4-dioxane. Analyze 5 μL of this solution by thin-layer chromatography using a 27:20:3 mixture of methanol/chloroform/water as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography and 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 the plate with a 20:1 mixture of water/sulfuric acid, heat at 200°C for 10 minutes, and examine it. A major spot is observed at an R_f value of about 0.7.

Purity Related substances Prepare a test solution by dissolving 5 mg of steviolbioside in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC). Analyze 10 μL of the test solution by liquid chromatography using the operating condition specified in the Assay for Stevia Extract in the Monographs. Continue the chromatography for 40 minutes and exclude the solvent peak from measurement. Measure the areas of all the peaks to determine the percentage of the main peak by the peak area percentage method. It is not less than 95.0%.

Stevioside $C_{38}H_{60}O_{18}$ [57817-89-7] A white powder.

Identification (1) Determine the spectrum of stevioside as directed in the Disk Method under Infrared Spectrophotometry. The spectrum exhibits absorptions at wavenumbers of about 2940 cm^{-1},1750 cm^{-1}, 1660 cm^{-1}, 1450 cm^{-1}, 1230 cm^{-1}, 1170 cm^{-1},

1080 cm^{-1},1040 cm^{-1}, 890 cm^{-1}, and 630 cm^{-1}.

(2) Dissolve 10 mg of stevioside by adding 0.5 mL of methanol, 0.5 mL of chloroform, and 0.1 mL of water. Using 5 μL of the resulting solution, proceed as directed in Identification (2) for steviolbioside. The main spot is observed at an R_f value of around 0.6.

Purity Related substances Prepare a test solution by dissolving 5 mg of stevioside in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC). Analyze 10 μL of the test solution by liquid chromatography using operating conditions specified in the Assay for Stevia Extract in the Monographs. Continue the chromatography for 30 minutes and exclude the solvent peak from measurement. Measure the areas of all the peaks to determine the percentage of the main peak by the peak area percentage method. It is not less than 95.0%.

Stevioside for Assay $C_{38}H_{60}O_{18}$ [57817-89-7] A white powder.

Identification Proceed as directed in Identification (1) and (2) for stevioside.

Purity Related substances Prepare a test solution by dissolving 5 mg of stevioside for assay in 5 mL of a 7:3 mixture of water/acetonitrile (for HPLC). Analyze 10 μL of the test solution by liquid chromatography using the operating conditions specified in thr Assay for Stevia Extract in the Monographs. Continue the chromatography for 30 minutes of injection and exclude the solvent peak from measurement. Measure the areas of all the peaks to determine the percentage of the main peak by the peak area percentage method. It is not less than 99.0%.

Loss on drying Not more than 5.0% (50 mg, 105°C, 2 hours).

Stigmasterol See Stigmasterol for Assay.

Stigmasterol for Assay $C_{29}H_{48}O$ [83-48-7] A white crystalline powder.

Identification Dissolve 5 mg of stigmasterol for assay in 2 mL of hexane, add 1 mL of acetic anhydride and 1 drop of sulfuric acid, and shake. The lower layer is red-purple, which changes green through blue.

Melting point 165–170°C.

Purity Related substances Prepare a test solution by dissolving 80 mg of stigmasterol for assay in 20 mL of acetone. Prepare a control solution by diluting exactly measured 1.5 mL of the test solution to exactly 50 mL with acetone. Analyze 2 μl potions of the test solution and the control solution by gas chromatography using the operating conditions specified in the Assay for Vegetable Sterol (High Concentration of Free Sterol) in the Monographs. Continue the chromatography for two times the retention time of the main peak and exclude the solvent peaks from measurement. The sum of the areas of all the peaks of the test solution, excluding the main peak, is not larger than the area of the main peak of the control solution.

Strongly Acidic Cation-exchange Resin The sodium salt of strongly acidic polystyrene sulfonic acid. A light yellow to yellow-brown powder. Passes through a 600-μm standard sieve, and hardly passes a 425-μm sieve.

Weigh about 50 g of strongly acidic cation-exchange resin, immerse in water for 30 minutes. Pour the resin with water into a glass tube for chromatography (about 25 mm internal diameter) to prepare a resin column. Pour 250 mL of diluted hydrochloric acid (1 in 4) into the column, and allow it to pass through at a rate of about 4 mL per minute. Then pour water to wash the column until the color of the washings becomes green to blue with bromocresol green TS, and perform the following test:

Measure 10 mL of the resin, pour it with water into a glass tube for chromatography (15 mm internal diameter), and allow 80 mL of 0.1 mol/L sodium hydroxide to pass through at a rate of about 2 mL per minute. The pH of the effluent is 5.0–6.5.

Strongly Acidic Cation-exchange Resin (Fine) A hydrogen ion type of strongly acidic polystyrene sulfonic acid. A light yellow to yellow-brown powder. Passes through a 150-μm standard sieve and hardly passes a 75-μm sieve.

Weigh about 50 g of strongly acidic cation-exchange resin (Fine), immerse in water for about 1 hour, and decant 2 or 3 times until the supernatant becomes clear. Pour the resin with water into a glass tube for chromatography (about 25 mm internal diameter) to prepare resin column. Pour 250 mL of diluted hydrochloric acid (1 in 4) into the column, and allow to pass through at a rate of about 4 mL per minute. Then pour water to wash the column until the color of the washings becomes green to blue with bromocresol green TS, and perform the following test:

Measure 10 mL of the resin, pour it with water into a glass tube for chromatography (15 mm internal diameter), and allow 80 mL of 0.1 mol/L sodium hydroxide to pass through at a rate of about 2 mL per minute. The pH of the effluent is 4.0–6.5.

Strongly Acidic Phosphorylated Cellulose Cation Exchanger (–O–PO_3H_2 Form) Use a strongly acidic cation exchanger prepared by introducing phosphoric groups into porous cellulose.

Strongly Basic Anion-exchange Resin A strongly basic quaternary ammonium salt of polystyrene. A yellow to yellow-brown powder. Passes through a 600-μm standard sieve but hardly passes through a 425-μm sieve.

Weigh about 50 g of strongly basic anion-exchange resin, immerse in water, allow to stand for 30 minutes, and pour the resin with water into a glass tube for chromatography (about 2.5 cm internal diameter) to prepare a resin column. Pour 2000 mL of sodium hydroxide solution (1 in 25) into the column, and allow to pass through at a rate of about 30 mL per minute. Then, wash the resin with water until the washings are neutral to phenolphthalein TS, and perform the following test:

Measure 10 mL of the resin, pour it with water into a glass tube for chromatography (15 mm internal diameter), and allow 70 mL of 0.1 mol/L hydrochloric acid to pass through the column at a rate of about 2 mL per minute. The pH of the effluent is 4.0–8.0.

Strontium Nitrate $Sr(NO_3)_2$ [K8554, Special Grade] [10042-76-9]

Styrene–Divinylbenzene Resin for Adsorption A porous resin made as adsorbent.

Substrate Solution for Protease Use an appropriate one of the following solutions.

1. Casein TS (pH 2.6 or pH 3.0)

Dry about 1 g of casein (milk), accurately weighed, at 105°C for 2 hours, and determine the loss on drying. Weigh the amount of casein (milk) equivalent to 0.60 g of the dried casein, add 6 mL of lactic acid TS and 75 mL of water, and dissolve while warming in a water bath. After cooling in running water, adjust the pH to 2.6 or 3.0 with sodium hydroxide TS (1 mol/L), and make up to 100 mL with water.

2. Casein TS (pH 6.0, pH 7.0, pH 8.0, or pH 10.0)

Dry about 1 g of casein (milk), accurately weighed, at 105°C for 2 hours, and determine the loss on drying. Weigh the amount of casein (milk) equivalent to 0.60 g of the dried casein, add 80 mL of disodium hydrogenphosphate TS (0.05 mol/L), and dissolve while warming in a water bath. After cooling in running water, adjust the pH to 6.0, 7.0, 8.0, or 10.0 with hydrochloric acid TS (1 mol/L) or sodium hydroxide TS (1 mol/L), and make up to 100 mL with water.

3. Dimethyl casein TS ((pH 7.0 or pH 8.0))

Dissolve 3.2 g of *N,N*-dimethyl casein in 200 mL of hot water. Separately, dissolve 25.9 g of sodium tetraborate decahydrate and 13.3 g of sodium dihydrogen phosphate decahydrate in 400 mL of water. To this solution, add the prepared solution of *N,N*-dimethyl casein and 0.6 mL of polyoxyethyelen(23) lauryl ether (3 in 10), and mix. Adjust the pH to 7.0 or 8.0 with hydrochloric acid TS (1 mol/L) or sodium hydroxide TS (1 mol/L), and make up to 1000 mL.

Substrate TS for Lysozyme To an appropriate amount of dried cells of *Micrococcus luteus (use a product suitable for the corresponding enzyme activity tests)*, add phosphate buffer (pH 6.2) to suspend uniformly. Adjust so that its transmittance is 10% at 640 nm. Prepare fresh before use.

Succinyl Trialanine *p*-Nitroanilide $C_{19}H_{25}N_5O_8$

N-Succinyl-L-alanyl-L-alanyl-L-alanine4-nitroanilide Use a product suitable for the corresponding enzyme activity tests.

Sucrose $C_{12}H_{22}O_{11}$ [K8383] [57-50-1] Use sucrose specified in the Japanese Pharmacopoeia.

Sulfanilic Acid $NH_2C_6H_4SO_3H$ [K8586, Special Grade] [121-57-3]

Sulfanilic Acid Azo β-Naphthol $C_{16}H_{11}N_2NaO_4S$ [633-96-5] Monosodium 4-(2-hydroxy-1-naphthylazo)benzenesulfonate. A yellow-red to reddish yellow powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 481–487 nm): Not less than 500. Weigh accurately about 10 mg of sulfanilic acid azo β-naphthol, previously dried for 24 hours in a vacuum desiccator, dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits an absorption maximum at wavelength of 481–487 nm. Measure the absorbance of this solution at the maximum between 481–487 nm against ammonium acetate TS (0.02 mol/L).

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add ammonium acetate TS (0.02 mol/L) to about 5 mg of sulfanilic acid azo β-naphthol to make exactly 25 mL. Analyze 10 μL each of the test solution and ammonium acetate TS (0.02 mol/L) by liquid chromatography using the operating conditions given in Purity (2) for aniline azo Schaeffer's salt. Measure the area of each peak that appears within 40 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the ammonium acetate TS in the test solution.

Water Not more than 10.0% (50 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

Sulfanilic Acid Azo G Salt $C_{16}H_9N_2Na_3O_{10}S$ [84030-17-1] Trisodium 7-hydroxy-8-(4-sulfophenylazo)-1,3-naphthalenesulfonate. An orange-red powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 472–478 nm): Not less than 303. Weigh accurately about 10 mg of sulfanilic acid azo G salt, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits an absorption maximum at a wavelength of 472–478 nm. Measure the absorbance of this solution at the maximum between 472–478 nm against ammonium acetate TS (0.02 mol/L) to determine the specific absorbance.

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add the mobile phase to 5 mg of sulfanilic acid azo G salt to make exactly 25 mL. Analyze 10 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 20 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the mobile phase in the test solution.

Operating conditions

Detector: Visible spectrophotometer (wavelength: 490 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 3:2 mixture of ammonium acetate–tetra-*n*-butylammonium bromide TS/acetonitrile (for HPLC).

Flow rate: 1.0 mL/min.

Water Not more than 10.0% (50 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

Sulfanilic Acid Azo R Salt $C_{16}H_9N_2Na_3O_{10}S_3$ [50880-65-4] Trisodium 3-hydroxy-4-(4-sulfophenylazo)-2,7-naphthalenesulfonate. A red to yellow-red powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 485–491 nm): Not less than 410. Weigh accurately about 10 mg of sulfanilic acid azo R salt, previously dried for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits an absorption maximum at a wavelength of 485–491 nm. Measure the absorbance of this solution at the maximum between 485–491 nm against ammonium acetate TS (0.02 mol/L).

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: Add the mobile phase to 5 mg of sulfanilic acid azo R salt to make exactly 25 mL. Analyze 10 μL each of the test solution and the mobile phase by liquid chromatography using the operating conditions given in Purity (2) for sulfanilic acid azo G salt. Measure the area of each peak that appears within 20 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from the mobile phase in the test solution.

Water Not more than 10.0% (10 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

Sulfur Dioxide SO_2 [7446-09-5] A colorless gas having a characteristic odor. Prepare by adding dropwise sulfuric acid to a concentrated solution of sodium hydrogen sulfite.

Sulfuric Acid H_2SO_4 [K8951, Special Grade] [7664-93-9]

Sulfuric Acid for the Readily Carbonizable Substances Test To sulfuric acid whose content is previously determined by the following procedure, add water carefully to prepare 94.4–95.5% sulfuric acid (H_2SO_4). Do not use the sulfuric acid if the concentration has changed by absorbing moisture during storage.

Assay Weigh accurately about 2 g of sulfuric acid into a stoppered flask, and add 30 mL of water. Weighing should be done promptly. After cooling, titrate with 1 mol/L sodium hydroxide (indicator: 2–3 drops of bromothymol blue).

Each mL of 1 mol/L sodium hydroxide = 49.04 mg of H_2SO_4

15% Sulfuric Acid–Methanol TS To 20 mL of methanol, add 8.2 mL of sulfuric acid gradually, and cool. Add methanol to make 100 mL.

85% Sulfuric Acid TS Determine the content of sulfuric acid to be used by the following methods, and prepare a 85% solution of sulfuric acid by adding sulfuric acid to water.

Measure the mass of a 100-mL ground glass stoppered Erlenmeyer flask to the digit of 0.1 mg. Place 1.0 g of sulfuric acid to the flask, and again measure the mass to the digit of 0.1 mg. Add 20 mL of water gradually while cooling the flask. Titrate with 1 mol/L sodium hydroxide (indicator: a few drops of bromothymol blue TS). The endpoint is when the color of solution changes from yellow to bluish green. Caluclate the content of sulfuric acid by the formula:

Content (%) of sulfuric acid = $V \times f \times 0.04904 \times 100/(m_1 - m_2)$

V = the amount of 1 mol/L sodium hydroxide consumed,

f = the factor of 1 mol/L sodium hydroxide,

m_1 = the mass (g) of the empty Erlenmeyer flask containing the sample,

m_2 = the mass (g) of the Erlenmeyer flask.

10% Sulfuric Acid TS Add 5.7 mL of sulfuric acid gradually to 10 mL of water. After cooling, make up with water to 100 mL.

70% (vol) Sulfuric Acid TS Add 70 mL of sulfuric acid gradually to 30 mL of water while cooling in icy water.

Sulfuric Acid TS (2.5 mol/L) Add 140 mL of sulfuric acid gradually to water. After cooling, make up with water to 1000 mL.

Sulfuric Acid TS (2 mol/L) Add 110 mL of sulfuric acid gradually to water. After cooling, make up with water to 1000 mL.

Sulfuric Acid TS (1 mol/L) Add 56 mL of sulfuric acid gradually to water. After cooling, make up with water to 1000 mL.

Sulfuric Acid TS (0.5 mol/L) Add 28 mL of sulfuric acid gradually to water. After cooling, make up with water to 1000 mL.

Sulfuric Acid TS (0.25 mol/L) Add 15 mL of sulfuric acid gradually to 1000 mL of water while stirring, and allow to cool.

Sulfuric Acid TS (0.05 mol/L) Add water to 100 mL of sulfuric acid TS (0.5 mol/L) to make 1000 mL.

Sulfuric Acid TS (0.025 mol/L) Add water to 100 mL of sulfuric acid TS (0.25 mol/L) to make 1000 mL.

Sulfuric Acid TS (5.5 mmol/L) Add 0.3 mL of sulfuric acid gradually to water. After cooling, make up with water to 1000 mL.

Sulfuric Acid TS (0.005 mol/L) Add water to 10 mL of sulfuric acid TS (0.5 mol/L) to make 1000 mL.

Sulfurous Acid Solution H_2SO_3 [7782-99-2] A colorless, clear liquid having a pungent odor. It is oxidized gradually in air.

Content Not less than 5.0% as SO_2.

Assay Add exactly 25 mL of 0.05 mol/L iodine to 10 mL of water, immediately stopper, and weigh. Add 1 mL of sulfurous acid solution, stopper, and weigh. Titrate with 0.1 mol/L sodium thiosulfate. Add 3 mL of starch TS as the indicator near the endpoint, when the color of solution becomes light yellow. The endpoint is when the color of the

solution disappears.

Each mL of 0.05 mol/L iodine = 3.203 mg of SO_2

Synthetic Zeolite for Desiccation A mixture of $6(Na_2O)\cdot 6(Al_2O_3)\cdot 12(SiO_2)$ and $6(K_2O)\cdot 6(Al_2O_3)\cdot 12(SiO_2)$ that was produced for drying purposes. Usually, it is produced with the addition of a binding agent in spherical shape with the diameter of about 2 mm. It is originally white to grayish white. Some products contain discoloring agents that change in color when they absorb moisture. The average pore size is 0.3 nm and the surface area is 500−700 m^2/g.

Loss on Ignition Not more than 2.0% (2 g, 550–600°C, 4 hours, allowing to cool in a desiccator containing phosphorus oxide(V)).

Tannic Acid–Acetic Acid TS Dissolve 10 mg of tannic acid *n*-hydrate in 80 mL of acetic acid by shaking, and add 32 mL of phosphoric acid. Prepare fresh before use.

Tannic Acid *n*-Hydrate $C_{14}H_{10}O_9\cdot nH_2O$ [1401-55-4] A white to light yellow powder or almost colorless, luster leaflet flakes.

Identification (1) Dissolve 2 g of tannic acid *n*-hydrate in water while heating in a water bath to make 10 mL. To 5 mL of this solution, add 1 mL of 10% (w/v) iron(III) chloride–hydrochloric acid TS. A blue-black color develops, and a blue-black precipitate forms when it is allowed to stand.

(2) Determine the spectrum of tannic acid *n*-hydrate as directed in the Paste Method under Infrared Spectrophotometry. The spectrum exhibits absorptions at wavenumbers of about 1710 cm^{-1}, 1610 cm^{-1}, 1540 cm^{-1}, 1180 cm^{-1}, 1080 cm^{-1}, 1020 cm^{-1}, 870 cm^{-1}, and 760 cm^{-1}.

Purity Sugar and dextrin Weigh 2 g of tannic acid *n*-hydrate, add 10 mL of water and 100 mL of ethanol (95), allow to stand for 1 hour. The solution is clear. When 5 mL of diethylen ether is added, the solution does not immediately get turbid.

Loss on drying Not more than 12.0% (1 g, 105, 2 hours).

Residue on ignition Not more than 1.0%. Weigh 1 g of tannic acid *n*-hydrate into a platinum crucible, add 0.2 mL of sulfuric acid, and gradually heat to carbonize it. Then heat strongly by a gas burner to incinerate it, and weigh the residue.

Tannic Acid TS Dissolve 1.0 g of tannic acid *n*-hydrate in 1 mL of ethabol (95), and add water to make 10 mL. Prepare fresh before use.

L(+)-Tartaric Acid HOOCCH(OH)CH(OH)COOH [K8532, Special Grade] [87-69-4]

Tetra-*n*-butylammonium Bromide $[CH_3(CH_2)_3]_4NBr$ [1643-19-2] White crystals or powder.

Content Not less than 98.0%.

Melting point 102−106°C.

Purity Clarity Almost clear (1.0 g, water 20 mL).

Residue on ignition Not more than 0.1%. Ignite a platinum crucible at 500 ± 50°C for at least 30 minutes, leave it to cool in a desiccator, and weigh accurately. Weigh 1 g of the sample in the crucible, and weigh accurately. Heat the crucible on a hot plate by

gradually increasing the temperature to sublime or decompose the sample. Remove it from the plate, and leave to cool at room temperature. Add about 0.2 mL of sulfuric acid, heat again gently, and continue heating until white fumes are no longer evolved. Heat the crucble in an electric furnace at 500 ± 50°C for 1 hour, remove it from the furnace, leave to cool in a desiccator, and weigh. If the value does not meet the specified limit, repeat the abovementioned steps (the addition of sulfuric acid, heating, and weighing) until the difference of two consequent constant weights is 0.3 mg or the resulting value is not more than the specified limit.

Assay Weigh accurately about 0.5 g of tetra-*n*-butylammonium bromide, dissolve it in 50 mL of water, add 5 mL of diluted nitric acid (1 in 3). Titrate the resulting solution with 0.1 mol/L silver nitrate while shaking vigorously. Separately, perform a blank test to make any necessary correction.

Each mL of 0.1 mol/L silver nitrate = 32.24 mg of $C_{16}H_{36}NBr$

Tetra-*n*-butylammonium Dihydrogen Phosphate TS (0.5 mol/L) A colorless to pale yellow, clear liquid.

Identification (1) To 10 mL of Add tetra-*n*-butylammonium dihydrogen phosphate TS (0.5 mol/L), add 1 mL of diluted ammonia solution (2 in 5) and 2 mL of magnesia TS, and mix. A white precipitate is formed.

(2) Heat 10 mL of tetra-*n*-butylammonium dihydrogen phosphate TS (0.5 mol/L) with 1 mL of sodium hydroxide solution (1 in 10). An odor of ammonia is evolved.

Absorbance Measure the absorbance of tetra-*n*-butylammonium dihydrogen phosphate TS (0.5 mol/L) as directed under Visible-Ultraviolet Spectrophotometry. The absorbance values at wavelengths of 240 nm, 245 nm, 300 nm, and 350 nm are not more than 0.50, 0.30, 0.15, and 0.10, respectively.

Purity (1) Bromide Not more than 0.1%. Prepare a test solution by dissolving 0.2 g of tetra-*n*-butylammonium dihydrogen phosphate TS (0.5 mol/L) in water to make 20 mL, adding 5 mL of diluted nitric acid (2 in 3) and 1 mL of silver nitrate solution (1 in 10), and leaving for 15 minutes. Prepare a control solution by adding water to 2 mL of Bromide Ion Standard Stock Solution to make 20 mL, then adding 5 mL of diluted nitric acid (2 in 3) and 1 mL of silver nitrate solution (1 in 10), and leaving for 15 minutes. The turbidity produced in the test solution is not stronger than that produced in the control solution.

(2) Molar concentration 0.45–0.55 mol/L. Dilute 25 mL of tetra-*n*-butylammonium dihydrogen phosphate TS (0.5 mol/L), measured exactly, to 50 mL. Titrate this solution with 1 mol/L sodium hydroxide. To confirm the endpoint, use a potentiometer with a glass indicator electrode and a silver-silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes.

Each mL of 1 mol/L sodium hydroxide = 339.45 mg of $[CH_3(CH_2)_3]_4NH_2PO_4$

Calculate the concentration by the formula:

$$A = \frac{0.33945 \times a \times f}{25 \times 1000}$$

$$B = \frac{A}{339.45}$$

A = Concentration (g/L),

B = molar concentration (mol/L),

a = amount (mL) of 1 mol/L sodium hydroxide consumed,

f = factor of 1 mol/L sodium hydroxide.

Tetrabutylammonium Hydrogensulfate $[(C_4H_9)_4N]HSO_4$ [32503-27-8] A white crystalline powder.

Content Not less than 98.0% of tetrabutylammonium hydrogensulfate ($[(C_4H_9)_4N]HSO_4$).

Purity (1) Clarity of solution Almost clear (1.0 g, water 20 mL).

(2) Chloride Not more than 0.001% as Cl. To a solution of 2 g of tetrabutylammonium hydrogensulfate (1 in 10), add 5 mL of diluted nitric acid (1 in 3) and 1 mL of silver nitrate solution (1 in 50), and allow to stand for 15 minutes. The white turbidity formed is not higher than that formed when 5 mL of diluted nitric acid (1 in 3) and 1 mL of silver nitrate solution (1 in 50) are added to 2 mL of Chloride Ion Standard Stock Solution (1 in 10) and the solution obtained is allowed to stand for 15 minutes.

Assay Weigh accurately about 0.7 g of tetrabutylammonium hydrogensulfate, and dissolve it in 100 mL of water (carbon dioxide-removed). Titrate the solution prepared with 0.1 mol/L sodium hydroxide solution (indicator: bromocresol green–methyl red TS).

Each mL of 0.1 mol/L sodium hydroxide = 0.03395g of $[(C_4H_9)_4N]HSO_4$

Tetrabutylammonium Hydrogensulfate TS (0.01 mol/L) Dissolve 3.4 g of tetrabutylammonium hydrogensulfate in water to make 1000 mL.

Tetrabutylammonium Hydroxide–Methanol TS A colorless to slightly pale yellow liquid.

Content Not less than 10%.

Weigh 5 g of tetrabutylammonium hydroxide–methanol TS, add 50 mL of water, and titrate with 0.1 mol/L hydrochloric acid. To confirm the endpoint, use a potentiometer with a glass indicator electrode and a silver-silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes. Separately, perform a blank test to make necessary correction.

Each mL of 0.1 mol/L hydrochloric acid = 25.947 mg of $[(CH_3CH_2CH_2CH_2)_4N]OH$

Tetrahydrofuran C_4H_8O [K9705, Special Grade] [109-99-9]

Tetrahydrofuran (containing BHT) [K9705, Special Grade] [109-99-9] Use tetrahydrofuran containing 0.025% of butylated hydroxytoluene.

Tetramethylammonium Bromide $C_4H_{12}BrN$ [64-20-0]

Content Not less than 98.0%.

Description White to yellowish white, volatile crystals.

Identification (1) Dissolve 1 g of tetramethylammonium bromide in 20 mL of water. To 10 mL of this solution, add 1 mL of diluted hydrochloric acid (1 in 6) and 1 mL of sodium *p*-toluenesulfonchloramide TS, then add 5 mL of ethyl acetate, and shake. The ethyl acetate layer is brown.

(2) Determine the absorption spectrum as directed in the Paste Method under s Spectrophotometry. It exhibits main absorptions at about 1490 cm^{-1}, 1400 cm^{-1}, and 950 cm^{-1}.

Purity Clarity Clear (1g, 20 mL).

Loss on drying Not more than 0.5% (1 g, 105°C, 2 hours).

Assay Dissolve 0.3 g of tetramethylammonium bromide by adding 50 mL of water and 5 mL of diluted nitric acid (1 in 3), and 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. A combined electrode can also be used for the indicator and reference electrodes.

Each mL of 0.1 mol/L silver nitrate = 0.015405 g of $[N(CH_3)_4]Br$

Tetrasodium Ethylenediaminetetraacetate Tetrahydrate $C_{10}H_{12}N_2Na_4O_8 \cdot 4H_2O$ Use a product suitable for the corresponding enzyme activity tests.

2,2′-Thiodiethanol $S(CH_2CH_2OH)_2$ [111-48-8] Use 2,2′-thiodiethanol produced for amino acid analysis.

Description A clear, colorless to pale yellow liquid.

Specific gravity d_{20}^{20}: 1.178–1.188.

Water Not more than 0.7% (0.1 g, Coulometric Titration).

β-Thujaplicin for Assay $C_{10}H_{12}O_2$ [499-44-5]

Boiling point 140–141°C (1.3 kPa).

Melting point 51–53°C.

Purity Related substances Prepare a test solution by dissolving 0.2 g of β-thujaplicin for assay in ethanol (95) to make 100 mL. Prepare the control solution by diluting 1 mL of the test solution, exactly measured, with ethanol (95) to 100 mL. Analyze 0.5 μL each of the test solution and the control solution by gas chromatography using the operating conditions specified in the Assay for Thujaplicin in the Monographs, and measure the peak areas. Continue the chromatography for two times the retention time of the main peak. Exclude the solvent peaks from measurement. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Thymol $C_{10}H_{14}O$ [89-83-8] Use thymol specified in the Japanese Pharmacopoeia.

Thymol Blue $C_{27}H_{30}O_5S$ [K8643, Special Grade] [76-61-9]

Thymol Blue TS Dissolve 0.1 g of thymol blue in 100 mL of ethanol (95). Filter if necessary.

Thymolphthalein $C_{28}H_{30}O_4$ [K8642, Special Grade] [125-20-2]

Thymolphthalein TS Dissolve 0.1 g of thymolphthalein in 100 mL of ethanol (95). Filter

if necessary.

Thymol–Sulfuric Acid TS Dissolve 0.5 g of thymol in 5 mL of sulfuric acid, and add ethanol (95) to make 100 mL.

Tin(II) Chloride Dihydrate $SnCl_2 \cdot 2H_2O$ [K8136, Special Grade, Reagent for Mercury Analysis] [10025-69-1]

Tin(II) Chloride–Hydrochloric Acid TS Dissolve 10 g of tin(II) chloride dihydrate in hydrochloric acid to make 100 mL. Store a tightly stoppered bottle.

Tin(II) Chloride–Sulfuric Acid TS Dissolve 10 g of tin(II) chloride dihydrate in diluted sulfuric acid (3 in 200) to make 100 mL.

Tin(II) Chloride TS Dissolve 0.1 g of tin(II) chloride dihydrate in 6.2 mL of citric acid–sodium hydroxide buffer (0.2 mol/L) at pH 5.0. Prepare fresh before use.

Tin(II) Chloride TS (Acidic) Dissolve 4 g of tin(II) chloride dihydrate in 125 mL of hydrochloric acid (arsenic-free), and add water to make 250 mL. Store in a tightly stoppered bottle. Use within 1 month of preparation

Titanium(III) Chloride Solution $TiCl_3$ [K8401, Special Grade] [7705-07-9]

Titanium(IV) Oxide TiO_2 [K8703, Special Grade] [13463-67-7]

Tocopherol Acetate $C_{31}H_{52}O_3$ [7695-91-2] Use tocopherol acetate specified in the Japanese Pharmacopoeia.

***d*-α-Tocopherol for Assay** $C_{29}H_{50}O_2$ [59-02-9] A light yellow, viscous liquid.

Identification Weigh accurately about 5 mg of *d*-α-tocopherol for assay, and dissolve it in ethanol (99.5) to make exactly 10 mL. Measure exactly 1 mL of this solution, and add ethanol (99.5) to make exactly 10 mL. Measure the absorbance of this solution. The solution exhibits an absorption maximum near 292 nm.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 292 nm): 67–82.

Weigh accurately about 5 mg of *d*-α-tocopherol for assay, and dissolve it in ethanol (99.5) to make exactly 10 mL. Measure exactly 1 mL of this solution, and add ethanol (99.5) to make exactly 10 mL. Measure the absorbance of this solution.

Purity Related substances Prepare a test solution as follows: Dissolve about 50 mg of *d*-α-tocopherol for assay, weighed accurately, in hexane to make exactly 100 mL. Prepare a control solution as follows: Add hexane to 1.5 mL of the test solution, measured exactly, to make exactly 100 mL. Analyze 20 μL each of the test solution and the control solution by liquid chromatography using the operating conditions below. Continue the chromatography for twice the retention time of the main peak and measure the peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 292 nm).

Column: A stainless steel tube (3–6 mm internal diameter and 15–25 cm length).

Packing material of column: 5- to 10-μm silica gel for liquid chromatography.

Column temperature: Room temperature (constant).

Mobile phase: A 200:1 mixture of hexane/2-propanol.

Flow rate: Adjust the retention time of the main peak to about 5 minutes.

d-β-Tocopherol for Assay $C_{28}H_{48}O_2$ [16698-35-4] A light yellow, viscous liquid.

Identification Weigh accurately about 5 mg of *d*-β-tocopherol for assay, and dissolve it in ethanol (99.5) to make exactly 10 mL. Measure exactly 1 mL of this solution, and add ethanol (99.5) to make exactly 10 mL. Measure the absorbance of this solution. The solution exhibits an absorption maximum near 296 nm.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 296 nm): 77–95.

Weifdgh accurately about 5 mg of *d*-β-tocopherol for assay, and dissolve it in ethanol (99.5) to make exactly 10 mL. Measure exactly 1 mL of this solution, and add ethanol (99.5) to make exactly 10 mL. Measure the absorbance of this solution.

Purity Related substances Prepare a test solution as follows: Dissolve about 50 mg of d-β-tocopherol for assay, weighed accurately, in hexane to make exactly 100 mL. Prepare a control solution as follows: Add hexane to 1.5 mL of the test solution, measured exactly, to make exactly 100 mL. Analyze 20 μL each of the test solution and the control solution by liquid chromatography using the operating conditions below. Continue the chromatography for about twice the retention time of the main peak, and measure the peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 292 nm).

Column: A stainless steel tube (3–6 mm internal diameter and 15–25 cm length).

Packing material of column: 5- to 10-μm silica gel for liquid chromatography.

Column temperature: Room temperature (constant).

Mobile phase: A 200:1 mixture of hexane/2-propanol.

Flow rate: Adjust the retention time of the main peak to about 10 minutes.

d-γ-Tocopherol for Assay $C_{28}H_{48}O_2$ [7616-22-0] A light yellow, viscous liquid.

Identification Weigh accurately about 5 mg of *d*-γ-tocopherol for assay, and dissolve it in ethanol (99.5) to make exactly 10 mL. Measure exactly 1 mL of this solution, and add ethanol (99.5) to make exactly 10 mL. Measure the absorbance of this solution. The solution exhibits an absorption maximum near 297 nm.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 297 nm): 83–103. Weigh accurately about 5 mg of *d*-γ-tocopherol for assay, and dissolve it in ethanol (99.5) to make exactly 10 mL. Measure exactly 1 mL of this solution, and add ethanol (99.5) to make exactly 10 mL. Measure the absorbance of this solution.

Purity Related substances Prepare a test solution as follows: Dissolve about 50 mg of *d*-γ-tocopherol for assay, weighed accurately, in hexane to make exactly 100 mL. Prepare a control solution as follows: Add hexane to 1.5 mL of the test solution, exactly measured, to make exactly 100 mL. Analyze 20 μL each of the test solution and the control solution by liquid chromatography using the operating conditions below.

Continue the chromatography for about twice the retention time of the main peak, and measure the peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution, other than the main peak, is not greater than the area of the main peak from the control solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 292 nm).

Column: A stainless steel tube (3－6 mm internal diameter and 15–25 cm length).

Packing material of column: 5- to 10-μm silica gel for liquid chromatography.

Column temperature: Room temperature (constant).

Mobile phase: A 200:1 mixture of hexane/2-propanol.

Flow rate: Adjust the retention time of the main peak to about 11 minutes.

d-δ-Tocopherol for Assay $C_{27}H_{46}O_2$ [119-13-1] A light yellow, viscous liquid.

Identification Weigh accurately about 5 mg of *d*-δ-tocopherol for assay, and dissolve it in ethanol (99.5) to make exactly 10 mL. Measure exactly 1 mL of this solution, and add ethanol (99.5) to make exactly 10 mL. Measure the absorbance of this solution. The solution exhibits an absorption maximum near 298 nm.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 298 nm): 83–101. Weigh accurately about 5 mg of d-δ-tocopherol for assay, and dissolve it in ethanol (99.5) to make exactly 10 mL. Measure exactly 1 mL of this solution, and add ethanol (99.5) to make exactly 10 mL. Measure the absorbance of this solution.

Purity Related substances Prepare a test solution as follows: Dissolve about 50 mg of *d*-δ-tocopherol for assay, weighed accurately, in hexane to make exactly 100 mL. Prepare a control solution as follows: Add hexane to 1.5mL of the test solution, measured exactly, to make exactly 100 mL. Analyze 20 μL each of the test solution and the control solution by liquid chromatography using the conditions below. Continue the chromatography for about twice the retention time of the main peak, and measure the peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution is not greater than the area of the main peak from the control solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 292 nm).

Column: A stainless steel tube (3–6 mm internal diameter and 15–25 cm length).

Packing material for column: 5- to 10-μm silica gel for liquid chromatography.

Column temperature: Room temperature (constant).

Mobile phase: A 200:1 mixture of hexane/2-propanol.

Flow rate: Adjust the retention time of the main peak to about 20 minutes.

Toluene $C_6H_5CH_3$ [K8680, Special Grade] [108-88-3]

***o*-Toluenesulfonamide** $C_7H_9NO_2S$ [88-19-7] Colorless crystals, or a white crystalline powder.

Melting point 157–160°C.

Purity *p*-Toluenesulfonamide Analyze a solution (1 in 5000) of *o*-toluene-

sulfonamide in ethyl acetate by gas chromatography using the operating conditions specified in the Purity (6) for Sodium Saccharin in the Monographs. Only one peak of *o*-toluenesulfonamide is observed.

_p_-Toluenesulfonamide $CH_3C_6H_4SO_2NH_2$ [70-55-3] White to slightly light brown crystals or crystalline powder.

Melting point 135–140°C.

Purity o-Toluenesulfonamide Analyze a solution (1 in 5000) of *p*-toluenesulfonamide in ethyl acetate by gas chromatography using the operating conditions specified in the Purity (5) for Calcium Saccharin in the Monographs. Only one peak of *p*-toluenesulfonamide is observed.

Trehalose Dihydrate $C_{12}H_{22}O_{11}\cdot 2H_2O$ Use a product suitable for the corresponding enzyme activity tests.

Tributyrin $(C_3H_7COO)_3C_3H_5$ Use a product suitable for the corresponding enzyme activity tests.

Trichloroacetic Acid CCl_3COOH [K8667, Special Grade] [76-03-9]

Trichloroacetic Acid–Sodium Dodecyl Sulfate TS Dissolve 100g trichloroacetic acid and 100 g of sodium dodecyl sulfate (for enzyme) in water to make 1000 mL.

Trichloroacetic Acid–Sulfuric Acid TS

Solution 1: Dissolve 163 g of trichloroacetic acid in water to make 1000 mL.

Solution 2: Add gradually 49.0 g of sulfuric acid to about 700 mL of water to make 1000 mL.

Mix 400 mL of Solution 1 and 250 mL of Solution 2, and make up to 1000 mL with water.

Trichloroacetic Acid TS Dissolve 18 g of sodium acetate, 110 mL of 1 mol/L trichloroacetic acid solution, and 19 mL of acetic acid in about 600 mL of water. Adjust the pH to 4.0 with sodium hydroxide TS (1 mol/L), and dilute with water to make 1000 mL.

Trichloroacetic Acid TS (for protease activity test) Dissolve 18.0 g trichloroacetic acid and 18.0 g of sodium acetate by adding 55 mL of acetic acid TS (6 mol/L) and water to make 1000 mL.

Triethylamine $(C_2H_5)_3N$ [121-44-8] A colorless, clear liquid having a strong odor of amine. Miscible with methanol, with ethanol (95), and with diethyl ether.

Specific gravity d_4^{25}: 0.722–0.730.

Boiling point 89–90°C.

Trifluoroacetic Acid CF_3COOH [76-05-1] A colorless, transparent liquid having pungent odor. Very soluble in water.

Content Not less than 99.0% of trifluoroacetic acid (CF_3COOH).

Identification (1) Trifluoroacetic acid is acidic.

(2) Determine the absorption spectrum of trifluoroacetic acid as directed in the Liquid Film Method under Infrared Spectrophotometry. It exhibits absorptions at

wavenumbers of about 3180 cm^{-1}, 1785 cm^{-1}, 1458 cm^{-1}, 1170 cm^{-1}, 811 cm^{-1} and 687 cm^{-1}.

Purity Unvolatile matter Not more than 0.02%. Weigh 10.0 g of trifluoroacetic acid, evaporate, and dry at 100°C for 2 hours. Cool in a desiccator for about 30 minutes, and weigh the residue.

Assay Weigh accurately about 3 g of trifluoroacetic acid, and add 30 mL of water. Titrate with 1 mol/L sodium hydroxide (indicator: 2–3 drops of phenolphthalein).

Each mL of 1 mol/L sodium hydroxide = 114.0 mg of CF_3COOH

Trimethylaminopropyl-bonded Silica Gel Use trimethylaminopropyl-bonded silica gel produced for ion-exchange absorbent.

Trimethylchlorosilane $(CH_3)_3SiCl$ [75-77-4] A colorless or almost colorless liquid having a pungent odor. Reacts to water.

Content Not less than 98.0%.

Assay Analyze 0.5 μL of trimethylchlorosilane by gas chromatography using the operating conditions given below. Determin the content of trimethylchlorosilane from the area of the trimethylchlorosilane peak and the sum of the areas of all the peaks of this substance.

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: 30°C.

Injection port temperature: 80°C.

Detector temperature: 250°C.

Carrier gas: Helium.

Flow rate: 1.33 mL minute.

Injection method: Split.

Split ratio: 1:100.

Measurement time: Three times the retention time of the main peak.

2,2,4-Trimethylpentane $CH_3C(CH_3)_2CH_2CH(CH_3)CH_3$ [K9703, Special Grade] [540-84-1] A colorless liquid. Practically insoluble in water, and miscible in chloroform and in diethyl ether.

Purity Measure the absorbance of this substance as directed under Ultraviolet-Visible Spectrophotometry. Use water as the reference. The absorbance is not more than 0.050 at 230 nm, 0.010 at 250 nm, and 0.005 at 280 nm.

2,2,4-Trimethylpentane for Ultraviolet Absorption Spectrum Measurement $CH_3C(CH_3)_2CH_2CH(CH_3)CH_3$ [K9703, Special Grade] [540-84-1]

Absorbance Prepare a test solution as follows: To 180 mL of 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement, add 1 mL of hexadecane for ultraviolet absorption spectrum measurement, evaporate the mixture on a water bath under

nitrogen to 1 mL, dissolve the residue by adding 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement to make exactly 25 mL. Measure the absorbance of the test solution in a 5-cm path length cell against 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement as the reference solution. It is not more than 0.01 (absorbance/cm light pass length) at 280–400 nm.

2,2,4-Trimethylpenthane TS Place 300 mL of dimethyl sulfoxide for ultraviolet absorption spectrum measurement in a 1-L separating funnel, add 75 mL of phosphoric acid, shake, and allow to stand for 10 minutes. Add 150 mL of 2,2,4-trimethylpentane for ultraviolet absorption spectrum measurement, shake, and allow to stand for 10 minutes. Collect the upper layer, and store in a tightly stoppered glass bottle.

Triphenylchloromethane $(C_6H_5)_3CCl$ [76-83-5] White to grayish white or yellowish crystals or crystalline powder. Soluble in acetic acid, and soluble in water with decomposition.

Content Not less than 98.0%.

Assay Weigh accurately about 0.4 g of triphenylchloromethane, add 40 mL of ethanol (95) and 10 mL of sodium hydroxide solution (1 in 10), cover with a watch dish, and heat the mixture on a water bath for 3 hours. After cooling, neutralize the solution with diluted nitric acid (1 in 3), and add 3 mL of diluted nitric acid (1 in 3). Titrate the resulting solution 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. A combined electrode can also be used for the indicator and reference electrodes. Perform a blank test to make any necessary correction.

Each mL of 0.1 mol/L silver nitrate = 27.878 mg of $(C_6H_5)_3CCl$

Triphenylphosphine Oxide $C_{18}H_{15}OP$ [791-28-6] A very slightly brownish white powder.

Melting point 156–158°C.

Purity (1) Clarity of solution Light brown, clear (1 g, 10 mL acetone).

(2) Related substances Weigh accurately 10 mg of triphenylphosphine oxide, previously dried for 24 hours in a vacuum desiccator, and dissolve it in methanol to make exactly 100 mL. Measure exactly 1 mL of this solution, and add a 67:33 mixture of acetonitrile/water to make exactly 100 mL. Use the resultant solution as the test solution. Measure exactly 2 mL of the test solution, and add a 67:33 mixture of acetonitrile/water to make exactly 100 mL. Use this solution as the control solution. Analyze 20 μL each of the test solution and the control solution by liquid chromatography using the operating conditions specified in Purity (6) for Sucralose in the Monographs. Continue the chromatography for two times the retention time of the main peak, and measure peak areas. The sum of the areas of all the peaks, other than the main peak, from the test solution, is not greater than the area of the main peak from the control solution.

Tris Buffer (1 mol/L) Dissolve 121 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 600 mL of water, adjust the pH with hydrochloric acid TS (1 mol/L) to the value specified

in the corresponding section of this publication, and add water to make 1000 mL.

Tris Buffer (1 mol/L, pH 8.0, containing tetrasodium ethylenediaminetetraacetate) Dissolve 22.6 g of tetrasodium ethylenediaminetetraacetate tetrahydrate in Tris buffer (1 mol/L) at pH 8.0 to make 1000 mL.

Tris Buffer (0.2 mol/L) Dissolve 24.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water, adjust the pH with hydrochloric acid TS (4 mol/L) to the value specified in the corresponding section of this publication, and add water to make 1000 mL.

Tris Buffer (1/7 mol/L) Dissolve 17.3 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water, adjust the pH with hydrochloric acid TS (1 mol/L) to the value specified in the corresponding section of this publication, and add water to make 1000 mL.

Tris Buffer (0.1 mol/L) Dissolve 12.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water, and adjust the pH with hydrochloric acid TS (1 mol/L) to the value specified in the corresponding section of this publication, and add water to make 1000 mL.

Tris Buffer (0.1 mol/L, pH 7.8, containing calcium chloride) Mix 4mL of a solution of calcium chloride dihydrate (1 in 80), 200 mL of a solution of 2-amino-2-hydroxymethyl-1,3-propanediol (97 in 2000), and 600 mL of water. Adjust the pH of the mixture to 7.8 with hydrochloric acid TS (1 mol/L), and add water to make 1000 mL.

Tris Buffer (0.1 mol/L, pH 8.0, containing calcium chloride) Dissolve 12.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 1.47 g of calcium chloride dihydrate in water, adjust the pH to 8.0 with hydrochloric acid TS (1 mol/L), and add water to make 1000 mL.

Tris Buffer (0.05 mol/L) Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 600 mL of water, adjust the pH with 10% hydrochloric acid TS to the value specified in the corresponding section of this publication, and add water to make 1000 mL.

Tris Buffer (0.05 mol/L pH7.5, containing calcium chloride and polyethylene glycol) Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 0.11 g of calcium chloride dihydrate, and 10 g of polyethylene glycol 8000 in 800 mL of water, adjust the pH to 7.5 with hydrochloric acid TS (0.5 mol/L) or sodium hydroxide solution (0.5 mol/L), and add water to make 1000 mL.

Tris Buffer (0.005 mol/L pH7.0, containing calcium chloride) Dissolve 0.61 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 0.56 g of calcium chloride dihydrate in 800 mL of water, adjust the pH to 7.0 with hydrochloric acid TS (0.1 mol/L), and add water to make 1000 mL.

Tris Buffer (pH 7.0) for Pectin Determination Dissolve 6.055 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 0.147 g of calcium chloride dihydrate in about 750 mL of water, and adjust the pH to 7.0 with 1 mol/L hydrochloric acid. Add water to make exactly 1000 mL.

Tris–Maleate Buffer Dissolve 1.21 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 1.16 g of maleic acid in water to make 100 mL. Measure 25 mL of this solution, adjust the pH with sodium hydroxide TS (0.1 mol/L) to the value specified in the corresponding

section of this publication, and add water to100 mL.

Tris–Phosphate Buffer Dissolve 36.3g of 2-amino-2-hydroxymethyl-1,3-propanediol and 50.0 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH with hydrochlonic acid TS (2 mol/L) to the value specified in the corresponding section of this publication, and add water to 100 mL.

Trisodium Citrate Dihydrate $Na_3C_6H_5O_7 \cdot 2H_2O$ [K8288, Special Grade] [6132-04-3]

Trisodium Citrate TS (1 mol/L) Dissolve 294 g of trisodium citrate dihydrate in water to make 1000 mL.

Trisodium 7-Hydroxy-1,3,6-naphthalenetrisulfonate $C_{10}H_5Na_3O_{10}S_3$ [31894-34-5] A white to light gray powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength between 285–291 nm): Not less than 105. Weigh accurately about 10 mg of trisodium 7-hydroxy-1,3,6-naphthalenetrisulfonate, dried previously for 24 hours in a vacuum desiccator, and dissolve it in ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. Refer to this solution as Solution A. Measure exactly 10 mL of Solution A, and add ammonium acetate TS (0.02 mol/L) to make exactly 100 mL. This solution exhibits absorption maxima in the ranges 237–243 nm, 285–291 nm, and 341–347 nm, respectively. Measure the absorbance of this solution at the maximum between 285–291 nm against ammonium acetate TS (0.02 mol/L).

Purity (1) Clarity of solution Almost clear (10 mg, ammonium acetate TS (0.02 mol/L) 100 mL).

(2) Related substances Prepare a test solution as follows: To 5 mg of trisodium 7-hydroxy-1,3,6-naphthalenetrisulfonate, add ammonium acetate–tetra-*n*-butylammonium bromide TS to make exactly 50 mL. Analyze 10 μL each of the test solution and ammonium acetate–tetra-*n*-butylammonium bromide TS by liquid chromatography using the operating conditions given below. Measure the area of each peak that appears within 60 minutes of injection. The area percentage of the main peak of the test solution is not less than 95.0% of the total area of all the peaks, excluding those from mobile phase A in the test solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (wavelength: 240 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: Ammonium acetate–tetra-*n*-butylammonium bromide TS.

B: Acetonitrile (for HPLC).

Concentration gradient (A/B): Maintain 70/30 for 30 minutes, run a linear gradient from 70/30 to 50/50 in 10 minutes, and maintain 50/50 for 20 minutes.

Flow rate: 1.0 mL/min.

Water Not more than 15.0% (10 mg, Coulometric Titration). The anode solution and catholyte solution used for water determination should contain propylene carbonate and diethanolamine, and methanol and ethylene glycol, respectively.

TS A for Free Fatty Acid Determination A solution that contains acyl-CoA synthetase (microorganism-derived), coenzyme A (microorganism-derived), adenosine 5′-triphosphate disodium salt trihydrate (microorganism-derived), 4-aminoantipyrine, ascorbate oxidase (pumpkin-derived), and phosphate buffer (pH7.0). Use a product suitable for the corresponding enzyme activity tests.

TS B for Free Fatty Acid Determination A solution that contains acyl-CoA synthetase (microorganism-derived), peroxidase (horse radish-derived), and 3-methyl-N-ethyl-N-(2-hydroxyethyl)-aniline. Use a product suitable for the corresponding enzyme activity tests.

TS for D-Glucose and D-Fructose Determination It contains hexokinase, glucose-6-phosphate dehydrogenase, triethanolamine buffer (pH 7.6), nicotinamide adenine dinucleotide (oxidized form), adenosine triphosphate, and magnesium sulfate. Use a product suitable for the corresponding enzyme activity tests.

TS for D-Glucose Determination (containing glucose oxidase and peroxidase) Dissolve 550 unit of glucose oxidase (*Aspergillus*-derived) and 125 unit of peroxidase (horse radish-derived, pyrogallol substrate) in 40 mL of Tris–phosphate buffer at pH 7.2. Add 1 mL of 0.4% (w/v) 4-aminoantipirine and 1.4 mL of phenol solution (1 in 20), and add pH 7.2 Tris–phosphate buffer to make 50 mL. Prepare fresh before use.

TS for D-Glucose Determination (containing hexokinase) It contains hexokinase, glucose-6-phosphate dehydrogenase, adenosine triphosphate, and nicotinamide adenine dinucleotide (oxidized form). Use a product suitable for the corresponding enzyme activity tests.

TS for D-Glucose Determination (containing mutarotase) It contains mutarotase (porcine liver-derived), glucose oxidase (*Penicillium* genus-derived), peroxidase (horse radish-derived), ascorbic acid oxidase (pumpkin-derived), 4-aminoantipyrine, and phenol. Use a product suitable for the corresponding enzyme activity tests.

TS for α-D-Glucose 1-Phosphoric Acid Determination Weigh 0.199 g of β-nicotinamide adenine dinucleotide (oxidized form), 0.305 g of magnesium chloride hexahydrate, and 0.51 mg of α-D-Glucose 1,6-Diphospate Potassium Salt *n*-Hydrate, and mix them with 50 mL of water and 40 mL of Tris buffer (0.05 mol/L, pH 7.0). Add 1.5 mL of disodium dihydrogen ethylenediaminetetraacetate (0.2 mol/L), 0.3 mL of phosphoglucomitase, and 0.4 mL of glucose-6-phosphate dehydrogenase. Make the solution up to 100 mL with water.

TS for L-Glutamic Acid Determination It contains L-glutamic acid oxidase (*Streptomyces* genus-derived), peroxidase, 4-aminoantipyrine, and *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethylaniline sodium salt. Use a product suitable for the

corresponding enzyme activity tests.

TS for Phospholipid Determination Dissolve 3 units of choline oxidase, 6 units peroxidase (horse radish-derived, guaiacol substrate), 1 mg of phenol, and 0.6 mg of 4-aminoantipyrine in 4 mL of HEPES buffer (0.05 mol/L) at pH 7.4.

Urea NH_2CONH_2 [K8731, Special Grade] [57-13-6]

Uranine $C_{20}H_{10}Na_2O_5$ [K8830, Special Grade] [518-47-8]

Uranine TS Dissolve 0.20 g of uranine in water to make 100 mL. Store a brown glass bottle.

Vanadic Acid–Molybdic Acid TS Dissolve 1.12 g of ammonium vanadate(V) in about 300 mL of warm water, and add 250 mL of nitric acid. Mix this solution with a solution prepared by dissolving 27 g of powdered hexaammonium heptamolybdate tetrahydrate in about 400 mL of warm water. After cooling, add water to make 1000 mL. Store in a brown bottle, and use 3 or 4 days after preparation.

Vanadic Acid TS Dissolve2.5 g of ammonium vanadate(V) in 600 mL of boiling water, cool to 60–70°C, and add 20 mL of nitric acid. Leave the solution to cool to room tempe rature, and add water to make 1000 mL.

Vanillin $C_8H_8O_3$ [121-33-5]

Content Not less than 98.0%.

Description A white to light yellow crystalline powder having a characteristic odor.

Identification Determine the absorption spectrum of vanillin as directed in the Disk Method under Infrared Spectrophotometry. It exhibits absorptions at wavenumbers of about 3180 cm^{-1}, 1670 cm^{-1}, 1590 cm^{-1}, 1510 cm^{-1}, 1270 cm^{-1}, 1160 cm^{-1}, and 860 cm^{-1}.

Melting point 80.5–83.5°C.

Assay To 5 g of hydroxylammonium chloride, add 10 mL of water and 50 mL of ethanol (95). Add 5 drops of phenol blue TS, then add 1 mol/L sodium hydroxide until the color of solution becomes light green, and add about 3 g of vanillin, accurately weighed. Allow this solution to stand for 20 minutes, and titrate with 1 mol/L sodium hydroxide. The endpoint is when the color of the solution becomes light green.

Each mL of 1 mol/L sodium hydroxide = 152.15 mg of $C_8H_8O_3$

Vinyl Acetate $CH_3COOCHCH_2$ [K6724, Special Grade] [108-05-4] A colorless liquid. Soluble in toluene.

Refractive index n_D^{20}: 1.393–1.397.

1-Vinyl-2-pyrrolidone C_6H_9NO [88-12-0] A clear liquid.

Purity Analyze 0.5-μL portions of 1-vinyl-2-pyrrolidone by gas chromatography using the operating conditions given below. Measure the peak areas of the components contained, and determine the content of 1-vinyl-2-pyrrolidone as directed under the peak area percentage method. It is not less than 99.0%. Adjust the detection sensitivity, so that the peak height of 1-vinyl-2-pyrrolidone obtained from 0.5 μL of the sample is about 70% of the full scale.

Operating conditions

Detector: Flame-ionization detector.

Column: A silicate glass capillary tube (0.53 mm internal diameter and 30 m length) coated with a 1.0 μm thick layer of polyethylene glycol for gas chromatography.

Column temperature: Maintain the temperature at 80°C for 1 minute, raise it to 190°C at a rate of 10°C/minute, and maintain at 190°C for 20 minutes.

Inlet temperature: 190°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the peak of 1-vinyl-2-pyrrolidoe appears about 15 minutes after injection.

Water (carbon dioxide removed) Use water prepared by one of the following methods or a mixture of waters prepared by more than one of the following methods. Prepare fresh before use.

1. Heat water in a flask, keep boiling for 5 minutes or more, and stop heating. Loosely cover the flask with a watch glass, and leave it until effervescence stops. Cool the water while protecting from carbon dioxide in the air by connecting the flask with a gas washing bottle containing calcium hydroxide solution (1 in 4) or with a soda lime tube.
2. Place water into a flask, and pass nitrogen gas through the water for 15 minutes or more.
3. Remove carbon dioxide from water using a gas separator with a carbon dioxide separation membrane.
4. Collect deionized water with the resistance of 18MΩ/cm or more in an Erlenmeyer flask through which nitrogen gas is passed, with care not to allow effervescence. Use it immediately after collection.

Water (dissolved oxygen removed) Use water prepared by one of the following methods or a mixture of waters prepared by more than one of the following methods. Prepare fresh before use.

1. Heat water in a flask, keep boiling for 5 minutes or more, and stop heating. Loosely cover the flask with a watch glass, and leave it until effervescence stops. Cool the water while protecting from oxygen in the air by connecting the flask with a gas washing bottle containing pyrogallol–sodium hydroxide TS or by other appropriate method.
2. Place water into a flask, and pass nitrogen gas through the water for 15 minutes or more.
3. Remove oxygen from water using a gas separator with an oxygen separation membrane.
4. Remove air well using an ultrasonic vibrator.
5. Collect deionized water with the resistance of 18MΩ/cm or more in an Erlenmeyer flask through which nitrogen gas is passed, with care not to allow effervescence. Use it immediately after collection.

Water Determination TS Prepare by any of the following methods. A TS prepared by

another method can be used if it is equivalent or superior in accuracy to the specified TS.

Method 1. Dissolve 63 g of iodine in 100 mL of pyridine for water determination, and cool in ice. Pass dry sulfur dioxide through the solution until the weight of the solution increases by 32 g. Then, add chloroform for water determination to make 500 mL, and allow to stand for at least 24 hours. Store in a cold place, protected from light and moisture. Since this solution deteriorates with time, standardize before use.

Method 2. Dissolve 102 g of imidazole for water determination in 350 mL of diethylene glycol monoethyl ether for water determination, and cool in ice. While maintaining the solution temperature at 25–30°C, pass dry sulfur dioxide through the solution until the weight of the solution increases by 64 g. Dissolve 50 g iodine in the resulting solution, and allow to stand for at least 24 hours. Store in a cold place, protected from light and moisture. Since this solution deteriorates with time, standardize before use.

Method 3. Pass dry sulfur dioxide through 220 mL of propylene carbonate for water determination until the weight of the solution increases by 32 g. Add a solution prepared by dissolving 81g of 2-(methylamino)pyridine in 180 mL of propylene carbonate for water determination or diethylene glycol monoethyl ether for water determination and cooling in icy, and then add 36 g of iodine to dissolve it. Allow to stand for at least 24 hours. Store in a cold place, protected from light and moisture. Since this solution deteriorates with time, standardize before use.

Standardization As directed in the Water Determination Test, transfer an appropriate amount of methanol for water determination into a dry titration flask, and add Water Determination TS dropwise to the endpoint to make the inside of the flask anhydrous. Add quickly about 30 mg of water, accurately weighed, and titrate with Water Determination TS while stirring vigorously. The number of mg (f) of water (H_2O) equivalent to 1 mL of Water Determination TS is obtained by the formula:

f = Weight (mg) of added water (H_2O)/Volume (mL) of Water Determination TS consumed

Waxy Corn Starch Use a product suitable for the corresponding enzyme activity tests.

Waxy Corn Starch (lintner soluble) Use a product suitable for the corresponding enzyme activity tests. It is obtained by acid-treating starch derived from the seeds of *Zea mays* L. var. *ceratina* Sturt. and then defatting the treated starch.

Description An odorless white to pale yellow powder.

Identification (1) Boil 1 g of Waxy Corn Starch in 50 mL of water, and leave to cool. It almost dissolves, and becomes a colorless, clear or slightly turbid viscose liquid.

(2) Waxy Corn Starch, when iodine TS (0.005 mol/L) is add dropwise, turns red-purple.

Purity When Waxy Corn Starch is observed microscopically as directed in "Microscopic examination," "Tests for Crude Drugs," "General Tests" in *The Japanese Pharmacopeia*, no other starch particles are found. It may contain part of tissues of source plants but the amount is very little.

Loss on drying Not more than 5% (4 g, 105°C, 6 hours).

Weakly Acidic Cation-exchange Resin (fine) A hydrogen ion type of weakly acidic methacrylic carboxylic acid. A white powder. Passes through a 150-μm standard sieve and hardly passes through a 75-μm sieve.

Weigh about 50 g of weakly acidic cation-exchange resin (Fine), immerse in water, allow to stand for about 1 hour, and decant 2 or 3 times until the supernatant becomes clear. Pour the resin with water into glass tube for chromatography (about 25 mm internal diameter) to prepare a resin column. Pour 250 mL of diluted hydrochloric acid (1 in 4) into the column, and allow to pass through at a rate of about 4 mL per minute. Then, pour water to wash the column until the color of the washings changes to green to blue with bromocresol green TS, and perform the following test:

Measure 10 mL of the resin, pour it with water into a glass tube for chromatography (15 mm internal diameter), and allow 80 mL of 0.1 mol/L sodium hydroxide to pass through at a rate of about 2 mL per minute. The pH of the effluent is 4.0–6.5.

Weakly Basic Anion-exchange Resin (free form) Weakly basic polystyrene polyamine. A yellow to yellow-brown a granular substance. Passes through a 600-μm standard sieve but hardly passes through a 425-μm sieve.

Identification Measure 10 mL of the resin, pour it with water into a glass tube for chromatography (15 mm internal diameter), and pass 70 mL of 0.1 mol/L hydrochloric acid through the column at a rate of about 2 mL per minute. The pH of the effluent is 4.0–8.0.

Total ion-exchange amount 1.2 milliequivalent/mL. Weigh 5.0 mL of the resin, remove the water on the surface with filter paper, add 500 mL of 0.2 mol/L hydrochloric acid, and allow to stand for 12 hours with an occasional stirring. Titrate 10 mL of the supernatant, measured exactly, with 0.1 mol/L sodium hydroxide (indicator: 3 drops of phenolphthalein TS). Separately, perform a blank test to determine the total ion-exchange amount by the following formula. The solid content (%) refers to the mass fraction of 10.0 g of the resin by drying it in a reduced-pressure desiccator (4 kPa) at 40 for 12 hours and weigh the weight before drying.

$$\text{Total ion-exchange amount} = \frac{a - b}{\text{Amount (mL) of the sample} \times \text{Solid content (\%)}/100} \times 5$$

a = volume (mL) of 0.1 mol/L sodium hydroxide consumed in the blank test,

b = volume (mL) of 0.1 mol/L sodium hydroxide consumed in the test.

Weakly Basic DEAE-Cellulose Anion Exchanger ($-O-C_2H_4-N(C_2H_5)_2$ form) Use a weakly basic anion-exchanger prepared by introducing diethylaminoethyl groups into porous cellulose.

Wijs TS Dissolve 7.9 g of iodine trichloride and 8.9 g of iodine separately in a small volume of acetic acid each. Mix the two solutions, and add acetic acid to make 1000 mL. Store in a light-resistant, glass-container.

Xylan Poly(β-D-xylopyranose[1 → 4]) Use a product suitable for the corresponding enzyme activity tests.

Xylene $C_6H_4(CH_3)_2$ [K8271, First Grade] [1330-20-7]

***o*-Xylene** $C_6H_4(CH_3)_2$ [95-47-6] A clear, colorless liquid.

Refractive index n_D^{20}: 1.501–1.506.

Specific gravity d_4^{20}: 0.875–0.885.

Distillation test 143–146°C, not less than 95% vol.

Xylene Cyanol FF $C_{25}H_{27}N_2NaO_6S_2$ [K8272, Special Grade] [2650-17-1]

Xylenol Orange $C_{31}H_{30}N_2Na_2O_{13}S$ [K9563, Special Grade] [1611-35-4]

Xylenol Orange TS Dissolve 0.1 g of xylenol orange in water to make 100 mL.

Xylose $C_5H_{10}O_5$ Use a product suitable for the corresponding enzyme activity tests.

Zinc Zn [K8012, Special Grade] [7440-66-6]

Zinc, Arsenic Analysis Zn [K8012, Arsenic Analysis Grade] [7440-66-6]
Use sandy zinc. Do not use porous zinc because it dissolves too rapidly in general. Suitable one is such that when the operation is finished, a small amount of zinc remains undissolved and hydrogen gas still evolves.

Zinc (Reference Material) Zn [Reference Material for Volumetric Analysis, K 8005] [7440-66-6] In addition to Reference Material for Volumetric Analysis specified in JIS K8005, a certified reference material capable of being used for volumetric analysis is usable.

Zinc Acetate Dihydrate $Zn(CH_3COO)_2 \cdot 2H_2O$ [K8356, Special Grade] [5970-45-6]

Zinc Acetate TS Dissolve 120 g of zinc acetate dihydrate in 880 mL of water. Filter the resulting solution before use through a filter paper for quantitative analysis (5C).

Zinc Chloride $ZnCl_2$ [K8111, Special Grade] [7646-85-7]

Zinc Chloride TS Dissolve 27 mg of zinc chloride in water, and add 0.75 mL of polyoxyethylene(23) lauryl ether (3 in 10) and water to make 1000 mL.

Zinc Chloride TS (pH 3.0) Dissolve 1.0 g of zinc chloride in 19 mL of water, and adjust the pH to 3.0 with diluted hydrochloric acid (1 in 2).

Zinc for Arsenic Analysis See Zinc, Arsenic Analysis.

Zinc Iodide–Starch TS Boil 100 mL of water, and add 5 mL of potassium iodide solution (3 in 20) and 10 mL of zinc chloride solution (1 in 5). Keep boiling, and with continuous stirring, add a uniform suspension prepared by adding 30 mL of cold water to 5 g of starch (soluble). Continue to boil for 2 minutes, and cool. Stopper tightly, and store in a cold place.

Zinc Powder Zn [K8013, Arsenis Analysis Grade] [7440-66-6]

Zinc Sulfate Heptahydrate $ZnSO_4 \cdot 7H_2O$ [K8953, Special Grade] [7446-20-0]

Zinc Sulfate–Sodium Chloride–Potassium Iodide TS Dissolve 50 g of sodium chloride, 10 g of zinc sulfate heptahydrate, and 5.0 g of potassium iodide in water to make 200 mL.

2. Volumetric Solutions

Volumetric standard solutions are prepared according to the corresponding methods given below. The degree of deviation from the specified concentration (mol/L) is expressed by the factor (f). Usually, standard solutions are prepared so that the factor is in the range of 0.970–1.030. When volumetric standard solutions are used, the actual consumption amount of a solution is determined by multiplying the volume of the solution used for titration by the factor of the solution.

0.1 mol/L Ammonium Iron(II) Sulfate

This solution contains 39.21 g of ammonium iron(II) sulfate hexahydrate ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, molecular weight: 392.14) per 1000 mL.

Add 30 mL of sulfuric acid gradually to 300 mL of water while stirring, and cool. Then add 40 g of ammonium iron(II) sulfate hexahydrate and water to make 1000 mL.

Standardization Proceed as directed in either of the procedures below.

1. Weigh accurately 0.12 g of potassium dichromate (reference material), dried as directed in its certificate, dissolve it in 100 mL of water, add 30 mL of sulfuric acid gradually while stirring, and cool. Titrate with the prepared ammonium iron(II) sulfate solution (indicator: about 0.2 mL of ferroin TS). The endpoint is when the color of the solution changes from blue-green to red-brown.

Each mL of 0.1 mol/L ammonium iron(II) sulfate = 4.903 mg of $K_2Cr_2O_7$

The factor is calculated by the formula:

$f = m/(0.004903 \times V) \times A/100$

f = factor of 0.1 mol/L ammonium iron(II) sulfate,

m = weight (g) of potassium dichromate (reference material),

A = content (%) of potassium dichromate (reference material),

V = volume (mL) of 0.1 mol/L ammonium iron(II) sulfate consumed.

2. Measure exactly 25 mL of the prepared ammonium iron(II) sulfate, and add 25 mL of water and 5 mL of phosphoric acid. Titrate with 0.02 mol/L potassium permanganate. The endpoint is when a faint red color persists for 15 seconds.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.1 mol/L ammonium iron(II) sulfate,

f_1 = factor of 0.02 mol/L potassium permanganate,

V = volume (mL) of 0.02 mol/L potassium permanganate consumed.

0.1 mol/L Ammonium Thiocyanate

This solution contains 7.612 g of ammonium thiocyanate (NH_4SCN, molecular weight: 76.12) per 1000 mL.

Dissolve 8 g of ammonium thiocyanate in 1000 mL of water. Stopper tightly and store.

Standardization Measure exactly 25 mL of 0.1 mol/L silver nitrate, and add 25 mL of water, 2 mL of nitric acid, 10 mL of nitrobenzene. Titrate this solution with the prepared ammonium thiocyanate while shaking well (indicator: ammonium iron(III) sulfate–nitric acid TS). The endpoint is when the solution turns brown.

The factor is calculated by the formula:

$f = f_1 \times 25/V$

f = factor of 0.1 mol/L ammonium thiocyanate,

f_1 = factor of 0.1 mol/L silver nitrate,

V = volume (mL) of 0.1 mol/L ammonium thiocyanate consumed.

0.05 mol/L Ammonium Thiocyanate

This solution contains 3.806 g of ammonium thiocyanate (NH_4SCN, molecular weight: 76.12) per 1000 mL.

Dissolve 4 g of ammonium thiocyanate in 1000 mL of water. Stopper tightly and store.

Standardization Measure exactly 25 mL of 0.05 mol/L silver nitrate, and add 25 mL of water, 2 mL of nitric acid, 10 mL of nitrobenzene. Titrate this solution with the prepared ammonium thiocyanate while shaking well (indicator: ammonium iron(III) sulfate–nitric acid TS). The endpoint is when the color of the solution turns brown. Standardize before use.

The factor is calculated by the formula:

$f = f_1 \times 25/V$

f = factor of 0.05 mol/L ammonium thiocyanate,

f_1 = factor of 0.05 mol/L silver nitrate,

V = volume (mL) of 0.05 mol/L ammonium thiocyanate consumed.

0.01 mol/L Bismuth Nitrate

This solution contains 4.851 g of bismuth nitrate pentahydrate ($Bi(NO_3)_3 \cdot 5H_2O$, molecular weight: 485.07) per 1000 mL.

Dissolve 4.9 g of bismuth nitrate pentahydrate by adding 20 mL of diluted nitric acid (1 in 3) and 1000 mL of water. Stopper tightly and store.

Standardization Measure exactly 25 mL of the prepared bismuth nitrate solution, and adjust its pH to 1–2 with diluted nitric acid. Titrate with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: a few drops of xylenol orange TS). The endpoint is when the color of the solution changes from red to yellow.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.01 mol/L bismuth nitrate,

f_1 = factor of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate,

V = volume (mL) of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate consumed.

0.05 mol/L Bromine

This solution contains 7.990 g of bromine (Br_2, molecular weight: 159.81) per 1000 mL.

Dissolve 3 g of potassium bromate and 15 g of potassium bromide in water to make 1000 mL. Store in a tightly stoppered brown bottle.

Standardization Measure exactly 25 mL of the prepared bromine solution, add 100 mL of water and 10 mL of diluted sulfuric acid (1 in 5), stopper immediately, and shake gently. Add 2 g of potassium iodide, stopper immediately, shake gently, and leave in a dark place for 2–3 minutes. Titrate 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. The endpoint is when the blue color of the solution disappears. Separately, perform a blank test to make any necessary correction.

The factor is calculated by the formula:

$f = f_1 \times (V–V_0)/25$

f = factor of 0.05 mol/L bromine,

f_1 = factor of 0.1 mol/L sodium thiosulfate,

V = volume (mL) of 0.1 mol/L d sodium thiosulfate consumed,

V_0 = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the blank test.

0.1 mol/L Cerium(IV) Sulfate

This solution contains 40.43 g of cerium(IV) sulfate tetrahydrate ($Ce(SO_4)_2 \cdot 4H_2O$, molecular weight: 404.30) per 1000 mL.

To about 40.4 g of cerium(IV) sulfate tetrahydrate, add 50 mL of sulfuric acid, and mix them. To the mixture, add 900 mL of water in 20-mL portions while stirring, being careful about heat generation. Allow to stand for 24 hours, filter the solution through a glass filter, and add water to make 1000 mL.

Standardization Measure exactly 25 mL of 0.1 mol/L cerium(IV) sulfate, add 30 mL of diluted sulfuric acid (1 in 6), and titrate with 0.1 mol/L ammonium ferrous sulfate (indicator: about 0.2 mL of ferroin TS). The endpoint is when the color of the solution changes blue-green to yellow-red. Calculate the normality factor by the formula:

$f = f_1 \times V/25$

f = factor of 0.1 mol/L cerium(IV) sulfate,

f_1 = factor of 0.1 mol/L ammonium ferrous sulfate,

V = volume (mL) of 0.1 mol/L ammonium ferrous sulfate consumed.

0.1 mol/L Diammonium Cerium(IV) Nitrate

This solution contains 54.82 g of diammonium cerium(IV) nitrate ($Ce(NH_4)_2(NO_3)_6$), molecular weight: 548.22) per 1000 mL.

Dissolve 57 g of diammonium cerium(IV) nitrate in 500 mL of diluted sulfuric acid (3 in 53), and add water to make 1000 mL. Allow to stand for about 18 hours, and filter this

solution if necessary. Stopper tightly and store.

Standardization Measure exactly 25 mL of 0.1 mol/L iron(II) ammonium sulfate, add 5 mL of phosphoric acid, and titrate with the prepared diammonium cerium(IV) nitrate solution (indicator: about 0.2 mL of ferroin TS). The endpoint is when the color of the solution changes from red-brown to blue-green.

The factor is calculated by the formula:

$f = f_1 \times 25/V$

f = factor of 0.1 mol/L diammonium cerium(IV) nitrate,

f_1 = factor of 0.1 mol/L iron(II) ammonium sulfate,

V = volume (mL) of 0.1 mol/L diammonium cerium(IV) nitrate consumed.

0.1 mol/L Disodium Dihydrogen Ethylenediaminetetraacetate

This solution contains 37.22 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, molecular weight: 372.24) per 1000 mL.

Dissolve 38 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate in water to make 1000 mL. Store a plastic bottle made of polyethylene or the like.

Standardization Measure exactly 25 mL of 0.1 mol/L zinc, and add 75 mL of water and 10 mL of ammonia solution–ammonium chloride TS. Titrate with the prepared solution of disodium dihydrogen ethylenediaminetetraacetate (indicator: 50 mg of eriochrome black T–sodium chloride indicator). The endpoint is when the color of the solution changes from red to blue.

The factor is calculated by the formula:

$f = f_1 \times 25/V$

f = factor of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate,

f_1 = factor of 0.1 mol/L zinc,

V = volume (mL) of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate consumed.

0.05 mol/L Disodium Dihydrogen Ethylenediaminetetraacetate

This solution contains 18.61 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, molecular weight: 372.24) per 1000 mL.

Dissolve 18.6 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate in water to make 1000 mL. Store in a plastic bottle made of polyethylene or the like.

Standardization Measure exactly 25 mL of 0.05 mol/L zinc, and add 75 mL of water and 5 mL of ammonia solution–ammonium chloride TS. Titrate with the prepared solution of disodium dihydrogen ethylenediaminetetraacetate (indicator: 50 mg of eriochrome black T–sodium chloride indicator). The endpoint is when the color of the solution changes from red to blue.

The factor is calculated by the formula:

$f = f_1 \times 25/V$

f = factor of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate,

f_1 = factor of 0.05 mol/L zinc,

V= volume (mL) of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate consumed.

0.02 mol/L Disodium Dihydrogen Ethylenediaminetetraacetate

This solution contains 7.445 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, molecular weight: 372.24) per 1000 mL.

Dissolve 7.5 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate in water to make 1000 mL. Store a plastic bottle made of polyethylene or the like.

Standardization Measure exactly 25 mL of 0.02 mol/L zinc, and add 75 mL of water and 5 mL of ammonia solution–ammonium chloride TS. Titrate with the prepared solution of disodium dihydrogen ethylenediaminetetraacetate (indicator: 50 mg of eriochrome black T–sodium chloride indicator). The endpoint is when the color of the solution changes from red to blue.

The factor is calculated by the formula:

$f = f_1 \times 25/V$

f = factor of 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate,

f_1 = factor of 0.02 mol/L zinc,

V = volume (mL) of 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate consumed.

0.01 mol/L Disodium Dihydrogen Ethylenediaminetetraacetate

This solution contains 3.722 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O3$, molecular weight: 372.24) per 1000 mL.

Dissolve 3.8 g of disodium dihydrogen ethylenediaminetetraacetate dihydrate in water to make 1000 mL. Store a plastic bottle made of polyethylene or the like.

Standardization Measure exactly 25 mL of 0.01 mol/L zinc, and add 75 mL of water and 5 mL of ammonia solution–ammonium chloride TS. Titrate with the prepared solution of disodium dihydrogen ethylenediaminetetraacetate (indicator: 50 mg of eriochrome black T–sodium chloride indicator). The endpoint is when the color of the solution changes from red to blue.

The factor is calculated by the formula:

$f = f_1 \times 25/V$

f = factor of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate,

f_1 = factor of 0.01 mol/L zinc,

V= volume (mL) of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate consumed.

2 mol/L Hydrochloric Acid

This solution contains 72.92 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1000 mL.

Measure 180 mL of hydrochloric acid, and add water to make 1000mL.

Standardization Weigh accurately 2.6–2.8 g of sodium carbonate (reference material), dried as directed in its certificate, dissolve it in 50 mL of water, and titrate with the prepared hydrochloric acid (indicator: 2 drops of bromophenol blue TS). When the titration is close to the endpoint, boil the solution to expel carbon dioxide, and immediately continue the titration. The endpoint is when the color of the solution changes from blue-purple to bluish green. Be careful about a large amount of carbon dioxide emissions while in titration.

Each mL of 2 mol/L hydrochloric acid = 105.99 mg of Na_2CO_3

The factor is calculated by the formula:

$f = m/(0.10599 \times V) \times A/100$

f = factor of 2 mol/L hydrochloric acid,

m = weight (g) of sodium carbonate (reference material),

A = content (%) of sodium carbonate (reference material),

V = volume (mL) of 2 mol/L hydrochloric acid consumed.

1 mol/L Hydrochloric Acid

This solution contains 36.46 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1000 mL.

Measure 90 mL of hydrochloric acid, and add water to make 1000 mL.

Standardization Weigh accurately 1.3–1.4 g of sodium carbonate (reference material), dried as directed in its certificate, and proceed as directed in either of the two procedures below.

1. Dissolve it in 70 mL of water, and titrate with the prepared hydrochloric acid. While in titration, keep stirring the sodium carbonate solution vigorously and do not boil it. Confirm the endpoint using a potentiometer with a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes.

2. Dissolve it in 50 mL of water, and titrate with the prepared hydrochloric acid (indicator: 2 drops of bromophenol blue TS). Near the endpoint, boil to expel the carbon dioxide, and immediately continue the titration. The endpoint is when the color of the solution changes from blue-purple to bluish green.

Each mL of 1 mol/L hydrochloric acid = 52.99 mg of Na_2CO_3

The factor is calculated by the formula:

$f = m/(0.05299 \times V) \times A/100$

f = factor of 1 mol/L hydrochloric acid,

m = weight (g) of sodium carbonate (reference material),

A = content (%) of sodium carbonate (reference material),

V = volume (mL) of 1 mol/L hydrochloric acid consumed.

0.5 mol/L Hydrochloric Acid

This solution contains 18.23 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1000 mL.

Prepare the solution, as directed for 1 mol/L Hydrochloric Acid, using 45 mL of hydrochloric acid.

Standardize the the prepared solution, as directed for 1 mol/L Hydrochloric Acid, using 0.6–0.7 g of sodium carbonate (reference material).

Each mL of 0.5 mol/L hydrochloric acid = 26.497 mg of Na_2CO_3

The factor is calculated by the formula:

$f = m/(0.026497 \times V) \times A/100$

f = factor of 0.5 mol/L hydrochloric acid,

m = weight (g) of sodium carbonate (reference material),

A = content (%) of sodium carbonate (reference material),

V = volume (mL) of 0.5mol/L hydrochloric acid consumed.

0.2 mol/L Hydrochloric Acid

This solution contains 7.292 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1000 mL.

Prepare by diluting 1 mol/L hydrochloric acid with water to 5 times its original volume, or prepare as directed for 1 mol/L Hydrochloric Acid, using 18 mL of hydrochloric acid. Standardize as directed for 1 mol/L Hydrochloric Acid, using accurately weighed 0.26–0.30 g of sodium carbonate (reference material).

Each mL of 0.2 mol/L hydrochloric acid = 10.60 mg of Na_2CO_3

The factor is calculated by the formula:

$f = m/(0.01060 \times V) \times A/100$

f = factor of 0.2 mol/L hydrochloric acid,

m = weight (g) of sodium carbonate (reference material),

A = content (%) of sodium carbonate (reference material),

V = volume (mL) of 0.2mol/L hydrochloric acid consumed.

0.1 mol/L Hydrochloric Acid

This solution contains 3.646 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1000 mL.

Prepare by diluting 1 mol/L hydrochloric acid with water to 10 times its original volume, or prepare as directed for 1 mol/L Hydrochloric Acid, using 9.0 mL of hydrochloric acid. Standardize as directed for 1 mol/L Hydrochloric Acid, using accurately weighed 0.13–0.16 g of sodium carbonate (reference material).

Each mL of 0.1 mol/L hydrochloric acid = 5.299 mg of Na_2CO_3

The factor is calculated by the formula:

$f = m/(0.005299 \times V) \times A/100$

f = factor of 0.1 mol/L hydrochloric acid,

m = weight (g) of sodium carbonate (reference material),

A = content (%) of sodium carbonate (reference material),

V = volume (mL) of 0.1mol/L hydrochloric acid consumed.

0.05 mol/L Hydrochloric Acid

This solution contains 1.823 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1000 mL.

Prepare by diluting 0.1 mol/L hydrochloric acid with water to 2 times its original volume, and use the factor of 0.1 mol/L hydrochloric acid; or prepare by diluting 1 mol/L hydrochloric acid with water to 20 times its original volume, and use the factor of 1 mol/L hydrochloric acid. Either solution does not require standardization.

0.02 mol/L Hydrochloric Acid

This solution contains 0.7292 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1000 mL.

Prepare by diluting 0.1 mol/L hydrochloric acid with water to 5 times its original volume, and use the factor of 0.1 mol/L hydrochloric acid; or prepare by diluting 1 mol/L hydrochloric acid with water to 50 times its original volume, and use the factor of 1 mol/L hydrochloric acid. Either solution does not require standardization.

0.01 mol/L Hydrochloric Acid

This solution contains 0.3646 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1000 mL.

Prepare by diluting 0.1 mol/L hydrochloric acid with water to 10 times its original volume, and use the factor of 0.1 mol/L hydrochloric acid; or prepare by diluting 1 mol/L hydrochloric acid with water to 100 times its original volume, and use the factor of 1 mol/L hydrochloric acid. Either solution does not require standardization.

0.5 mol/L Hydrochloric Acid, Methanolic

This solution contains 18.23 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1000 mL.

Measure 45 mL of hydrochloric acid, add 45 mL of water and methanol to make 1000 mL. Standardize as directed for 0.5 mol/L Hydrochloric Acid.

Each mL of 0.5 mol/L methanolic hydrochloric acid = 26.497 mg of Na_2CO_3

The factor is calculated by the formula:

$f = m/(0.026497 \times V) \times A/100$

f = factor of 0.5 mol/L methanolic hydrochloric acid,

m = weight (g) of sodium carbonate (reference material),

A = content (%) of sodium carbonate (reference material).

V = volume (mL) of 0.5mol/L methanolic hydrochloric acid consumed.

0.5 mol/L Hydroxylammonium Chloride

This solution contains 34.75 g of hydroxylammomium chloride ($NH_2OH \cdot HCl$, molecular weight: 69.49) per 1000 mL.

Dissolve 35 g of hydroxylammonium chloride in 40 mL of water by warming to about 65°C. Cool, add 15 mL of bromophenol blue–sodium hydroxide TS, and add ethanol (95) to make exactly 1000 mL. Prepare fresh before use.

0.05 mol/L Iodine

This solution contains 12.69 g of iodine (I_2, molecular weight: 253.81) per 1000 mL.

To 40 g of potassium iodide, add 25 mL of water, and then add 13 g of iodine to dissolve it. Add water to make 1000 mL. Add 3 drops of hydrochloric acid, and mix. Stopper tightly, and store protected from light in a dark place. Restandardize frequently.

Standardization Measure exactly 25 mL of the prepared iodine solution, and add 1mL of hydrochloric acid TS (1 mol/L). Titrate with 0.1 mol/L sodium thiosulfate (indicator: 3 mL of starch TS). Add the indicator near the endpoint, when the solution becomes light yellow. The endpoint is when the blue color of the solution disappears.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.05 mol/L iodine,

f_1 = factor of 0.1 mol/L sodium thiosulfate,

V = volume (mL) of 0.1 mol/L sodium thiosulfate consumed.

0.05 mol/L Iodine for Sodium Hydrosulfite

This solution contains 12.69 g of iodine (I_2, molecular weight: 253.81) per 1000 mL.

To 40 g of potassium iodide, add 25 mL of water, and then add 13 g of iodine to dissolve it. Add water to make 1000 mL. Add 3 drops of hydrochloric acid, and mix. Stopper tightly, and store, protected from light, in a dark place.

Standardization Measure exactly 25 mL of the prepared iodine solution , and add 1 mL of hydrochloric acid TS (1 mol/L). Titrate with 0.1 mol/L sodium thiosulfate (indicator: 3 mL of starch TS). Add the indicator near the endpoint, when the color of the solution changes to pale yellow. The endpoint is when the blue color of the solution disappears.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.05 mol/L iodine,

f_1 = factor of 0.1 mol/L sodium thiosulfate,

V = volume (mL) of 0.1 mol/L sodium thiosulfate consumed.

0.005 mol/L Iodine

This solution contains 1.269 g of iodine (I_2, molecular weight: 253.81) per 1000 mL.

Prepare by diluting 0.05 mol/L iodine with water to 10 times its original volume. Use the factor of 0.05 mol/L iodine instead of standardization. Prepare fresh before use.

0.05 mol/L Lead(II) Nitrate

This solution contains 16.56 g of lead(II) nitrate ($Pb(NO_3)_2$, molecular weight: 331.21) per 1000 mL.

Weigh 17.0 g of lead(II) nitrate, transfer it into a 1000-mL volumetric flask, dissolve it in 25 mL of diluted nitric acid (1 in 51), and add water to the volume.

Standardization Measure exactly 25 mL of the prepared lead(II) nitrate, add 10 mL of hexamethylenetetramine solution (1 in 10), and adjust its pH to 5.2–5.4 with diluted nitric acid (1 in 11). Titrate this solution with 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: a few drops of xylenol orange). The endpoint is when the color of the solution changes from red-purple to yellow.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.05 mol/L lead(II) nitrate,

f_1 = 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate,

V = volume (mL) of 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate consumed.

0.1 mol/L Magnesium Acetate

This solution contains 21.45 g of magnesium acetate tetrahydrate ($Mg(CH_3COO)_2 \cdot 4H_2O$, molecular weight: 214.45) per 1000 mL.

Dissolve 21.5 g of magnesium acetate tetrahydrate in water to make 1000 mL.

Standardization Measure exactly 25 mL of the prepared magnesium acetate solution, add about 50 mL of water and 3 mL of ammonia solution–ammonium chloride TS. Add 50 mg of eriochrome black T–sodium chloride indicator while heating to about 40°C, and titrate with 0.05 mol/L disodium dihydrogen ethylenediaminetetracetate. The endpoint is when the color of the solution changes from red to blue.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.1 mol/L magnesium acetate,

f_1 = factor of 0.1 mol/L disodium dihydrogen ethylenediaminetetracetate,

V = volume (mL) of 0.1 mo/L disodium dihydrogen ethylenediaminetetracetate consumed.

0.05 mol/L Magnesium Chloride

This solution contains 10.17 g of magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, molecular weight: 203.30) per 1000 mL.

Dissolve 10.2 g of magnesium chloride hexahydrate in water (carbon dioxide-removed) to make 1000 mL.

Standardization Measure exactly 25 mL of the prepared magnesium chloride solution, add 50 mL of water, 2 mL of ammonia solution–ammonium chloride TS, and 50 mg of eriochrome black T-sodium chloride indicator. Titrate with 0.05 mol/L disodium dihydrogen ethylenediaminetetraacetate while keeping the solution temperature at 40°C. The endpoint is when the color of the solution changes from red to blue.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.05 mol/L magnesium chloride,

f_1 = factor of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate,

V = volume (mL) of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate consumed.

0.5 mol/L Morpholine, Methanolic

This solution contains 43.56 g of morpholine (C_4H_9NO, molecular weight: 87.12) per 1000 mL.

Measure 11 mL of morpholine, and add methanol to make 250 mL.

0.1 mol/L Nitric Acid

This solution contains 6.301 g of nitric acid (HNO_3, molecular weight: 63.01) in 1000 mL.

Measure 7 mL of nitric acid, and dissolve in water to make 1000 mL.

Standardization Weigh accurately 0.13–0.16 g of sodium carbonate (reference material), dried as directed in its certificate, dissolve it in 50 mL of water, and titrate with the prepared nitric acid solution. While titration, keep the sodium carbonate solution stirring and do not boil it. Confirm the endpoint, using a potentiometer or an indocator (2 drops of bromophenol blue TS). When a potentiometer is used, use a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes.

When the indicator is used, boil near the endpoint to expel the carbon dioxide, and continue the titration. The endpoint is when the color of the solution changes from blue-purple to bluish green.

Each mL of 1 mol/L hydrochloric acid = 5.299 mg of Na_2CO_3

The factor is calculated by the formula:

$f = m/(0.005299 \times V) \times A/100$

f = factor of 0.1 mol/L nitric acid,

m = weight (g) of sodium carbonate (reference material),

A = content (%) of sodium carbonate (reference material),

V = volume (mL) of 0.1 mol/L nitric acid consumed.

0.05 mol/L Oxalic Acid

This solution contains 6.303 g of oxalic acid dihydrate ($C_2H_2O_4 \cdot 2H_2O$, molecular weight: 126.07) per 1000 mL.

Dissolve 6.4 g of oxalic acid dihydrate in water to make 1000 mL. Tightly stopper and store.

Standardization Measure exactly 25 mL of the prepared oxalic acid solution, add 200 mL of diluted sulfuric acid (1 in 21), and heat to about 70°C. Add an appropriate volume (about 2 mL less than that required for titration) of 0.02 mol/L potassium permanganate while shaking gently. Allow to stand until the red color of the solution disappears, and continue the titration with the potassium permanganate. The endpoint is when the faint red color of the solution persists for about 30 seconds. Separately, perform a blank test to make any necessary correction. In the titration, the solution temperature at the endpoint should not be 60°C or lower.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.05 mol/L oxalix aicd,

f_1 = factor of 0.02 mol/L potassium permanganate,

V = volume (mL) of 0.02 mol/L potassium permanganate consumed.

0.1 mol/L Perchloric Acid

This solution contains 10.05 g of perchloric acid ($HClO_4$, molecular weight: 100.46) per 1000 mL.

Weigh 1000 g of acetic acid for nonaqueous titration whose water content has been determined, and add 14 g of perchloric acid whose content (70–72%) is known. Add the amount, calculated by the following formula, of acetic anhydride, stopper tighly, and store. Before use, allow to stand at least for 1 hours. Be careful about storage conditions, because it may freeze in winter time.

$m = \{(1000 \times W_1/100 + 14/W_2/100) - 0.5\} \times 5.7$

m = weight (g) of acetic anhydride (amount required to adjust the water content to 0.05%),

W_1 = water content (%) of acetic acid for nonaqueous titration,

W_2 = water content (%) of perchloric acid that is calculated by the formula, [100 – perchloric acid content (%)].

Standardization Weigh accurately 0.5–0.6 g of potassium hydrogen phthalate (reference material), dried as directed in its certificate, add 50 mL of acetic acid for nonaqueous titration, and titrate with the prepared perchloric acid. Confirm the endpoint using a potentiometer with a glass indicator electrode and a silver–silver

chloride reference electrode. A combined electrode can also be used for the indicator and refrence electrodes. Perform a blank test, using 50 mL of acetic acid for nonaqueous titration to correct the titration volume.

Each mL of 0.1 mol/L perchloric acid = 20.422 mg of potassium hydrogen phthalate

The factor is calculated by the formula:

$f = m/\{0.020422 \times (V - V_0)\} \times A/100$

f = factor of 0.1 mol/L perchloric acid,

m = weight (g) of potassium hydrogen phthalate (reference material),

A = purity (%) of potassium hydrogen phthalate (reference material),

V = volume (mL) of 0.1 mol/L perchloric acid consumed,

V_0 = volume (mL) of 0.1 mol/L perchloric acid consumed in the blank test.

1/60 mol/L Potassium Dichromate

This solution contains 4.903 g of potassium dichromate ($K_2Cr_2O_7$, molecular weight: 294.18) per 1000 mL.

Proceed as directed in either of the procedures below.

1. Weigh accurately 4.9–5.0 g of potassium dichromate (reference material), dried as directed in its certificate, and dissolve it in water to make exactly 1000 mL. Stopper tightly and store.

Each mL of 1/60 mol/L potassium dichromate = 4.903 mg of $K_2Cr_2O_7$

The factor is calculated by the formula:

$f = m/4.903 \times A/100$

f = factor of 1/60 mol/L potassium dichromate,

m = weight (g) of potassium dichromate (reference material),

A = content (%) of potassium dichromate(reference material).

2. Dissolve 5 g of potassium dichromate in water (dissolved oxygen-removed) to make 1000 mL.

Standardization Transfer exactly 25 mL of the prepared solution in a 300-mL ground stoppered Erlenmeyer flask, add 50 mL of water and 2 g of potassium iodide to dissolve, and add 6 mL of diluted sulfuric acid (1 in 6). Immediately stopper, shake gently, and allow to stand in dark place for 5 minutes. Titrate with 0.1 mol/L sodium thiosulfate (indicator: 3 mL of starch TS). Add the indicator near the endpoint, when the solution turns light yellow. The endpoint is when the color of solution becomes blue-green. Separately, perform a blank test to make necessary correction. The factor is calculated by the formula:

$f_1 = f_2 \times (V - V_0)/25$

f_1 = factor of 1/60 mol/L potassium dichromate,

f_2 = factor of 0.1 mol/L sodium thiosulfate,

V = volume (mL) of 0.1 mol/L sodium thiosulfate consumed,

V_0 = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the blank test.

1 mol/L Potassium Hydroxide

This solution contains 56.11 g of potassium hydroxide (KOH, molecular weight: 56.11) per 1000 mL.

Weigh 70 g of potassium hydroxide or an amount of potassium hydroxide solution (high purity) or potassium hydroxide solution (for semiconductors), equivalent to 70 g of potassium hydroxide, into a plastic container made of polyethylene or the like, and dissolve it in 1000 mL of water (carbon dioxide-removed). Stopper tightly to protect it from carbon dioxide, and leave for 4–5 days. Store the supernatant in a tightly stoppered plastic container.

Standardization Weigh accurately 2.4–2.6 g of amidosulfuric acid (reference material), dried as directed in its certificate, and dissolve it in 70 mL of water, and titrate with the prepared potassium hydroxide solution. To confirm the endpoint, use a potentiometer or an indicator (a few drops of bromothymol blue TS). When a potentiometer is used, use a glass indicator electrode and a silver–silver chloride reference. A combined electrode can also be used for the indicator and reference electrodes. When the indicator is used, the endpoint is when the color of the solution changes from yellow to bluish green.

Each mL of 1 mol/L potassium hydroxide = 97.09 mg of $HOSO_2NH_2$

The factor is calculated by the formula:

$f = m/(0.09709 \times V) \times A/100$

f = factor of 1 mol/L potassium hydroxide,

m = weight (g) of amidosulfuric acid (reference material),

A = content (%) of amidosulfuric acid (reference material),

V = volume (mL) of 1 mol/L potassium hydroxide consumed.

0.1 mol/L Potassium Hydroxide

This solution contains 5.611 g of potassium hydroxide (KOH, molecular weight: 56.11) per 1000 mL.

Weigh 7 g of potassium hydroxide or an amount of potassium hydroxide solution (high purity) or potassium hydroxide solution (for semiconductors), equivalent to 7 g of potassium hydroxide. Prepare as directed for 1 mol/L Potassium Hydroxide.

Standardization Proceed as directed for 1 mol/L Potassium Hydroxide using 0.24–0.26 g of the dried amidosulfuric acid (reference material).

Each mL of 0.1 mol/L potassium hydroxide = 9.709 mg of $HOSO_2NH_2$

The factor is calculated by the formula:

$f = m/(0.009709 \times V) \times A/100$

f = factor of 0.1 mol/L potassium hydroxide,

m = weight (g) of amidosulfuric acid (reference material),

A = content (%) of amidosulfuric acid (reference material),

V = volume (mL) of 0.1 mol/L potassium hydroxide consumed.

0.5 mol/L Potassium Hydroxide, Ethanolic

This solution contains 28.05 g of potassium hydroxide (KOH, molecular weight: 56.11) per 1000 mL.

Weigh 35 g of potassium hydroxide into a plastic container made of high-density polyethylene or the like, dissolve it in 20 mL of water (carbon dioxide-removed), add aldehyde-free ethanol to make 1000 mL, and mix. Stopper tightly to protect this solution from carbon dioxide, and leave for 2–3 days. Store the supernatant in a tightly stoppered plastic container.

Standardization Measure exactly 25 mL of 0.25 mol/L sulfuric acid, add 50 mL of water (carbon dioxide-removed), and titrate with the prepared potassium hydroxide solution in ethanol. To confirm the endpoint, use a potentiometer or an indicator (3 drops of phenolphthalein TS). When a potentiometer is used, use a glass indicator electrode (for nonaqueous titration) and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes. When the indicator is used, the endpoint is when a faint red color persists for about 30 seconds. Standardize before use.

The factor is calculated by the formula:

$f = f_1 \times 25/V$

f = factor of 0.5 mol/L ethanolic potassium hydroxide,

f_1 = factor of 0.25 mol/L sulfuric acid,

V = volume (mL) of 0.5 mol/L ethanolic potassium hydroxide consumed.

0.1 mol/L Potassium Hydroxide, Ethanolic

This solution contains 5.611 g of potassium hydroxide (KOH, molecular weight: 56.11) per 1000 mL.

Weigh 7 g of potassium hydroxide into a plastic container made of high-density polyethylene or the like, dissolve it in 20 mL of water (carbon dioxide-removed), add aldehyde-free ethanol (a product that will not interfere with the titration) to make 1000 mL, and mix. Stopper tightly to protect the solution from carbon dioxide, and leave for 2–3 days. Store the supernatant in a tightly stoppered plastic container.

Standardization Measure exactly 25 mL of 0.05 mol/L sulfuric acid, add 50 mL of water (carbon dioxide-removed), and titrate with the prepared potassium hydroxide solution in ethanol. To confirm the endpoint, use a potentiometer or an indicator (3 drops of phenolphthalein TS). When a potentiometer is used, use a glass indicator electrode (for nonaqueous titration) and a silver–silver chloride reference electrode. A combined electrode can also be used for indicator and reference electrodes. When the indicator is used, the endpoint is when a faint red color persists for about 30 seconds. Standardize before use.

The factor is calculated by the formula:

$f = f_1 \times 25/V$

f = factor of 0.1 mol/L ethanolic potassium hydroxide,

f_1 = factor of 0.05 mol/L sulfuric acid,

V = volume (mL) of 0.1 mol/L ethanolic potassium hydroxide consumed.

0.02 mol/L Potassium Hydroxide, Ethanolic

This solution contains 1.122 g of potassium hydroxide (KOH, molecular weight: 56.11) per 1000 mL.

Dilute 0.1 mol/L ethanolic potassium hydroxide with aldehyde-free ethanol (a product that will not interfere with the titration) to 5 times its original volume.

Standardization Measure exactly 25 mL of 0.01 mol/L sulfuric acid, add 50 mL of water (carbon dioxide-removed), and titrate with the prepared potassium hydroxide solution in ethanol. To confirm the endpoint, use a potentiometer or an indicator (3 drops of phenolphthalein TS). When a potentiometer is used, use a glass indicator electrode (for nonaqueous titration) and a silver–silver chloride reference electrode. A combined electrode can also be used for indicator and reference electrodes. When the indicator is used, the endpoint is when a faint red color persists for about 30 seconds. Standardize before use.

The factor is calculated by the formula:

$f = f_1 \times 25/V$

f = factor of 0.02 mol/L ethanolic potassium hydroxide,

f_1 = factor of 0.01 mol/L sulfuric acid,

V = volume (mL) of 0.02 mol/L ethanolic potassium hydroxide consumed.

0.05 mol/L Potassium Iodate

This solution contains 10.70 g of potassium iodate (KIO_3, molecular weight: 214.00) per 1000 mL.

Proceed as directed in either of the procedures below.

1. Weigh accurately 10.7–10.8 g of potassium iodate (reference material), dried as directed in its certificate, transfer it into a 1000-volumetric flask, and dissolve by adding water (dissolved oxgen-removed). Add water to the volume, and mix. Stopper tightly and store.

The factor is calculated by the formula:

$f = m/10.700 \times A/100$

f = factor of 0.05 mol/L potassium iodate,

m = weight (g) of potassium iodate (reference material),

A = content (%) of potassium iodate (reference material).

2. Dissolve 10.7 g of potassium iodate in water (dissolved oxygen-removed), and add water (dissolved oxgen-removed) to make 1000 mL volume. Stopper tightly and store.

Standardization Transfer exactly 10 mL of the prepared potassium iodate solution into a 200-mL glass-stoppered Erlenmeyer flask, and add 30 mL of water. Add 3 g of

potassium iodide, then add 5 mL of diluted sulfuric acid (1 in 6), immediately stopper the flask, and dissolve by stirring gently. Allow to stand for 5 minutes in a dark place. Titrate with 0.1 mol/L sodium thiosulfate (indicator: 3 mL of starch TS). Add the indicator near the endpoint, when the solution becomes light yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test to make any necessary correction.

The factor is calculated by the formula:

$f_1 = f_2 \times (V–V_0)/30$

f_1 = factor of 0.05 mol/L potassium iodate,

f_2 = factor of 0.1 mol/L sodium thiosulfate,

V = volume (mL) of 0.1 mol/L sodium thiosulfate consumed,

V_0 = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the blank test.

0.02 mol/L Potassium Permanganate

This solution contains 3.161 g of potassium permanganate ($KMnO_4$, molecular weight: 158.03) per 1000 mL.

Dissolve 3.2 g of potassium permanganate in 1050 mL of water, and boil gently for 1–2 hours. Allow to stand in a dark place for about 18 hours, and filter the supernatant through a glass-filter (G4). Do not wash the glass-filter with water before filtration. Store in a brown glass-stoppered bottle, previously washed with hot water.

Standardization Weigh accurately 0.20–0.24 g of sodium oxalate (reference material), dried as directed in its certificate, and dissolve it in about 200 mL of water. Add 20 mL of diluted sulfuric acid (1 in 2), and heat to about 70°C. To this solution, immediately add a volume about 2 mL less than that required for titration of the prepared potassium permanganate. Allow to stand until the red color of the solution disappears, and continue the titration with the potassium permanganate. The endpoint is when the faint red color of the solution persists for about 15 seconds. To confirm the endpoint, a potentiometer can be used. For electrodes, use a platinum indicator electrode and a silver-silver chloride or glass reference electrode. A combined electrode can also be used for the indicator and reference electrodes. Separately, perform a blank test to make any necessary correction.

In the titration, the solution temperature at the endpoint should be 60°C or higher.

Each mL of 0.02 mol/L $KMnO_4$ = 6.700 mg of $Na_2C_2O_4$

The factor is calculated by the formula:

$f = m/\{0.006700 \times (V–V_0)\} \times A/100$

f = factor of 0.02 mol/L potassium permanganate,

m = weight (g) of sodium oxalate (reference material),

A = content (%) of sodium oxalate (reference material),

V = volume (mL) of 0.02 mol/L potassium permanganate consumed,

V_0 = volume (mL) of 0.02 mol/L potassium permanganate consumed in the blank

test.

0.1 mol/L Silver Nitrate

This solution contains 16.99 g of silver nitrate ($AgNO_3$, molecular weight: 169.87) per 1000 mL.

Dissolve about 17 g of silver nitrate in water to make 1000 mL. Stopper tightly and store in a dark place, protected from light.

Standardization Weigh accurately 0.14–0.17 g of sodium chloride (reference material), dried as directed in its certificate, and dissolve it in 70 mL of water, and titrate with the prepared silver nitrate solution. Confirm the endpoint using a potentiometer or an indicator (a few drops of uranine TS). When a potentiometer is used, use a platinum or silver indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes. When the indicator is used, the endpoint is when the solution becomes reddish.

Each mL of 1 mol/L silver nitrate = 5.844 mg of NaCl

The factor is calculated by the formula:

$f = m/(0.005844 \times V) \times A/100$

f = factor of 0.1 mol/L silver nitrate,

m = weight (g) of sodium chloride (reference material),

A = content (%) of sodium carbonate (reference material),

V = volume (mL) of 0.1 mol/L silver nitrate consumed.

0.05 mol/L Silver Nitrate

This solution contains 8.4954 g of silver nitrate ($AgNO_3$, molecular weight: 169.87) per 1000 mL.

Dissolve about 8.5 g of silver nitrate in water to make 1000 mL. Stopper tightly and store in a dark place, protected from light.

Standardization Weigh accurately 0.07–0.09 g of sodium chloride (reference material), dried as directed in its certificate, and proceed as directed in either of the two procedures below:

1. Dissolve it in 70 mL of water, and titrate with the prepared silver nitrate solution. Confirm the endpoint using a potentiometer with a silver indicator electrode and a silver-silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes.

2. Dissolve it in 50 mL of water, and titrate with the prepared silver nitrate solution (indicator: a few drops of uranine TS). The endpoint is when the solution becomes reddish.

Each mL of 0.05 mol/L silver nitrate = 2.922 mg of NaCl

The factor is calculated by the formula:

$f = m/(0.002922 \times V) \times A/100$

f = factor of 0.05 mol/L silver nitrate,

m = weight (g) of sodium chloride (reference material),
A = content (%) of sodium carbonate (reference material),
V = volume (mL) of 0.05 mol/L silver nitrate consumed.

0.005 mol/L Silver Nitrate

This solution contains 8.495 g of silver nitrate ($AgNO_3$, molecular weight:169.87) per 1,000 mL. Prepare by diluting 0.1 mol/L silver nitrate with water to 20 times its original volume. Use the factor of 0.1 mol/L silver nitrate instead of standardization. Prepare fresh before use.

0.1 mol/L Sodium Acetate

This solution contains 8.203 g of sodium acetate (CH_3COONa, molecular weight: 82.03) per 1000 mL.

Dissolve 8.20 g of sodium acetate in 1000 mL of acetic acid for nonaqueous titrtation. Stopper tightly and store.

Standardization Titrate 25 mL of the prepared sodium acetate solution, measured exactly, with 0.1 mol/L perchloric acid. Confirm the endpoint using a potentiometer with a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for the indicator and reference electrodes.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.1 mol/L sodium acetate,
f_1 = factor of 0.1 mol/L perchloric acid,
V = volume (mL) of 0.1 mo/L perchloric acid consumed.

0.1 mol/L Sodium Chloride

This solution contains 5.844 g of sodium chloride (NaCl, molecular weight: 58.44) per 1000 mL.

Prepare the solution using either of the following methods.

1. Weigh accurately 5.844 g of sodium chloride (reference material), dried as directed in its certificate, and dissolve it in water to make exactly 1000 mL. Stopper tightly and store.

The factor is calculated by the formula:

$f = m/5.844 \times A/100$

f = factor of 0.1 mol/L sodium chloride,
m = weight (g) of sodium chloride (reference material),
A = content (%) of sodium chloride (reference material).

2. Dissolve 5.9 g of sodium chloride in water to make 1000 mL. Standardize this solution. Stopper tightly and store.

Standardization Measure exactly 25 mL of the prepared solution, add 50 mL of

water, and shake well. 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. A combined electrode can also be used for indicator and reference electrodes. An indicator is used to confirm the endpoint, add 15 of water to exactly 25 mL of the prepared solution, shake well, and titrate with 0.1 mol/L silver nitrate. The endpoint is when the color of the solution becomes reddish.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.1 mol/L sodium chloride,

f_1 = factor of 0.1 mol/L silver nitrate,

V = volume (mL) of 0.1 mo/L silver nitrate consumed.

1 mol/L Sodium Hydroxide

This solution contains 40.00 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1000 mL.

Prepare the solution by one of the three methods below.

1. Weigh 40 g of sodium hydroxide into a plastic container made of high-density polyethylene or the like, and dissolve it in 100 mL of water (carbon dioxide-removed). After cooling, transfer this solution into an air-tight, high-density plastic container, and allow to stand for at least 24 hours. Transfer it into a high-density plastic container, add water (carbon dioxide-removed) to make 1000 mL, and mix. Store in a tightly stoppered plastic container.

2. Dissolve an amount of sodium hydroxide solution (high purity) or sodium hydroxide solution (for semiconductors), equivalent to 40 g of sodium hydroxide, in 1000 mL of water (carbon dioxide-removed). Stir this solution for about 1 hour. If necessary, leave it for about 24 hours, and filter through a 0.2-μm filter. Store in a tightly stoppered high-density plastic container.

3. Weigh 165 g of sodium hydroxide into a plastic container made of polyethylene or the like, and dissolve it in 150 mL of water (carbon dioxide-removed). Stopper tightly to protect the solution from carbon dioxid, and leave for 4–5 days. Place 54 mL of the supernatant in a 1000-mL plastic container, add water (carbon dioxide-removed) to make 1000 mL, and mix. Store in a tightly stoppered, high-density, plastic container.

Standardization Weigh accurately 2.4–2.6 g of amidosulfuric acid (reference material), dried as directed in its certificate, dissolve it in 70 mL of water, and titrate with the prepared sodium hydroxide solution. To confirm the endpoint, use a potentiometer or an indicator (a few drops of bromothymol blue TS). When a potentiometer is used, use a glass indicator electrode and a silver–silver chloride reference electrode. A combined electrode can also be used for indicator and reference electrodes. When the indicator is used, the endpoint is when the color of the solution changes from yellow to bluish green.

Each mL of 1 mol/L sodium hydroxide = 97.09 mg of $HOSO_2NH_2$

The factor is calculated by the formula:

$f = m/(0.09709 \times V) \times A/100$

f = factor of 1 mol/L sodium hydroxide,

m = weight (g) of amidosulfuric acid (reference material),

A = content (%) of amidosulfuric acid (reference material),

V = volume (mL) of 1 mol/L sodium hydroxide consumed.

0.5 mol/L Sodium Hydroxide

This solution contains 20.00 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1000 mL.

Prepare as directed for 1 mol/L Sodium Hydroxide, using 20 g of sodium hydroxide or 27 mL of the supernatant of sodium hydroxide solution (high purity) or sodium hydroxide solution (for semiconductors).

Standardization Proceed as directed for 1 mol/L Sodium Hydroxide using 1.2–1.3 g of dried amidosulfuric acid (reference material).

Each mL of 0.5 mol/L sodium hydroxide = 48.55 mg of $HOSO_2NH_2$

The factor is calculated by the formula:

$f = m/(0.04855 \times V) \times A/100$

f = factor of 0.5 mol/L sodium hydroxide,

m = weight (g) of amidosulfuric acid (reference material),

A = content (%) of amidosulfuric acid (reference material),

V = volume (mL) of 0.5 mol/L sodium hydroxide consumed.

0.45 mol/L Sodium Hydroxide

This solution contains 18.00 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1000 mL.

Prepare as directed for 1 mol/L Sodium Hydroxide, using 18 g of sodium hydroxide or 24.3 mL of the supernatant of sodium hydroxide solution (high purity) or sodium hydroxide solution (for semiconductors).

Standardization Proceed as directed for 1 mol/L Sodium Hydroxide using 1.08–1.17 g of dried amidosulfuric acid (reference material).

Each mL of 0.45 mol/L sodium hydroxide = 43.69 mg of $HOSO_2NH_2$

The factor is calculated by the formula:

$f = m/(0.04369 \times V) \times A/100$

f = factor of 0.45 mol/L sodium hydroxide,

m = weight (g) of amidosulfuric acid (reference material),

A = content (%) of amidosulfuric acid (reference material),

V = volume (mL) of 0.45 mol/L sodium hydroxide consumed.

0.25 mol/L Sodium Hydroxide

This solution contains 9.999 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1000 mL.

Prepare the solution by either of the methods below.

1. Prepare by diluting 1 mol/L sodium hydroxide with water (carbon dioxide-removed) to 4 times its original volume.

2. Prepare as directed for 1 mol/L Sodium Hydroxide, using about 10 g sodium hydroxide or 13.5 mL of the supernatant of sodium hydroxide solution (high purity) or sodium hydroxide solution (for semiconductors).

Standardization Proceed as directed for 1 mol/L Sodium Hydroxide using 0.60– 0.65 g of dried amidosulfuric acid (reference material).

Each mL of 0.25 mol/L sodium hydroxide = 24.27 mg of $HOSO_2NH_2$

The factor is calculated by the formula:

$f = m/(0.02427 \times V) \times A/100$

f = factor of 0.25 mol/L sodium hydroxide,

m = weight (g) of amidosulfuric acid (reference material),

A = content (%) of amidosulfuric acid (reference material),

V = volume (mL) of 0.25 mol/L sodium hydroxide consumed.

0.2 mol/L Sodium Hydroxide

This solution contains 7.999 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1000 mL.

Prepare the solution by either the methods below.

1. Prepare by diluting 1 mol/L sodium hydroxide with water (carbon dioxide-removed) to 5 times its original volume.

2. prepare as directed for 1 mol/L Sodium Hydroxide, using about 8 g of sodium hydroxide or 10.8 mL of the supernatant of sodium hydroxide solution (high purity) or sodium hydroxide solution (for semiconductors).

Standardization Proceed as directed for 1 mol/L Sodium Hydroxide using 0.48– 0.52 g of dried amidosulfuric acid (reference material).

Each mL of 0.2 mol/L sodium hydroxide = 19.42 mg of $HOSO_2NH_2$

The factor is calculated by the formula:

$f = m/(0.01942 \times V) \times A/100$

f = factor of 0.2 mol/L sodium hydroxide,

m = weight (g) of amidosulfuric acid (reference material),

A = content (%) of amidosulfuric acid (reference material),

V = volume (mL) of 0.2 mol/L sodium hydroxide consumed.

0.1 mol/L Sodium Hydroxide

This solution contains 4.000g of sodium hydroxide (NaOH, molecular weight: 40.00) per

1000 mL.

Prepare the solution by either of the methods below.

1. Prepare by diluting 1 mol/L sodium hydroxide with water (carbon dioxide-removed) to 10 times its original volume.

2. prepare as directed for 1 mol/L Sodium Hydroxide, using about 4.5 g of sodium hydroxide or 5.4 mL of the supernatant of sodium hydroxide solution (high purity) or sodium hydroxide solution (for semiconductors).

Standardization Proceed as directed for 1 mol/L Sodium Hydroxide using 0.24– 0.26 g of dried amidosulfuric acid (reference material).

Each mL of 0.1 mol/L sodium hydroxide = 9.709 mg of $HOSO_2NH_2$

The factor is calculated by the formula:

$f = m/(0.009709 \times V) \times A/100$

f = factor of 0.1 mol/L sodium hydroxide,

m = weight (g) of amidosulfuric acid (reference material),

A = content (%) of amidosulfuric acid (reference material),

V = volume (mL) of 0.1 mol/L sodium hydroxide consumed.

0.05 mol/L Sodium Hydroxide

This solution contains 2.000 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1000 mL.

Prepare by diluting 1 mol/L sodium hydroxide with water (carbon dioxide-removed) to 20 times its original volume.

Standardization Use the factor of 1 mol/L sodium hydroxide instead of standardization, or proceed as directed for 1 mol/L Sodium Hydroxide using 0.12– 0.13 g of dried amidosulfuric acid (reference material).

Each mL of 0.05 mol/L sodium hydroxide = 4.855 mg of $HOSO_2NH_2$

The factor is calculated by the formula:

$f = m/(0.004855 \times V) \times A/100$

f = factor of 0.05 mol/L sodium hydroxide,

m = weight (g) of amidosulfuric acid (reference material),

A = content (%) of amidosulfuric acid (reference material),

V = volume (mL) of 0.05 mol/L sodium hydroxide consumed.

0.02 mol/L Sodium Hydroxide

This solution contains 0.7999 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1000 mL.

Prepare by diluting 0.1 mol/L sodium hydroxide with water (carbon dioxide-removed) to 5 times its original volume.

Standardization Use the factor of 0.1 mol/L sodium hydroxide instead of standardization, or proceed as directed for 1 mol/L Sodium Hydroxide using 48–52 mg of

dried amidosulfuric acid (reference material).

Each mL of 0.02 mol/L sodium hydroxide = 1.942 mg of $HOSO_2NH_2$

The factor is calculated by the formula:

$f = m/(0.001942 \times V) \times A/100$

f = factor of 0.01 mol/L sodium hydroxide,

m = weight (g) of amidosulfuric acid (reference material),

A = content (%) of amidosulfuric acid (reference material),

V = volume (mL) of 0.02 mol/L sodium hydroxide consumed.

0.01 mol/L Sodium Hydroxide

This solution contains 0.400 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1000 mL.

Prepare by diluting 0.1 mol/L sodium hydroxide with water (carbon dioxide-removed) to 10 times its original volume.

Standardization Use the factor of 0.1 mol/L sodium hydroxide instead of standardization, or proceed as directed for 1 mol/L Sodium Hydroxide using 24–26 mg of dried amidosulfuric acid (reference material).

Each mL of 0.01 mol/L sodium hydroxide = 0.9709 mg of $HOSO_2NH_2$

The factor is calculated by the formula:

$f = m/(0.0009709 \times V) \times A/100$

f = factor of 0.01 mol/L sodium hydroxide,

m = weight (g) of amidosulfuric acid (reference material),

A = content (%) of amidosulfuric acid (reference material),

V = volume (mL) of 0.01 mol/L sodium hydroxide consumed.

Standardize and store, as directed for 1 mol/L sodium hydroxide. Restandardize frequently.

0.1 mol/L Sodium Thiosulfate

This solution contains 24.82 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$, molecular weight: 248.18) per 1000 mL.

Dissolve 26 g of sodium thiosulfate pentahydrate and 0.2 g of sodium carbonate in 1000 mL of water (dissolved oxygen-removed). Store the prepared solution in a tightly stoppered container. Before use, leave it two days.

Standardization Weigh accurately 0.9–1.1 g of potassium iodate (reference material), dried as directed in its certificate, and dissolve it in water to make exactly 250 mL. Measure exactly 25 mL of this solution, add 75 mL of water, 2 g of potassium iodide, and 2 mL of diluted sulfuric acid (1 in 2), immediately stopper, and shake gently. Allow to stand for 5 minutes in a dark place. Titrate with the prepared sodium thiosulfate solution (indicator: 3 mL of starch TS). Add the indicator near the endpoint, when the solution becomes light yellow. The endpoint is when the blue color of the solution disappears.

Perform a blank test with 100 mL of water to make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate = 3.5667 mg of KIO_3

The factor is calculated by the formula:

$f = (m \times 25/250)/\{0.0035667 \times (V-V_0)\} \times A/100$

f = factor of 0.1 mol/L sodium thiosulfate,

m = weight (g) of potassium iodate (reference material),

A = content (%) of potassium iodate (reference material),

V = volume (mL) of 0.1 mol/L sodium thiosulfate consumed,

V_0 = volume (mL) of 0.1 mol/L sodium thiosulfate consumed in the blank test.

0.05 mol/L Sodium Thiosulfate

This solution contains 12.41 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$, molecular weight: 248.18) per 1000 mL.

Dissolve 13 g of sodium thiosulfate pentahydrate and 0.2 g of sodium carbonate in 1000 mL of water (dissolved oxygen-removed). Store the prepared solution in a tightly stoppered container. Before use, leave it two days.

Standardization Weigh accurately 0.4–0.5 g of potassium iodate (reference material), dried as directed in its certificate, and dissolve it in water to make exactly 250 mL. Measure exactly 25 mL of this solution, add 75 mL of water, 1 g of potassium iodide, and 2 mL of diluted sulfuric acid (1 in 2), immediately stopper, and shake gently. Allow to stand for 5 minutes in a dark place. Titrate with the prepared sodium thiosulfate solution (indicator: 3 mL of starch TS). Add the indicator near the endpoint, when the solution becomes light yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test with 100 mL of water to make any necessary correction.

Each mL of 0.05 mol/L sodium thiosulfate = 1.7833 mg of KIO_3

The factor is calculated by the formula:

$f = (m \times 25/250)/\{0.0017833 \times (V-V_0)\} \times A/100$

f = factor of 0.05 mol/L sodium thiosulfate,

m = weight (g) of potassium iodate (reference material),

A = content (%) of potassium iodate (reference material),

V = volume (mL) of 0.05 mol/L sodium thiosulfate consumed,

V_0 = volume (mL) of 0.05 mol/L sodium thiosulfate consumed in the blank test.

0.01 mol/L Sodium Thiosulfate

This solution contains 2.482 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$, molecular weight: 248.18) per 1000 mL.

Dissolve 2.6 g of sodium thiosulfate pentahydrate and 0.2 g of sodium carbonate in 1000 mL of water (dissolved oxygen-removed). Store the prepared solution in a tightly stoppered container. Before use, leave it two days.

Standardization Weigh accurately 0.3–0.4 g of potassium iodate (reference material),

dried as directed in its certificate, and dissolve it in water to make exactly 250 mL. Measure exactly 25 mL of this solution, add 75 mL of water, 1 g of potassium iodide, and 2 mL of diluted sulfuric acid (1 in 2), immediately stopper, and shake gently. Allow to stand for 5 minutes in a dark place. Titrate with the prepared sodium thiosulfate solution (indicator: 3 mL of starch TS). Add the indicator near the endpoint, when the solution becomes light yellow. The endpoint is when the blue color of the solution disappears. Perform a blank test with 100 mL of water to make necessary correction.

Each mL of 0.01 mol/L sodium thiosulfate = 0.35667 mg of KIO_3

The factor is calculated by the formula:

$f = (m \times 25/250)/\{0.0035667 \times (V - V_0)\} \times A/100$

f = factor of 0.01 mol/L sodium thiosulfate,

m = weight (g) of potassium iodate (reference material),

A = content (%) of potassium iodate (reference material),

V = volume (mL) of 0.01 mol/L sodium thiosulfate consumed,

V_0 = volume (mL) of 0.01 mol/L sodium thiosulfate consumed in the blank test.

0.005 mol/L Sodium Thiosulfate

This solution contains 1.241 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$, molecular weight: 248.18) per 1000 mL.

Transfer exactly 10 mL of 0.1 mol/L sodium thiosulfate into a 200-mL volumetric flask, add water (dissolved oxygen-removed) to the volume, and mix. Prepare fresh before use. Use the factor of 0.1 mol/L sodium thiosulfate instead of standardization.

0.5 mol/L Sulfuric Acid

This solution contains 49.04 g of sulfuric acid (H_2SO_4, molecular weight: 98.08) per 1000 mL.

Measure about 1000 mL of water, add slowly 30 mL of sulfuric acid while stirring, and allow to cool to 20°C. Stopper tightly and store.

Standardization Weigh accurately 1.3–1.6 g of sodium carbonate (reference material), dried as directed in its certificate, dissolve it in 70 mL of water, and titrate with the prepared sulfuric acid solution. Use a potentiometer or an indicator (a few drops of bromophenol blue TS) to confirm the endpoint. When a potentiometer is used, use a glass indicator electrode and a silver–silver chloride reference electrode. While in titration, keep shaking the sodium carbonate solution vigorously and do not boil. The endpoint is the second inflection point. When the indicator is used, boil the solution near the endpoint to expel carbon dioxide. Cool it, and continue the titration. The endpoint is when the color of the solution changes from blue-purple to bluish green.

Each mL of 0.5 mol/L sulfuric acid = 52.99 mg of Na_2CO_3

The factor is calculated by the formula:

$f = m/(0.05299 \times V) \times A/100$

f = factor of 0.5 mol/L sulfuric acid,
m = weight (g) of sodium carbonate (reference material),
A = content (%) of sodium carbonate (reference material),
V = 0.5 mol/L sulfuric acid consumed.

0.25 mol/L Sulfuric Acid

This solution contains 24.52 g of sulfuric acid (H_2SO_4, molecular weight: 98.08) per 1000 mL.

Prepare and standardize as directed for 0.5 mol/L Sulfuric Acid, using 15 mL of sulfuric acid.

Standardization Proceed as directed for 0.5 mol/L Sulfuric Acid, using 0.65–0.80 g of dried sodium carbonate (reference material).

Each mL of 0.25 mol/L sulfuric acid = 26.497 mg of Na_2CO_3

The factor is calculated by the formula:

$f = m/(0.026497 \times V) \times A/100$

f = factor of 0.25 mol/L sulfuric acid,
m = weight (g) of sodium carbonate (reference material),
A = content (%) of sodium carbonate (reference material),
V = 0.25 mol/L sulfuric acid consumed.

0.1 mol/L Sulfuric Acid

This solution contains 9.808 g of sulfuric acid (H_2SO_4, molecular weight: 98.08) per 1000 mL.

Prepare as directed for 0.5 mol/L Sulfuric Acid, using 6 mL of sulfuric acid.

Standardization Proceed as directed for 0.5 mol/L Sulfuric Acid, using 0.26–0.32 g of dried sodium carbonate (reference material).

Each mL of 0.1 mol/L sulfuric acid = 10.599 mg of Na_2CO_3

The factor is calculated by the formula:

$f = m/(0.010599 \times V) \times A/100$

f = factor of 0.1 mol/L sulfuric acid,
m = weight (g) of sodium carbonate (reference material),
A = content (%) of sodium carbonate (reference material),
V = 0.1 mol/L sulfuric acid consumed.

0.05 mol/L Sulfuric Acid

This solution contains 0.4904 g of sulfuric acid (H_2SO_4, molecular weight: 98.08) per 1000 mL.

Prepare by diluting 0.5 mol/L sulfuric acid with water to 10 times its original volume, or prepare as directed for 0.5 mol/L Sulfuric Acid, using 3 mL of sulfuric acid.

Standardization Proceed as directed for 0.5 mol/L Sulfuric Acid, using 0.13–0.16 g

of dried sodium carbonate (reference material).

Each mL of 0.05 mol/L sulfuric acid = 5.299 mg of Na_2CO_3

The factor is calculated by the formula:

$f = m/(0.005299 \times V) \times A/100$

f = factor of 0.05 mol/L sulfuric acid,

m = weight (g) of sodium carbonate (reference material),

A = content (%) of sodium carbonate (reference material),

V = 0.05 mol/L sulfuric acid consumed.

0.025 mol/L Sulfuric Acid

This solution contains 2.452 g of sulfuric acid (H_2SO_4, molecular weight: 98.08).

Prepare by diluting 0.05 mol/L sulfuric acid with water to 2 times its original volume. Use the factor of 0.05 mol/L sulfuric acid instead of standardization. Prepare fresh before use.

0.01 mol/L Sulfuric Acid

This solution contains 0.9808 g of sulfuric acid (H_2SO_4, molecular weight: 98.08) per 1000 mL. Prepare fresh before use.

Prepare by diluting 0.1 mol/L sulfuric acid with water to 10 times its original volume. Use the factor of 0.1 mol/L sulfuric acid instead of standardization.

0.005 mol/L Sulfuric Acid

This solution contains 0.4904 g of sulfuric acid (H_2SO_4, molecular weight: 98.08) per 1000 mL.

Prepare by diluting 0.05 mol/L sulfuric acid with water to 10 times its original volume. Use the factor of 0.05 mol/L sulfuric acid instead of standardization. Prepare fresh before use.

0.1 mol/L Titanium(III) Chloride

This solution contains 15.42 g of titanium(III) chloride ($TiCl_3$, molecular weight: 154.24) per 1000 mL.

Measure 75 mL of titanium(III) chloride solution, and add 75 mL of hydrochloric acid and freshly boiled and cooled water to make 1000 mL. Transfer into a light-resistant bottle equipped with a burette, replace the air in the bottle with nitrogen or hydrogen, and use. Standardize before use.

Standardization Weigh 3 g of ammonium iron(II) sulfate, transfer it into a wide-mouthed 500-mL flask, and dissolve in 50 mL of water (carbon dioxide-removed) while passing carbon dioxide or nitrogen. Add 25 mL of diluted sulfuric acid (27 in 100), and add quickly 40 mL of 0.02 mol/L potassium permanganate, exactly measured, while passing carbon dioxide or nitrogen. Titrate with the prepared titanium(III) chloride

solution to almost the endpoint, add immediately 5 g of ammonium thiocyanate, and continue the titration. The endpoint is when the color of the solution disappears. Perform a blank test to make any necessary correction.

The factor is calculated by the formula:

$f = f_1 \times 40/V$

f = factor of 0.1 mol/L titanium(III) chloride,

f_1 = factor of 0.02 mol/L potassium permanganate,

V = volume (mL) of 0.1 mol/L titanium(III) chloride consumed.

0.1 mol/L Zinc

This solution contains 6.538 g of zinc (Zn, molecular weight: 65.38) per 1000 mL.

Weigh accurately 3.3 g of zinc (reference material), dried as directed in its certificate, add 25 mL of water and 40 mL of diluted nitric acid (1 in 3), and heat under a reflux condenser on a water bath to dissolve. Boil the mixture gently to remove nitrogen oxides, allow to cool, and transfer into a 500-mL volumetric flask. Wash the Erlenmeyer flask and reflux condenser with water, and add the washings to the volumetric flask. Add water to the marked line, and mix well. Stopper tightly and store.

The factor is caliculated by the formula:

$f = m/3.2690 \times A/100$

f = factor of 0.1 mol/L zinc,

m = weight (g) of zinc (reference material),

A = content (%) of zinc (reference material).

0.05 mol/L Zinc

This solution contains 3.269 g of zinc (Zn, molecular weight: 65.38) per 1000 mL.

Weigh accurately about 1.7 g of zinc (reference material), dried as directed in its certificate, add 25 mL of water and 25 mL of diluted nitric acid (1 in 3), and heat under a reflux condenser on a water bath to dissolve. Boil the mixture gently to remove nitrogen oxides, allow to cool, and transfer into a 500-mL volumetric flask. Wash the Erlenmeyer flask and reflux condenser with water, and add the washings to the volumetric flask. Add water to the marked line, and mix well. Stopper tightly and store.

The factor is calculated by the formula:

$f = m/1.6345 \times A/100$

f = factor of 0.05 mol/L zinc,

m = weight (g) of zinc (reference material),

A = content (%) of zinc (reference material).

0.02 mol/L Zinc

This solution contains 1.3076 g of zinc (Zn, molecular weight: 65.38) per 1000 mL.

Proceed as directed for 0.05 mol/L Zinc using 0.66 g of zinc (reference material).

The factor is calculated by the formula:

$f = m/0.6538 \times A/100$

f = factor of 0.02 mol/L zinc,

m = weight (g) of zinc (reference material),

A = content (%) of zinc (reference material).

0.01 mol/L Zinc

This solution contains 0.6538 g of zinc (Zn, molecular weight: 65.38) per 1000 mL.

Proceed as directed for 0.05 mol/L Zinc using 0.33 g of zinc (reference material).

The factor is calculated by the formula:

$f = m/0.3269 \times A/100$

f = factor of 0.01 mol/L zinc,

m = weight (g) of zinc (reference material),

A = content (%) of zinc (reference material).

0.1 mol/L Zinc Acetate

This solution contains 21.95 g of zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$, molecular weight: 219.50) per 1000 mL.

Dissolve about 22 g of zinc acetate dihydrate by adding 2 mL of acetic acid and 1000 mL of water. Stopper tightly and store.

Standardization Measure exactly 25 mL of the prepared zinc acetate solution, and add 75 mL of water and 2 mL of ammonia solution–ammonium chloride TS. Titrate with 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 50 mg of eriochrome black T–sodium chloride indicator). The endpoint is when the color of solution changes from red to blue.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.1 mol/L zinc acetate,

f_1 = factor of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate,

V = volume (mL) of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate consumed.

0.02 mol/L Zinc Acetate

This solution contains 4.390 g of zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$, molecular weight: 219.50) per 1000 mL.

Dissolve 4.43 g of zinc acetate dihydrate by adding 2 mL of acetic acid and 1000 mL of water. Stopper tightly and store.

Standardization Measure exactly 25 mL of the prepared zinc acetate solution, and add 75 mL of water and 2 mL of ammonia solution–ammonium chloride TS. Titrate with 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 50 mg of

eriochrome black T–sodium chloride indicator). The endpoint is when the color of solution changes from red to blue.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.02 mol/L zinc acetate,

f_1 = factor of 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate,

V = volume (mL) of 0.02 mol/L disodium dihydrogen ethylenediaminetetraacetate consumed.

0.01 mol/L Zinc Acetate

This solution contains 2.195 g of zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$, molecular weight: 219.50) per 1000 mL.

Dissolve 2.2 g of zinc acetate dihydrate by adding 2 mL of acetic acid and 1000 mL of water. Stopper tightly and store.

Standardization Measure exactly 25 mL of the prepared zinc acetate, and add 75 mL of water and 2 mL of ammonia solution–ammonium chloride TS. Titrate with 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate (indicator: 50 mg of eriochrome black T–sodium chloride indicator). The endpoint is when the color of solution changes from red to blue.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.01 mol/L zinc acetate,

f_1 = factor of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate,

V = volume (mL) of 0.01 mol/L disodium dihydrogen ethylenediaminetetraacetate consumed.

0.1 mol/L Zinc Sulfate

This solution contains 28.76 g of zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$, molecular weight: 287.55) per 1000 mL.

Dissolve 29 g of zinc sulfate heptahydrate in water to make 1000 mL.

Standardization Measure exactly 25 mL of the prepared zinc sulfate, and add 5 mL of ammonium buffer (pH 10.7) and 40 mg of eriochrome black T–sodium chloride indicator. Titrate with 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate. The endpoint is when the red-purple color of the solution changes to blue-purple.

The factor is calculated by the formula:

$f = f_1 \times V/25$

f = factor of 0.1 mol/L zinc sulfate,

f_1 = factor of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate,

V = volume (mL) of 0.1 mol/L disodium dihydrogen ethylenediaminetetraacetate consumed.

3. Standard Solutions

When standard solutions are prepared using standard solutions specified in the Measurement Act of Japan (MA), the solutions shall fit the purpose of use, in terms of necessary parameters, such as acid concentration and the presence or absence of a stabilizer.

Aluminum Standard Stock Solution

Weigh 17.6 g of aluminum potassium sulfate dodecahydrate, dissolve it by adding 10 mL of water and 15 mL of diluted hydrochloric acid (2 in 3), and add water to make 1000 mL. Each mL of this solution contains 1 mg of aluminum (Al). Store in a plastic bottle made of polyethylene or the like.

The standard solution [aluminum (Al) concentration of 1000 mg/L] specified in the MA may be used.

Ammonium Standard Solution

Weigh 2.97 g of ammonium chloride, and dissolve it in water to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add water to make exactly 1000 mL. Each mL of this solution contains 10 μg of ammonium (NH_4).

A solution prepared by exactly diluting the standard solution (ammonium (NH_4) concentration of 1000 mg/L) specified in the MA with water—so that each mL of it contains 10 μg of ammonium (NH_4)—may be used.

Arsenic Standard Stock Solution

Weigh 1.32 g of arsenic trioxide, and dissolve it in 6 mL of sodium hydroxide solution (1 in 10). Adjust its pH to 3–5 with 500 mL of water or diluted hydrochloric acid (1 in 4), and add water to make 1000 mL. To exactly 10 mL of this solution, add water to make exactly 100 mL. Each mL of this solution contains 0.1 mg of arsenic (As).

A solution prepared by exactly diluting the standard solution [arsenic (As) concentration of 1000 mg/L or 100 mg/L] specified in the MA with water—so that each mL of it contains 0.1 mg of arsenic (As)—may be used.

Arsenic Standard Solution

Measure exactly 5 mL of Arsenic Standard Stock Solution, add 10 mL of diluted sulfuric acid (1 in 20) and freshly boiled and cooled water to make exactly 1000 mL. Each mL of this solution contains 0.5 μg of arsenic (As).

Barium Standard Solution

Weigh 1.779 g of barium chloride dihydrate, and dissolve it in water to make exactly 1000 mL. Each mL of this solution contains 1 mg of barium (Ba).

The standard solution [barium (Ba) concentration of 1000 mg/L] specified in the MA may be used.

Bromide Ion Standard Stock Solution

Weigh 0.129 g of sodium bromide, previously dried at 110°C for 2 hours, and dissolve it in water to make exactly 1000 mL. Each mL of this solution contains 0.1 mg of Bromide ion (Br^-).

A solution prepared by diluting the standard solution [bromide ion (Br^-) concentration of 1000 mg/L] specified in the MA with water—so that each mL of it contains 0.1 mg of bromide iron (Br^-)—may be used.

Calcium Standard Solution (0.1 mg/mL)

Weigh 2.50 g of calcium carbonate, and dissolve it by adding 50 mL of water and 15 mL of diluted hydrochloric acid (2 in 3). Heat it gently without boiling to expel carbon dioxide, cool. Add water to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add water to make exactly100 mL. Each mL of this solution contains 0.1 mg of calcium (Ca). Store in a plastic bottle made of polyethylene or the like.

A solution prepared by diluting the standard solution [calcium (Ca) concentration of 1000 mg/L or 100 mg/L] specified in the MA with water—so that each mL of it contains 0.1 mg of calcium (Ca)—may be used.

Chloride Ion Standard Stock Solution

Weigh 0.165 g of sodium chloride (reference material), dried as directed in its certificate, dissolve it in water to make exactly 1000 mL. Each mL of this solution contains 0.1 mg of chloride ion (Cl^-).

A solution prepared by exactly diluting the ion standard solution [chloride ion (Cl^-) concentration of 1000 mg/L] specified in the MA with water—so that each mL of it contains 0.1 mg of chloride ion (Cl^-)—may be used.

Chromium Standard Solution

Weigh 2.83 g of potassium dichromate, dissolve it by adding 50 mL of water and 5 mL of diluted nitric acid (1 in 3), and add water to make exactly 1000 mL. Measure exactly 25 mL of this solution, and add water to make exactly 1000 mL. Dilute exactly measured 10 mL of this solution with water to exactly 100 mL. Each mL of this solution contains 2.5 μg of chromium (Cr).

A solution prepared by exactly diluting the standard solution [chromium (Cr) concentration of 1000 mg/L or 100 mg/L] specified in the MA with water—so that each mL of it contains 2.5 μg of chromium (Cr)—may be used.

Cyanide Standard Stock Solution

Weigh 2.50 g of potassium cyanide (a mass fraction of 100%), and dissolve it in water to make exactly 1000 mL. Each mL of this solution contains 1 mg of cyanide ion (CN^-). Stopper tightly, and store in a cold, dark place.

Cyanide Standard Solution

Measure exactly 10 mL of Cyanide Standard Stock Solution and add 100 mL of sodium hydroxide solution (1 in 25) and water to make exactly 1000 mL. Prepare fresh before use. Each mL of this solution contains 10 μg of cyanide (CN).

Food Blue No.1 Color Precursor Standard Stock Solution

Weigh accurately about 0.5 g of food blue No. 1 (not more than 0.5% of color precursor), and dissolve it in water to make exactly 50 mL. Using this solution, proceed as directed in Titanium(III) Chloride Method (ii) in the Assay in the Coloring Matter Tests to obtain the volume (V mL) of 0.1 mol/L titanium(III) chloride consumed. After titration, add 1–2 drops of 0.1 mol/L titanium(III) chloride, stir well, and allow to cool. Add water to make exactly 500 mL. Use the resulting solution as Food Blue No. 1 Color Precursor Standard Stock Solution. Proceed as directed in the following procedure to determine the concentration, D (mg/mL), of color precursor in this standard stock solution.

(i) Determine the concentration, A (mg/mL), of color precursor produced by reduction titration with 0.1 mol/L titanium(III) chloride by the formula:

$$A\ (mg/mL) = \frac{V \times 0.1 \times F \times 408.4}{500}$$

V = volume (mL) of 0.1 mol/L titanium(III) chloride consumed,

F = factor of 0.1 mol/L titanium(III) chloride,

(ii) Determine the content, C (%), of color precursor in food blue No.1 used to prepare Food Blue No. 1 Color Precursor Standard Stock Solution.

Test Solution Weigh accurately about 0.1 g of the same food blue No. 1 used to prepare Food Blue No. 1 Color Precursor Standard Stock Solution, and dissolve it in ammonium acetate TS (0.02 mol/L) to make 100 mL.

Standard Solutions Add ammonium acetate TS (0.02 mol/L) to exactly measured 10 mL of Food Blue No. 1 Color Precursor Standard Stock Solution to make 100 mL (Standard Solution 1). To exactly 25 mL, 5 mL, and 0.5 mL, of Standard Solution 1, add ammonium acetate TS (0.02 mol/L) to make 50 mL each. Refer to them as Standard Solutions 2, 3, and 4. Then add ammonium acetate TS (0.02 mol/L) to exactly 2 mL of solution 4 to make 20 mL (standard solution 5).

Determination of the concentration, B (mg/mL), of color precursor in the test solution Analyze equal portions of the test solution and the five standard solutions by liquid chromatography using the following operating conditions.

Operating conditions

Detector: Ultraviolet spectrophotometer or photodiode array detect or (wavelength: 254 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 about 40°C.

Flow rate: 1 mL/min.

Mobile phase

A: Ammonium acetate (0.02 mol/L).

B: A 7:3 mixture of acetonitrile/water.

Concentration gradient (A/B): Run a linear gradient from 90/10 to 40/60 in 25 minutes, and maintain 40/60 for 5 minutes.

Obtain the peak area of color precursor in each standard solution and derive the concentration of color precursor in each solution from A (mg/mL). Prepare a calibration curve by plotting the values obtained on a graph, with the concentration on the x axis and the peak area on the y axis. Determine the concentration, B (mg/mL), of color precursor in the test solution from the peak area of color precursor in the test solution and the calibration curve. Then calculate the content, C (%), of color precursor in food blue No.1 used to prepare Food Blue No. 1 Color Precursor Standard Stock Solution by the formula:

$$C\ (\%) = \frac{B \times 10}{M_t} \times \left(1 + \frac{B}{M_t \times 10}\right)$$

M_t = Weight (g) of food blue No. 1 used to prepare the test solution.

Calculate the concentration, D (mg/mL), of color precursor in Food Blue No. 1 Color Precursor Standard Stock Solution by the formula:

$$D\ (mg/mL) = \frac{(V \times 0.1 \times F \times 408.4) + (C \times M_t \times 0.01 \times 1000)}{500}$$

V = volume (mL) of 0.1 mol/L titanium(III) chloride consumed,

F = factor of 0.1 mol/L titanium(III) chloride,

C = content (%) of color precursor in food blue No.1 used to prepare Food Blue No. 1 Color Precursor Standard Stock Solution,

M_t = weight of food blue No. 1 used to prepare Food Blue No. 1 Color Precursor Standard Stock Solution.

Note: Food Blue No.1 Color Precursor Standard Stock Solution is stable for one year after preparation if it is stored in a cold, dark place.

Food Green No. 3 Color Precursor Standard Stock Solution

Weigh accurately about 0.5 g of food green No. 3 (not more than 0.5% of color

precursor), and dissolve it in water to make exactly 50 mL. Using this solution, proceed as directed in Titanium(III) Chloride Method (ii) in the Assay in the Coloring Matter Tests to obtain the volume (V mL) of 0.1 mol/L titanium(III) chloride consumed. After titration, add 1–2 drops of 0.1 mol/L titanium(III) chloride, stir well, and allow to cool. Add water to make exactly 500 mL. Use the resulting solution as Food Green No. 3 Color Precursor Standard Stock Solution. Proceed as directed in the following procedure to determine the concentration, D (mg/mL), of color precursor in this standard stock solution.

(i) Determine the concentration, A (mg/mL), of color precursor produced by reduction titration with 0.1 mol/L titanium(III) chloride by the formula:

$$\text{A (mg/mL)} = \frac{\text{V} \times 0.1 \times \text{F} \times 416.4}{500}$$

V = volume (mL) of 0.1 mol/L titanium(III) chloride consumed,

F = factor of 0.1 mol/L titanium(III) chloride.

(ii) Determine the content, C (%), of color precursor in food green No. 3 used to prepare Food Green No. 3 Color Precursor Standard Stock Solution.

Test Solution Weigh accurately about 0.1 g of the same food green No. 3 used to prepare Food Green No. 3 Color Precursor Standard Stock Solution, and dissolve it in ammonium acetate TS (0.02 mol/L) to make 100 mL.

Standard Solutions Add ammonium acetate TS (0.02 mol/L) to exactly 10 mL of Food Green No. 3 Color Precursor Standard Stock Solution to make 100 mL (Solution 1). To exactly 25 mL, 5 mL, and 0.5 mL of Solution 1, add ammonium acetate TS (0.02 mol/L) to make 50 mL each. Refer to them as standard solutions 2, 3, and 4. Then add ammonium acetate TS (0.02 mol/L) to exactly 2 mL of solution 4 to make 20 mL (solution 5).

Determination of the concentration, B (mg/mL), of color precursor in the test solution Analyze equal portions of the test solution and the five standard solutions by liquid chromatography using the following operating conditions.

Operating conditions

Detector: Ultraviolet spectrophotometer or photodiode array detector (wavelength: 254 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 about 40°C.

Flow rate: 1 mL/minute.

Mobile phase

A: Ammonium acetate (0.02 mol/L).

B: A 7:3 mixture of acetonitrile/water.

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.

Obtain the peak area of color precursor in each standard solution and derive the concentration of color precursor in each solution from A (mg/mL). Prepare a calibration curve by plotting the values obtained on a graph, with the concentration on the x axis and the peak area on the y axis. Determine the concentration, B (mg/mL), of color precursor in the test solution from the peak area of color precursor in the test solution and the calibration curve. Then calculate the content, C (%), of color precursor in food green No. 3 used to prepare Food Green No. 3 Color Precursor Standard Stock Solution by the formula:

$$C\ (\%) = \frac{B \times 10}{M_t} \times \left(1 + \frac{B}{M_t \times 10}\right)$$

M_t = Weight (g) of food green No. 3 used to prepare the test solution.

Calculate the concentration, D (mg/mL), of color precursor in Food Green No. 3 Color Precursor Standard Stock Solution by the formula:

$$D\ (mg/mL) = \frac{(V \times 0.1 \times F \times 416.4) + (C \times M_t \times 0.01 \times 1000)}{500}$$

V = volume (mL) of 0.1 mol/L titanium(III) chloride consumed,

F = factor of 0.1 mol/L titanium(III) chloride,

C = content (%) of color precursor in food green No. 3 used to prepare Food Green No. 3 Color Precursor Standard Stock Solution,

M_t = weight of food green No. 3 used to prepare Food Green No. 3 Color Precursor Standard Stock Solution.

Note: Food Green No. 3 Color Precursor Standard Stock Solution is stable (usable) for one year after preparation if it is stored in a cold, dark place.

Formaldehyde Standard Solution (2 μg/mL)

Weigh 0.54 g of formaldehyde (equivalent to HCHO mass fraction of 37%), and add water to make exactly 1000 mL. Measure exactly 10 mL of the solution, and add water to make exactly 1000 mL. Each mL of this solution contains 2 μg of formaldehyde (HCHO). Prepare fresh before use.

A solution prepared by exactly diluting the standard solution [formaldehyde (HCHO) concentration of 1000 mg/L] specified in the MA with water—so that each mL of it contains 2 μg of formaldehyde (HCHO)—may be used.

Fluoride Ion Standard Stock Solution

Weigh 2.210 g of sodium fluoride, dried at 110°C for 2 hours, into a polyethylene beaker,

and dissolve it in 200 mL of water while stirring. Transfer the solution into a 1000-mL volumetric flask, and dilute with water to the volume. Each mL of this solution contains 1 mg of fluorine (F). Store in a polyethylene container.

Iodide Ion Standard Stock Solution

Weigh 0.118 g of sodium iodide, previously dried at 110°C for 2 hours, and dissolve it in water to make exactly 1000 mL. Each mL of this solution contains 0.1 mg of iodide ion (I^-). Prepare fresh before use.

Iron Standard Stock Solution

Weigh 8.63 g of ammonium iron(III) sulfate dodecahydrate, dissolve it by adding 25 mL of diluted nitric acid (1 in 3) and water, and further add water to make exactly 1000 mL. Each mL of this solution contains 1 mg of iron (Fe). Store, protected from light.

Iron Standard Solution

To exactly 10 mL of Iron Standard Stock Solution, add 25 mL of diluted nitric acid (1 in 3) and water to make exactly 1000 mL. Each mL of this solution contains 10 μg of iron (Fe). Store, protected from light.

A solution prepared by exactly diluting the standard solution [iron (Fe) concentration of 1000 mg/L or 100 mg/L] specified in the MA with 25 mL of diluted nitric acid (1 in 3) and water—so that each mL of it contains 10 μg of iron (Fe)—may be used

Lead Standard Stock Solution

Weigh 0.16 g of lead(II) nitrate, dissolve it in 10 mL of diluted nitric acid (1 in 10), and add water to make exactly 1000 mL. Each mL of this solution contains 0.1 mg of lead (Pb). For the preparation and storage of this solution, use glass instruments free from soluble lead(II) salts.

A solution prepared by exactly diluting the standard solution [lead (Pb) concentration of 1000 mg/L or 100 mg/L] specified in the MA with water—so that each mL of it contains 0.1 mg of lead (Pb)—may be used.

Lead Standard Solution

Measure exactly 1 mL of Lead Standard Stock Solution, and add diluted nitric acid (1 in 100) to make exactly 100 mL. Each mL of this solution contains 1 μg of lead (Pb). Prepare fresh before use.

Lead Standard Solution (for heavy metals limit test)

Measure exactly 10 mL of Lead Standard Stock Solution, and add water to make exactly 100 mL. Each mL of this solution contains 10 μg of lead (Pb). Prepare fresh before use.

Lithium Lactate Standard Solution

Weigh 0.1066 g of lithium lactate, previously dried at 105°C for 4 hours, and dissolve it in water to make exactly 1000 mL. Each mL of this solution contains 0.1 mg of lactic acid ($C_3H_6O_3$). Prepare fresh before use.

Manganese Standard Solution

Weigh 3.60 g of manganese(II) chloride tetrahydrate, dissolve it by adding 15 mL of diluted nitric acid (1 in 2) and water, and add water to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add 15 mL of diluted hydrochloric acid (2 in 3) and water to make exactly 1000 mL. Each mL of this solution contains 10 μg of manganese (Mn).

A solution prepared by exactly diluting the standard solution [manganese (Mn) concentration of 1000 mg/L or 100 mg/L] specified in the MA with water—so that each mL of it contains 10 μg of manganese (Mn)—may be used.

Matching Fluids

According to the table below, transfer the prescribed volumes of Colorimetric Standard Stock Solutions and water into a test tube, and mix them. To transfer them, use a burette or pipette with precise graduations of 0.1 mL or less. The preparation of each Colorimetric Standard Stock Solution (CSSS) is given below.

Symbol for Matching Fluid	Volume (mL) of Cobalt(II) Chloride CSSS	Volume (mL) of Iron(III) Chloride CSSS	Volume (mL) of Copper(II) Sulfate CSSS	Volume (mL) of Water
A	0.1	0.4	0.1	4.4
B	0.3	0.9	0.3	3.5
C	0.1	0.6	0.1	4.2
D	0.3	0.6	0.4	3.7
E	0.4	1.2	0.3	3.1
F	0.3	1.2	0.0	3.5
G	0.5	1.2	0.2	3.1
H	0.2	1.5	0.0	3.3
I	0.4	2.2	0.1	2.3
J	0.4	3.5	0.1	1.0
K	0.5	4.5	0.0	0.0
L	0.8	3.8	0.1	0.3
M	0.1	2.0	0.1	2.8
N	0.0	4.9	0.1	0.0
O	0.1	4.8	0.1	0.0

P	0.2	0.4	0.1	4.3
Q	0.2	0.3	0.1	4.4
R	0.3	0.4	0.2	4.1
S	0.2	0.1	0.0	4.7
T	0.5	0.5	0.4	3.6

Colorimetric Standard Stock Solution (CSSS)

Prepare each CSSS as directed below, and store in a ground glass-stoppered bottle.

Cobalt(II) Chloride CSSS

Weigh about 59.5 g of cobalt(II) chloride hexahydrate (mass fraction 100%), dissolve it in diluted hydrochloric acid (1 in 40), and add diluted hydrochloric acid (1 in 40) to make exactly 1000 mL. Or, weigh 65 g of cobalt(II) chloride hexahydrate, and dissolve in diluted hydrochloric acid (1 in 40) to make 1000 mL. Measure exactly 5 mL of the resulting solution, transfer into a 250-mL ground-glass stoppered flask, add 5 mL of hydrogen peroxide TS and 15 mL of sodium hydroxide solution (1 in 5), and boil for 10 minutes. Cool, and add 2 g of potassium iodide and 20 mL of diluted sulfuric acid (1 in 4). After the precipitate is dissolved, titrate with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add the indicator near the endpoint, when the color of the solution becomes light yellow. The endpoint is when the blue color of the solution disappears. Each mL of 0.1 mol/L sodium thiosulfate is equivalent to 23.79 mg of cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$, molecular weight: 237.93). To the remaining portion of the cobalt(II) chloride hexahydrate solution, add diluted hydrochloric acid (1 in 40) to make a solution containing 59.5 mg of cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) per mL.

Copper(II) Sulfate CSSS

Weigh 62.4 g of copper(II) sulfate pentahydrate (mass fraction 100%), dissolve it in diluted hydrochloric acid (1 in 40), and add diluted hydrochloric acid (1 in 40) to make exactly 1000 mL. Or, weigh about 65 g of copper(II) sulfate pentahydrate, and dissolve it in diluted hydrochloric acid (1 in 40) to make 1000 mL. Measure exactly 10 mL of the resulting solution, transfer into a 250-mL ground-glass stoppered flask, and add 40 mL of water. Add 4 mL of diluted acetic acid (1 in 4) and 3 g of potassium iodide, and titrate with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add the indicator near the endpoint, when the color of the solution is light yellow. The endpoint is when the blue color of the solution disappears. Each mL of 0.1 mol/L sodium thiosulfate is equivalent to 24.97 mg of copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, molecular weight: 249.69). To the remaining portion of the copper (II) sulfate pentahydrate solution, add diluted hydrochloric acid (1 in 40) to make a solution containing 62.4 mg of copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) per mL.

Iron(III) Chloride CSSS

Weigh 45.0 g of iron(III) chloride hexahydrate (mass fraction 100%), dissolve it in diluted hydrochloric acid (1 in 40), and add diluted hydrochloric acid (1 in 40) to make exactly 1000 mL. Or, weigh about 55 g of iron(III) chloride hexahydrate, and dissolve in diluted hydrochloric acid (1 in 40) to make 1000 mL. Measure exactly 10 mL of the solution, transfer into a 250-mL ground-glass stoppered flask, and add 15 mL of water and 3 g of potassium iodide. Stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 mL of water, and titrate with 0.1 mol/L sodium thiosulfate (indicator: 1–3 mL of starch TS). Add the indicator near the endpoint, when the color of the solution becomes light yellow. The endpoint is when the blue color of the solution disappears. Each mL of 0.1 mol/L sodium thiosulfate is equivalent to 27.03 mg of iron(II) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, molecular weight: 270.30). To the remaining portion of the iron(II) chloride hexahydrate solution, add diluted hydrochloric acid (1 in 40) to make a solution containing 45.0 mg of ferric chloride ($FeCl_3 \cdot 6H_2O$) per mL.

Mercury Standard Solution

Weigh 1.35 g of mercury(II) chloride, and dissolve it by adding 25mL of diluted nitric acid (1 in 3) and water, then add water to make exactly 1000 mL. Measure exactly 10 mL of the solution, and add 25mL of diluted nitric acid (1 in 3) and water to make exactly 1000 mL. Measure exactly 10 mL of the resulting solution, and add 25 mL of diluted nitric acid (1 in 3) and water to make exactly 1000 mL. Each mL of this solution contains 0.1 μg of mercury (Hg). Prepare fresh before use. A solution prepared by diluting the standard solution [mercury (Hg) concentration of 1000 mg/L or 100 mg/L] specified by the MA with 25 mL of diluted nitric acid (1 in 3) and water—so that each mL of it contains 0.1 μg of mercury (Hg)—may be used.

Nickel Standard Solution

Weigh 4.05 g of nickel(II) chloride hexahydrate (mass fraction 100%), dissolve it by adding 10 mL of diluted hydrochloric acid (2 in 3) and water, and add water to make exactly 1000 mL. Measure exactly 5 mL of the solution, and add water to make exactly 1000 mL. Each mL of this solution contains 5 μg of nickel (Ni).

A solution prepared by diluting the standard solution [nickel (Ni) concentration of 1000 mg/L or 100 mg/L] specified in the MA with water—so that each mL of it contains 5 μg of nickel (Ni)—may be used.

Nitrate Ion Standard Stock Solution

See Nitrate Standard Solution.

Nitrate Standard Solution

Weigh 1.63 g of potassium nitrate and dissolve it in water to make exactly 1000 mL. Measure exactly 10 mL of the solution, and add water to make exactly 100 mL. Each mL of this solution contains 0.1 mg of nitrate ion (NO_3^-).

A solution prepared by diluting the standard solution [nitrate iron (NO_3^-) concentration of 1000 mg/L] specified in the MA with water—so that each mL of it contains 0.1 mg of nitrate iron (NO_3^-)—may be used.

Phosphate Standard Solution

Weigh 0.1433 g of potassium dihydrogen phosphate, and dissolve it in water to make exactly 100 mL. Measure exactly 10 mL of the solution, and add water to make exactly 1000 mL. Each mL of this solution contains 10 μg of the phosphate ion (PO_4^{3-}).

A solution prepared by diluting the standard solution [phosphate ion (PO_4^{3-}) concentration of 1000 mg/L or 100 mg/L] specified in the MA with water—so that each mL of it contains 10 μg of the phosphate ion (PO_4^{3-})—may be used.

Phosphorus Standard Solution

Weigh 4.394 g of potassium dihydrogen phosphate, and dissolve it in water to make exactly 1000 mL. Each mL of this solution contains 1 mg of phosphorus (P).

Potassium Standard Solution (0.1 mg/mL)

Weigh 1.91 g of potassium chloride, dissolve it in water to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add water to make exactly 100 mL. Each mL of this solution contains 0.1 mg of potassium (K). Store in a plastic bottle made of polyethylene or the like.

A solution prepared by diluting the standard solution [potassium (K) concentration of 1000 mg/L or 100 mg/L] specified in the MA with water—so that each mL of it contains 0.1 mg of K—may be used.

Selenium Standard Stock Solution

Weigh 2.19 g (mass fraction of 100%) of sodium selenite, and dissolve it in water to make exactly 1000 mL. Each mL of this solution contains 1 mg of selenium (Se). The standard solution [selenium (Se) concentration of 1000 mg/L] specified in the MA may be used.

Selenium Standard Solution

Measure exactly 10 mL of Selenium Standard Stock Solution, and add water to make exactly 1000 mL. Each mL of this solution contains 10 μg of selenium (Se).

Silicon Standard Stock Solution

Weigh 0.214 g of silicon dioxide, ignited at 900–1000°C and cooled, into a platinum crucible, and fuse it by heating with 1 g of sodium carbonate. After cooling, dissolve it in

water to make exactly 100 mL. Each mL of this solution contains 1 mg of silicon (Si). Store in a plastic bottle made of polyethylene or the like.

Sodium Standard Solution (0.1 mg/mL)
Dissolve 2.54 g of sodium chloride in water to make exactly 1000 mL. To 10 mL of this solution, add water to make exactly 100 mL. Each mL of this solution contains 0.1 mg of sodium (Na). Store a plastic bottle made of polyethylene or the like.

A solution prepared by diluting the standard solution [sodium (Na) concentration of 1000 mg/L or 100 mg/L] specified in the MA with water—so that each mL of it contains 0.1 mg of sodium (Na)—may be used.

Strontium Standard Solution (1.0 mg/mL)
Dissolve 2.42 g of strontium nitrate in water to make exactly 1000 mL. Each mL of this solution contains 1 mg of strontium (Sr). The standard solution [strontium (Sr) concentration of 1000 mg/L] specified in the MA may be used.

Sulfate Ion Standard Stock Solution
Weigh 0.148 g of sodium sulfate decahydrate, previously dried at 110°C for 2 hours, and dissolve it in water to make exactly 1000 mL. Each mL of this solution contains 0.1 mg of sulfate ion (SO_4^{2-}).

A solution prepared by diluting the standard solution [sulfate ion (SO_4^{2-}) concentration of 1000 mg/L or 100 mg/L] specified in the MA with water—so that each mL of it contains 0.1 mg of sulfate ion (SO_4^{2-})—may be used.

Titanium Standard Stock Solution
Weigh 0.167 g of titanium(IV) oxide, add 5 g of ammonium sulfate and 10 mL of sulfuric acid, and dissolve the mixture by heating. After cool, add water to make 100 mL. Each mL of this solution contains 1 mg of titanium (Ti).

Titanium Standard Solution
Measure exactly 10 mL of Titanium Standard Stock Solution, and add water to make exactly 1000 mL. Each mL of this solution contains 10 μg of titanium (Ti). Prepare fresh before use.

Tyrosine Standard Solution
Weigh 50 mg of Tyrosine Reference Standard, previously dried at 105°C for 3 hours, and dissolve it in 0.1 mol/L hydrochloric acid to make exactly 50 mL. Measure exactly 5 mL of this solution, and add hydrochloric acid TS (0.1 mol/L) to make exactly 100 mL. Each mL of this solution contains 50 μg of tyrosine ($C_9H_{11}NO_3$).

Water–Methanol Standard Solution

Measure 500 mL of methanol for water determination, transfer into a dry 1000-mL volumetric flask, add 2 mL of water, and add methanol for water determination again to make 1000 mL. The standardization of this solution is done immediately after water determination TS is standardized. Store in a cold place, protected from light and moisture.

Standardization According to the procedure directed under Water Determination, transfer 25 mL of methanol for water determination into a dry titration flask, and add carefully water determination TS to the end point. Add exactly 10 mL of water determination TS, and titrate with Water-Methanol Standard Solution to the end point. Calculate the number of mg of water (H_2O), f′, contained in 1 mL of Water-Methanol Standard Solution by the following formula:

$$f' = \frac{f \times 10}{\text{Volume (mL) of Water-Methanol Standard Solution consumed}}$$

f′ = number of mg of water (H_2O) equivalent to 1 mL of water determination TS.

A water-methanol standard solution that is traceable to the International System of Units may be usable.

Yttrium Standard Stock Solution

Each mL of this solution contains 1 mg of yttrium (Y). Use a product prepared for inductively coupled plasma-atomic emission spectrometry.

Zinc Standard Solution

Weigh 4.40 g of zinc sulfate hepthydrate, and dissolve it in water to make exactly 1000 mL. Measure exactly 10 mL of the solution, and add water to make exactly 1000 mL. Each mL of this solution contains 10 μg/mL of zinc (Zn).

A solution prepared by diluting the standard solution [zinc (Zn) concentration of 1000 mg/L or 100 mg/L] specified in the MA with water—so that each mL of it contains 10 μg of zinc (Zn)—may be used.

4. Reference Standards

(1) For Reference Standards listed in this section, use products manufactured by persons who are registered with the Minister of Health, Labour and Welfare, as specified by the Minister.

Food Blue No. 1 Reference Standard

Food Blue No. 2 Reference Standard

Food Green No. 3 Reference Standard

Food Red No. 2 Reference Standard
Food Red No. 3 Reference Standard
Food Red No. 40 Reference Standard
Food Red No. 102 Reference Standard
Food Red No. 104 Reference Standard
Food Red No. 105 Reference Standard
Food Red No. 106 Reference Standard
Food Yellow No. 4 Reference Standard
Food Yellow No. 5 Reference Standard
Natamycin Reference Standard
Nisin Reference Standard
Xylitol Reference Standard

(2) ***p*-Aminobenzoylglutamic Acid Reference Standard**

Use *p*-Aminobenzoyl Glutamic Acid RS for Purity specified in the Japanese Pharmacopoeia.

(3) **Cyanocobalamin Reference Standard**

Use Reference Standard specified in the Japanese Pharmacopoeia.

(4) **Folic Acid Reference Standard**

Use Reference Standard specified in the Japanese Pharmacopoeia.

(5) **Glycyrrhizic Acid Reference Standard**

Use Reference Standard specified in the Japanese Pharmacopoeia.

(6) **Lysozyme Reference Standard**

Use Reference Standard specified in the Japanese Pharmacopoeia.

(7) **Nicotinamide Reference Standard**

Use Reference Standard specified in the Japanese Pharmacopoeia.

(8) **Riboflavin Reference Standard**

Use Reference Standard specified in the Japanese Pharmacopoeia.

(9) **Saccharated Pepsin Reference Standard**

Use Reference Standard specified in the Japanese Pharmacopoeia.

(10) **Thiamine Hydrochloride Reference Standard**

Use Reference Standard specified in the Japanese Pharmacopoeia.

(11) ***dl*-α-Tocopherol Reference Standard**

Use Reference Standard specified in the Japanese Pharmacopoeia.

(12) **Tocopheryl Acetate Reference Standard**

Use Tyrosine RS for Digestion Test specified in the Japanese Pharmacopoeia.

(13) **Tyrosine Reference Standard**

Use Reference Standard specified in the Japanese Pharmacopoeia.

5. Stationary Phases and Packing Materials for Chromatography

Aminated Polyvinyl Alcohol Gel for Liquid Chromatography
Use a product prepared for liquid chromatography.

Amino-bonded Silica Gel for Liquid Chromatography
Use a product prepared for liquid chromatography.

Aminopropyl-bonded Silica Gel for Liquid Chromatography
Use a product prepared for liquid chromatography.

Butylated Polyvinyl Alcohol Polymer Gel for Liquid Chromatography
Use a product prepared for liquid chromatography.

Cation Exchange Resin for Liquid Chromatography, Ag-form
Use a product prepared for liquid chromatography.

Cation Exchange Resin for Liquid Chromatography, Ca-form
Use a product prepared for liquid chromatography.

Cation Exchange Resin for Liquid Chromatography, H-form
Use a product prepared for liquid chromatography.

Cation Exchange Resin for Liquid Chromatography, Na-form
Use a product prepared for liquid chromatography.

Diatomaceous Earth for Chromatography
Use white to grayish white, high-quality diatomaceous earth.

Diatomaceous Earth for Gas Chromatography
Use a high-quality product prepared for gas chromatography by purifying diatomaceous earth.

Dimethylsilanized Silica Gel for Thin-Layer Chromatography (containing fluorescent indicator)
Use dimethylsilanized silica gel prepared for thin-layer chromatography to which a fluorescent indicator is added.

Hexadecylamidopropylsilanized Silica Gel for Liquid Chromatography
Use a product prepared for liquid chromatography.

Methyl Silicone Polymer

Use a high-quality product prepared for gas chromatography.

Microcrystalline Cellulose for Thin-Layer Chromatography
Use a product prepared for thin-layer chromatography.

Octadecylsilanized Silica Gel for Liquid Chromatography
Use a product prepared for liquid chromatography.

Octadecylsilanized Silica Gel for Thin-Layer Chromatography
Use a product prepared for thin-layer chromatography.

Octylsilanized Silica Gel for Liquid Chromatography
Use a product prepared for liquid chromatography.

Phenyl-bonded Silica Gel for Liquid Chromatography
Use a product prepared for liquid chromatography.

Polyethylene Glycol 20M
Use a high-quality product prepared for thin-layer chromatography.

Polyethylene Glycol 6000 [25322-68-3]
Use a high-quality product prepared for gas chromatography.

Porous Anion Exchanger
Use a product prepared for ion chromatography.

Silica Gel for Gas Chromatography
Use a product prepared for gas chromatography.

Silica Gel for Liquid Chromatography
Use a product prepared for liquid chromatography.

Silica Gel for Thin-Layer Chromatography
Use a high-quality product prepared for thin-layer chromatography.

Silica Gel for Thin-Layer Chromatography (containing fluorescent indicator)
Use silica gel prepared for thin-layer chromatography to which fluorescent indicator is added.

Strongly Acidic Cation-exchange Resin for Liquid Chromatography

Use a high-quality product prepared for liquid chromatography.

Strongly Basic Anion-exchange Resin for Liquid Chromatography
Use a product prepared for liquid chromatography.

Styrene–Divinylbenzene Porous Polymer for Gas Chromatography
Use a product prepared for gas chromatography.

Thin-Layer Plate for Yucca Form Extract
Use a 10 cm × 10 cm plate coated with 5 to 7-μm silica gel for thin-layer chromatography.

Weakly Acidic Cation-exchange Resin for Liquid Chromatography
Use a product prepared for liquid chromatography.

Zeolite for Gas Chromatography $AlNaO_6Si_2$ [1318-02-1]
Use natural or synthetic zeolite prepared for gas chromatography.

6. Thermometers

Unless otherwise specified, use rod thermometers with an immersion line or total immersion mercury-filled rod thermometers calibrated under the Japanese Industrial Standards. For the tests directed under Congealing Point, Boiling Point and Distillation Range Tests, and Melting Point (Method 1), use thermometers with an immersion line. The rod thermometers with an immersion line are shown in the following table.

Standards for Thermometers with an Immersion Line

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Liquid	Mercury	Mercury	Mercury	Mercury	Mercury	Mercury
Gas filled above liquid	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon
Temperature range	−17 to 50°C	40 to 100°C	90 to 150°C	140 to 200°C	190 to 250°C	240 to 320°C
Minimum graduation	0.2°C	0.2°C	0.2°C	0.2°C	0.2°C	0.2°C
Longer graduation lines	each 1°C	each 1°C	each 1°C	each 1°C	each 1°C	each 1°C
Graduation number	each 2°C	each 2°C	each 2°C	each 2°C	each 2°C	each 2°C
Total length (mm)	280–300	280–300	280–300	280–300	280–300	280–300
Stem diameter (mm)	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3
Bulb length (mm)	12–18	12–18	12–18	12–18	12–18	12–18
Distance from the bottom of bulb to the lowest graduation line (mm)	75–90	75–90	75–90	75–90	75–90	75–90
Distance from the top of thermometer to the highest graduation line (mm)	35–65	35–65	35–65	35–65	35–65	35–65
Distance from the bottom of bulb to immersion line (mm)	58–62	58–62	58–62	58–62	58–62	58–62
Form of top of thermometer	loop	loop	loop	loop	loop	loop
Test temperature	–15°C 15°C 45°C	45°C 70°C 95°C	95°C 120°C 145°C	145°C 170°C 195°C	195°C 220°C 245°C	245°C 280°C 315°C
Allowable limit of error	0.2°C	0.2°C	0.2°C	0.2°C	0.3°C (0.2°C when the temperature tested is 195°C)	0.4°C (0.5°C when the temperature tested is 315°C)

Note. For auxiliary thermometers, use appropriate types of mercury thermometers with a temperature range of 0°C to 360°C and a minimum graduation of not more than 1°C.

7. Filter Papers

Use filter papers conforming to the specifications given below. Unless otherwise specified, when the term “filter paper” is given alone, use filter papers for qualitative analysis.

Filter papers must be stored, protected from gases and other contaminants.

Filter Paper for Qualitative Analysis

Use filter papers conforming to the specifications for filter papers for qualitative analysis under the Japanese Industrial Standards (for chemical analysis).

Filter Paper for Quantitative Analysis

Use filter papers conforming to the specifications for filter papers for quantitative analysis under the Japanese Industrial Standards (for chemical analysis).

Filter Paper for Chromatography

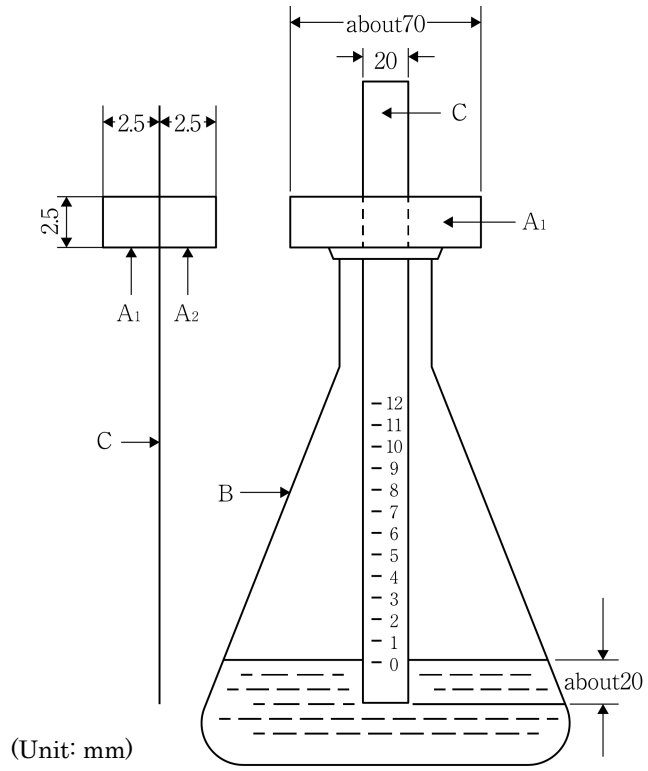
Use filter papers conforming to the specifications for filter papers for the quantitative analysis and the specifications given in the table below. The tests for α-cellulose content, copper value, pH, ash content, filtration time, and wet burst strength should be performed as directed under the Japanese Industrial Standards. The test for water absorption should be performed as directed below.

Class	No. 1	No. 2	No. 3	No. 4
α-Cellulose content (%)	Not less than 90	Not less than 95	Not less than 95	Not less than 95
Copper value (%)	Not more than 1.6	Not more than 1.4	Not more than 1.4	Not more than 1.4
pH	5–8	5–8	5–8	5–8
Ash content (%)	Not more than 0.02	Not more than 0.12	Not more than 0.12	Not more than 0.12
Filtration time (sec)	330 ± 132	240 ± 96	120 ± 48	100 ± 40
Wet burst strength (cm)	Not less than 13	Not less than 20	Not less than 12	Not less than 15
Water absorption (cm)	6 ± 1.2	5.5 ± 1.1	7 ± 1.4	7.5 ± 1.5

Test for Water Absorption

Apparatus

Use the apparatus as illustrated in the following figure:



A_1 and A_2: Glass block to hold the filter paper

B: Erlenmeyer flask (Capacity: about 1000 mL)

C: Sample filter paper

Procedure

Transfer about 300 mL of water into Erlenmeyer flask B, and place 2 pieces of glass blocks (A_1 and A_2) in parallel on the mouth of the Erlenmeyer flask to hold the filter paper. Insert a sample filter paper, previously marked with 1-cm graduations using a pencil, between the two glass blocks. Gently slip down the filter paper in water until its lower edge reaches the surface of the water, then quickly slip it down until the zero mark is at the water-level, and fix the filter paper. Measure the height of water absorbed by the filter paper in 10 minutes.

Membrane Filter

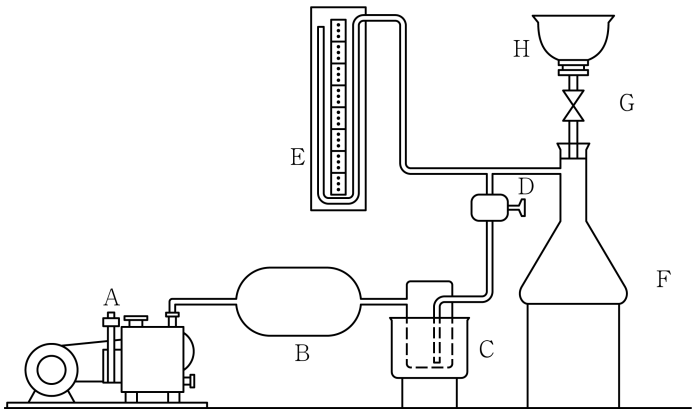
Use membrane filters conforming to the specifications given in the following table. The tests for thickness should be performed, according to the testing methods for paper thickness and paper density under the Japanese Industrial Standards. The tests for water flow rate and bubble point should be performed as directed below:

Pore size (μm)	Thickness (μm)	Water flow rate (mL/min/cm²)	Bubble point (N/mm²)
1.0 or 1.2	100–170	150–300	5.9×10^{-2}–14.7×10^{-2}
0.45	130–170	20–60	16.7×10^{-2}–34.3×10^{-2}
0.10	90–150	1.0–5.0	49.0×10^{-2}–294.2×10^{-2}
0.05	70–150	0.1–2.0	98.1×10^{-2}–490.3×10^{-2}

Water Flow Rate Test

Apparatus

Use the apparatus as illustrated in the following figure:



A: Vacuum pump
B: Reservoir (Capacity: not less than 10 liters)
C: Cold trap
D: Vacuum regulator
E: Manometer
F: Suction filter bottle (Capacity: 1–4 liters)
G: Valve
H: Filter device (1000-mL container, equipped with a filter holder 47 mm in internal diameter, supported by a stainless steel screen)

Procedure

Close valve G and open vacuum regulator D fully to reduce the pressure in the system with vacuum pump A. Then, using D, adjust the pressure in the system to 69 ± 0.7 kPa. Place the sample membrane filter into the filter holder. The filter should be previously moistened with water, taking care not to allow air to enter the filter. Then assemble the filter device. Measure 500 mL of water, previously filtered twice through a membrane filter with the same pore size as the sample filter or with a smaller pore size, and pour into the filter device. Open valve G, measure the time it takes to finish filtering, and calculate the water flow rate by the formula:

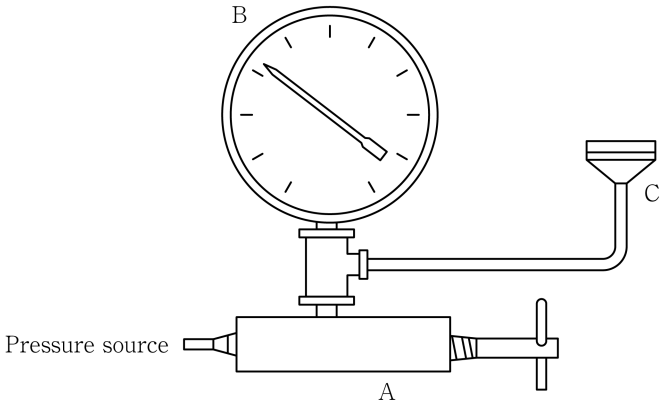
Water flow rate (mL/min/cm^2)

$$= \frac{500 \text{ mL} \times 60}{\text{Filtration time (sec)} \times \text{Effective filtration area (cm}^2\text{)}}$$

Bubble Point Test

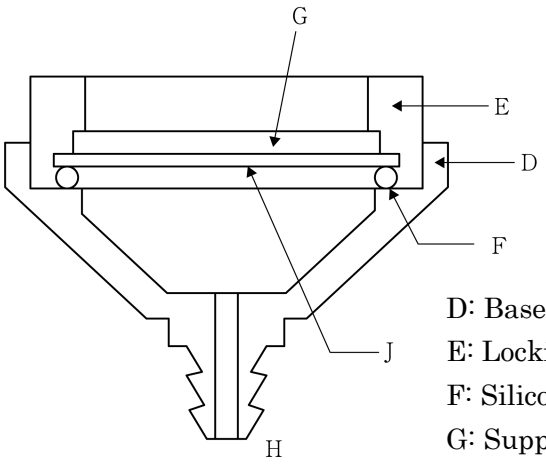
Apparatus

Use the apparatus as illustrated in Figures 1 and 2.



A: Regulator
B: Pressure gauge
C: Filter holder (9.5 ± 0.5 cm in effective filtration area; the enlarged detail illustrated in Figure 2.)

Fig. 1



D: Base
E: Locking ring
F: Silicone O-ring
G: Supporting disk
H: Air inlet
J: Sample membrane filter

Fig. 2

Procedure

Moisten completely the sample membrane filter with water, fit it in the filter holder. Put water into the holder until 2 to 3 mm above supporting disk G. Adjust the pressure to a point not exceeding the expected bubble point, using regulator A, and increase the pressure at a rate of 0.14×10^{-2}N/mm^2 per second. Regard the pressure at which a stable effervescence occurs at the center of the sample membrane filter.

8. Filters

Glass Filter

Use a glass filter conforming to the specifications for chemical analysis-grade glass filters under the Japanese Industrial Standards.

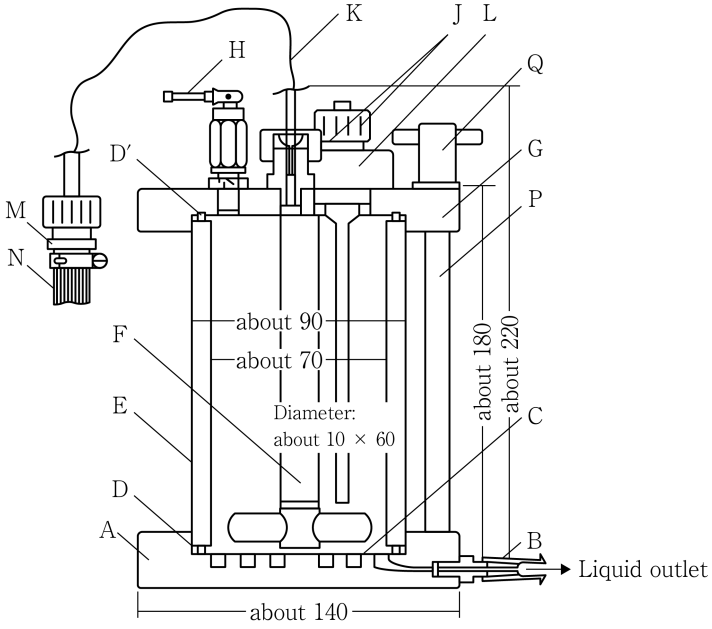
Pressure Filter

Operate a pressure filter as directed in the procedure given below.

Apparatus

Generally, apparatus is as illustrated in the figure.

A: Base plate
B: Liquid outlet tube
C: Supporting screen
D, D′: Silicone O-ring
E: Cell
F: Stirring column
G: Cover
H: Safety valve
J: Tube joint cap
K: Pressure-resistant tube
L: Sample inlet
M: Pressure source connector
N: Pressure-resistant hose
P: Clamping shaft
Q: Cross nut for clamping



(Unit: mm)

Procedure

Attach liquid outlet tube B to base plate A, place the membrane filter on supporting screen C, and attach silicone O-ring D to the surface of the membrane filter. Place cell E on D, attach silicone O-ring D′ to cover G, to which stirring column F , safety valve H,

and other parts are attached, and place on E. Set up clamping shaft P to G, and tighten uniformly with cross nut Q. Place the pressure filter on the stirrer, and pour the sample liquid through sample inlet L. Connect the pressure source (such as a nitrogen cylinder) and the pressure filter, using pressure-resistant hose N and pressure-resistant tube K, increase gradually the pressure to the specified level, and filter the sample. During filtration, stir slowly to the extent that effervescence ceases.

9. Sieves

Use sieves conforming to the specifications for sieves under the Japanese Industrial Standards.

10. Detector Tube Type Gas-Measuring Instruments

Use a detector tube type gas-measuring device that meets the Japan Industrial Standards.

11. Infrared Reference Spectra

The infrared reference spectra contained in this section were obtained at a resolution of 4 cm^{-1}, using a Fourier-transform infrared spectrophotometer, under the conditions specified in the individual monographs. The horizontal axis indicates the wavenumber (cm^{-1}) and the vertical axis indicates the transmittance (%). As a reference, a potassium bromide disk without any sample was used in the disk method (10 mm in diameter), and an optical plate was used in the paste, thin film, and liquid film methods.
(See Annex 1)

12. Measurement Instruments

Capacitance Moisture Meter

Use a unit meeting the performances specified in Japanese Industrial Standards K1105.

Yellow Phosphor Luminescent Oxygen Analyzer

Use a unit meeting the performances specified in Japanese Industrial Standards K1105.