Atazanavir Sulphate

Published on: 07 February, 2024 **Last date for comments:** 22 March, 2024

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Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Manufacturers are also invited to submit samples of their products to the IPC to ensure that the proposed monograph adequately controls the quality of the product(s) they manufacture. Comments and samples received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in, with a copy to Dr. Gaurav Pratap Singh (email: gpsingh.ipc@gov.in) before the last date for comments.

Document History and Schedule for the Adoption Process

Description	Details
Document version	1.0
First draft published on IPC website for public comments	February 7, 2024
Last date for comments	**March 22, 2024**
Monograph revisions proposed for inclusion in	**IP 2026**
Tentative effective date of monograph revisions	**July, 2026**
Draft revision published on IPC website for public comments	--
Further follow-up action as required.	

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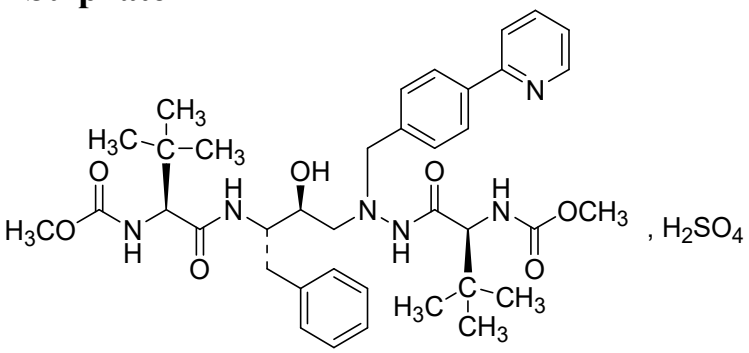
Change **to**: **Atazanavir Sulphate**



$C_{38}H_{52}N_6O_7,H_2SO_4$ Mol. Wt. 802.9

Atazanavir Sulphate is salt with sulphuric acid of (3*S*,8*S*,9*S*,12*S*)-3,12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl)phenyl]methyl]-2,5,6,10,13-pentaazatetradecanedioic acid dimethyl ester.

Atazanavir Sulphate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{38}H_{52}N_6O_7,H_2SO_4$, calculated on the anhydrous basis.

Category. Antiretroviral.

Description. A white to pale yellow crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *atazanavir sulphate IPRS* or with the reference spectrum of atazanavir sulphate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

C. It gives reaction (A) of sulphates (2.3.1).

Tests

Specific optical rotation (2.4.22). -44° to -40°, determined in 1.0 per cent w/v solution in *methanol*.

Related substances. *NOTE- On the basis of the synthetic route, perform either test A or test B. Test B is recommended if atazanavir formyl, ethyl, amine, or valine analogs are potential impurities.*

A. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 2.73 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 3.5 with *orthophosphoric acid*.

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution. Dissolve 20 mg of the substance under examination in the solvent mixture, with the aid of ultrasound and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.04 per cent w/v solution of *atazanavir sulphate IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture. Further dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (c). A 0.04 per cent w/v solution of *atazanavir system suitability mixture IPRS (containing atazanavir sulphate, atazanavir R,S,S,S-diastereomer, atazanavir S,R,S,S-diastereomer)* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm) (Such as Ymc-pack ODS-AQ),
- mobile phase: A. a mixture of 75 volumes of the buffer solution and 25 volumes of *acetonitrile*,
  B. a mixture of 25 volumes of the buffer solution and 75 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
5	100	0
45	0	100
50	100	0
60	100	0

Name	Relative retention time	Correction factor
1-Methyl-2-pyrrolidone[#]	0.06	---
Atazanavir related compound A[1*]	0.17	---
Pyridinyl benzoic acid[2]	0.30	0.63
Pyridinyl benzaldehyde[3]	0.55	0.43
Dealkyl atazanavir[4]	0.66	1.7
Atazanavir benzylidenehydrazine analog[5]	0.76	0.67
Atazanavir *S*,*R*,*S*,*S*-diastereomer[6]	0.97	---
Atazanavir *R*,*S*,*S*,*S*-diastereomer[7]	0.99	---
Atazanavir	1.0	---
Atazanavir *S*,*S*,*S*,*R*-diastereomer[8]	1.03	---
Atazanavir *S*,*R*,*R*,*S*-diastereomer[9]	1.06	---
Atazanavir benzylidenehydrazine carbamate[10]	1.3	0.67
Atazanavir di-tert-butyl analog[11]	1.4	---

[#]For information only; disregard if not used in the process,
[*] This impurity is determined by the Limit of Atazanavir related compound A test,
[1](*S*)-2-[(Methoxycarbonyl)amino]-3,3-dimethylbutanoic acid,
[2]4-(Pyridin-2-yl)benzoic acid,
[3]4-(Pyridin-2-yl)benzaldehyde,
[4]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,
[5](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,
[6]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,
[7]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,
[8]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,
[9]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,
[10]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,
[11]*tert*-Butyl 2-{(2*S*,3*S*)-3-[(*tert*-butoxycarbonyl)amino]-2-hydroxy-4-phenylbutyl}-2-[4-(pyridin-2-yl)benzyl]hydrazinecarboxylate.

Inject reference solution (b) and (c). The test is not valid unless the resolution between the peaks due to atazanavir *R*,*S*,*S*,*S*-diastereomer and atazanavir is not less than 1.5 in the chromatogram obtained reference solution (c) and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to pyridinyl benzoic acid, pyridinyl benzaldehyde, dealkyl atazanavir, atazanavir benzylidenehydrazine

analog, atazanavir *S*,*R*,*S*,*S*-diastereomer, atazanavir *R*,*S*,*S*,*S*-diastereomer, atazanavir *S*,*S*,*S*,*R*-diastereomer, atazanavir *S*,*R*,*R*,*S*-diastereomer, atazanavir benzylidenehydrazine carbamate and atazanavir di-tert-butyl analog, each of, is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

The sum of all the secondary peaks including atazanavir related compound A is not more than 0.5 per cent.

B. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 23 mg of the substance under examination in the mobile phase, with the aid of ultrasound and dilute to 100.0 ml with the mobile phase.

Reference solution. A 0.0023 per cent w/v solution of *atazanavir sulphate IPRS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Zorbax RX-C8),
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by dissolving 1.6 g of *ammonium formate* in 1000 ml of *water*, adjusted to pH 4.0 with *formic acid*, add 50 ml of *methanol* and 40 volumes of *acetonitrile,*
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume: 10 µl.

Name	Relative retention time
Atazanavir amine analog[1]	0.27
Atazanavir formyl analog[2]	0.55
Atazanavir valine analog[3]	0.73
Atazanavir	1.0
Atazanavir ethyl analog[4]	1.45
Atazanavir dipeptide analog[5]	1.60

[1]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,

[2]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,

[3]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 sulphate,

[4]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.

[5]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.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. Run the chromatogram 4.7 times the retention time of the principal peak. The area of any peak corresponding to atazanavir amine analog, atazanavir formyl analog, atazanavir valine analog, atazanavir ethyl analog and atazanavir dipeptide analog, each of, is not more than 0.015 times the area of the principal peak in the chromatogram obtained with the reference solution (0.15 per cent), the area of any other secondary peak is not more than 0.01 times the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any peak with an area less than 0.005 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Limit of Atazanavir related compound A. Determine by liquid chromatography (2.4.14). *(NOTE- Perform this test when Related substances A is used).*

Solvent mixture. Equal volumes of mobile phase A and mobile phase B.

Test solution. Dissolve 114 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.0001 per cent w/v solution of *atazanavir related compound A IPRS* in the solvent mixture (*Note- Store this solution at 5°*).

Reference solution (b). A 0.11 per cent w/v solution of *atazanavir sulphate IPRS* in reference solution (a).

Use chromatographic system as described under Related substances A with the following modifications
– injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
5	95	5
8	0	100
14	0	100
15	95	5
25	95	5

The relative retention time with reference to atazanavir for atazanavir related compound A is about 0.4.

Inject reference solution (a) and (b). The test is not valid unless the tailing factor is not less than 0.8 and not more than 1.5 for atazanavir peak in the chromatogram obtained reference solution (b) and the relative standard deviation for replicate injections is not more than 5.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to atazanavir related compound A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). Ignore the peak if the area is less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent) and do not include in calculation of total impurities.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 2.5 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography as described under Related substances A with the following modifications.

– mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 2.73 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 3.5 with *orthophosphoric acid* and 50 volumes of *acetonitrile.*

Inject reference solution (a) and (c). The test is not valid unless the resolution between the peaks due to atazanavir *R*,*S*,*S*,*S*-diastereomer and atazanavir is not less than 1.5, the tailing factor is not less than 0.8 and not more than 1.5 for atazanavir peak in the chromatogram obtained reference solution (c) and the relative standard deviation for replicate injections is not more than 1.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution.

Calculate the content of $C_{38}H_{52}N_6O_7,H_2SO_4$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Labelling. The label states, if a test for related substances other than method A is used, the test with which the article complies.