CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.7548247

Available online at: http://www.iajps.com *Research Article*

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANIOUS ESTIMATION OF ATAZANAVIR AND COBICISTAT IN TABLET DOSAGE FORM

M.Swathi, V.Kiran Kumar
Mother Teresa College of Pharmacy, Ghatkesar, NFC Nagar, Hyderabad, Telangana, India

Abstract:
A simple sensitive, and precise high performance liquid chromatographic method for the analysis of atazanavir and cobicistat has been developed and validated for the simultaneous determination of compounds in commercial pharmaceutical products. The compounds were well separated on ODS intersil C18 reverse phase column by the use of mobile phase of Orthophosphoric acid acetonitrile in a ratio of 45:55 v/v at a flow rate of 1.0 ml/min with detection wavelength at 272 nm. The retention time of atazanavir and cobicistat was found to be 4.135min and1.668min the method was validated in terms of linearity, precision, accuracy, and specificity, robustness, ruggedness and solution stability Degradation studies like acid, base, peroxide, thermal, uv and water. The method dies require only 10 min as run time for analysis which prove the adoptability of the method for the routine quality control analysis of the drug.

Keywords: *Atazanavir and Cobicistat, RP-HPLC, Simultaneous estimation.*

Corresponding author:
M. Swathi,
M. Pharmacy,
Mother Teresa College of Pharmacy,
Department of pharmaceutical Analysis,
Ghatkesar, NFC Nagar
Hyderabad, Telangana, India
E-mail: anithaorsu0826@gmail.com

QR code

Please cite this article in press M. Swathi et al, ***Formulation, RP-HPLC Method Development And Validation For Simultaneous Estimation Of Atazanavir And Cobicistat In Tablet Dosage Form.,*** *Indo Am. J. P. Sci, 2022; 09(12).*

INTRODUCTION:

Atazanavir is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults and pediatric patients 3 months of age and older weighing at least 5kg.[1] Atazanavir is also indicated in combination with cobicistat and other antiretrovirals for the treatment of HIV-1 infection in adults and pediatric patients weighing at least 35kg.[2] Atazanavir selectively inhibits the virus-specific processing of viral Gag and Gag-Pol polyproteins in HIV-1 infected cells by binding to the active site of HIV-1 protease, thus preventing the formation of mature virions. Atazanavir is not active against HIV-2. IUPAC name methyl N-[(1S)-1-{N'-[(2S,3S)-2-hydroxy-3-[(2S)-2-[(methoxycarbonyl)amino]-3,3-dimethylbutanamido]-4-phenylbutyl]-N'- {[4-(pyridin-2 yl) phenyl]methyl}hydrazinecarbonyl}-2,2-dimethylpropyl]carbamate. Molecular formula $C_{38}H_{52}N_6O_7$. Molecular Weight 704.8.

Cobicistat is a CYP3A inhibitor indicated to increase systemic exposure of atazanavir or darunavir (once daily dosing regimen) in combination with other antiretroviral agents in the treatment of HIV-1 infection.[3] It is not interchangeable with ritonavir to increase systemic exposure of darunavir 600 mg twice daily, fosamprenavir, saquinavir, or tipranavir due to lack of exposure data.[4] Cobicistat is a mechanism-based inhibitor of cytochrome P450 3A (CYP3A) isoforms. Inhibition of CYP3A-mediated metabolism by cobicistat increases the systemic exposure of CYP3A substrates atazanavir and darunavir and therefore enables increased anti-viral activity at a lower dosage. Cobicistat does not have any anti-HIV activity on its own. IUPAC Name 1,3-thiazol-5-ylmethyl *N*-[(2*R*,5*R*)-5-[[(2*S*)-2-[[methyl-[(2-propan-2-yl-1,3-thiazol-4-yl)methyl]carbamoyl]amino]-4-morpholin-4ylbutanoyl]amino]-1,6 diphenylhexan-2-yl]carbamate. Molecular Formula $C_{40}H_{53}N_7O_5S_2$. Molecular Weight 776.

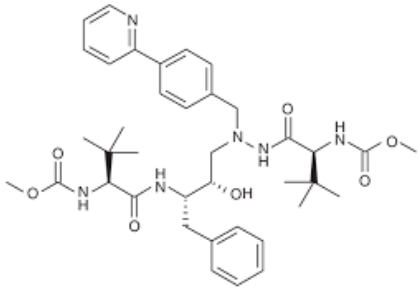

Figure 1: Structure of Atazanavir

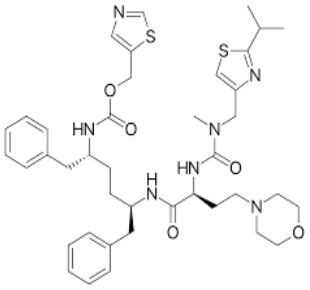

Figure 2: Structure of Cobicistat

The literature survey revealed that There are very few methods reported in the literature for analysis of Atazanavir and Cobicistat alone or in combination with other drugs in the pure form and pharmaceuticals formulations by RP-HPLC.[5-14] In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Atazanavir and Cobicistat Simultaneous estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Atazanavir and Cobicistat. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Atazanavir and Cobicistat in pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Atazanavir and Cobicistat were Purchased from Sun Pharma India Limited. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment's: The Waters HPLC system with a UV or photo diode array detector was used for method development and validation. The output signal was monitored and processed by using Empower software. Chromatographic condition: The mobile phase used 0.1% Orthophosphoric acid, buffer and Acetonitrile in the gradient mode employing at a flow rate of 1.2 ml/min. The analytical column used Inertsil ODS 3V (4.0 x 250mm, 5µm). The detection was carried out at a wavelength of 270nm for a run time of 10 min. Diluent used as Acetonitrile and hplc grade water %B 0.00 90.0 10.0 3.00 40.0 60.0 5.00

10.0 90.0 8.00 10.0 90.0 8.10 90.0 10.0 12.00 90.010.0

preparation of standard solution

Accurately Weighed and transferred working Standards of 15 mg of Cobicistat and 30 mg of Atazanavir into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solution was taken into a 10ml volumetric flask and made up to 10ml.

Assay of Pharmaceutical Dosage form: (Sample Preparation)

1tablet was weighed, powdered and then the weight was transferred into a 100mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Method development

Chromatographic parameters were preliminary optimized to develop HPLC method for simultaneous atazanavir and cobicistat with short analyses time (10min), and acceptable resolution (> 2). The isoabsoprtive point of atazanavir and cobicistat selected was 270 nm. In order to identify a suitable organic modifier, various compositions of acetonitrile and methanol were tested along with different buffers. Different columns like X-terra, Inertsil, inspire columns were tried. Resolution was the major problem while we are developing method. Resolution was less very less when we are using one organic phase, to increase resolution acetonitrile were used in isocratic mode.

Finally, separation for simultaneous determination of atazanavir and cobicistat was carried out by gradient elution with a flow rate of 1 ml/min inertsil (ODS 250 x 4.6 mm, 5µm.)

System suitability: All the system suitability parameters are within range and satisfactory as per ICH guidelines.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Atazanavir and Cobicistat in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 37.5 ppm to 225 ppm and 75 ppm to 450 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Atazanavir and Cobicistat and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The resulte are shown in table 6.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The results are shown in table 7.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 8.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

RESULTS AND DISCUSSION:

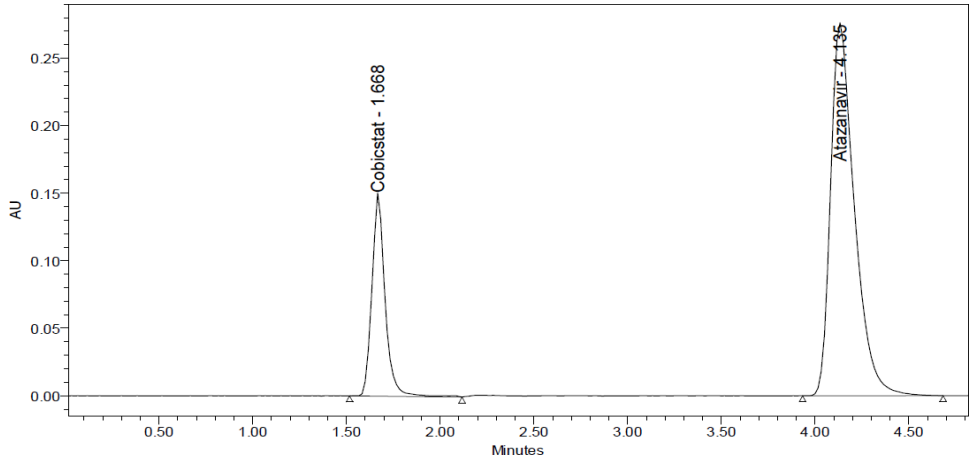

Figure 3: Standard chromatogram

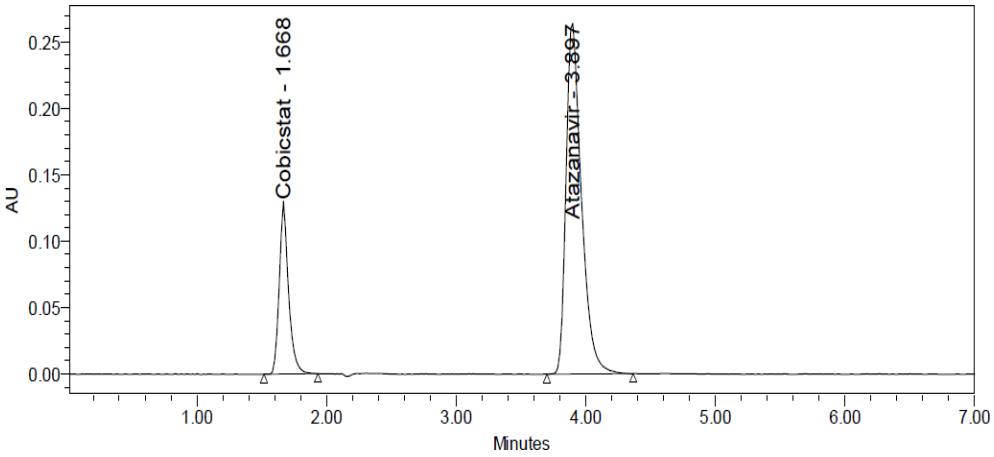

Figure 4: Sample chromatogram

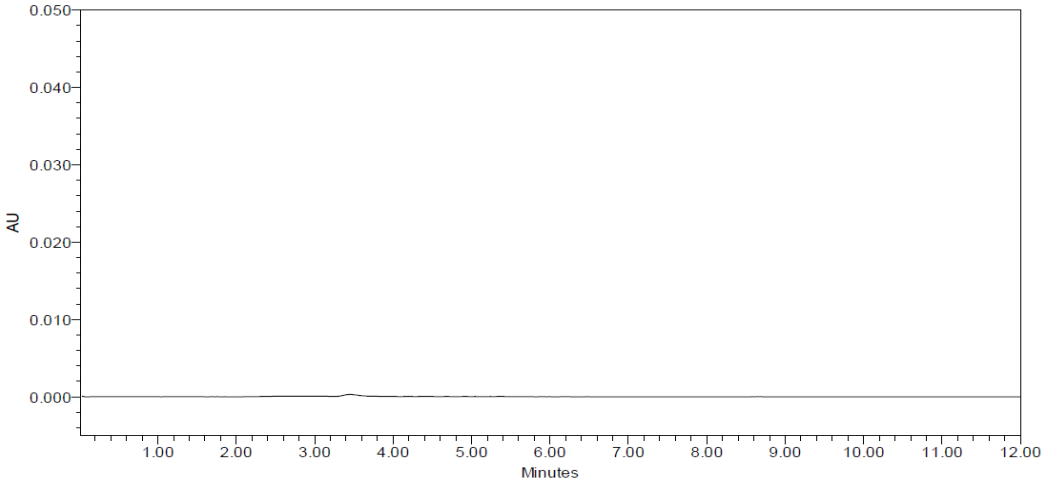

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameters	Atazanavir	Cobicistat
Retention time	4.34	2.23
USP Plate count	2614	2632
USP Tailing	1.6	1.8

Table 2: Assay results for Atazanavir and Cobicistat

S. No.	Cobicistat %Assay	Atazanavir %Assay
1	99.54	99.00
2	100.17	99.91
3	101.07	100.84
4	101.14	99.79
5	100.35	100.84
6	101.25	99.97
AVG	100.59	100.1
STDEV	0.6815	0.6987
%RSD	0.68	0.70

Table 3: Linearity results of Cobicistat and Atazanavir

S.no	Concentration Cobicistat (µg/ml)	Response	Concentration Atazanavir (µg/ml)	Response
1	0	0	0	0
2	37.5	191498	6.25	225114
3	75	390010	12.5	414984
4	112.5	546140	18.75	622694
5	150	738926	25	837022
6	187.5	946014	31.25	1049477
7	225	1125330	37.5	1273064

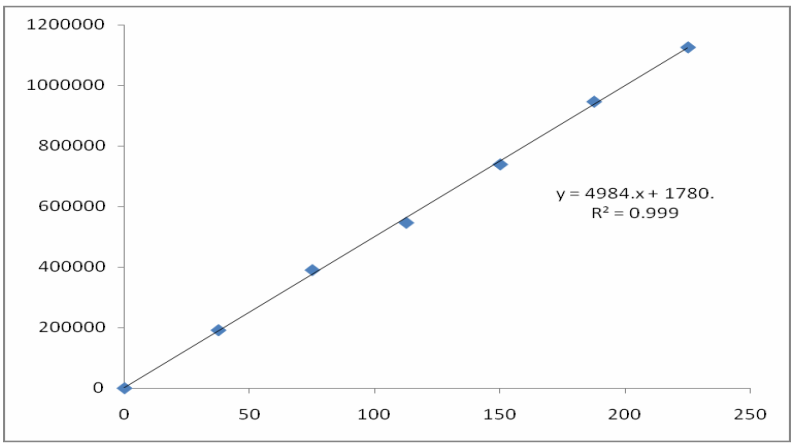

Figure 6: Linearity graph for Cobicista

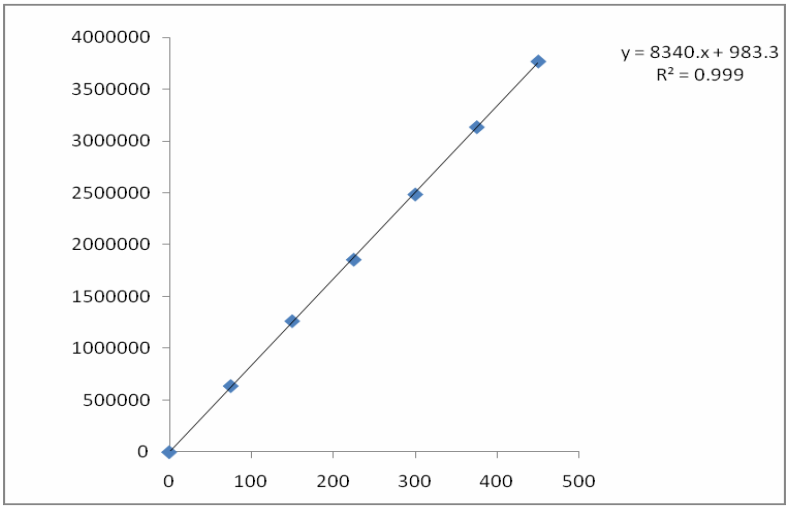

Figure 7: Linearity graph for Atazanavir

Table 4: Showing accuracy results for Cobicistat and **Atazanavir**

Sample	**Amount added (µg/ml)**	**Amount Recovered (µg/ml)**	**Recovery (%)**	**% RSD**
Cobicistat	75	75.47	100.63	1.01
	150	151.7	101.13	0.56
	225	224.01	99.56	1.75
Atazanavir	150	151.19	100.80	0.37
	300	305.36	101.79	1.18
	450	455.3	101.18	0.25

Table 5: Precision results for Cobicistat and Atazanavir

Sr. No.	Cobicistat	Atazanavir
1	718678	2466350
2	715658	2466490
3	738088	2456197
4	740141	2453992
5	739309	2453388
6	740816	2447690
Mean	732115	2457351
Std. Dev.	11652.8	7565
%RSD	1.592	0.3

Table 6. Ruggedness results of Cobicistat and Atazanavir.

Sr. No.	Cobicistat	Atazanavir
1	736679	2470266
2	738088	2463991
3	747936	2460592
4	739309	2454003
5	721714	2455955
Mean	737722	2461678.5
Std. Dev.	8811.1	6079
%RSD	1.19	0.2

Table 7: Robustness data of Cobicistat and Atazanavir method.

S.NO	Robustness condition	Cobicistat %RSD	Atazanavir %RSD
1	Flow minus	0.8	1.3
2	Flow Plus	0.2	0.4
3	Mobile phase minus	0.2	2.5
4	Mobile phase Plus	0.1	0.7
5	Temperature minus	0.3	2.5
6	Temperature Plus	0.6	0.4

Table 8: LOD, LOQ of Cobicistat and Atazanavir

Drug	LOD	LOQ
Cobicistat	1.32	4.01
Atazanavir	0.40	1.22

CONCLUSION:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Cobicistat and Atazanavir in Tablet dosage form. Retention time of Cobicistat and Atazanavir were found to be 1.668 min and 4.135 min. %RSD of the Cobicistat and Atazanavir were and found to be 0.68 and 0.70 respectively. %Recover was Obtained as 100.59% and 100.1% for Cobicistat and Atazanavir. LOD, LOQ values were obtained from regression equations of Cobicistat and Atazanavir were 1.32 ppm, 4.01 ppm and 0.40ppm, 1.22 ppm respectively. Regression

equation of Cobicistat is y = 4984.x + 1780, and of Atazanavir is y = 8340.x +983.3. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

REFERENCES:

1. FDA Approved Drug Products: Reyataz (atazanavir) for oral use
2. FDA Approved Drug Products: Evotaz (atazanavir and cobicistat) oral tablets
3. Mathias AA, German P, Murray BP, Wei L, Jain A, West S, Warren D, Hui J, Kearney BP: Pharmacokinetics and pharmacodynamics of GS-9350: a novel pharmacokinetic enhancer without anti-HIV activity. Clin Pharmacol Ther. 2010 Mar;87(3):322-9. doi: 10.1038/clpt.2009.228. Epub 2009 Dec 30. [Article]
4. Nanda K, Stuart GS, Robinson J, Gray AL, Tepper NK, Gaffield ME: Drug interactions between hormonal contraceptives and antiretrovirals. AIDS. 2017 Apr 24;31(7):917-952. doi:
5. Sindu Priya, D., and Gowri Sankar, D., Stability-indicating reversed-phase high performance liquid chromatography method for the simultaneous estimation of darunavir and ritonavir. American Journal of Pharmatech Research, 2015. 6(1): 527-540.
6. Masthannamma, S.K., et al. Asian J Pharm Clin Res, 2016. 9(3): 62-70.
7. Panigrahy, U.P., Sunil Kumar A., A novel validated RP-HPLC-DAD method for the simultaneous estimation of Netupitant and Palonosetron in bulk and pharmaceutical dosage form with forced degradation studies Int J Pharm Sci, Rev. Res 2016. 36(2), 82-89.
8. Dnyaneshwar, S.P., et al. Development and validation of RP-HPLC method for the simultaneous estimation of atazanavir sulphate and ritonavir in bulk and formulations International Journal of Pharmacy and Pharmaceutical Sciences, 2013. 5(3): 33-38.
9. Purnima, V.B., et al. RP-HPLC method development for the assay and degradation study of cobicistat and atazanavir sulphate in bulk and combined dosage form. European Journal of Biomedical and Pharmaceutical sciences, 2016. 3(5): 450-461.
10. Pratik Vora, K., Ameta, S.C., Shirsat, M.K., Forced degration studies on atazanavir and cobicistat by RP-HPLC method, International Journal of Pharma and Bio Sciences, 2017. 8(1): 75-84.
10. Kumar, B.K., International Journal of Pharmacy and Industrial Research, 2015. 5 (4), 167-177
11. Nagaraju, P., et al. World Journal of Pharmacy and Pharmaceutical Sciences, 5(8): 650-671.
12. Venkata, S.S.N.M., Haribabu, B., and Rama Krishna Veni, P., Stability indicating RP-HPLC method for simultaneous estimation of atazanavir and cobicistat in tablets. British Journal of Pharmaceutical Research, 2016. 11(6): 1-10.
13. Pal, N., Rao, AS., and Ravikumar, P., Asian Journal of Chemistry, 2016. 28 (2): 273-276.