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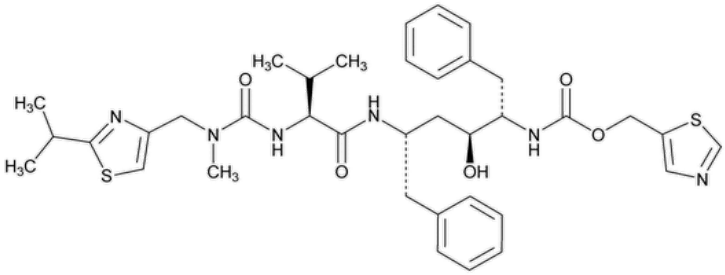
Ritonavir



$C_{37}H_{48}N_6O_5S_2$ 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 CAS RN®: 155213-67-5; UNII: O3J8G9O825.

DEFINITION

Ritonavir contains NLT 97.0% and NMT 102.0% of ritonavir ($C_{37}H_{48}N_6O_5S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K**▲ (CN 1-May-2020)

• **B.** The retention time of the major peak of the *Sample solution* is within 2% of the retention time of the major peak of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Solution A: 4.1 mg/mL of monobasic potassium phosphate in water

Solution B: Acetonitrile, tetrahydrofuran (inhibitor-free), *n*-butanol, and *Solution A* (18:8:5:69)

Mobile phase: *Solution B*

Diluent: Acetonitrile and *Solution A* (1:1)

Standard stock solution: 2.0 mg/mL of USP Ritonavir RS in *Diluent*. [Note—This solution may be kept for 5 days if refrigerated.]

Standard solution 1: 0.10 mg/mL of USP Ritonavir RS from the *Standard stock solution* diluted with *Diluent*

Standard solution 2: 0.025 mg/mL of USP Ritonavir RS from *Standard solution 1* diluted with *Diluent*

Sample solution: 0.025 mg/mL of Ritonavir in *Diluent*

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; 3-µm packing L26

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time: 40 min

System suitability

Sample: *Standard solution 2*

Suitability requirements

Capacity factor, *k'*: NLT 13

Column efficiency: NLT 5000 theoretical plates

Tailing factor: 0.8–1.2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution 2* and *Sample solution*

Calculate the percentage of ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the portion of Ritonavir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Ritonavir RS in *Standard solution 2* (mg/mL)

C_U = concentration of Ritonavir in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.2%, determined on 1.0 g

• **Organic Impurities**

Ritonavir is alkali sensitive. All glassware should be prerinsed with distilled water before use to remove residual detergent contamination.

Solution A, Solution B, Mobile phase, Diluent, Standard stock solution, and **Standard solution 1:** Prepare as directed in the *Assay*.

Solution C: Acetonitrile, tetrahydrofuran (inhibitor-free), *n*-butanol, and *Solution A* (47:8:5:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
60	100	0
120	0	100
120.1	100	0
155	100	0

Identity solution: 1 mg/mL of USP Ritonavir Related Compounds Mixture RS in *Diluent*

Standard solution 2: 5 µg/mL of USP Ritonavir RS from *Standard solution 1* in *Diluent*. [Note—This is stable for 48 h.]

Sample solution: 1 mg/mL of Ritonavir in *Diluent*

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; 3-µm packing L26

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time

Standard solution 2: 40 min

System suitability

Samples: *Identity solution* and *Standard solution 2*

[Note—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.0 between hydroxyritonavir and hydantoin aminoalcohol peaks, *Identity solution*

Peak-to-valley ratio: NLT 1 for ritonavir and the 4-hydroxy isomer, *Identity solution*

Capacity factor, *k'*: NLT 13, *Standard solution 2*

Column efficiency: NLT 5000 theoretical plates, *Standard solution 2*

Tailing factor: 0.8–1.2, *Standard solution 2*

Relative standard deviation: NMT 3.0%, *Standard solution 2*

Analysis

Samples: *Diluent*, *Identity solution*, *Standard solution 2*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Ritonavir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of each impurity from the *Sample solution*

r_S = peak response from *Standard solution 2*

C_S = concentration of *Standard solution 2* (mg/mL)

C_U = concentration of Ritonavir in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Mixture of ureidovaline and *N*-deacylvaline ritonavir[a]	0.07	1.0	0.1
Acetamidoalcohol[b]	0.15	1.0	0.1
2,5-Thiazolylmethyl dicarbamate[c]	0.24	1.37	0.1
Hydroxyritonavir[d]	0.36	1.0	0.3
Hydantoin aminoalcohol[e]	0.39	0.73	0.1
Ritonavir hydroperoxide[f]	0.45	1.0	0.1
Hydantoin-oxazolidinone derivative[g]	0.47	0.76	0.1
Ethyl analog[h]	0.64	1.0	0.1
Mixture of BOC-aminoalcohol and isobutoxycarbonyl aminoalcohol[i]	0.81	0.74	0.1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Oxazolidinone derivative[j]	0.87	0.53	0.1
Ureidovaline isobutyl ester[k]	0.94	1.0	0.1
4-Hydroxy isomer[l]	1.05	1.0	0.1
3*R*-Epimer[m]	1.11	1.0	0.3
Aminoalcohol urea derivative[n]	1.14	1.0	0.1
3*R*,5*R*-Diastereomer[o]	1.23	1.0	0.1
5*R*-Epimer[p]	1.32	1.0	0.1
Diacyl valine urea[q]	1.62	1.0	0.1
Divalinyl analog[r]	2.87	0.73	0.2
O-Acyl ritonavir[s]	3.20	1.0	0.1
Any other individual impurity	—	1.0	0.1
Total impurities	—	—	1.0

[a] Ureidovaline is [*N*-methyl[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl-L-valine and *N*-deacylvaline ritonavir is thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

[b] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-acetamido-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

[c] Bis(thiazol-5-ylmethyl) (2*S*,3*S*,5*S*)-3-hydroxy-1,6-diphenylhexane-2,5-diyldicarbamate.

[d] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-2-(3-{[2-(2-hydroxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

[e] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-3-hydroxy-5-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-1,6-diphenylhexan-2-ylcarbamate.

[f] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-(3-{[2-(2-hydroperoxypropan-2-yl)thiazol-4-yl]methyl}-3-methylureido)-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

[g] (4*S*,5*S*)-Thiazol-5-ylmethyl 4-benzyl-5-{(*S*)-2-[(*S*)-4-isopropyl-2,5-dioxoimidazolidin-1-yl]-3-phenylpropyl}-2-oxooxazolidine-3-carboxylate.

[h] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-{3-[(2-ethylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

[i] BOC-aminoalcohol is thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-*t*-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate and isobutoxycarbonyl aminoalcohol is thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-isobutoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

[j] (*S*)-*N*-[(*S*)-1-[(4*S*,5*S*)-4-Benzyl-2-oxooxazolidin-5-yl]-3-phenylpropan-2-yl]-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamide.

[k] (*S*)-Isobutyl 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.

[l] Thiazol-5-ylmethyl (2*S*,4*S*,5*S*)-4-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

[m] Thiazol-5-ylmethyl (2*S*,3*R*,5*S*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

[n] Bis(thiazol-5-ylmethyl) (2*S*,2'*S*,3*S*,3'*S*,5*S*,5'*S*)-5,5'-carbonylbis(azanediyl)bis(3-hydroxy-1,6-diphenylhexane-5,2-diyl)dicarbamate.

[o] Thiazol-5-ylmethyl (2*S*,3*R*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

[p] Thiazol-5-ylmethyl (2*S*,3*S*,5*R*)-3-hydroxy-5-[(*S*)-2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamido]-1,6-diphenylhexan-2-ylcarbamate.

[q] (3*S*,4*S*,6*S*,10*S*,13*S*,15*S*,16*S*)-Bis(thiazol-5-ylmethyl)-4,15-dihydroxy-10-isopropyl-8,11-dioxo-3,6,13,16-tetrabenzyl-2,7,9,12,17-pentaazaoctadecanedioate.

[r] (2*S*,2'*S*)-*N*,*N*'-[(2*S*,3*S*,5*S*)-3-Hydroxy-1,6-diphenylhexane-2,5-diyl]bis(2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanamide).

[s] (*S*)-[(5*S*,8*S*,10*S*,11*S*)-8,11-Dibenzyl-5-isopropyl-1-(2-isopropylthiazol-4-yl)-2-methyl-3,6,13-trioxo-15-(thiazol-5-yl)-14-oxa-2,4,7,12-tetraazapentadecan-10-yl] 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate.

SPECIFIC TESTS

• **Water Determination ⟨921⟩, *Method I*:** NMT 0.5%, determined on 0.500 g

ADDITIONAL REQUIREMENTS

• **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

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
RITONAVIR	Documentary Standards Support	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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