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WORLD HEALTH ORGANIZATION
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SAQUINAVIR
Final text for inclusion in *The International Pharmacopoeia*

SAQUINAVIRUM

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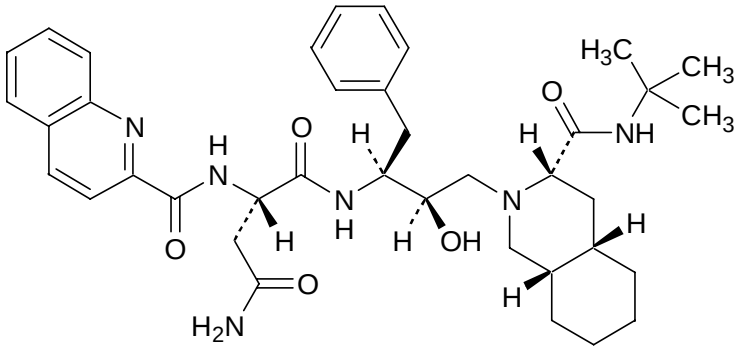

$C_{38}H_{50}N_6O_5$

Relative molecular mass. 670.8

Chemical name. (2*S*)-N^1-[(1*S*,2*R*)-1-benzyl-3-[(3*S*,4a*S*,8a*S*)-3-[(1,1-dimethylethyl)carbamoyl]octahydroisoquinolin-2(1*H*)-yl]-2-hydroxypropyl]-2-[(quinolin-2-ylcarbonyl)amino]butanediamide**;** CAS Reg. NO.127779-20-8.

Description. A white or almost white powder.

Solubility. Practically insoluble in water and soluble in methanol.

Category. Antiretroviral (Protease Inhibitor).

Storage. Saquinavir should be kept at 2-8°C in a tightly-closed container, protected from light.

Additional information. Saquinavir is slightly hygroscopic.

Requirements

Saquinavir contains not less than **98.5 %** and not more than **101.0 %** of $C_{38}H_{50}N_6O_5$, calculated with reference to the dried substance.**Identity tests**

- ***Either tests A and B or test C may be applied.***

A. Carry out test A.1. or, where UV detection is not available, test A.2.

A.1. Carry out the test as described under ‘‘Thin-layer chromatography’’ (Vol. 1, p. 83), using silica gel R6 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 1 mg of the test substance per ml and (B) 1 mg of saquinavir RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

A.2. Carry out the test as described under ‘‘Thin-layer chromatography’’ (Vol. 1, p. 83), using silica gel R5 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 1 mg of the test substance per ml and (B) 1 mg of saquinavir RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in air or in a current of cool air. Dip the plate in dilute basic potassium permanganate (1 g/l) TS. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance, and intensity with that obtained with solution B.

B. The absorption spectrum of a 20 µg/ml solution in methanol R, when observed between 220 nm and 280 nm, exhibits one maximum at about 238 nm; the specific absorbance ($A^{1\%}_{1cm}$) is 670 to 730.

C. Carry out the examination as described under ‘‘Spectrophotometry in the infrared region’’ (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from saquinavir RS or with the *reference spectrum* of saquinavir.

Specific optical rotation. Use a 5.0 mg/ml solution in methanol R; $[\alpha]_D^{20\,°C}$ = - . - 50 ° to - 56 °

Heavy metals. Use 1.0 g in 30 ml of methanol R for the preparation of the test solution as described under ‘‘ Limit test for heavy metals’’, Procedure 2, (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 10 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 5 hours at 105 °C; it loses not more than 20 mg/g.

Related substances. Carry out the test as described under “High–performance liquid chromatography” (Vol. 5, p. 257), using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography R (5 µm).

Use the following conditions for gradient elution:

Mobile phase A: 50 volumes of a mixture of 5 parts of acetonitrile R and 2 parts methanol R, 15 volumes of phosphate buffer pH 3.4 and 35 volumes of purified water.

Mobile phase B: 70 volumes of acetonitrile R, 15 volumes of phosphate buffer pH 3.4 and 15 volumes of purified water.

Prepare the phosphate buffer pH 3.4 by dissolving 4.88 g of anhydrous sodium dihydrogen phosphate in 800 ml of purified water, adjust the pH to 3.4 by adding phosphoric acid (105 g/l) and dilute to 1000 ml with purified water.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-25	100	0	Isocratic
25-45	100 to 45	0 to 55	Linear gradient
45-55	45	55	Isocratic
55-60	45 to 100	55 to 0	Linear gradient
60-70	100	0	Isocratic re-equilibration

Prepare the following solutions using mobile phase A as diluent. For solution (1) use 0.5 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 0.5 µg of saquinavir per ml.

For the system suitability test: prepare solution (3) using 2 ml of solution (1) and 5 ml of sulfuric acid (475 g/l), heat carefully in a boiling water-bath for 30 minutes.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 220 nm.

Maintain the column temperature at 30 °C using, for example, a water-bath.

Inject 20 µl of solution (3). The test is not valid unless the resolution between the peak due to saquinavir (retention time = about 21 minutes) and the peak of similar size with a retention time of about 0.45 relative to the saquinavir peak is not less than 14. The test is also not valid unless the resolution between two smaller peaks of similar size, eluted after the saquinavir peak and which increase during decomposition, is not less than 4.0. The ratio of the retention times of these two peaks relative to the saquinavir peak is about 1.8 and 1.9 respectively. If necessary adjust the amount of acetonitrile in both mobile phases A and B, or adjust the gradient program.

Inject alternatively 20 µl each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2). In the chromatograms obtained with solution (1), the area of any peak, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution (2) (0.2%) and the area of not more than one such peak is greater than the area of the principal peak obtained with solution (2) (0.1 %). The sum of the areas of all peaks, other than the principal peak, is not greater than five times the area of the principal peak obtained with solution (2) (0.5 %). Disregard any peak with an

area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

Assay. Dissolve 0.300 g, accurately weighed, in 50 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS, determine the end point potentiometrically as described under ‘‘Non aqueous titration’’ method A (Vol.1, p. 131). Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 33.54 mg of $C_{38}H_{50}N_6O_5$; calculate with reference to the dried substance.

Impurities *Note: A list of known and potential impurities that have been shown to be controlled by the tests in this monograph will be included for information, if and when the relevant information is available.*

Reagents

Silica gel for chromatography, octadecylsilyl, base deactivated
A very finely divided silica gel, pre-treated before the bonding of octadecylsilyl groups to minimize the interaction with basic compounds.

Potassium permanganate, basic, dilute (1 g/l) TS
A solution of potassium permanganate R containing about 1 g of $KMnO_4$ per litre of sodium hydroxide (1 mol/l).

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