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Ritonavir Oral Solution

DEFINITION
Ritonavir Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$).

IDENTIFICATION
• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY
• **Procedure**

Buffer: 4.1 g/L of monobasic potassium phosphate

Mobile phase: Acetonitrile, methanol, tetrahydrofuran (stabilizer-free), and *Buffer* (17.5:10:10:62.5). Filter the required solutions individually prior to use.

Diluent: Acetonitrile and *Buffer* (50:50)

Standard solution: 25 µg/mL of USP Ritonavir RS in *Diluent*

Sample solution: Nominally 25 µg/mL of ritonavir in *Diluent* from a measured volume of Oral Solution

Chromatographic system

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

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ritonavir ($C_{37}H_{48}N_6O_5S_2$) in the portion of Oral Solution 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 the *Standard solution* (µg/mL)

C_U = nominal concentration of ritonavir in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS
• **Deliverable Volume ⟨698⟩**

For multiple-unit containers

Acceptance criteria: Meets the requirements

IMPURITIES

• **Organic Impurities**

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

Buffer A: 4.1 g/L of monobasic potassium phosphate

Buffer B: 3.8 g/L of monobasic potassium phosphate and 0.25 g/L of dibasic potassium phosphate

Solution A: Acetonitrile and *Buffer A* (50:50)

Solution B: Acetonitrile and *Buffer A* (65:35)

Solution C: Butyl alcohol and *Buffer A* (8:92)

Mobile phase: Acetonitrile, butyl alcohol, tetrahydrofuran (stabilizer-free), and *Buffer B* (18:5:8:69). Adjust with 1 M phosphoric acid or 1 M potassium hydroxide to a pH of 6.3 ± 0.1, if necessary.

Cleaning solution: Acetonitrile, butyl alcohol, tetrahydrofuran (stabilizer-free), and *Buffer A* (30:8:13:49)

Peak identification solution: Transfer 5–10 g of Oral Solution to a suitable sealed container. Add an amount of citric acid equivalent to 1% by weight of Oral Solution taken, and mix until dissolved. Seal the container, and heat at 70° for 24 h. Transfer 5.0 mL of the thermally degraded sample to a 200-mL volumetric flask. Dissolve and dilute with *Solution B* to volume.

Standard stock solution: 0.1 mg/mL of USP Ritonavir RS in *Solution A*

Standard solution: 10 µg/mL of USP Ritonavir RS in *Solution C* from the *Standard stock solution*

Sample stock solution: Nominally 2 mg/mL of ritonavir in *Solution B* prepared as follows. Transfer a measured volume of Oral Solution equivalent to 400 mg of ritonavir to a 200-mL volumetric flask, and dilute with *Solution B* to volume.

Sample solution: Nominally 1 mg/mL of ritonavir prepared as follows. Transfer 25.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Solution C* to volume. Transfer 15.0 mL of the solution to a 50-mL centrifuge tube that has been previously rinsed with methanol and dried. Add 20.0 mL of heptane, then stopper the tube. Shake the tube vigorously until a uniform emulsion is obtained, making sure to vent periodically. Centrifuge the resulting emulsion for about 5 min. Carefully aspirate off the top layer (heptane), leaving the clear bottom layer (*Sample solution*) in the tube. The centrifuged emulsion will have three distinct layers. The top layer (clear heptane) and the bottom layer (*Sample solution*) are separated by a viscous white cloudy layer. The middle layer should be considered part of the top layer for removal by aspiration. Repeat the extraction steps, and analyze the *Sample solution*.

Chromatographic system

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

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; 3-µm packing L26. Wash the column with the *Cleaning solution* after each injection of the *Peak identification solution* and each injection of the *Sample solution* for about 26 min, and equilibrate with *Mobile phase* for about 30 min. Store in the *Cleaning solution* after the analysis is completed.

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time: 1.8 times the retention time of ritonavir

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.2

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Peak identification solution*, *Standard solution*, and *Sample solution*

[Note—Determine the relative retention value (*r*) for the components listed in *Table 1* as directed in *Chromatography ⟨621⟩*, using the time measured at the baseline deflection of the *Standard solution* chromatogram as the void volume (t_M).]

Calculate the percentage of each impurity in the portion of Oral Solution 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 of ritonavir from the *Standard solution*

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

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

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. Disregard peaks less than 0.05%.

Table 1

Name	Relative Retention (*r*)	Relative Response Factor	Acceptance Criteria, NMT (%)
Ureidovaline[a,b]	0.03	—	—
N-Deacylvaline ritonavir[c,d]	0.11	1.0	0.7
Acetamidoalcohol[b,e]	0.15	1.0	0.1
Hydroxypropyl carbamate analog[d]	0.24	0.59	0.4
2,5-Thiazolylmethyldicarbamate[b,f]		1.37	0.1
Hydroxyritonavir[d,g]	0.36	1.0	0.5
Hydantoin aminoalcohol[d,h]	0.39	0.73	0.5
Ritonavir hydroperoxide[d,i]	0.44	1.0	0.2
Ethyl carbamate analog[d,j]	0.45	0.66	1.0
Hydantoin-oxazolidinone derivative[b,k]	0.50	0.76	0.2
Ethyl analog[b,l]	0.64	1.0	0.1
O-Acyl isomer[d,m]	0.74	1.0	0.2
BOC-aminoalcohol[b,n]	0.81	—	—
Isobutoxycarbonyl aminoalcohol[b,o]		0.74	0.1
Oxazolidinone derivative[d,p]	0.87	0.53	0.2
Ureidovaline isobutyl ester[b,q]	0.94	1.0	0.1
Ritonavir	1.00	—	—

Name	Relative Retention (*r*)	Relative Response Factor	Acceptance Criteria, NMT (%)
4-Hydroxy isomer[b,r]	1.05	1.0	0.1
3*R*-Epimer[b,s]	1.11	1.0	0.3
Aminoalcohol urea derivative[b,t]	1.14	1.0	0.1
3*R*,5*R*-Diastereomer[b,u]	1.23	1.0	0.1
5*R*-Epimer[b,v]	1.32	1.0	0.1
Diacyl valine urea[b,w]	1.70	1.0	0.1
Any individual unspecified degradation product	—	1.0	0.2
Total process impurity	—	—	1.2
Total impurities	—	—	3.0

[a] {*N*-Methyl[(2-isopropyl-4-thiazolyl)methyl]amino}carbonyl-L-valine (not quantified by this method due to solvent front and placebo interferences; controlled as a synthetic impurity in the drug substance).

[b] Process impurity.

[c] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-amino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate.

[d] Degradation product.

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

[f] Bis(thiazol-5-ylmethyl) (2*S*,3*S*,5*S*)-3-hydroxy-1,6-diphenylhexane-2,5-diyldicarbamate. (If two peaks appear with a relative retention of 0.24, the second peak is identified as the 2,5-thiazolylmethyl dicarbamate impurity. If a single peak with a relative retention of 0.24 appears, report as *Hydroxypropyl carbamate analog*.)

[g] 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.

[h] 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.

[i] 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 (unspecified degradant; report as *Ethyl carbamate analog* due to possible coelution).

[j] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-5-[(*S*)-2-ethoxycarbonylamino-3-methylbutanamido]-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate (possible coelution with ritonavir hydroperoxide).

[k] (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.

[l] 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.

[m] (*S*)-{(2*S*,3*S*,5*S*)-5-Amino-1,6-diphenyl-2-[(thiazol-5-ylmethoxy)carbonylamino]hexan-3-yl} 2-{3-[(2-isopropylthiazol-4-yl)methyl]-3-methylureido}-3-methylbutanoate (unspecified degradant).

[n] Thiazol-5-ylmethyl (2*S*,3*S*,5*S*)-(5-*t*-butoxycarbonylamino)-3-hydroxy-1,6-diphenylhexan-2-ylcarbamate (may coelute with isobutoxycarbonyl aminoalcohol; report as isobutoxycarbonyl aminoalcohol using a relative response factor of 0.74).

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

[p] (*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 (unspecified degradant).

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

[r] 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.

[s] 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.

[t] 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.

[u] 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.

[v] 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.

[w] (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.

SPECIFIC TESTS

• **Alcohol Content**

Internal standard solution: Transfer 10.0 mL of butyl alcohol to a 200-mL volumetric flask and dilute with methanol to volume.

Internal standard identity solution: Dilute 5.0 mL of the *Internal standard solution* with methanol to 100 mL.

Standard stock solution: 4.0% (v/v) of dehydrated alcohol in methanol

Standard solution: 0.4% (v/v) of dehydrated alcohol prepared as follows. Transfer 10.0 mL of the *Standard stock solution* and 5 mL of the *Internal standard solution* to a 100-mL volumetric flask, and dilute with methanol to volume.

Sample stock solution: Transfer 5.0 mL of Oral Solution to a 50-mL volumetric flask with the aid of several portions of methanol, and dilute with methanol to volume.

Sample solution: Transfer 10.0 mL of the *Sample stock solution* and 5.0 mL of the *Internal standard solution* to a 100-mL volumetric flask, and dilute with methanol to volume.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m fused silica capillary; 1-µm layer of phase G16

Temperatures

Injection port: 185°

Detector: 220°

Column: See *Table 2*.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	5
40	10	145	6
145	20	200	9.75

Carrier gas: Helium

Flow rate: 4.5 mL/min

Makeup gas flow: 30 mL/min

Injection volume: 1 µL

Injection type: Split injection with a split ratio, 4:1

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–1.2 for the alcohol peak

Relative standard deviation: NMT 3.0% for the peak area ratio of alcohol to butyl alcohol

Analysis

Samples: *Internal standard identity solution*, *Standard solution*, and *Sample solution*

Calculate the percentage of alcohol in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times C \times D$$

R_U = peak area ratio of alcohol to butyl alcohol from the *Sample solution*

R_S = average peak area ratio of alcohol to butyl alcohol from the *Standard solution*

C = concentration of dehydrated alcohol in the *Standard solution* (% v/v)

D = dilution factor used to prepare the *Sample solution*, 100

Acceptance criteria: 40%–47% (v/v) of alcohol (C_2H_6O)

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total aerobic microbial count does not exceed 10^2 cfu/mL.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers. Store at room temperature.

• **USP Reference Standards ⟨11⟩**

USP Ritonavir RS

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

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

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