Calcium Pantothenate 30 mg/g, Riboflavin 3 mg/g, Pyridoxine Hydrochloride 5 mg/g, Nicotinamide 30 mg/g, Ascorbic Acid 200 mg/g and Thiamine Nitrate 3 mg/g Granules

Dissolution *<6.10>* Conduct this procedure without exposure to light. Weigh accurately about 0.5 g of Calcium Pantothenate 30 mg/g, Riboflavin 3 mg/g, Pyridoxine Hydrochloride 5 mg/g, Nicotinamide 30 mg/g, Ascorbic Acid 200 mg/g and Thiamine Nitrate 3 mg/g Granules, and perform the test at 75 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Start the test, withdraw not less than 20 mL of the medium at the specified minute after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add a solution of metaphosphoric acid (1 in 50) to make exactly 10 mL, and use this solution as the sample solution.

Riboflavin, Nicotinamide and Thiamine Nitrate

Weigh accurately about 17 mg of Riboflavin RS, previously dried at 105ºC for 2 hours, add 1 mol/L hydrochloric acid, and dissolve by warming in a boiling water bath. After cooling, add 1 mol/L hydrochloric acid to make exactly100 mL, and use this solution as the standard stock solution (1). Weigh accurately about 17 mg of Nicotinamide RS, previously dried for 4 hours using silica gel as a desiccant, dissolve in a solution of metaphosphoric acid (1 in 50) to make exactly 100 mL, and use this solution as the standard stock solution (2). Further, weigh accurately about 17 mg of Thiamine Chloride Hydrochloride RS (separately, determine the water *<2.48>* with 30 mg by coulometric titration), dissolve in a solution of metaphosphoric acid (1 in 50) to make exactly 100 mL, and use this solution as the standard stock solution (3). Pipet 1 mL of the standard stock solution (1) and (3) and 10 mL of the standard stock solution (2), and add a solution of metaphosphoric acid (1 in 50) to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and calculate the peak areas, A_{Ta} and A_{Sa}, of riboflavin, A_{Tb} and A_{Sb}, of nicotinamide and, A_{Tc}, and A_{Sc} of thiamine of both solutions.

Dissolution rate (%) with respect to the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$)

$= M_{Sa}/M_T \times A_{Ta}/A_{Sa} \times 1/C_a \times 9$

M_{Sa}: Amount (g) of Riboflavin RS

M_T: Amount (g) of sample

C_a: Labeled amount (g) of riboflavin ($C_{17}H_{20}N_4O_6$) in 1 g

Dissolution rate (%) with respect to the labeled amount of nicotinamide ($C_6H_6N_2O$)

$= M_{Sb}/M_T \times A_{Tb}/A_{Sb} \times 1/C_b \times 90$

M_{Sb}: Amount (g) of Nicotinamide RS

M_T: Amount (g) of sample

C_b: Labeled amount (g) of nicotinamide ($C_6H_6N_2O$) in 1 g

Dissolution rate (%) with respect to the labeled amount of thiamine nitrate ($C_{12}H_{17}N_5O_4S$)

$= M_{Sc}/M_T \times A_{Tc}/A_{Sc} \times 1/C_c \times 9 \times 0.9706$

M_{Sc}: Amount (g) of Thiamine Chloride Hydrochloride RS, calculated on the anhydrous basis

M_T: Amount (g) of sample

C_c: Labeled amount (g) of thiamine nitrate ($C_{12}H_{17}N_5O_4S$) in 1 g

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40ºC.

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate and 0.94 g of sodium 1-hexane sulfonate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nicotinamide is about 5 minutes.

System suitability

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, nicotinamide, thiamine and riboflavin are eluted in this order with the resolutions between the peaks of nicotinamide and thiamine, and between the peaks of thiamine and riboflavin being not less than 13, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of each component is not more than 3.0%.

Calcium Pantothenate and Pyridoxine Hydrochloride

Weigh accurately about 17 mg of Calcium Pantothenate RS, previously dried at 105ºC for 4 hours, dissolve in a solution of metaphosphoric acid (1 in 50) to make exactly 100 mL, and use this solution as the standard stock solution (4). Weigh accurately about 27 mg of Pyridoxine Hydrochloride RS,

previously dried for 4 hours using silica gel as a desiccant, dissolve in a solution of metaphosphoric acid (1 in 50) to make exactly 100 mL, and use this solution as the standard stock solution (5). Pipet 10 mL of the standard stock solution (4) and 1 mL of the standard stock solution (5), and add a solution of metaphosphoric acid (1 in 50) to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_{Td} and A_{Sd}, of pantothenic acid and, A_{Te}, and A_{Se}, of pyridoxine of both solutions.

Dissolution rate (%) with respect to the labeled amount of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$)
$= M_{Sd}/M_T \times A_{Td}/A_{Sd} \times 1/C_d \times 90$

M_{Sd}: Amount (g) of Calcium Pantothenate RS
M_T: Amount (g) of sample
C_d: Labeled amount (g) of calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) in 1 g

Dissolution rate (%) with respect to the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3$·HCl)
$= M_{Se}/M_T \times A_{Te}/A_{Se} \times 1/C_e \times 9$

M_{Se}: Amount (g) of Pyridoxine Hydrochloride RS
M_T: Amount (g) of sample
C_e: Labeled amount (g) of pyridoxine hydrochloride ($C_8H_{11}NO_3$·HCl) in 1 g

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40ºC.

Mobile phase: Dissolve 2.72 g of potassium dihydrogen phosphate and 0.94 g of sodium 1-hexane sulfonate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To 950 mL of this solution add 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pantothenic acid is about 8 minutes.

System suitability

System performance: When the procedure is run with 40 μL of the standard solution under the above operating conditions, calcium pantothenate and pyridoxine hydrochloride are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of each component is not more than 3.0%.

Ascorbic acid

Pipet 5 mL of the sample solution, shake with 5 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS, and titrate <*2.50*> with a solution of 2,6-dichloroindophenol sodium until a light red color persists for 5 seconds. Perform a blank determination in the same manner, and make any necessary correction.

Dissolution rate (%) with respect to the labeled amount of ascorbic acid ($C_6H_8O_6$)
$= 1/M_T \times V \times 1/C_f \times A \times 36000$

M_T: Amount (g) of sample
V: Titration volume (mL)
C_f: Labeled amount (mg) of ascorbic acid ($C_6H_8O_6$) in 1 g
A: Amount (mg) of ascorbic acid ($C_6H_8O_6$), corresponding to 1 mL of a solution of 2,6-dichloroindophenol sodium

A is decided by the following standardization of a solution of 2,6-dichloroindophenol sodium.

2,6-Dichloroindophenol Sodium Solution:

Preparation−Dissolve 52 mg of sodium hydrogen carbonate in 50 mL of water, then dissolve 64 mg of 2,6-dichloroindophenol sodium, add water to make 1000 mL, and filter. Prepare before use.

Standardization−Weigh accurately about 11 mg of Ascorbic Acid RS, previously dried for 24 hours using silica gel as a desiccant, and dissolve in metaphosphoric acid-acetic acid TS to make exactly 100 mL. Pipet 2 mL of this solution, shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide, and titrate <*2.50*> with a solution of 2,6-dichloroindophenol sodium until a light red color persists for 5 seconds. Perform a blank determination in the same manner, and make any necessary correction. Calculate the quantity (*A* mg) of ascorbic acid ($C_6H_8O_6$), equivalent to 1 mL of this test solution.

Dissolution Requirements

	Labeled amount	Specified minute	Dissolution rate
Riboflavin	3 mg/g	30 minutes	Not less than 75%
Nicotinamide	30 mg/g		Not less than 85%
Thiamine Nitrate	3 mg/g		Not less than 85%
Calcium Pantothenate	30 mg/g		Not less than 85%
Pyridoxine Hydrochloride	5 mg/g		Not less than 85%
Ascorbic acid	200 mg/g		Not less than 70%

Calcium Pantothenate RS Calcium Pantothenate (JP). When dried, it contains from 5.83 to 5.94% of Nitrogen (N:14.01).