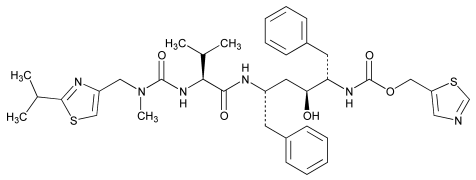
Ritonavir



$C_{37}H_{48}N_6O_5S_2$ 720.94

2,4,7,12-Tetraazatridecan-13-oic acid, 10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-5-thiazolylmethyl ester [5*S*-(5*R**,8*R**,10*R**,11*R**)]-.

5-Thiazolylmethyl [(α*S*)-α-[(1*S*,3*S*)-1-hydroxy-3-[(2*S*)-2-[3-[(2-isopropyl-4-thiazolyl)methyl]-3-methylureido]-3-methylbutyramido]-4-phenylbutyl]phenethyl]carbamate [*155213-67-5*].

» Ritonavir contains not less than 97.0 percent and not more than 102.0 percent of $C_{37}H_{48}N_6O_5S_2$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight, light-resistant containers. Store between 5° and 30°.

USP Reference standards ⟨11⟩**—***USP Ritonavir RS. USP Ritonavir Related Compounds Mixture RS.*

Identification—

A: *Infrared Absorption* ⟨197⟩—

Test specimen—Dissolve 50 mg of Ritonavir in 1.0 mL of chloroform. Add 1 drop of this solution to the surface of a potassium bromide or a sodium chloride disk, and evaporate to dryness.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* is within 2% of the retention time of the major peak in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

X-ray diffraction ⟨941⟩—The X-ray diffraction pattern conforms to that of USP Ritonavir RS if the drug substance is used for the solid dosage forms.

Heavy metals, *Method II* ⟨231⟩**:** not more than 0.002%, using 1.0 g of Ritonavir and 2 mL of *Standard Lead Solution* (10 ppm Pb) in the *Standard Preparation.*

Water, *Method I* ⟨921⟩**:** not more than 0.5%, determined on 0.500 g.

Residue on ignition ⟨281⟩**:** not more than 0.2%, determined on 1.0 g.

Organic volatile impurities ⟨467⟩**:** meets the requirements.

(Official until July 1, 2008)

Related compounds—[NOTE—Ritonavir is alkali sensitive. All glassware should be prerinsed with distilled water prior to use to remove residual detergent contamination.]

Monobasic potassium phosphate solution (0.03M), Diluent, Solution A, Solution B, and *Mobile phase*—Prepare as directed in the *Assay.*

Standard stock solution and *Intermediate stock solution*—Prepare as directed for *Standard stock preparation* and *Intermediate standard preparation* in the *Assay.*

Ritonavir identity standard solution—Transfer about 50 mg of USP Ritonavir Related Compounds Mixture RS, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

Standard solution—Transfer 5.0 mL of the *Intermediate standard solution* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. [NOTE—This solution may be used for 48 hours if stored at room temperature.]

Test solution—Transfer about 50 mg of Ritonavir, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 15-cm column that contains 3-µm packing L26 and is maintained at a constant temperature of about 60°. The flow rate is about 1.0 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0	100	0	equilibrium
0–60	100	0	isocratic
60–120	100→0	0→100	gradient
120.1	0→100	100→0	step gradient
120.1–155	100	0	isocratic

The run time for the *Standard solution* is 40 minutes, and the run time for the *Test solution* is 155 minutes. Chromatograph the *Ritonavir identity standard solution* and the *Standard solution,* and record the responses as directed for *Procedure:* the retention time of ritonavir is between 30 and 35 minutes; the resolution, *R,* between impurity E and impurity F (see *Table 1)* in the *Ritonavir identity standard solution* is not less than 1.0; the ratio of peak (H_p) to valley (H_v) of Ritonavir and impurity N (regioisomer) is not less than 1; the capacity factor, *k′,* using the main component peak of the first *Standard solution* injection, is not less than 13; the column efficiency, using the main component peak of the first *Standard solution* injection, is not less than 5000 theoretical plates; the tailing factor, using the main component peak of the first *Standard solution* injection, is between 0.8 and 1.2; and the relative standard deviation of the peak area response of the main component peak, for replicate injections of the *Standard solution,* is not more than 3.0%.

Table 1. Approximate Relative Retention Time (RRT) for Known Related Impurities

Impurity Identity	Common Name	Response Factor	RRT
A + B	Mixture of 2,4-Wing acid and monoacyl valine	—	0.07
C	Monoacylacetamide	—	0.15
D	5-Wing diacyl	1.37	0.24
E	Oxidation impurity	—	0.36
F	Acid hydrolysis product	0.73	0.39
G	Ritonavir hydroperoxide	—	0.45
H	Acid/base by-product	0.76	0.47
I	Ethyl analog	—	0.64
J + K	Mixture of Boc-monoacyl and monoacyl isobutyl carbamate	0.74	0.81
L	Base cyclization product	0.53	0.87
M	2,4-Wing isobutyl ester	—	0.94
N	Regioisomer	—	1.05
O	Isomer #2	—	1.11
P	Di-monoacyl urea	—	1.14

Table 1. Approximate Relative Retention Time (RRT) for Known Related Impurities *(Continued)*

Impurity Identity	Common Name	Response Factor	RRT
Q	Isomer #4	—	1.23
R	Isomer #1	—	1.32
S	Di-monoacyl valine urea	—	1.62
T	2,4-Wing diacyl	0.73	2.87
U	Triacyl impurity	—	3.20

Procedure—Separately inject equal volumes (about 50 µL) of the *Diluent, Ritonavir identity standard solution, Standard solution,* and *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses. Calculate the percentage of each impurity in the portion of Ritonavir taken by the formula:

$$0.0025(W_S / W_T)\ (R_T / R_S)(1/F)P$$

in which W_S is the the weight, in mg, of USP Ritonavir RS taken to prepare the *Standard solution;* W_T is the weight, in mg, of Ritonavir taken to prepare the *Test solution;* R_T is the area of the impurity peak obtained from the *Test solution;* R_S is the average peak area of ritonavir obtained from the six injections of the *Standard solution;* F is the response factor for the impurity (see values in *Table 1*); and P is the purity, in percentage, of USP Ritonavir RS taken to prepare the *Standard solution.* Not more than 0.3% of impurity E and O is found; not more than 0.2% of impurity T is found; not more than 0.1% of any other impurity is found; and not more than 1.0% of total impurities is found.

Assay—

Monobasic potassium phosphate solution (0.03M)—Dissolve about 8.2 of monobasic potassium phosphate in 2.0 L of water. Mix well, and filter through a 0.45-µm nylon membrane.

Diluent—Prepare a mixture of *Monobasic potassium phosphate solution (0.03M)* and acetonitrile (1 : 1). Mix well, and filter through a 0.45-µm nylon membrane.

Solution A—Prepare a mixture of the filtered *Monobasic potassium phosphate solution (0.03M),* acetonitrile, tetrahydrofuran (inhibitor-free), and *n*-butanol (69 : 18 : 8 : 5).

Solution B—Prepare a mixture of acetonitrile, the filtered *Monobasic potassium phosphate solution (0.03M),* tetrahydrofuran (inhibitor-free), and *n*-butanol (47 : 40 : 8 : 5).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system.* Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩). [NOTE—Because of the high dependence of retention time and selectivity on the *Mobile phase* composition, the volumes should be accurately measured. Excessive or continued helium sparging must be avoided. Store the *Mobile phase* in a tightly sealed container when not in use.]

Standard stock preparation—Transfer about 100 mg of USP Ritonavir RS, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix. [NOTE—This solution may be kept for 5 days if refrigerated.]

Intermediate standard preparation—Transfer 5.0 mL of the *Standard stock preparation* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Standard preparation—Transfer 25.0 mL of the *Intermediate standard preparation* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

Assay preparation—Transfer 5.0 mL of the *Test solution,* prepared as directed in the test for *Related compounds,* to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix. Dilute 25.0 mL of this solution with *Diluent* to 100-mL, and mix.

Chromatographic system—Proceed as directed in the test for *Related compounds.* The run time for the *Standard preparation* and *Assay preparation* is 40 minutes. Chromatograph the *Standard preparation,* and record the responses as directed for *Procedure:* the capacity factor, k', using the main component peak of the first *Standard preparation* injection, is not less than 13; the column efficiency, using the main component peak of the first *Standard preparation* injection, is not less than 5000 theoretical plates; the tailing factor, using the main component peak of the first *Standard preparation* injection, is between 0.8 and 1.2; and the relative standard deviation of the peak area response of the main component peak, for replicate injections of the *Standard preparation,* is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak area responses. Calculate the percentage, on the as-is basis, of $C_{37}H_{48}N_6O_5S_2$ in the portion of Ritonavir taken by the formula:

$$0.5(W_S / W_T)(\ r_T / r_S)P$$

in which W_S is the weight, in mg, of USP Ritonavir RS taken to prepare the *Standard preparation;* W_T is the weight, in mg, of Ritonavir taken to prepare the *Assay preparation;* r_T is the peak area of the impurity obtained from the chromatogram of the *Assay preparation;* r_S is the average peak area of ritonavir obtained from the chromatograms of the five injections of the *Standard preparation;* and P is the purity, in percentage, of USP Ritonavir RS taken to prepare the *Standard preparation.*

Calculate the percentage, on the anhydrous basis, of $C_{37}H_{48}N_6O_5S_2$ in the portion of Ritonavir taken by the formula:

$$100A/(100 - B)$$

in which A is the percentage of $C_{37}H_{48}N_6O_5S_2$ on the as-is basis, as calculated above; and B is the percentage of water content.