Ritonavir tablets (Ritonaviri compressi)

Category. Antiretroviral (Protease Inhibitor).

Storage. Ritonavir tablets should be stored in a tightly closed container, at temperatures not exceeding 30 °C.

Additional information. Strength in the current WHO Model list of essential medicines: 25 mg, 100 mg. Ritonavir 100 mg tablets (co-packaged with Nirmatrelvir 150 mg tablets) are listed on the 8th Invitation to Manufacturers of therapeutics against COVID-19 to submit an Expression of Interest (EOI) for Product Evaluation to the WHO Prequalification Unit.

Requirements

Comply with the monograph for "Tablets".

Definition. Ritonavir tablets contain not less than 90.0% and not more than 110.0% of the amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) stated on the label.

Manufacture. Ritonavir tablets are manufactured using Ritonavir in the amorphous form in order to ensure suitable solubility properties.

Identity tests

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 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions in methanol R. For solution (A) shake a quantity of the powdered tablets containing 25 mg of Ritonavir with 5 mL and filter. Add 0.5 mL of ammonia (~260 g/l) TS to 2 mL of the filtrate and shake. For solution (B) use 2 mL of a 5 mg/mL solution of ritonavir RS. Add 0.5 mL of ammonia (~260 g/l) TS and shake. 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 *1.14.1 Chromatography*, Thin-layer chromatography,

using the conditions described above under test A.1 but using silica gel R5 as the coating substance. Spray lightly with basic potassium permanganate (5 g/l) TS and 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. See the test described under Assay. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that 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 hydrochloric acid (0.1 mol/l) VS and rotating the paddle at 75 revolutions per minute. At 90 minutes withdraw a sample of 10 mL of the medium through an in-line filter. Allow the filtered sample to cool down to room temperature.

Determine the content of ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the medium by *1.14.1 Chromatography*, High-performance liquid chromatography, using the conditions described under Assay and a suitable solution of ritonavir RS as a reference solution.

For each of the six tablets tested calculate the total amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the medium. The amount in solution for each tablet is not less than 80% of the amount stated on the label. If the amount obtained for one of the six tablets is less than 80%, repeat the test using a further six tablets; the average amount for all 12 tablets tested is not less than 75% and no tablet releases less than 60%.

Related substances

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using the conditions described under Assay.

Prepare the following solutions using a mixture of 70 volumes of mobile phase A and 30 volumes of mobile phase B as diluent. For solution (1) shake a quantity of the powdered tablets containing 25 mg of Ritonavir with 50 mL of diluent, filter and use the clear filtrate. For solution (2) dilute a suitable volume of solution (1) to obtain a concentration of 0.5 µg of Ritonavir per mL.

For the system suitability test: prepare solution (3) using 5 mL of solution (1) and 1 mL of sulfuric acid (475 g/l) TS, heat in a

boiling water-bath for 20 minutes.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 240 nm.

Maintain the column temperature at 35 °C.

Inject 20 µl of solution (3). The test is not valid unless the resolution between the principal peak (retention time about 22 minutes) and the peak with a relative retention of about 0.8 is at least 2.0. The test is also not valid unless the resolution between the principal peak and the peak with a relative retention of about 1.5 is at least 6.5. If necessary adjust the amount of acetonitrile in both mobile phases A and B or adjust the gradient programme.

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

In the chromatogram obtained with solution (1), the area of any peak, other than the principal peak, is not greater than three times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%); the area of not more than two such peaks is greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (0.2%); the area of not more than four such peaks is greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than ten times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%). 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%). Disregard any peak with a retention time less than the retention time of the peak obtained with solution (3) with a relative retention of about 0.5.

Assay

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with base-deactivated particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm).

Use the following conditions for gradient elution:

Mobile phase A: 35 volumes of acetonitrile R, 28 volumes of sodium phosphate buffer

pH 4.0 and 37 volumes of water R.

Mobile phase B: 70 volumes of acetonitrile R, 28 volumes of sodium phosphate buffer

pH 4.0 and 2 volumes of water R.

Prepare the sodium phosphate buffer pH 4.0 by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate R and 1.88 g of sodium hexanesulfonate R in 800 mL of water R, adjust the pH to 4.0 by adding phosphoric acid (~105 g/l) TS and dilute to 1000 mL with water R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-20	70	30	Isocratic
20-30	70 to 0	30 to 100	Linear gradient
30-40	0	100	Isocratic
40-45	0 to 70	100 to 30	Linear gradient
45-50	70	30	Isocratic re-equilibration

Prepare the following solutions using a mixture of 70 volumes of mobile phase A and 30 volumes of mobile phase B as diluent. For solution (1) weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing 25 mg of Ritonavir with 50 mL of diluent, filter and use the clear filtrate. For solution (2) use 0.5 mg of ritonavir RS per mL of diluent.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 240 nm.

Maintain the column temperature at 35 °C.

For the system suitability test: prepare solution (3) using 5 mL of solution (1) and 1 mL of sulfuric acid (475 g/l) TS, heat in a boiling water-bath for 20 minutes.

Inject 20 µl of solution (3). The test is not valid unless the resolution between the principal peak (retention time about 22 minutes) and the peak with a relative retention of about 0.8 is at least 2.0. The test is also not valid unless the resolution between the principal peak and the peak with a relative retention of about 1.5 is at least 6.5.

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

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2) and calculate the content of ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the tablets.