Sobuzoxane Fine Granules

Dissolution <*6.10*> Weigh accurately an amount of Sobuzoxane Fine Granules, equivalent to about 80 mg of sobuzoxane ($C_{22}H_{34}N_4O_{10}$) according to the labeled amount, and perform the test at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution of sodium lauryl sulfate (1 in 250) 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 0.45 μm. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add the mobile phase to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Sobuzoxane RS, previously dried at 105ºC for 1 hour, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 25 mL. Then, pipet 2 mL of this solution, add the mobile phase to make exactly 25 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 determine the peak areas, A_T and A_S, of sobuzoxane of both solutions.

The requirements are met if Sobuzoxane Fine Granules conform to the dissolution requirements.

Dissolution rate (%) with respect to the labeled amount of sobuzoxane ($C_{22}H_{34}N_4O_{10}$)
$= M_S/M_T \times A_T/A_S \times 1/C \times 360$

M_S: Amount (mg) of Sobuzoxane RS
M_T: Amount (g) of sample
C: Labeled amount (mg) of sobuzoxane ($C_{22}H_{34}N_4O_{10}$) in 1 g

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 211 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 40ºC.

Mobile phase: A mixture of acetonitrile for liquid chromatography and water (3:2).

Flow rate: Adjust the flow rate so that the retention time of sobuzoxane is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of sobuzoxane are not less than 6000 and not more than 1.5, 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 sobuzoxane is not more than 1.5%.

Dissolution Requirements

Labeled amount	Specified minute	Dissolution rate
800 mg/g	30 minutes	Not less than 70%

Sobuzoxane RS $C_{22}H_{34}N_4O_{10}$: 514.53 1,1'-ethylenedi-4-isobutoxycarbonyloxymethyl-3,5-dioxopiperazin. It meets the following requirement.

Purification method−Dissolve about 3 g of sobuzoxane in 8 mL of chloroform, previously wet filling 200 g of silica gel for column chromatography in a glass chromatography tube, 3.5 cm in inside diameter and 50 cm in length, with chloroform, put a filter paper on the upper end of the silica gel, and add on the silica gel column prepared by pressing lightly by a small amount of sea sand. Wash the container with three 5-mL of portions of chloroform, and add the washings on the column. Then, elute with ethyl acetate, and fractionate the effluent from the point at which the color of all silica gel changes from clear to white, discard the first 10 mL of the effluent, and collect 100 mL of the subsequent effluent. Evaporate the filtrate in a water bath at 40ºC under reduced pressure, repeat recrystallization of the residue with ethanol (95) until the requirement for Related substances are met, and dry for 8 hours under reduced pressure.

Description−Sobuzoxane RS occurs as white crystals.

Identification (1) Infrared absorption spectrum−Determine the infrared absorption spectrum of Sobuzoxane RS, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 2970 cm^{-1}, 1754 cm^{-1}, 1732 cm^{-1}, 1707 cm^{-1}, 1249 cm^{-1}, 970 cm^{-1} and 790 cm^{-1}.

(2) Nuclear magnetic resonance spectrum−Determine the ^1H spectrum of a solution of Sobuzoxane RS, previously dried, in deuterated dimethyl sulfoxide for nuclear magnetic resonance spectroscopy (1 in 100), using tetramethylsilane for nuclear magnetic resonance spectoroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*>: it exhibits a double signal A at around δ 0.9 ppm, a multiple signal B at around δ 1.9 ppm, single signals C and D, at around δ 2.6 ppm and at around δ 3.6 ppm, a double signal E at around δ 3.9 ppm, and a single signal F at around δ 5.6 ppm. The ratio of integrated intensity of each signal, A:B:C:D:E:F, is about 6:1:2:4:2:2.

Melting point <*2.60*>: 133 − 134.5ºC

Related substances−Dissolve 0.10 g of Sobuzoxane RS in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of this solution, and add chloroform to make exactly 100 mL. Pipet 0.5 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Thin-layer Chromatography <2.03>. Develop the plate of silica gel for thin-layer chromatography with a mixture of chloroform and methanol (19:1) to a distance of about 15 cm, and dry at 105ºC for 5 minutes. After cooling, spot 10 µL of the sample solution and standard solution on the plate, and air-dry the plate with cold wind. Then, develop the plate with a mixture of chloroform and methanol (19:1) to a distance of about 10 cm, air-dry the plate, and dry at 105ºC for 3 minutes. Allow the plate to stand for 15 minutes in iodine vapor: the number of the spots other than the principal spot obtained from the sample solution is not more than 1, and not more intense than the spot from the standard solution.

Loss on drying <2.41>: not more than 0.30% (1 g, 105ºC, 1 hour).

Content: not less than 99.5%. Assay−Weigh accurately about 0.3 g of Sobuzoxane RS, previously dried, add 50 mL of a mixture of acetic acid (100) and acetic anhydride (7:3) to dissolve, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.45 mg of $C_{22}H_{34}N_4O_{10}$

Silica gel for column chromatography Prepared for column chromatography.