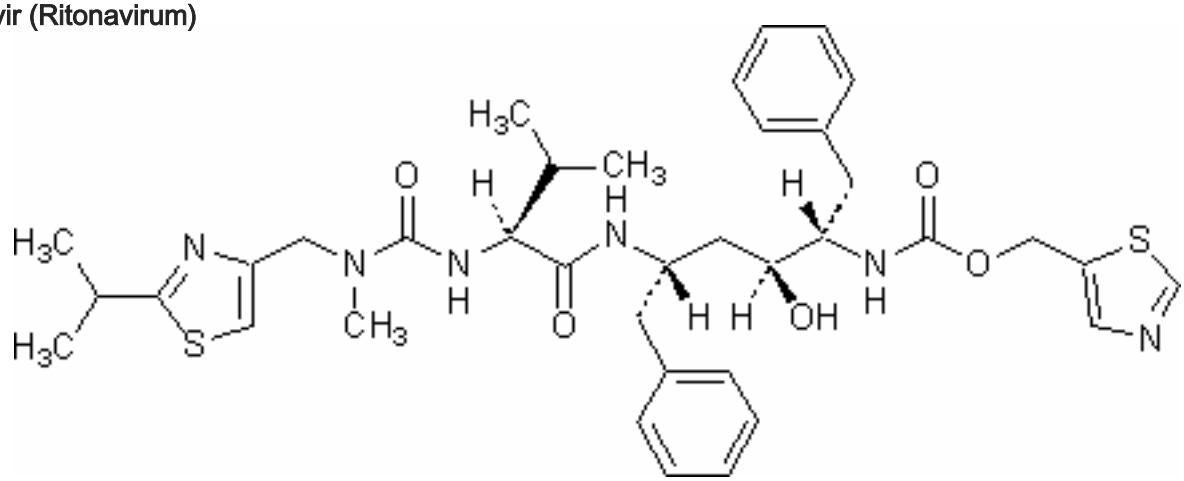
Ritonavir (Ritonavirum)



$C_{37}H_{48}N_6O_5S_2$

Relative molecular mass. 721.0

Chemical name. thiazol-5-ylmethyl [(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[[methyl[[2-(1-methylethyl)thiazol-4-yl]methyl]carbamoyl]amino]butanoyl]amino]-5-phenylpentyl]carbamate; CAS Reg. NO.155213-67-5.

Description. A white or almost white powder.

Solubility. Practically insoluble in water, freely soluble in methanol R, sparingly soluble in acetone R and very slightly soluble in acetonitrile R.

Category. Antiretroviral (Protease Inhibitor).

Storage. Ritonavir should be kept in a well-closed container, protected from light.

Additional information. Ritonavir may exhibit polymorphism.

Requirements

Ritonavir contains not less than **98.5 %** and not more than **101.0 %** of $C_{37}H_{48}N_6O_5S_2$, 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 *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 2 solutions in methanol containing (A) 5 mg of the test substance per mL and (B) 5 mg of ritonavir 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 *1.14.1 Chromatography*, Thin-layer chromatography, using silica gel R5 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 2 solutions in methanol containing(A) 5 mg of the test substance per mL and (B) 5 mg of ritonavir RS per mL. After removing the plate from the chromatographic chamber, allow it to dry exhaustively in a current of cool air. 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. The absorption spectrum of a 40 µg/mL solution in methanol R, when observed between 220 nm and 280 nm, exhibits one maximum at about 240 nm; the specific absorbance ($A^{1\%}_{1cm}$) is 116 to 128.

C. 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 ritonavir RS or with the *reference spectrum* of ritonavir. If the spectra obtained in the solid-state show differences, dissolve the test substance and the reference substance separately in a minimal amount of methanol R, crystallise by adding just enough water drop by drop, filter and dry for about one hour and record the spectra again.

Specific optical rotation. Use a 20.0 mg/mL solution in methanol R; $[\alpha]_D^{20\,°C}$ = +7° to +10°.

Heavy metals. Use 1.0 g in 30 mL of methanol R for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 2; determine the heavy metals content according to method A; not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 2 hours at 105 °C; it loses not more than 5 mg/g.

Related substances. 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 octadecylsilyl silica gel for chromatography R (5 µm).

Use the following conditions for gradient elution:

Mobile phase A: 35 volumes of acetonitrile R, 28 volumes sodium phosphate buffer pH 4.0 and 37 volumes of purified water.

Mobile phase B: 70 volumes of acetonitrile R, 28 volumes sodium phosphate buffer pH 4.0 and 2 volumes of purified water.

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 purified water, adjust the pH to 4.0 by adding phosphoric acid (~105 g/l) TS and dilute to 1000 mL with purified water.

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 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 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), 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 not less than 3.5. 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 not less than 9.0. If necessary adjust the amount of acetonitrile in both mobile phases A and B, or adjust the gradient programme.

Inject alternatively 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 obtained with solution (2) (0.3%). In the chromatogram obtained with solution (1), the areas of not more than two peaks, other than the principal peak, are greater than twice the area of the principal peak obtained with solution (2) (0.2%) and the areas of not more than four such peaks are 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 ten times the area of the

principal peak 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%).

Assay. Dissolve 0.25 g, accurately weighed, in 30 mL of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS, determine the end point potentiometrically as described under 2.6 Non-aqueous titration Method A. Each mL of perchloric acid (0.1 mol/l) VS is equivalent to 36.05 mg of $C_{37}H_{48}N_6O_5S_2$.