Atazanavir capsules (Atazanaviri capsulae)

2018-01

Category. Antiretroviral (Protease Inhibitor).

Storage. Atazanavir capsules should be kept in a tightly closed container.

Additional information. Strength in the current WHO Model List of Essential Medicines (EML): 100 mg, 300 mg of atazanavir (as sulfate). Strength in the current WHO EML for children: 100 mg of atazanavir (as sulfate). Additional strength available: 150 mg and 200 mg of atazanavir (as sulfate).

Requirements

Comply with the monograph for *Capsules*.

Definition

Atazanavir capsules contain Atazanavir sulfate. They contain not less than 90.0% and not more than 110.0% of the amount of atazanavir, $C_{38}H_{52}N_6O_7$, stated on the label.

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 *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) disperse a quantity of the content of the capsules containing 20 mg of atazanavir in 10 mL of methanol R, sonicate for 10 minutes, allow to cool to room temperature, dilute to 20 mL, filter and use the filtrate. For solution (B) use a solution containing 1.1 mg of atazanavir sulfate RS per mL.

After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or a current of air. Examine the chromatogram in ultraviolet light (254 nm).

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

A.2 Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography using the conditions described above under test A.1 but using a plate containing silica gel R5 as the coating substance. Spray 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 with that obtained with solution (B).

B. Disperse a quantity of the content of the capsules containing about 20 mg of atazanavir in 10 mL of methanol R, sonicate for 10 minutes, allow to cool to room temperature, dilute to 20 mL and filter. Dilute 1.0 mL of the filtrate to 100.0 mL with methanol R. The absorption spectrum *(1.6)* of the resulting solution, when observed between 230 and 340 nm, exhibits two maxima of similar intensity at about 250 nm and 280 nm.

C. Carry out the test as described under *1.14.4 High-performance liquid chromatography* using the conditions given under "Assay". Record the UV spectrum of the principal peak in the chromatograms with a diode array detector in the range of 200 nm to 400 nm.

The retention time and the UV spectrum of the principle peak in the chromatogram obtained with the solution (1) corresponds to the retention time and the UV spectrum of the peak due to atazanavir in the chromatogram obtained with solution (2).

Dissolution. Carry out the test as described under *5.5 Dissolution test for solid oral dosage forms* using as the dissolution medium 900 mL of dissolution buffer pH 2.5 TS and rotating the paddle at 50 revolutions per minute. At 45 minutes withdraw a sample of 10.0 mL of the medium through an in-line filter. Allow the filtered sample to cool to room temperature. If tablets are tested with a strength of more than 100 mg dilute a suitable volume of the filtrate with dissolution medium to obtain a solution containing about 0.11 mg of the labelled amount of atazanavir per mL. Measure the absorbance (*1.6*) of a 1 cm layer of the resulting solution at the maximum at about 250 nm, using the dissolution medium as the blank.

For each of the capsules tested, calculate the total amount of atazanavir ($C_{38}H_{52}N_6O_7$) in the medium, using an absorptivity value of 11.4 for atazanavir sulfate ($A^{1\%}_{1cm}$ = 114). Each mg of atazanavir sulfate ($C_{38}H_{52}N_6O_7 \cdot H_2O_4S$) is equivalent to 0.878 mg of atazanavir ($C_{38}H_{52}N_6O_7$).

Evaluate the results as described under *5.5 Dissolution test for solid dosage forms, Acceptance criteria*. The amount in solution for each capsule is not less than 75% (Q) of the amount stated on the label.

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) 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 acetonitrile R and water R. For solution (1) weigh and mix the content of 20 capsules. Transfer a quantity of the mixed contents equivalent to 20 mg of atazanavir into a 20 mL volumetric flask. Add 10 mL of the diluent, sonicate for 10 minutes, allow to cool to room temperature, make up to volume and filter. For solution (2) dilute 1.0 mL of solution (1) 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 alternatively 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) (1.0%);
- 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) (2.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

Assay

-Either test A or test B may be applied.

A. 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).

As the mobile phase use a solution prepared as follows: 60 volumes of acetonitrile R and 40 volumes of 0.02 M phosphate buffer pH 3.5. Prepare the 0.02 M phosphate buffer pH 3.5 as described in the related substances test.

Prepare the following solutions using as diluent a mixture of equal volumes of acetonitrile R and water R. For solution (1) weigh and mix the content of 20 capsules. Transfer a quantity equivalent to 20.0 mg of atazanavir, accurately weighed, into a 20 mL volumetric flask. Add 10 mL of the diluent, sonicate for 10 minutes, allow to cool to room temperature and make up to volume. Filter a portion of this solution, discarding the first few mL. Dilute 5.0 mL of the filtrate to 50.0 mL with the diluent. For solution (2) use a solution containing 0.11 mg of atazanavir sulfate RS per mL.

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 alternately 20 µL each of solutions (1) and (2) and record the chromatograms for 1.5 times the retention time of atazanavir

(retention time about 4 minutes).

Measure the areas of the peak responses obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of atazanavir ($C_{38}H_{52}N_6O_7$) in the capsules, using the declared content of $C_{38}H_{52}N_6O_7 \cdot H_2O_4S$ in atazanavir sulfate RS. Each mg of atazanavir sulfate ($C_{38}H_{52}N_6O_7 \cdot H_2O_4S$) is equivalent to 0.878 mg of atazanavir ($C_{38}H_{52}N_6O_7$).

B. Weigh and mix the content of 20 capsules. Transfer a quantity of the mixed contents equivalent to 20 mg of atazanavir, accurately weighed, to a 20 mL volumetric flask. Add about 10 mL of methanol R, sonicate for about 10 minutes, allow to cool to room temperature and make up to volume with methanol R. Filter a portion of this solution, discarding the first few mL of the filtrate. Dilute 1.0 mL of the filtrate to 10.0 mL with methanol R.

Measure the absorbance *(1.6)* of a 1 cm layer of this solution at the maximum at about 250 nm, using methanol R as a blank. Calculate the percentage content of $C_{38}H_{52}N_6O_7$ in the capsules using an absorptivity value of 14.5 for atazanavir sulfate ($A^{1\%}_{1cm}$ = 145). Each mg of atazanavir sulfate ($C_{38}H_{52}N_6O_7 \cdot H_2O_4S$) is equivalent to 0.878 mg of atazanavir ($C_{38}H_{52}N_6O_7$).