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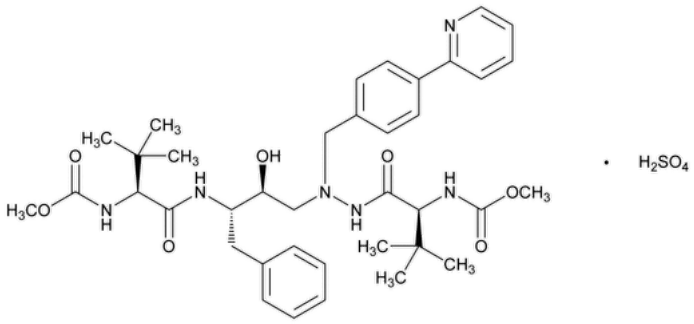
Add the following:

▲Atazanavir Sulfate



$C_{38}H_{52}N_6O_7 \cdot H_2SO_4$ 802.93

2,5,6,10,13-Pentaazatetradecanedioic acid, 3-12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[-4-(2-pyridinyl)phenyl]methyl]-, dimethyl ester, (3*S*,8*S*,9*S*,12*S*)-, sulfate (1:1) (salt);
Dimethyl (3*S*,8*S*,9*S*,12*S*)-9-benzyl-3,12,di-*tert*-butyl-8-hydroxy-4,11-dioxo-6-(*p*-2-pyridylbenzyl)-2,5,6,10,13-pentaazatetradecanedioate, sulfate (1:1) (salt) CAS RN®: 229975-97-7.

DEFINITION

Atazanavir Sulfate contains NLT 98.0% and NMT 102.0% of atazanavir sulfate ($C_{38}H_{52}N_6O_7 \cdot H_2SO_4$), calculated on the anhydrous and solvent-free basis.

IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197A or 197K
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. Identification Tests—General ⟨191⟩, *Chemical Identification Tests, Sulfate*:** Meets the requirements

ASSAY

- **Procedure**

Buffer: 2.73 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Acetonitrile and *Buffer* (50:50)

System suitability solution: 0.4 mg/mL of USP Atazanavir System Suitability Mixture RS in *Mobile phase*. Sonicate, if necessary, to dissolve prior to final dilution.

Standard solution: 0.4 mg/mL of USP Atazanavir Sulfate RS in *Mobile phase*. Sonicate, if necessary, to dissolve prior to final dilution.

Sample solution: 0.4 mg/mL of Atazanavir Sulfate in *Mobile phase*. Sonicate, if necessary, to dissolve prior to final dilution.

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: NLT 1.5 times the retention time of atazanavir

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for atazanavir *R*,*S*,*S*,*S*-diastereomer and atazanavir are 0.9 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.5 between atazanavir *R*,*S*,*S*,*S*-diastereomer and atazanavir, *System suitability solution*

Tailing factor: 0.8–1.5 for the atazanavir peak, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of atazanavir sulfate ($C_{38}H_{52}N_6O_7 \cdot H_2SO_4$) in the portion of Atazanavir Sulfate taken:

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

r_U = peak response of atazanavir from the *Sample solution*

r_S = peak response of atazanavir from the *Standard solution*

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

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

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.2%

Organic Impurities

[Note—Perform either *Procedure 1* or *Procedure 2*, depending on the synthetic route. *Procedure 2* is recommended if atazanavir formyl, ethyl, amine, or valine analogs are potential impurities.]

• **Procedure 1**

Buffer, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Solution A: Acetonitrile and *Buffer* (25:75)

Solution B: Acetonitrile and *Buffer* (75:25)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
45	0	100
50	100	0
60	100	0

Diluent: *Solution A* and *Solution B* (50:50)

Standard solution: 0.4 µg/mL of USP Atazanavir Sulfate RS in *Diluent*. Sonicate, if necessary, to dissolve prior to final dilution.

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between atazanavir *R,S,S,S*-diastereomer and atazanavir, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Atazanavir Sulfate taken:

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

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of atazanavir from the *Standard solution*

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

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

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

Acceptance criteria: See *Table 2*. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
1-Methyl-2-pyrrolidone	0.06	—	—[a]
Atazanavir related compound A	0.17	—	—[b]
Pyridinyl benzoic acid[c]	0.30	1.6	0.1
Pyridinyl benzaldehyde[d]	0.55	2.3	0.1
Dealkyl atazanavir[e]	0.66	0.59	0.1
Atazanavir benzylidenehydrazine analog[f]	0.76	1.5	0.1
Atazanavir *S,R,S,S*-diastereomer[g]	0.97	1.0	0.1
Atazanavir *R,S,S,S*-diastereomer[h]	0.99	1.0	0.1
Atazanavir	1.0	—	—
Atazanavir *S,S,S,R*-diastereomer[i]	1.03	1.0	0.1
Atazanavir *S,R,R,S*-diastereomer[j]	1.06	1.0	0.1
Atazanavir benzylidenehydrazine carbamate[k]	1.3	1.5	0.1
Atazanavir di-*tert*-butyl analog[l]	1.4	1.0	0.1
Any individual unspecified impurity	—	1.0	0.1
Total impurities[m]	—	—	0.5

[a] For information only; disregard if not used in the process.

[b] This impurity is determined by the *Limit of Atazanavir Related Compound A* test.

[c] 4-(Pyridin-2-yl)benzoic acid.

[d] 4-(Pyridin-2-yl)benzaldehyde.

[e] Methyl [(5*S*,10*S*,11*S*,14*S*)-11-benzyl-5-(*tert*-butyl)-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-2-oxa-4,7,8,12-tetraazahexadecan-14-yl]carbamate.

[f] (2*S*,3*S*)-3-Amino-4-phenyl-1-{1-[4-(pyridin-2-yl)benzyl]-2-[4-(pyridin-2-yl)benzylidene]hydrazinyl}butan-2-ol.

[g] Methyl {(5*S*,10*R*,11*S*,14*S*)-11-benzyl-5-(*tert*-butyl)-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[4-(pyridin-2-yl)benzyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl}carbamate.

[h] Methyl {(5*R*,10*S*,11*S*,14*S*)-11-benzyl-5-(*tert*-butyl)-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[4-(pyridin-2-yl)benzyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl}carbamate.

[i] Methyl {(5*S*,10*S*,11*S*,14*R*)-11-benzyl-5-(*tert*-butyl)-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[4-(pyridin-2-yl)benzyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl}carbamate.

[j] Methyl {(5*S*,10*R*,11*R*,14*S*)-11-benzyl-5-(*tert*-butyl)-10-hydroxy-15,15-dimethyl-3,6,13-trioxo-8-[4-(pyridin-2-yl)benzyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl}carbamate.

[k] Methyl [(*S*)-1-{[(2*S*,3*S*)-3-hydroxy-1-phenyl-4-{1-[4-(pyridin-2-yl)benzyl]-2-[4-(pyridin-2-yl)benzylidene]hydrazinyl}butan-2-yl]amino}-3,3-dimethyl-1-oxobutan-2-yl]carbamate.

[l] *tert*-Butyl 2-{(2*S*,3*S*)-3-[(*tert*-butoxycarbonyl)amino]-2-hydroxy-4-phenylbutyl}-2-[4-(pyridin-2-yl)benzyl]hydrazinecarboxylate.

[m] Total impurities includes the sum of all impurities found in the tests for *Limit of Atazanavir Related Compound A* and *Organic Impurities, Procedure 1*.

• **Procedure 2**

[Note—This procedure is recommended if atazanavir formyl, ethyl, amine, or valine analogs are potential impurities.]

Buffer: 1.6 g/L of ammonium formate in water. Adjust with formic acid to a pH of 4.0. Add 50 mL of methanol per liter of the solution.

Mobile phase: Acetonitrile and *Buffer* (40:60)

Standard stock solution: 0.23 mg/mL of USP Atazanavir Sulfate RS in *Mobile phase*. Sonicate, if necessary, to dissolve prior to final dilution.

Standard solution: 0.023 mg/mL of USP Atazanavir Sulfate RS in *Mobile phase* from *Standard stock solution*. Prepare this solution fresh.

Sample solution: 0.23 mg/mL of Atazanavir Sulfate in *Mobile phase*. Sonicate, if necessary, to dissolve prior to final dilution.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection volume: 10 µL

Run time: NLT 4.7 times the retention time of atazanavir

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Atazanavir Sulfate taken:

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

r_U = peak response of any individual impurity from the *Sample solution*

r_S = peak response of atazanavir from the *Standard solution*

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

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

Acceptance criteria: See *Table 3*. The reporting threshold is 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Atazanavir amine analog[a]	0.27	0.15
Atazanavir formyl analog[b]	0.55	0.15
Atazanavir valine analog[c]	0.73	0.15
Atazanavir	1.0	—
Atazanavir ethyl analog[d]	1.45	0.15
Atazanavir dipeptide analog[e]	1.60	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

[a] Methyl [(*S*)-1-{[(2*S*,3*S*)-4-{2-[(*S*)-2-amino-3,3-dimethylbutanoyl]-1-[4-(pyridin-2-yl)benzyl]hydrazinyl}-3-hydroxy-1-phenylbutan-2-

[a] Methyl [(*S*)-1-{[(2*S*,3*S*)-4-{2-[(*S*)-2-amino-3,3-dimethylbutanoyl]-1-[4-(pyridin-2-yl)benzyl]hydrazineyl}-3-hydroxy-1-phenylbutan-2-yl]amino}-3,3-dimethyl-1-oxobutan-2-yl]carbamate.

[b] Methyl [(*S*)-1-{[(2*S*,3*S*)-4-{2-[(*S*)-2-formamido-3,3-dimethylbutanoyl]-1-[4-(pyridin-2-yl)benzyl]hydrazineyl}-3-hydroxy-1-phenylbutan-2-yl]amino}-3,3-dimethyl-1-oxobutan-2-yl]carbamate.

[c] Methyl {(5*S*,10*S*,11*S*,14*S*)-11-benzyl-5-(*tert*-butyl)-10-hydroxy-15-methyl-3,6,13-trioxo-8-[4-(pyridin-2-yl)benzyl]-2-oxa-4,7,8,12-tetraazahexadecan-14-yl}carbamate sulfate.

[d] Methyl {(6*S*,11*S*,12*S*,15*S*)-12-benzyl-6-(*tert*-butyl)-11-hydroxy-16,16-dimethyl-4,7,14-trioxo-9-[4-(pyridin-2-yl)benzyl]-3-oxa-5,8,9,13-tetraazaheptadecan-15-yl}carbamate.

[e] Methyl {(5*S*,8*S*,13*S*,14*S*,17*S*)-14-benzyl-5,8-di-*tert*-butyl-13-hydroxy-18,18-dimethyl-3,6,9,16-tetraoxo-11-[4-(pyridin-2-yl)benzyl]-2-oxa-4,7,10,11,15-pentaazanonadecan-17-yl}carbamate.

• **Limit of Atazanavir Related Compound A**

[Note—Perform this test when *Organic Impurities, Procedure 1* is used.]

Buffer: 2.73 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Solution A: Acetonitrile and *Buffer* (25:75)

Solution B: Acetonitrile and *Buffer* (75:25)

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	95	5
5	95	5
8	0	100
14	0	100
15	95	5
25	95	5

Diluent: *Solution A* and *Solution B* (50:50)

System suitability solution: 1.1 mg/mL of USP Atazanavir Sulfate RS and 1 µg/mL of USP Atazanavir Related Compound A RS in *Diluent*. Sonicate, if necessary, to dissolve prior to final dilution.

Standard stock solution: 0.01 mg/mL of USP Atazanavir Related Compound A RS in *Diluent*

Standard solution: 1 µg/mL of USP Atazanavir Related Compound A RS in *Diluent* from *Standard stock solution*. Store this solution at 5°.

Sample solution: 1.14 mg/mL of Atazanavir Sulfate in *Diluent*. Sonicate, if necessary, to dissolve prior to final dilution.

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3-µm packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[Note—The relative retention times for atazanavir related compound A and atazanavir are 0.4 and 1.0, respectively.]

Suitability requirements

Tailing factor: 0.8–1.5 for the atazanavir peak, *System suitability solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of atazanavir related compound A in the portion of Atazanavir Sulfate taken:

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

r_U = peak response of atazanavir related compound A from the *Sample solution*

r_S = peak response of atazanavir related compound A from the *Standard solution*

C_S = concentration of USP Atazanavir Related Compound A RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: NMT 0.1%. Disregard the atazanavir related compound A peak when it is less than 0.05%, and do not include it in the total impurities calculation.

SPECIFIC TESTS

• **Water Determination ⟨921⟩:** NMT 2.5%

• **Optical Rotation ⟨781S⟩, *Procedures*, *Specific Rotation***

Sample solution: 10 mg/mL of Atazanavir Sulfate in methanol

Acceptance criteria: −40° to −44°

ADDITIONAL REQUIREMENTS

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

• **Labeling:** If a test for *Organic Impurities* other than *Organic Impurities, Procedure 1* is used, then the labeling states the test with which the article complies.

• **USP Reference Standards ⟨11⟩**

USP Atazanavir Related Compound A RS

(*S*)-2-[(Methoxycarbonyl)amino]-3,3-dimethylbutanoic acid.

$C_8H_{15}NO_4$ 189.21

USP Atazanavir Sulfate RS

USP Atazanavir System Suitability Mixture RS

This is a mixture of atazanavir sulfate, atazanavir *R,S,S,S*-diastereomer, and atazanavir *S,R,S,S*-diastereomer (other impurities may also be present).▲ (USP 1-Aug-2022)

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

Topic/Question	Contact	Expert Committee
ATAZANAVIR SULFATE	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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