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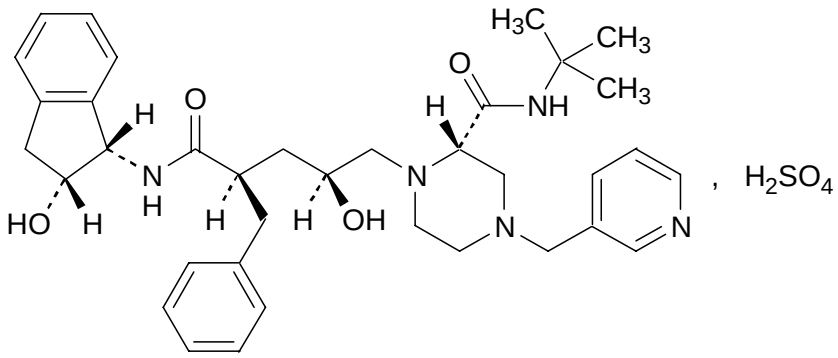
INDINAVIR SULFATE
Final text for inclusion in *The International Pharmacopoeia*

Indinaviri sulfas

Indinavir sulfate



$C_{36}H_{47}N_5O_4,H_2O_4S$

Relative molecular mass. 711.9

Chemical name. (2*S*)-1-[(2*S*,4*R*)-4-benzyl-2-hydroxy-5-[[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino]-5-oxopentyl]-*N*-(1,1-dimethylethyl)-4-(pyridin-3-ylmethyl)piperazine-2-carboxamide sulfate; CAS Reg. No. 157810-81-6.

Description. A white or almost white powder.

Solubility. Freely soluble in water, soluble in methanol.

Category. Antiretroviral (protease inhibitor).

Storage. Indinavir sulfate should be kept in a tightly closed container, protected from light.

Additional information. Indinavir sulfate occurs as the monoethanolate which is hygroscopic. It converts to the hydrate upon loss of ethanol and exposure to moist air.

Requirements

Indinavir sulfate contains not less than **98.5%** and not more than **101.0%** of $C_{36}H_{47}N_5O_4,H_2O_4S$ calculated on anhydrous, ethanol free basis.

Identity tests

- Either tests A, B and D, or tests C and D 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 as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in methanol containing (A) 1 mg of the test substance per ml and (B) 1 mg of indinavir sulfate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively 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 as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions in methanol containing (A) 1 mg of the test substance per ml and (B) 1 mg of indinavir sulfate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. Spray with vanillin/sulfuric acid TS1. Heat the plate for a few minutes at 120°C. 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 0.100 mg/ml solution, when observed between 220 nm and 280 nm, exhibits one maximum at about 260 nm; the specific absorbance ($A^{1\%}_{1cm}$) is between 56 and 65.

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 indinavir sulfate RS or with the *reference spectrum* of indinavir sulfate.

D. A 20 mg/ml solution yields reaction A described under “General identification tests” as characteristic of sulfates (Vol. 1, p. 115).

Specific optical rotation. Use a 10.0 mg/ml solution and calculate with reference to the anhydrous and ethanol free substance; $[\alpha]_D^{20°C}$ = +27° to +31°.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under "Limit test for heavy metals", Procedure 1 (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.

Water. Determine as described under ''Determination of water by the Karl Fischer method'', Method A (Vol. 1, p. 135), using 0.5 g of the substance; the water content is not more than 15 mg/g.

pH value. pH of a 10 mg/ml solution in carbon-dioxide-free water R, 2.8-3.2.

Ethanol content. Determine by "Gas chromatography with static head-space injection". Use a fused-silica capillary or wide bore column 30 m long and 0.32 mm or 0.53 mm in internal diameter coated with macrogol 20 000 R (film thickness: 0.25 µm).

As detector use a flame ionization detector.

Use nitrogen for chromatography R or helium for chromatography R as the carrier gas at an appropriate pressure and a split ratio 1:5 with a linear velocity of about 35 cm/sec.

The following head-space injection conditions may be used:

Equilibration temperature (°C)	80
Equilibration time (min)	60
Transfer line temperature (°C)	85
Pressurization time (s)	30
Injection volume (ml)	1

Maintain the temperature of the column at 30°C for 7 min, then raise the temperature at a rate of 35°C per min to 180°C and maintain for 10 min, maintaining the temperature of the injection port at 140°C and that of the flame ionization detector at 250°C.

Test solution. Dissolve 0.200 g of the test substance in purified water and dilute to 20.0 ml with the same solvent. Introduce 5.0 ml of this solution and 1.0 ml of purified water into a headspace vial. Prepare two more vials.

Reference solutions. Add 0.200 g of ethanol R to purified water and dilute to 200.0 ml with the same solvent. Transfer respectively 2.0 ml, 3.0 ml and 4.0 ml in separate headspace injection vials and bring the volume to 6.0 ml with purified water.

Blank solution. Introduce 6.0 ml of purified water into a headspace vial.

Analyse the blank solution and then alternatively three times the test solution and the three reference solutions.

The test is not valid unless the relative standard deviation on the areas of the peaks obtained from the test solutions is not more than 5%.

Calculate the ethanol content by using the results obtained with the test solution and with the reference solutions; the ethanol content is not less than 50 mg/g and not more than 80 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 (5µm).

Use the following conditions for gradient elution:

Mobile phase A: 30 volumes of acetonitrile R, 5 volumes of phosphate buffer pH 7.5 and 65 volumes of purified water.

Mobile phase B: 70 volumes of acetonitrile R, 5 volumes of phosphate buffer pH 7.5 and 25 volumes of purified water.

Prepare the phosphate buffer pH 7.5 by dissolving 1.4 g of anhydrous disodium hydrogen phosphate in 50 ml of purified water, adjust the pH to 7.5 by adding phosphoric acid (105 g/l) and dilute it to 100 ml with purified water.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-5	93	7	Isocratic
5-25	93 to 20	7 to 80	Linear gradient
25-30	20	80	Isocratic
30-35	20 to 93	80 to 7	Return to the initial conditions
35-45	93	7	Isocratic re-equilibration

Prepare the following solutions. For solution (1) use 2.0 mg of the test substance per ml. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration equivalent to 2 µg of Indinavir sulfate per ml.

For the system suitability test: prepare solution (3) using 2 ml of solution (1) and 2 ml of sulfuric acid (190 g/l), heat carefully in a water bath at 80°C for 60 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 40°C, using, for example, a water-bath.

Inject 20 µl of solution (3). The test is not valid unless the resolution factor between the two major peaks, with a retention time between 15 and 20 min, is not less than 3.5. If necessary adjust the amount of acetonitrile in mobile phase A, 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 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 withsolution (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 water and titrate with sodium hydroxide (0.1 mol/l) VS, determine the end point potentiometrically. Each ml of sodium hydroxide (0.1 mol/l) VS is equivalent to 35.59 mg of $C_{36}H_{47}N_5O_4,H_2O_4S$; calculate with reference to the anhydrous and ethanol free 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, pretreated before the bonding of octadecylsilyl groups to minimize the interaction with basic compounds.

Macrogol 20 000 R. Polyethyleneglycol 20 000

Description. White or almost white solid with a waxy or paraffin-like appearance.

Solubility. Very soluble in water and dichloromethane R. Practically insoluble in alcohol and in fatty oils and mineral oils.

Nitrogen for chromatography.
Contains not less than 99.95% v/v of N_2.

Helium for chromatography.
Contains not less than 99.995% v/v of He.

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