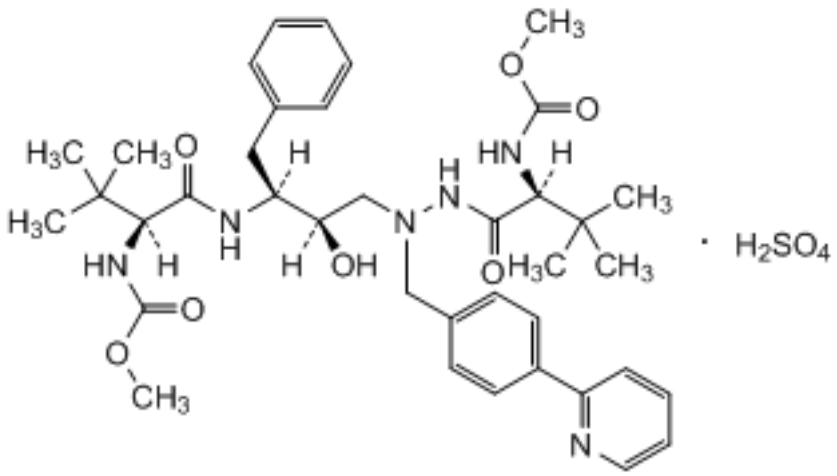
# Atazanavir sulfate (Atazanaviri sulfas)

**2019-01**

**Molecular formula.** $C_{38}H_{52}N_6O_7 \cdot H_2SO_4$

**Relative molecular mass.** 802.9

**Graphic formula.**



**Chemical names.** Dimethyl *N*,*N*'-[(3*S*,8*S*,9*S*,12*S*)-9-benzyl-8-hydroxy-2,2,13,13-tetramethyl-4,11-dioxo-6-{[4-(pyridin-2-yl)phenyl]methyl}-5,6,10-triazatetradecane-3,12-diyl]dicarbamate, monosulfate (*IUPAC*); 2,5,6,10,13-Pentaazatetradecanedioic acid, 3,12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl)phenyl]methyl]-, 1,14-dimethyl ester, (3*S*,8*S*,9*S*,12*S*)-, sulfate (1:1) (*CAS*); CAS Reg. No. 229975-97-7

**Description.** A white to a pale yellow powder.

**Solubility.** Freely soluble in methanol R, practically insoluble in water R.

**Category.** Antiretroviral (protease inhibitor).

**Storage.** Atazanavir sulfate should be kept in a tightly closed container.

**Additional information.** Atazanavir sulfate is slightly hygroscopic and may exhibit polymorphism.

## Requirements

Atazanavir sulfate contains not less than 98.0% and not more than 102.0% of $C_{38}H_{52}N_6O_7 \cdot H_2SO_4$, calculated with reference to the dried substance.

## Identity tests

-Either test A and D or test B, C and D should be applied.

A. Carry out the examination as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption spectrum is concordant with the spectrum obtained from atazanavir sulfate RS or with the *reference spectrum* of atazanavir sulfate.

If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the test substance and atazanavir sulfate RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from atazanavir sulfate RS.

B. Carry out test B.1, or where ultraviolet (UV) detection is not available, test B.2.

B.1 Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography using silica gel R6 as the coating substance and a mixture of 9.5 volumes of dichloromethane R and 0.5 volume of 2-propanol R as the mobile phase. Apply separately to the plate 10 µL of each of the following 2 solutions in methanol R. For solution (A) use a solution containing 1 mg of the test substance per mL. For solution (B) use a solution containing 1 mg of atazanavir sulfate RS per mL. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or in a current of air.

Examine the chromatogram in UV light (254 nm). The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).

B.2 Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography using the

conditions described under test B.1, but using a plate containing silica gel R5 as the coating substance. Spray the plate with basic potassium permanganate (~5 g/L) TS.

Examine the chromatogram in daylight. The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).

C. The absorption spectrum of a 10 µg/mL solution of the test substance in methanol R, when observed between 230 nm and 340 nm, exhibits two maxima of similar intensity at about 250 nm and 280 nm.

D. A 20 mg/mL solution of the test substance yields reaction A described under *2.1 General identification tests* as characteristic of sulfates.

**Heavy metals.** *2.2.3 Limit test for heavy metals*.

**Sulfated ash (*2.3*).** Not more than 2.0 mg/g.

**Loss on drying.** Dry for 3 hours at 105 °C; it loses not more than 10.0 mg/g.

**Specific optical rotation *(1.4)*** Use a 10 mg/mL solution in equal volumes of methanol R and water R at 22 °C and calculate with reference to the dried substance; the specific optical rotation is between -44 and -48.

**Related substances.** Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using a stainless steel column (150 mm x 4.6 mm) packed with end-capped, base-deactivated particles of silica gel, the surface of which has been modified with chemically-bonded octylsilyl groups (5 µm). Use the following conditions for gradient elution:

mobile phase A: 0.02 M phosphate buffer pH 3.5, acetonitrile R (70:30 *v/v*);

mobile phase B: 0.02 M phosphate buffer pH 3.5, acetonitrile R (30:70 *v/v*).

Prepare the 0.02 M phosphate buffer pH 3.5 by dissolving 2.72 g of anhydrous potassium dihydrogen phosphate R in 800 mL of water R, adjust the pH to 3.5 by adding phosphoric acid (~105 g/L) TS and dilute to 1000 mL with water R.

| Time (minutes) | Mobile phase A (% *v/v*) | Mobile phase B (% *v/v*) | Comments |
|---|---|---|---|
| 0–2 | 100 | 0 | Isocratic |
| 2–10 | 100 to 75 | 0 to 25 | Linear gradient |
| 10–30 | 75 to 50 | 25 to 50 | Linear gradient |
| 30–45 | 50 to 0 | 50 to 100 | Linear gradient |
| 45–50 | 0 | 100 | Isocratic |
| 50–52 | 0 to 100 | 100 to 0 | Linear gradient |
| 52–60 | 100 | 0 | Isocratic |

Prepare the following solutions using as diluent a mixture of equal volumes of water R and acetonitrile R. For solution (1) dissolve 50 mg of the test substance and dilute to 50.0 mL. For solution (2) dilute 10.0 mL of solution (1) to 200.0 mL. Dilute 10.0 mL of this solution to 100.0 mL. For solution (3) mix 1 mL of solution (1) with 4.5 mL of water R and 0.5 mL of sodium hydroxide (10 g/L) TS and heat the mixture in a water-bath at 85 °C for 15 minutes.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 250 nm. Maintain the column at a temperature of 30 °C.

Inject 20 µL of solution (3). The test is not valid unless the resolution between the peak due to atazanavir (retention time about 22 minutes) and the peak with a relative retention of about 1.2 is at least 4.

Inject alternately 20 µL each of solutions (1) and (2).

In the chromatograms obtained with test solution (1):

- the area of any impurity peak is not greater than the area of the peak due to atazanavir in the chromatogram obtained with solution (2) (0.5%);
- the sum of the areas of all impurity peaks is not greater than twice the area of the peak due to atazanavir in the chromatogram obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.1 times the area of the peak due to atazanavir in the chromatogram obtained with solution (2) (0.05%).

**Assay**

Dissolve 0.300 g in 30 mL of methanol R by sonication for 10 minutes. Add 30 mL of water and titrate with sodium hydroxide (0.1 mol/L) VS, determining the end-point potentiometrically. Each mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 40.15 mg of $C_{38}H_{52}N_6O_7 \cdot H_2SO_4$.