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Analytical Method Development and Validation Method for The Estimation of Related Impurities in Combined Dosage Form of Atazanavir and Cobicistat By RP-HPLC

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Abstract
A simple, economic, selective, and precise RP-HPLC method has been developed and validated for the estimation of related impurities of Atazanavir and Cobicistat in combined dosage form. Chromatographic separation has been achieved by using LC-20 AT C_{18} (250mm x 4.6mm, 5µm) column and using mobile phase buffer (potassium dihydrogen phosphate, pH 3.0): acetonitrile (60:40), 1mL/min was a flow rate. Samples were scanned at a wavelength of 224 nm. As per the ICH guidelines the method is validated. Retention time of Cobicistat and Atazanavir were found to be 5.247 and 3.513 respectively and the retention time of Atazanavir impurity and Cobicistat impurity were found to be 2.747 minute and 4.370 minute respectively. Validation of the method was successfully established by performing various validation parameters such as specificity, precision, linearity, accuracy, LOD, LOQ, robustness, ruggedness according to ICH guidelines. The linearity was observed in the range of 2.5-7.5 µg/mL for related impurities of Atazanavir and 2.5-7.5 µg/mL for related impurities of Cobicistat. The LOD value was found 0.206 µg/mL and 0.342 µg/mL for Atazanavir impurity and Cobicistat impurity respectively. The proposed method was found to be specific, linear, sensitive, precise, accurate and robust in nature.

Keywords
Atazanavir, Cobicistat, related impurities RP-HPLC Method, Validation.

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INTRODUCTION:
HIV medications can lower your chances of transmitting HIV. These medicines control the growth of the virus [1]. Atazanavir is used to treat human immunodeficiency virus (HIV-1) infection in adults. HIV is a retrovirus, and it differs from viruses, it is causing lifelong infections [2]. Atazanavir chemical name is methyl N[(1S)-1-{[(2S,3S)-3-hydroxy-4 [(2S)-2-[(methoxycarbonyl) amino]-3,3-dimethylN' {[-4-(pyridine-2yl) phenyl]methyl} butane hydrazido]-1-phenylbutan-2-yl]-carbamoyl}-2,2-dimethylpropyl]-carbamate.The related impurities of Atazanavir is 3R,4R,5S,6R)-5-(((4aR,7R,8R,8aR)-7,8-dihydroxy-2-(4-(pyridin-2yl)phenyl)

hexahydropyrano [3, 2-d][1,3]-dioxin-6-yl) oxy)-6-(hydroxy methyl) - tetrahydro-2H-pyran-2,3,4-triol. Structure of Atazanavir and its related impurities are given in **(figure 1 & 3).**

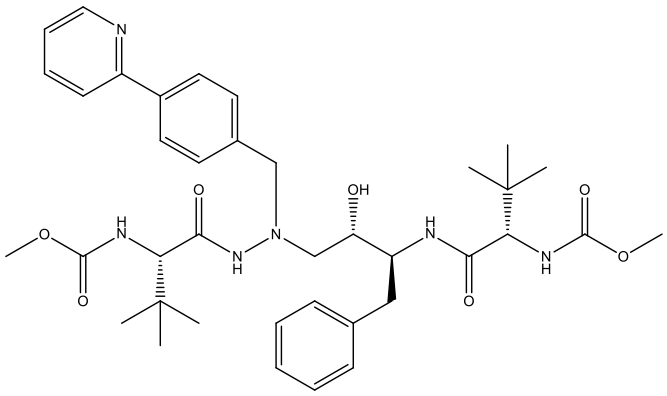

Fig.1: Structure of Atazanavir

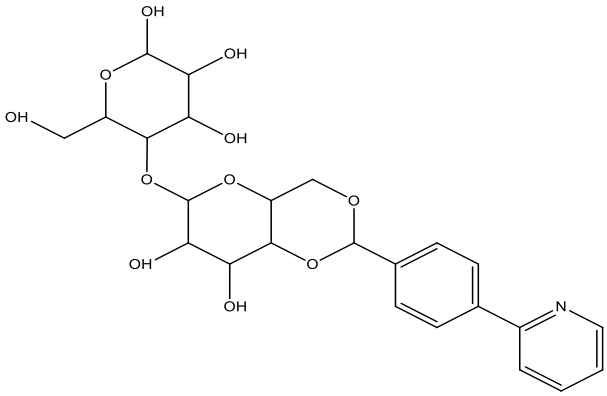

Fig.3: Structure of Atazanavir impurity

Atazanavir chemical formula is $C_{38}H_{52}N_6O_7$, and molecular weight is 704.856 g/mol. Atazanavir is drug of the protease inhibitors (PI) class [3, 4]. The U.S. food and drug administration (FDA) approved Atazanavir on June 20, 2003.

Cobicistat chemical name is Thiazol-5-ylmethylN- [1-benzyl-4- [[2- [[(2-isopropyl thiazol-4-yl) methyl carbamoyl] amino]-4-morpholinobutanoyl] amino]5-phenylpentyl] carbamate. The related impurities of Cobicistat is thiazol-5-ylmethyl ((2R, 5R)-5-((S)-2-(3-((2-(2-hydroxypropan-2-yl) thiazol-4-yl)-methyl)-3-methylureido)-4-morpholinobutanamido)-1,6diphendiphenylehexan2yl) carbamate. Structure of Cobicistat and its related impurities are given in **(figure 2 &4).**

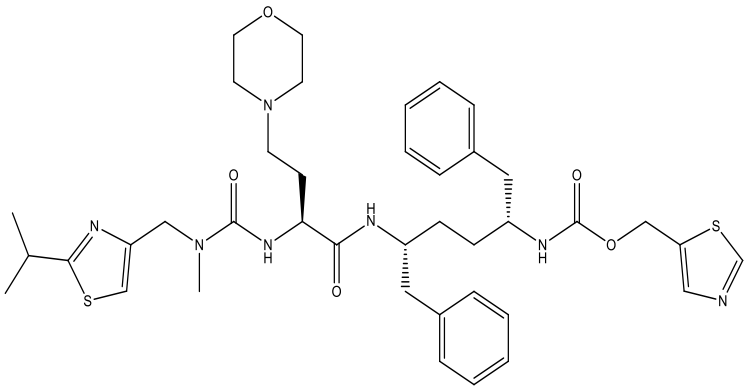

Fig.2: Structure of Cobicistat

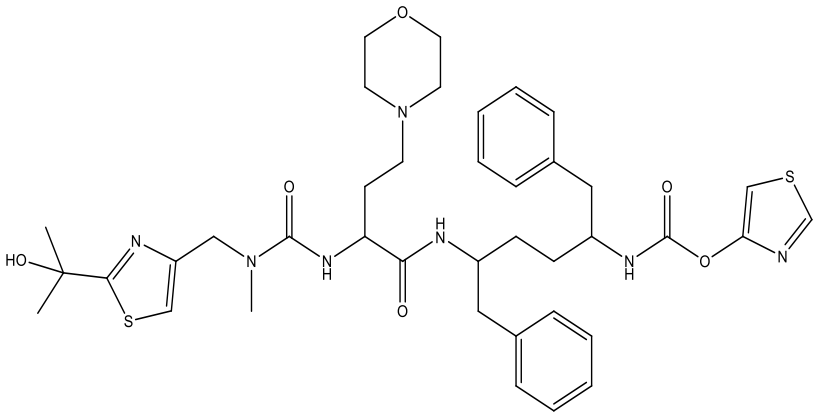

Fig.4: Structure of Cobicistat impurity

Cobicistat molecular formula is $CH_{53}N_7O_5S_2$, and a molecular weight is 776.023 g/mol [5, 6].

Atazanavir and Cobicistat combined marketed formulation under the name Evotaz (formerly GS-9350), it is indicated for treating infection with human immunodeficiency virus (HIV) combination with Atazanavir [7, 8]. Literature review revealed that various analytical method has been developed and validated for the estimation of Atazanavir and Cobicistat in single and combined dosage form. Literature review does not reveal related impurities method development and validation for Atazanavir and Cobicistat in combined dosage form by RP-HPLC. The present aim of my study is to develop and validated related impurities method development and validation for Atazanavir and Cobicistat in combined dosage form by RP-HPLC method [9, 10].

Simple, easy and facial various analytical related impurities method development and validation of various drugs like Ezetimibe, dipyridamole, Efavirenz and Fluradoline. Durga Babu has reported the HPLC method for the detection and evaluation of impurities in Ezetimibe. Also, Ramya has reported the analytical method development and validation for related substance in Dipyridamole by RP-HPLC. The estimation of related impurities in combined dosage form of Efavirenz by RP-HPLC has been reported by Kiran. Novel RP-HPLC method development and validation for determination and estimation of Fluradoline drug with its impurities has been reported by Srinivasa Rao have been published in several literatures. Atazanavir and Cobicistat combined marketed formulation under the name Evotaz (formerly GS-9350), it is indicated for treating infection with human immunodeficiency virus (HIV) combination with Atazanavir [11-14].

Related impurities play a very important role in analytical method development and validation field. The marketed product named Evotaz is most widely used as an antiviral agent which may contain known and unknown impurities of Atazanavir and Cobicistat. The main advantages of related impurities in pharmaceutical field in analytical method development and validation process is to calculate how much percentage of known impurities and unknown impurities is present in the marketed product named as Evotaz. In my present study, the known impurities of Atazanavir and Cobicistat is present which is within the limit criteria and there is absence of unknown impurities in marketed product named Evotaz which is estimated through analytical method development and validation of related impurities of Atazanavir and Cobicistat by RP-HPLC [15].

MATERIAL AND METHOD:

Instruments

The LC-20 AT (Shimadzu) system was used for HPLC method development and validation. As well as UV-visible detector, analysed at 224nm. Analytical weighing balance (Shimadzu ATX-224), sonicator (Frontline Ultrasonic Cleaner) and pH meter (Analab Scientific Pvt Ltd) was used during the experiment.

Chemical and Reagents

A grade gift sample of Atazanavir (established purity 99%) and its impurity were acquired from Yash Pharma. Cobicistat (established purity 98%) and its related impurities were kindly gifted by RPG Life Science. Acetonitrile, potassium di-hydrogen phosphate, orthophosphoric acid and methanol of HPLC and AR grade were procured from Merck and Rankem lab ltd. Water used to prepare buffers and other solutions was obtained from the Aquarch.

Optimization of Chromatographic Conditions

A mobile phase was prepared by dissolving 6.8-gram potassium dihydrogen phosphate into 1 litre water. Adjust pH 3.0 with o-phosphoric acid (OPA) and filter through 0.45-micron membrane filter, sonicated for 5 minutes for degassing. The isocratic mobile phase consisted of acetonitrile: 0.05M potassium dihydrogen phosphate buffer (pH 3.0) in the ratio of 75:25 (v/v). the mobile phase is use as a diluent. The

analysis was carried out on LC-20AT (Shimadzu) system. The analytes were separated on an analytical column Hypersil BDS C_{18} column (250mm x 4.6mm,5 μ m) column at 224nm wavelength. The column temperature was kept at 25°C. The volume of injection was 20 μL and the flow was sustained at 1mL/min. The run time was 15 minutes.

Preparation of Standard Solution:

Atazanavir standard stock solution: Weighed accurately about 500 mg of Atazanavir and transferred into 100 mL volumetric flask and dissolved in methanol up to the mark and get 5000 µg/mL of Atazanavir standard stock solution.

Atazanavir Impurity standard stock solution: Atazanavir impurity standard stock solution containing 50 µg/mL was prepared by dissolving 5mg of the drug in 100 mL of methanol up to prescribed concentration.

Cobicistat standard stock solution: Accurately weighed quantity of 500mg of Cobicistat was transferred into 100 mL volumetric flask and dissolved in methanol up to the mark and get 5000 µg/mL of Cobicistat standard stock solution.

Cobicistat Impurity standard stock solution: Cobicistat impurity standard stock solution containing 50 µg/mL was prepared by dissolving 5 mg of the drug in 100 mL of methanol up to prescribed concentration.

Working standard solution of Atazanavir: From above standard stock solution of Atazanavir, 1mL of solution was taken into 10mL volumetric flask and was made up to the mark with the mobile phase to get 500µg/mL of Atazanavir working standard solution.

Working standard solution of Atazanavir Impurity: From above Atazanavir impurity standard stock solution, 1mLof solution was taken into 10mL volumetric flask and was made up to the mark with the mobile phase to get 5µg/mL of Atazanavir impurity working standard solution.

Working standard solution of Cobicistat: To prepare the 500 µg/mL Cobicistat working standard solution, 1mL of the aforesaid Cobicistat standard stock solution was transferred into a 10mL volumetric flask and brought up to the required concentration with the mobile phase.

Working standard solution of Cobicistat Impurity: From above Cobicistat impurity standard stock solution, 1mL of solution was taken into 10mL volumetric flask and was made up to the mark with the mobile phase to get 5 µg/mL of Cobicistat impurity working standard solution.

Preparation of sample solution:

Weigh powdered 10 tablets and the average weight was determined. Tablets were crushed by mortar-pastel and mixed well. Accurately weighed tablet powder 500 mg equivalent of Atazanavir and Cobicistat into a 100mL volumetric flask. Add 60mL diluent, shake for 15 minutes and sonicate the solution for 10 minutes. Make up the volume with diluent. Filter the solution with 0.45-micron membrane filter.

RESULT AND DISCUSSION:

To develop an accurate and precise related impurities in combined dosage form of Atazanavir and Cobicistat by RP-HPLC. A Hypersil BDS, C_{18} column (250mm x 4.6mm,5μm) column was selected as the stationary phase for the separation and determination of related impurities method for Atazanavir and Cobicistat drug combinations. For the optimization of the mobile phase, sequential trails were performed by changing the ratio of water with methanol, water with acetonitrile and buffer (potassium dihydrogen phosphate) with acetonitrile by isocratic program and monitored at different ratios. Method optimized results are summarized in **Table 1.**

Table 1: Method development summary

S. No.	Mobile Phase	Remarks
1	Atazanavir and Cobicistat in Water: Methanol (50:50)	One Peak Observed
2	Atazanavir in Water: Methanol (50:50)	One peak confirmed by the injecting Atazanavir Solution
3	Cobicistat in Water: Methanol (50:50)	No Peak Observed
4	Atazanavir and Cobicistat in Water: Methanol (40:60)	Retention time is reduces. Still second peak did not find
5	Atazanavir and Cobicistat in Water: Methanol (20:80)	Retention time is reducing. Still second peak did not find

6	Atazanavir and Cobicistat in Water: Acetonitrile (50:50)	Still second peak did not find
7	Atazanavir and Cobicistat in Water: Acetonitrile (30:70)	Retention time is reducing. Still second peak did not find
8	Atazanavir and Cobicistat in water pH 3.0: methanol (30:70)	Both peaks are observed. But peak shape is not good
9	Atazanavir and Cobicistat in water pH 3.0: methanol (30:70)	Cobicistat peak confirmed
10	Atazanavir and Cobicistat in water pH 3.0: methanol (60:40)	Both peaks are observed. But peak shape is not good
11	Atazanavir and Cobicistat in water pH 3.0: methanol (40:60)	Retention time is reducing. But peak shape is not good
12	Atazanavir and Cobicistat in water pH 3.0: acetonitrile (60:40)	Retention time is reducing. Peak shape is good, but resolution is low between peaks

Based on the above trails, the mobile phase containing potassium dihydrogen phosphate (pH 3.00) and acetonitrile for with initial ratio 60:40 v/v.

System Suitability of RP-HPLC Method

System suitability test provides adequate evidence about the performance of the chromato- graphic system and ensures that it is capable of analysis of various samples accurately with- out causing any bias in the analysis. Chromatographic system must satisfy some of the pre- defined acceptance criteria in order to assure the ability of the system to perform the analysis of various samples. System suitability and chromatographic parameters were validated such as retention time, theoretical plates and the tailing factor was calculated. The results are summarized in **Table 2.**

Table 2: Results for System Suitability Test

Parameters	Atazanavir	Cobicistat	Atazanavir Impurity	Cobicistat Impurity
Retention Time	3.513	5.247	2.747	4.370
Theoretical plates per column	5662	7098	6018	6902
Tailing factor	1.583	1.382	1.579	1.393

Validation of RP-HPLC Method

The proposed method for estimation of related impurities of Atazanavir and Cobicistat was validated. As per the ICH guidelines, validated the developed method for the parameters like specificity, precision, linearity, accuracy, robustness and LOD and LOQ.

Specificity

Specificity is measure of relative separation of the individual components. The test was useful for showing separation/estimation of impurity peaks from the principal peak. Specificity was carried out to demonstrate that individual expected known peaks of the impurities were completely separated from Atazanavir and Cobicistat peak. The chromatogram of blank was not interfering with all known impurities of Atazanavir and Cobicistat. The Chromatogram of standard, sample and blank is shown in **Figs.5-7.**

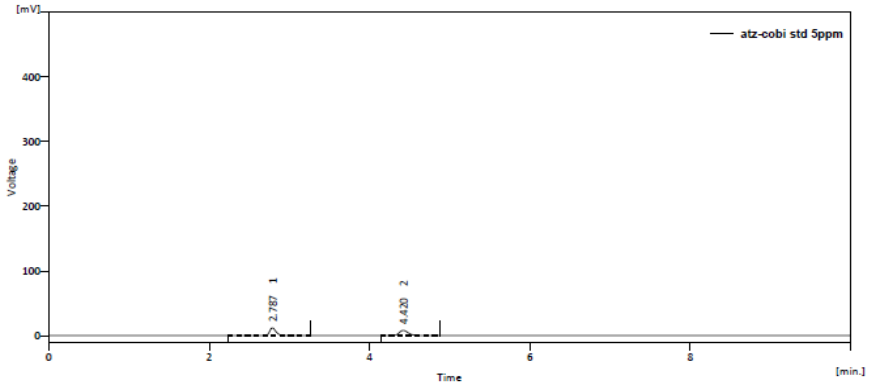

Fig.5: Chromatogram of Atazanavir Impurity and Cobicistat Impurity Standard

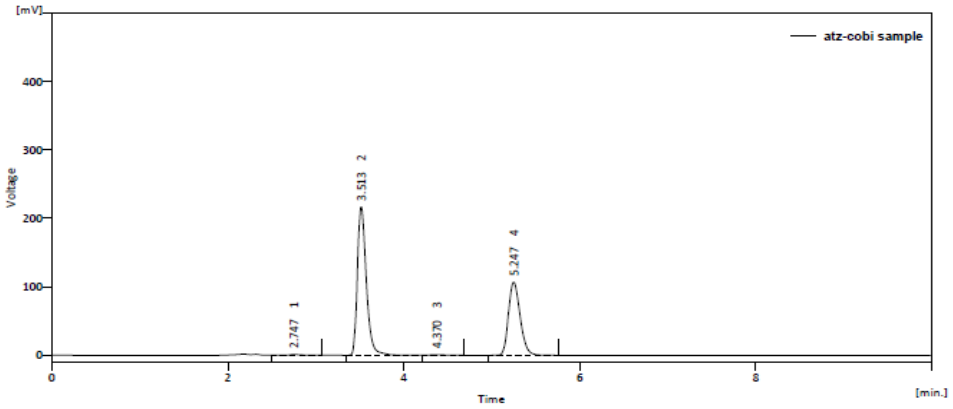

Fig.6: Chromatogram of Atazanavir and Cobicistat Sample

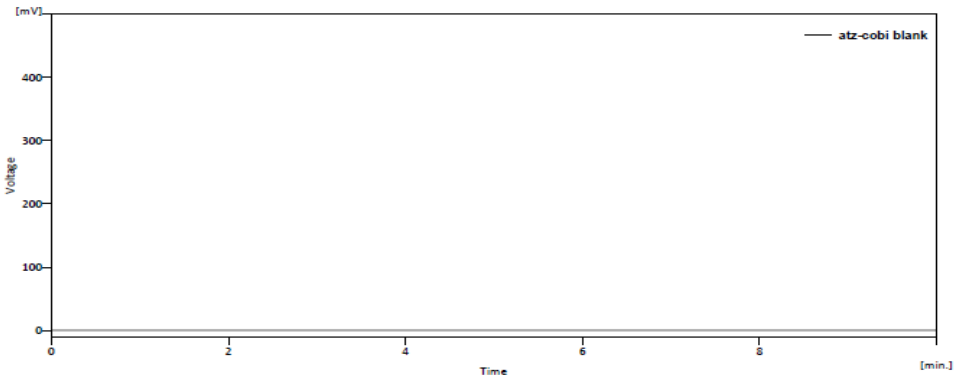

Fig.7: Chromatogram of Atazanavir and Cobicistat Blank

Linearity

Linearity was demonstrated by amazing six different concentrations of active compound. The linearity of an analytical procedure was its ability to obtain test results which were directly proportional to the concentration of analyte in the sample. The linearity of the method for all the related impurities was determined by analysing solution of related impurities of Atazanavir and Cobicistat at six different concentration levels 2.5 to 7.55µg/mL. Results for linearity were shown in **Table 3 and 4.**

Table 3: Linearity data for Atazanavir Impurity

Sr. No	Concentration(µg/mL)	Area
1	2.5	33.528
2	3.75	49.645
3	5	66.813

4	6.25	83.177
5	7.5	99.516

Table 4: Linearity Data for Cobicistat Impurity

Sr. No	Concentration(µg/mL)	Area
1	2.5	31.458
2	3.75	46.277
3	5	62.885
4	6.25	78.102
5	7.5	93.73

The overlay chromatogram of related impurity of Atazanavir and Cobicistat was shown in **Fig.8.**

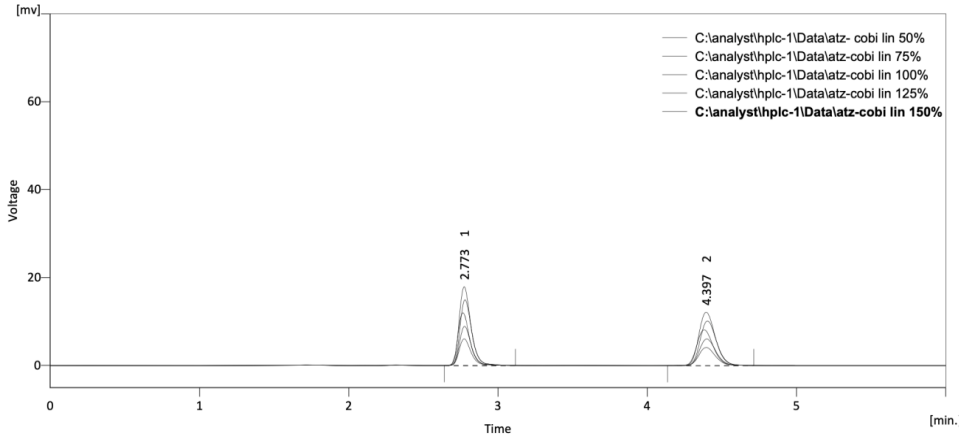

Fig.8: Chromatogram of Atazanavir and Cobicistat Standard overlay

The correlation coefficient was calculated for each known impurities **Figs. 9-10.**

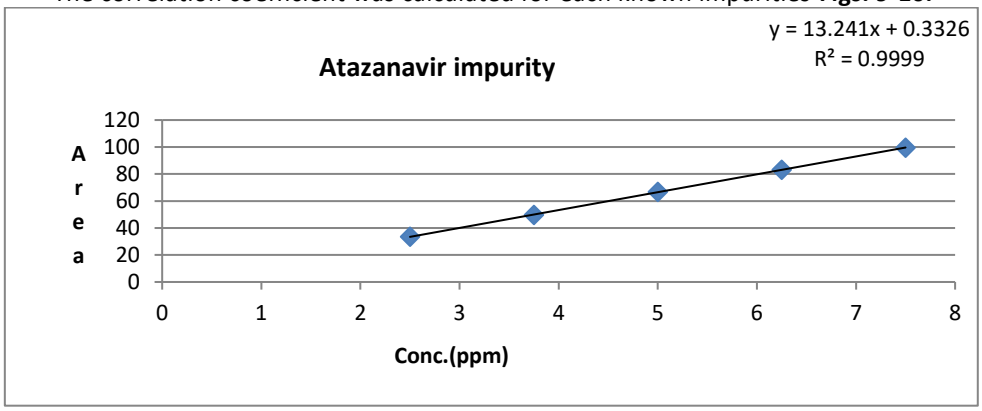

Fig.9: Calibration Curve of Atazanavir Impurity

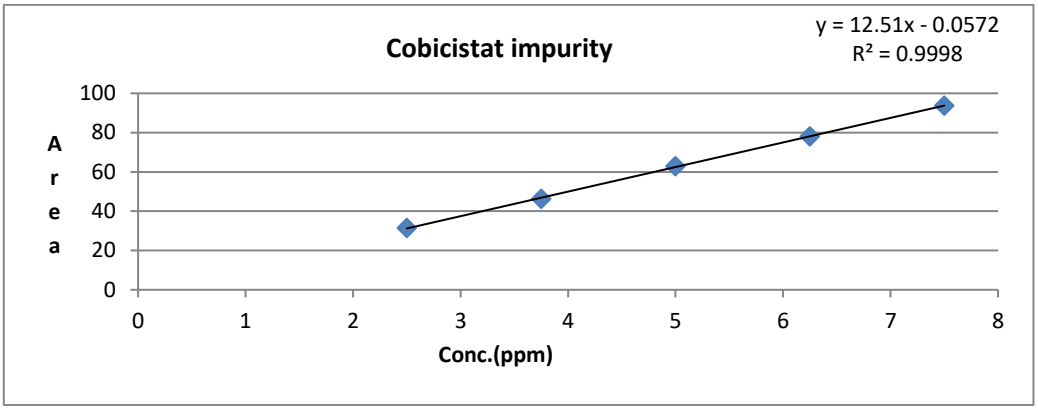

Fig.10: Calibration Curve of Cobicistat Impurity

Precision

The precision of analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. It was expected that an analytical method should generate outcomes that were reproducible. Precise analytical method leads to accurate results. Precision was carried out at three levels i.e., repeatability, intra-day precision and inter-day precision.

a) Repeatability

The working standard solution of related impurities of Atazanavir and Cobicistat was injected six times and areas of peaks were measured and % RSD was calculated. The results for repeatability of relative impurity of Atazanavir and Cobicistat are summarized in **(Table 5 & Table 6).**

Table 5: Repeatability Data for Atazanavir Impurity

Atazanavir Impurity				
Sr. No.	**Conc (µg/mL)**	**Area**	**Mean ± S.D (n=6)**	**% R.S. D**
1.	5	64.875 65.334 66.646 66.784 67.307 65.585	66.089±0.957	1.44

Table 6: Repeatability data for Cobicistat Impurity

Cobicistat Impurity				
Sr No.	**Conc (µg/mL)**	**Area**	**Mean ± S.D (n=6)**	**% R.S. D**
1.	5	60.895 61.334 62.183 62.697 63.181 61.578	61.978±0.866	1.39

b) Intraday precision

The working standard solution of related impurities of Atazanavir and Cobicistat were analysed three times on the same day and % R.S.D was calculated. It was carried out at three different level i.e., 2.5, 5 and 7.5 µg/mL under specified chromatographic conditions. The results for intraday precision of relative impurity of Atazanavir and Cobicistat are summarized in **Table 7.**

Table 7: Intraday precision data for Estimation of Atazanavir and Cobicistat Impurity

Sr. No.	Atazanavir Impurity			Cobicistat Impurity		
	Conc. (µg/mL)	Area Mean ± S.D. (n=3)	% R.S. D	Conc. (µg/mL)	Area Mean ± S.D. (n=3)	% R.S. D
1	LOQ	6.567±0.114	1.74	LOQ	6.242±0.122	1.95
2	5	66.370±1.400	2.10	5	62.207±1.189	1.91
3	7.5	99.099±1.753	1.76	7.5	93.237±1.730	1.85

c) Interday precision

The working standard solution of related impurities of Atazanavir and Cobicistat were analysed three times on the different day and % R.S.D was calculated. It was carried out at three different level i.e., 2.5, 5 and 7.5 µg/mL under specified chromatographic conditions. The results for interday precision of relative impurity of Atazanavir and Cobicistat are summarized in **Table 8.**

Table 8: Interday Precision data for Estimation of Atazanavir and Cobicistat Impurity

Sr. No.	Atazanavir Impurity			Cobicistat Impurity		
	Conc. (µg/mL)	Area Mean ± S.D. (n=3)	% R.S. D	Conc. (µg/mL)	Area Mean ± S.D. (n=3)	% R.S. D
1	LOQ	6.555±0.119	1.82	LOQ	6.202±0.158	2.55
2	5	65.091±1.720	2.64	5	61.662±1.116	1.81
3	7.5	99.855±1.504	1.52	7.5	92.929±1.129	1.21

Accuracy

Accuracy was performed by determining the % recovery of impurities by spiking them to the drug substance. Accuracy was a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Accuracy for all related impurities was determined by analysing Atazanavir and Cobicistat test solution spiked with all the related impurities at four different concentrations of levels of 80%,100% and 120% of each in triplicate at the specified limit. The % recovery of related impurities of Atazanavir and Cobicistat was calculated by injecting standard solution for each level. The results for accuracy of relative impurity of Atazanavir and Cobicistat are summarized in **Table 9 and 10.**

Table 9: Recovery Data for Atazanavir Impurity

S. No.	Conc. Level (%)	Sample amount (µg/mL)	Amount Added (µg/mL)	Amount recovered (µg/mL)	% Recovery	% R.S. D
1		2.5	0.25	0.250	99.862	
2	LOQ	2.5	0.25	0.254	101.767	1.071
3		2.5	0.25	0.254	101.708	
4		2.5	2	1.950	97.476	
5	80 %	2.5	2	2.014	100.684	1.692
6		2.5	2	1.965	98.261	
7		2.5	2.5	2.445	97.789	
8	100 %	2.5	2.5	2.462	98.495	0.367
9		2.5	2.5	2.450	98.016	
10		2.5	3	2.934	97.791	
11	120 %	2.5	3	2.960	98.674	0.705
12		2.5	3	2.975	99.161	

Table 10: Recovery Data for Cobicistat Impurity

Sr. No.	Conc. Level (%)	Sample amount (µg/mL)	Amount Added (µg/mL)	Amount recovered (µg/mL)	% Recovery	% R.S. D
1	LOQ	2.5	0.25	0.254	101.538	2.233
2		2.5	0.25	0.252	100.979	

3		2.5	0.25	0.244	97.422	
4		2.5	2	1.949	97.458	
5	80 %	2.5	2	2.009	100.427	1.875
6		2.5	2	2.018	100.906	
7		2.5	2.5	2.478	99.134	
8	100 %	2.5	2.5	2.494	99.767	0.334
9		2.5	2.5	2.482	99.281	
10		2.5	3	2.973	99.109	
11	120 %	2.5	3	3.002	100.062	0.778
12		2.5	3	3.019	100.650	

Robustness

The study of robustness was carried out to evaluate the influence of small but deliberate variations in the chromatogram conditions on the determinations of both drugs. Robustness of analytical method was the ability of a method to resist the change in its performance in spite of small, deliberate change in method parameters. It was expected that such change should not alter the performance of the analytical method. A study was conducted to determine the effect of variation in flow rate, mobile phase composition and pH of mobile phase. Working standard solutions was prepared of related impurities as per the test method wand was injected into the HPLC system using flow rates 0.8mL/min and 1.2 mL/min, mobile phase composition consists of +2% solvent and -2% solvent in gradient run and pH of buffer was sets at 2.8 and 3.2. Then % RSD of related impurities of Atazanavir and Cobicistat were calculated. The results for robustness of relative impurity of Atazanavir and Cobicistat are summarized in **Table 11 and 12**

Table 11: Robustness data for Atazanavir Impurity

Sr No.	Area at Flow rate (+ 0.2 mL/min)	Area at Flow rate (- 0.2 mL/min)	Area at pH (- 0.2)	Area at pH (+0.2)	Area at Mobile phase (- 2)	Area at Mobile phase (+2)
1	62.519	70.37	66.175	65.18	68.873	63.046
2	63.562	69.213	62.813	66.809	67.677	61.223
3	60.906	66.441	65.646	65.908	67.203	61.27
% R.S. D	2.14	0.80	2.78	1.23	1.26	1.68

Table 12: Robustness data for Cobicistat Impurity

Sr No.	Area at Flowrate (- 0.2 mL/min)	Area at Flow rate (+ 0.2 mL/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase (-2)	Area at Mobile phase (+2)
1	65.68	58.685	61.753	61.127	64.274	59.177
2	64.559	59.265	59.662	62.765	63.775	58.811
3	63.358	59.976	61.362	61.507	62.153	57.985
% R.S. D	1.79	1.09	1.82	1.38	1.74	1.04

Limit of detection (LOD) and limit of quantification (LOQ)

LOD (limit of detection) was the lowest amount of analyte present in sample that can be detected but not necessarily quantities, under stated condition. LOQ (Limit of Quantitation) was the lowest amount of analyte present in sample that can be determined with acceptable precision and accuracy under stated experimental conditions. LOD and LOQ were determined by measuring the standard deviation of the response and the slope which was obtain from the linearity data. The results for LOD and LOQ of relative impurity of Atazanavir and Cobicistat are summarized in **Table 13 and 14.**

Table 13: Limit of Detection Data for Atazanavir Impurity and Cobicistat Impurity

Atazanavir Impurity	Cobicistat Impurity.
LOD = 3.3 x (SD / Slope) = 3.3 x (0.273/13.241) = 0.068µg/mL	LOD = 3.3 x (SD / Slope) = 3.3 x (0.427/12.510) = 0.113µg/Ml

Table 14: Limit of Quantitation Data for Atazanavir Impurity and Cobicistat Impurity.

Atazanavir Impurity	Cobicistat Impurity
LOQ = 10 x (SD / Slope)	LOQ = 10 x (SD / Slope)
= 10 x (0.273/13.241)	= 10 x (0.427/12.510)
= 0.206 µg/mL	= 0.342 µg/Ml

Calculation of known Impurities of Atazanavir and Cobicistat

Analysed test solutions for three times and calculate % of each known impurities in comparison with standard preparations of Atazanavir and cobicistat impurities. The amount of known related impurities present in the formulation of Atazanavir and Cobicistat is calculated by using the formula given below.

For each known impurity of Atazanavir and Cobicistat:

% of each known impurities = (Cu/Cs) X (Ru/Rs) X 100

Where,

Cu= Concentration of each impurity in standard preparation

Cs= Concentration of each impurity in test preparation

Ru= Area of each impurity in test preparation

Rs= Area of each impurity in standard preparation

Results for % of each known impurities of Atazanavir and Cobicistat were shown in **Table 15.**

Table 15: Calculation of Known Impurities of Atazanavir and Cobicistat

Impurity	Conc (µg/mL)	Area	% Impurity	% R.S. D
Atazanavir	5	11.366	0.169	1.35
		11.231	0.167	
		11.539	0.172	
Cobicistat	5	9.272	0.148	1.24
		9.169	0.146	
		9.4	0.150	

Atazanavir is an antiretroviral drug of the protease inhibitor class used in combination with cobicistat which is used to treat infection of human immunodeficiency virus (HIV). Cobicistat is a licenced drug for use in the treatment of infection with the HIV. RP-HPLC method was developed for simultaneous estimation Atazanavir and Cobicistat. In RP-HPLC method, good resolution and separation of two drugs and its related impurities was achieved. As mobile phase used 0.05M potassium dihydrogen phosphate (pH 3.0): acetonitrile (60:40%v/v). The flow rate was 1ml/min. Retention time of Atazanavir and Cobicistat were found to be 3.515 and 5.247 minute respectively and the retention time of Atazanavir impurity and Cobicistat impurity were found to be 2.747 and 4.353 minute respectively. The proposed method was accurate and precise. So the proposed method can be used for routine analysis of atazanavir and cobicistat in tablets. As per ICH guidelines, the related impurities RP-HPLC method was developed and validated successfully in terms of specificity, linearity, precision, accuracy and robustness.

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