Octotiamine 25 mg, Riboflavin 2.5 mg, Pyridoxine Hydrochloride 40 mg and Cyanocobalamin 0.25 mg Tablets

Dissolution *<6.10>* Perform the test with 1 tablet of Octotiamine 25 mg, Riboflavin 2.5 mg, Pyridoxine Hydrochloride 40 mg and Cyanocobalamin 0.25 mg Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 as the dissolution medium. Withdraw exactly 20 mL of the medium at the specified minute after starting the test, and immediately fill up the dissolution medium with exactly 20 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, previously warmed to 37±0.5ºC. Filter these media through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, and use the solutions obtained from the medium collected 30 minutes and 90 minutes after starting the test as the sample solution (1) and the sample solution (2), respectively.

The requirements are met if Octotiamine 25 mg, Riboflavin 2.5 mg, Pyridoxine Hydrochloride 40 mg and Cyanocobalamin 0.25 mg Tablets conform to the dissolution requirements.

Octotiamine

Separately, weigh accurately about 27 mg of Octotiamine RS, previously dried in vacuum with silica gel for 4 hours, dissolve in 3 mL of methanol, and add 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 to make exactly 200 mL. Pipet 10 mL of this solution, add 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution (1), sample solution (2) and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_{T1}, A_{T2} and A_S, of octotiamine.

Dissolution rate (%) with respect to the labeled amount of octotiamine ($C_{23}H_{36}N_4O_5S_3$)

$= M_S \times (A_{T1}/A_S) \times 1/45 \ + \ (A_{T2}/A_S) \times 1/C \times 90$

M_S: Amount (mg) of Octotiamine RS

C: Labeled amount (mg) of octotiamine ($C_{23}H_{36}N_4O_5S_3$) in 1 tablet

Operating conditions —

Detector: An ultraviolet absorption photometer (wavelength: 236nm).

Column: A stainless steel column 4.6 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 25ºC.

Mobile phase: To 7.0 g of sodium perchlorate add 1000 mL of water to dissolve, and adjust to pH 3.0

with diluted phosphoric acid (1 in 10). To 900 mL of this solution add 1100 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of octotiamine is about 7 minutes.

System suitability —

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of octotiamine are not less than 1000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of octotiamine is not more than 1.5%.

Riboflavin and Pyridoxine Hydrochloride

Conduct this procedure without exposure to light. Separately, weigh accurately about 14 mg of Riboflavin RS, previously dried at 105ºC for 2 hours, dissolve in 0.05 mol/Lacetic acid-sodium acetate buffer solution, pH 4.0, to make exactly 200 mL, and use this solution as the standard stock solution (1). Separately, weigh accurately about 22 mg of Pyridoxine Hydrochloride RS, previously dried in vacuum with silica gel for 4 hours, dissolve in 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 to make exactly 50 mL, and use this solution as the standard stock solution (2). Pipet 4 mL of the standard stock solution (1) and 10 mL of the standard stock solution (2), add 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution (1) and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_{Ta} and A_{Sa}, of riboflavin, and A_{Tb} and A_{Sb}, of pyridoxine.

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

$= M_{Sa} \times A_{Ta}/A_{Sa} \times 1/C_a \times 18$

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

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

Dissolution rate (%) with respect to the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3$.HCl)

$= M_{Sb} \times A_{Tb}/A_{Sb} \times 1/C_b \times 180$

M_{Sb}: Amount (mg) of Pyridoxine Hydrochloride RS

C_b: Labeled amount (mg) of pyridoxine hydrochloride ($C_8H_{11}NO_3$.HCl) in 1 tablet

Operating conditions —

Detector: An ultraviolet absorption photometer (wavelength: 267 nm).

Column: A stainless steel column 4.6 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 25ºC.

Mobile phase: To 1.5 g of sodium 1-octanesulfonate add 825 mL of water to dissolve. To this solution add 175 mL of acetonitrile and 1 mL of phosphoric acid.

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

System suitability —

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, riboflavin and pyridoxine are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated with 10 μL of the standard solution according to the above operating conditions, the relative standard deviations of the peak areas of riboflavin and pyridoxine are not more than 1.5%, respectively.

Cyanocobalamin

Conduct this procedure without exposure to light. Separately, weigh accurately about 27 mg of Cyanocobalamin RS (previously determine the loss on drying *<2.41>*, previously dried in vacuum over phosphorus (V) oxide at 100ºC for 4 hours), and dissolve in 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 to make exactly 100 mL. Pipet 5 mL of this solution, and add 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 to make exactly 50 mL. Pipet 2 mL of this solution, add 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution (1) and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine the peak areas, A_T and A_S, of cyanocobalamin.

Dissolution rate (%) with respect to the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$)
$= M_S \times A_T/A_S \times 1/C \times 9/10$

M_S: Amount (mg) of Cyanocobalamin RS, calculated on the anhydrous basis

C: Labeled amount (mg) of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) in 1 tablet

Operating conditions —

Detector: An ultraviolet absorption photometer (wavelength: 361 nm).

Column: A stainless steel column 4.6 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 25ºC.

Mobile phase: Dissolve 0.49 g of phosphoric acid, 0.60 g of sodium dihydrogen phosphate dihydrate and 14 g of sodium perchlorate in water to make 1000 mL. To this solution add 500 mL of methanol.

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

System suitability —

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cyanocobalamin are not less than 1000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cyanocobalamin is not more than 1.5%.

Dissolution Requirements

	Labeled amount	Specified minute	Dissolution rate
Octotiamine	25 mg	90 minutes	Not less than 75%
Riboflavin	2.5 mg		
Pyridoxine Hydrochloride	40 mg	30 minutes	Not less than 85%
Cyanocobalamin	0.25 mg		

Octotiamine RS Octotiamine. When dried, it contains not less than 99.0% of octotiamine ($C_{23}H_{36}N_4O_5S_3$).