Benfotiamine, Pyridoxine Hydrochloride and Cyanocobalamin Capsules

Dissolution *<6.10>* Perform the test with 1 capsule of Benfotiamine, Pyridoxine Hydrochloride and Cyanocobalamin Capsules at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Withdraw exactly 20 mL of the medium at the specified minute after starting the test, and, at 30 minutes after start of the test, immediately add exactly 20 mL of water, previously warmed at 37±0.5ºC, carefully. Filter these media through a membrane filter with a pore size not exceeding 0.45 µm. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the mobile phase to make exactly V' mL so that each mL contains about 19 µg of benfotiamine ($C_{19}H_{23}N_4O_6PS$), about 14 µg of pyridoxine hydrochloride ($C_8H_{11}NO_3$.HCl) and about 0.14 µg of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$) according to the labeled amount, 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. Separately, weigh accurately about 28 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 (not more than 0.67 kPa)), and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the cyanocobalamin standard stock solution. Separately, weigh accurately about 28 mg of Pyridoxine Hydrochloride RS, previously dried in vacuum with silica gel for 4 hours, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the pyridoxine hydrochloride standard stock solution. Further, weigh accurately about 19 mg of Benfotiamine RS, previously dried at 105ºC for 2 hours, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the benfotiamine standard stock solution. To 5 mL each of cyanocobalamin standard stock solution and pyridoxine hydrochloride standard stock solution and 10 mL of benfotiamine standard stock solution add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 100 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, $A_{Ta(1)}$ and A_{Sa}, of pyridoxine, the peak areas, $A_{Tb(1)}$ and A_{Sb}, of cyanocobalamin, and the peak areas, $A_{Tc(1)}$, $A_{Tc(2)}$ and A_{Sc} of benfotiamine in each solution.

The requirements are met if Benfotiamine, Pyridoxine Hydrochloride and Cyanocobalamin Capsules conform to the dissolution requirements.

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

$$= M_{Sa} \times A_{Ta(1)}/A_{Sa} \times V'/V \times 1/C_a \times 45$$

Dissolution rate (%) with respect to the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$)

$$= M_{Sb} \times A_{Tb(1)}/A_{Sb} \times V'/V \times 1/C_b \times 9/20$$

Dissolution rate (%) with respect to the labeled amount of benfotiamine ($C_{19}H_{23}N_4O_6PS$)

$= M_{Sc} \times (A_{Tc(1)}/A_{Sc}) \times (1/45) + (A_{Tc(2)}/A_{Sc}) \times V'/V \times 1/C_c \times 90$

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

M_{Sb}: Amount (mg) of Cyanocobalamin RS, calculated on the anhydrous basis

M_{Sc}: Amount (mg) of Benfotiamine RS

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

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

C_c: Labeled amount (mg) of benfotiamine ($C_{19}H_{23}N_4O_6PS$) in 1 capsule

Operating conditions —

Detector: An ultraviolet absorption photometer (wavelength: 350 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 2.0 g of sodium 1-pentanesulfonate in 1000 mL of a mixture of water, acetonitrile and acetic acid (100) (171:27:2).

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, pyridoxine, cyanocobalamin and benfotiamine are eluted in this order with the resolutions between the peaks of pyridoxine and cyanocobalamin, and cyanocobalamin and benfotiamine being not less than 2.0, 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 2.0%.

Dissolution Requirements

	Labeled amount	Specified minute	Dissolution rate
Benfotiamine	34.58 mg	90 minutes	Not less than 75%
Pyridoxine Hydrochloride	25 mg	30 minutes	Not less than 75%
Cyanocobalamin	0.25 mg		Not less than 85%
Benfotiamine	69.15 mg	90 minutes	Not less than 75%
Pyridoxine Hydrochloride	50 mg	30 minutes	Not less than 75%

Cyanocobalamin	0.5 mg		Not less than 85%

Benfotiamine RS Benfotiamine. When dried, it contains not less than 99.0% of benfotiamine ($C_{19}H_{23}N_4O_6PS$).