Research Article

A Novel Validated RP-HPLC Method for the Simultaneous Estimation of Atazanavir Sulphate and Cobicistat in Bulk and Pharmaceutical Dosage Form

Uttam Prasad Panigrahy[*1], A. Sunil Kumar Reddy[2,3]

[1]Dept of Pharmaceutical Analysis and Quality Assurance, Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad, India.
[2]Department of Pharmaceutical Chemistry, Bharat Institute of Technology-Pharmacy, Ibrahimpatnam, Hyderabad, India.
[3]APL Research Centre-2, Aurobindo Pharma Ltd., Sanga Reddy, Medak, Telengana, India.
***Corresponding author's E-mail:** uttampanigrahy@gmail.com

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ABSTRACT

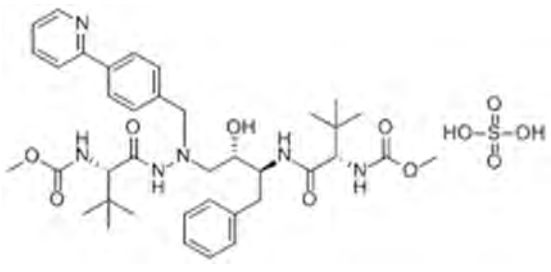
A novel approach was used to develop and validate a rapid isocratic Reversed Phase-High Performance Liquid Chromatographic method for the simultaneous estimation of Atazanavir Sulphate and Cobicistat in bulk and pharmaceutical dosage form. The separation was performed by Agilent ZORBAX eclipse plus C_{18} (100mm×4.6 mm, 3.5µm particle size) column, Agilent 1220 Infinity HPLC System with VWD detector and mobile phase contained a mixture of 0.01M Potassium dihydrogen phosphate (pH adjusted to 3.5 with orthophosphoric acid) and Acetonitrile (30:70, v/v). The flow rate was set to 1ml/min with responses measured at 260nm. The retention time of Atazanavir Sulphate and Cobicistat was 2.243min and 6.043min respectively with resolution of 4.806. Linearity was established in the range of 15-75µg/ml for Atazanavir Sulphate and 7.5-37.5µg/ml for Cobicistat with correlation coefficients (r^2=0.999). The percentage recoveries were between 99.92-100.03% and 99.91-100.05% for Atazanavir Sulphate and Cobicistat respectively. Validation parameters were evaluated according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. The developed method was successfully applied for the quantification and hyphenated instrumental analysis.

Keywords: Atazanavir Sulphate, Cobicistat, VWD detector, Hyphenated, ICH.

INTRODUCTION

Atazanavir Sulphate and Cobicistat combined dosage form is used for the treatment of human immunodeficiency virus (HIV-1). Atazanavir Sulphate is an azapeptide HIV-1 protease inhibitor that selectively inhibits the virus-specific processing of viral Gag and Gag-Pol polyproteins in HIV-1 infected cells, thus preventing formation of mature virions[1]. Cobicistat is a mechanism-based inhibitor of cytochrome P450 3A (CYP3A). Inhibition of CYP3A-mediated metabolism by Cobicistat increases the systemic exposure of the CYP3A substrate Atazanavir[2,3]. Atazanavir Sulphate is chemically known as (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, sulfate were shown in Figure 1a. Cobicistat is chemically known as 1, 3-thiazol-5-ylmethyl [(2*R*, 5R)-5-{[(2*S*)-2-[(methyl {[2-(propan-2-yl)-1, 3-thiazol-4-yl] methyl} carbamoyl) amino] -4- (morpholin-4-yl) butanoyl] amino}-1, 6 diphenylhexan-2-yl] carbamate was shown in Figure 1b.

(a)



(b)

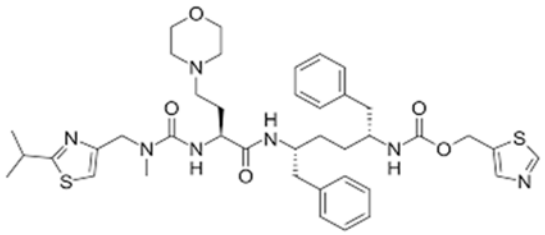


Figure 1: Chemical structure of (a) Atazanavir Sulphate (b) Cobicistat

Literature survey reveals that many analytical methods are reported for determination of Atazanavir Sulphate and Cobicistat individually and with other combination which includes high performance liquid chromatography (HPLC)[4-10], Liquid chromatography-mass spectroscopy (LC-MS)[11-13], UV-Spectrophotometry[14-19] and High performance thin layer chromatography (HPTLC)[20,21] methods. However, no method is reported for simultaneous estimation of Atazanavir Sulphate and Cobicistat in combined dosage form by reverse phase HPLC. The present study was aimed to develop a novel and validated method for the simultaneous estimation of Atazanavir Sulphate and Cobicistat in bulk and pharmaceutical dosage form according to ICH guidelines[22].

MATERIALS AND METHODS

Chemicals and reagents

Atazanavir Sulphate (API) was obtained from Hetero Drugs Ltd., Hyderabad, India and Cobicistat (API) was

obtained from Shilpa Medicare Limited, India. HPLC grade of Potassium dihydrogen phosphate was obtained from Rankem Ltd., India and HPLC grade of Acetonitrile was obtained from Merck Specialities Private Limited, India. HPLC grade of Water and Ortho phosphoric acid was obtained from Rankem Ltd., India. Evotaz tablet contains 300 mg of Atazanavir Sulphate and 150 mg of cobicistat were kindly supplied by Bristol-Myers Squibb.

Selection of UV-wavelength

In simultaneous estimation of Atazanavir Sulphate and Cobicistat isosbestic wavelength is used. Standard stock solutions of Atazanavir Sulphate and Cobicistat were prepared by dissolving 300mg of Atazanavir Sulphate and 150mg of Cobicistat in 100ml of diluent into a 100ml clean dry volumetric flask and the standard solutions was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 3000µg/ml of Atazanavir Sulphate and 1500µg/ml of Cobicistat. From the above standard stock solution of 3000µg/ml of Atazanavir Sulphate and 1500µg/ml of Cobicistat further pipette 10ml and transferred into a 100ml volumetric flask and dilute up to the mark with diluent to get the concentration of 300µg/ml of Atazanavir Sulphate and 150µg/ml of Cobicistat further pipette 1ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 30µg/ml of Atazanavir Sulphate and 15µg/ml of Cobicistat. The wavelength of maximum absorption (λmax) of 30µg/ml of Atazanavir Sulphate and 15µg/ml of Cobicistat were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against mobile phase as blank. The isosbestic wavelength (λmax) was found to be 260nm for the combination shown in Figure 2.

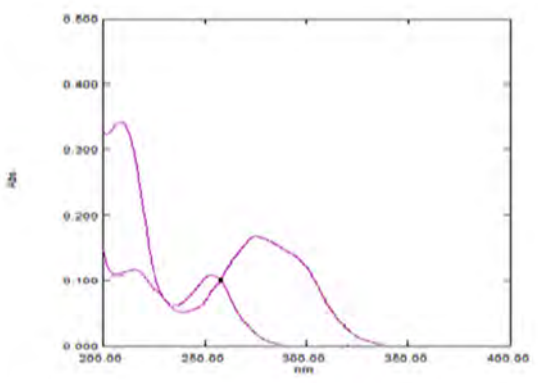


Figure 2: Isosbestic point of Atazanavir Sulphate and Cobicistat at 260nm

Instrumentation and chromatographic conditions

The analysis was performed by using a chromatographic system from Agilent 1220 Infinity HPLC System with VWD detector. The HPLC system was equipped with EZ chrome software. Semi-micro analytical balance (India), Ultrasonic bath sonicator (Frontline FS 4, Mumbai, India), Digital pH meter (Systronics model 802) and Whatmann filter paper No. 41 (Whatmann International Ltd., England) were used in the study. Atazanavir Sulphate and Cobicistat were analyzed in Agilent ZORBAX eclipse plus C_{18} (100mm×4.6 mm, 3.5µm particle size) column for the chromatographic separation. The mobile phase was composed of 0.01M Potassium dihydrogen phosphate (pH adjusted to 3.5 with orthophosphoric acid) and Acetonitrile (30:70, v/v). Filtered through 0.45µm nylon membrane filter under vacuum filtration and pumped at ambient temperature, at a flow rate of 1 ml/min with UV detection wavelength at 260nm. Injection volume was 20µl. The run time was 10 min and the retention time of Atazanavir Sulphate and Cobicistat was found to be 2.243min and 6.043min respectively with resolution of 4.806.

Chromatographic Parameters:

Equipment : Agilent 1220 Infinity HPLC System with VWD detector

Column : ZORBAX eclipse plus C_{18} (100mm×4.6 mm, 3.5µm particle size)

Flow rate : 1ml/min

Wavelength : 260 nm

Injection volume: 20 µl

Column oven : Ambient

Run time : 10 Minutes

Solutions and sample preparation

Preparation of Phosphate buffer

A 0.01M Potassium dihydrogen phosphate buffer was prepared by dissolving 1.368gm of Potassium dihydrogen phosphate in 1000ml of HPLC grade water and pH was adjusted to 3.5 with orthophosphoric acid. The buffer was filtered through 0.45µm nylon membrane filter to remove all fine particles and gases.

Preparation of mobile phase

The above prepared 0.01M Potassium dihydrogen phosphate buffer and Acetonitrile HPLC grade were mixed in the proportion of 30:70, v/v and was filtered through 0.45µm nylon membrane filter and degassed by sonication.

Preparation of diluent

Mobile phase was used as diluent.

Preparation of standard stock solutions of Atazanavir Sulphate and Cobicistat

Standard stock solutions of Atazanavir Sulphate and Cobicistat were prepared by dissolving 300mg of Atazanavir Sulphate (API) and 150mg of Cobicistat (API) in 100ml of diluent into a 100ml clean dry volumetric flask and the standard solutions was filtered through 0.45 µm nylon membrane filter and degassed by sonicator to get the concentration of 3000µg/ml of Atazanavir Sulphate

and 1500µg/ml of Cobicistat further pipette 10ml and transferred into a 100ml volumetric flask and dilute up to the mark with diluent to get the concentration of 300µg/ml of Atazanavir Sulphate and 150µg/ml of Cobicistat.

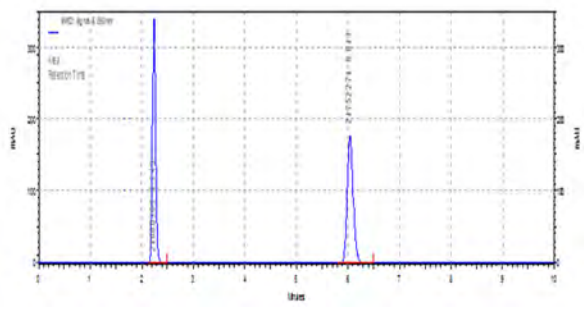
Preparation of standard solutions of Atazanavir Sulphate and Cobicistat for assay

From the above standard stock solution of 300µg/ml of Atazanavir Sulphate and 150µg/ml of Cobicistat further pipette 1ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 30µg/ml of Atazanavir Sulphate and 15µg/ml of Cobicistat.

Preparation of sample solutions of Atazanavir Sulphate and Cobicistat

Evotaz tablets contains equivalent amount of Atazanavir Sulphate 300mg and Cobicistat 150mg were taken into 100ml clean dry volumetric flask, diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent. Further pipette out 10ml from the above Atazanavir Sulphate and Cobicistat sample stock solution into a 100ml volumetric flask and diluted up to the mark with diluent to get the concentration of 300µg/ml of Atazanavir Sulphate and 150µg/ml of Cobicistat further pipette 1ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 30µg/ml of Atazanavir Sulphate and 15µg/ml of Cobicistat. 20µl from standard and sample solution were injected into the chromatographic system and the peak areas were measured for Atazanavir Sulphate and Cobicistat which was shown in Figure 3.

(a)



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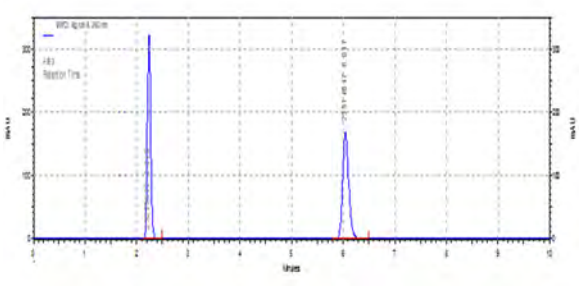


Figure 3: Atazanavir Sulphate and Cobicistat (a) Standard Chromatogram (b) Sample Chromatogram

The % assay was calculated by comparing the peak area of standard and sample chromatogram by using the formula given below and the assay results was shown in Table 1.

$$Assay\ \% = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt}{Label\ Claim} \times 100$$

Where:

AT = Average peak area of sample preparation

AS= Average peak area of standard preparation

WS = Weight of standard taken in mg

WT= Weight of sample taken in mg

P = Percentage purity of working standard

DS= Dilution factor for standard preparation

DT= Dilution factor for sample preparation

Method validation

The developed method for the simultaneous estimation of Atazanavir Sulphate and Cobicistat was validated as per the ICH guidelines for the parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

System suitability

At first the HPLC system was optimized as per the chromatographic conditions. One blank followed by six replicates of a single calibration standard solution of 30µg/ml of Atazanavir Sulphate and 15µg/ml of Cobicistat was injected to check the system suitability. To ascertain the system suitability for the proposed method, the parameters such as retention time, theoretical plates, peak asymmetry and resolution were taken and results were presented in Table 2.

Specificity

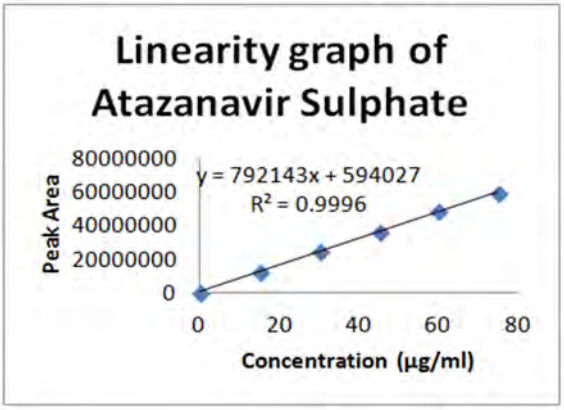
The effect of excipients and other additives usually present in the combined dosage form of Atazanavir Sulphate and Cobicistat in the determination under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting the blank and placebo solution into the HPLC system.

Linearity and range for Atazanavir Sulphate and Cobicistat

Aliquots of 0.5, 1, 1.5, 2 and 2.5ml of mixed standard working solutions of Atazanavir Sulphate and Cobicistat was pipette out from the standard stock solution of 300µg/ml of Atazanavir Sulphate and 150µg/ml of Cobicistat and transferred into a series of 10ml clean dry volumetric flask and make volume up to the mark with the same diluent to get the concentration of 15, 30, 45, 60 and 75µg/ml of Atazanavir Sulphate and 7.5, 15, 22.5, 30 and 37.5µg/ml of Cobicistat. The calibration standard

solutions of Atazanavir Sulphate and Cobicistat were injected using a 20µl Hamilton Rheodyne injector and the chromatograms were recorded at 260nm and a calibration graph was obtained by plotting peak area versus concentration of Atazanavir Sulphate and Cobicistat respectively. The linearity data is presented in Figure 4 and Table 3. Acceptance Criteria: Correlation coefficient should not be less than 0.999.

(a)



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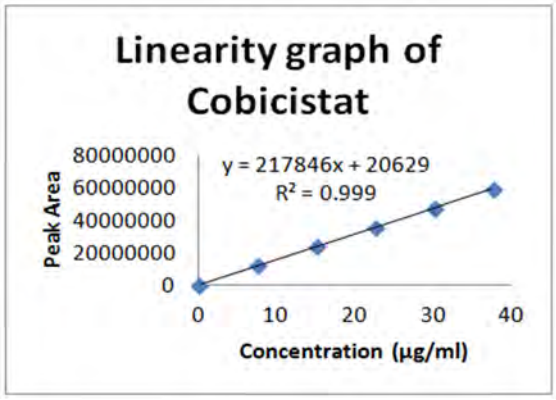

Figure 4: Linearity graph of (a) Atazanavir Sulphate (b) Cobicistat

Accuracy studies for Atazanavir Sulphate and Cobicistat

The accuracy of the method was determined by calculating recovery of Atazanavir Sulphate and Cobicistat by the method of standard addition. Known amount of standard solution of Atazanavir Sulphate and Cobicistat at 50%, 100% and 150% was added to a pre quantified sample solution and injected into the HPLC system. The mean percentage recovery of Atazanavir Sulphate and Cobicistat at each level was calculated and the results were presented in Table 4.

Preparation of pre quantified sample solution for accuracy studies

Evotaz tablets contains equivalent amount of Atazanavir Sulphate 300mg and Cobicistat 150mg were taken into 100ml clean dry volumetric flask, diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent. Further pipette out 10ml from the above Atazanavir Sulphate and Cobicistat sample stock solution into a 100ml volumetric flask and diluted up to the mark with diluent to get the concentration of 300µg/ml of Atazanavir Sulphate and 150µg/ml of Cobicistat further pipette 1ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 30µg/ml of Atazanavir Sulphate and 15µg/ml of Cobicistat.

Preparation of standard solution of Atazanavir Sulphate and Cobicistat for accuracy studies

Standard stock solutions of Atazanavir Sulphate and Cobicistat were prepared by dissolving 300mg of Atazanavir Sulphate (API) and 150mg of Cobicistat (API) in 100ml of diluent into a 100ml clean dry volumetric flask and the standard solutions was filtered through 0.45 µm nylon membrane filter and degassed by sonicator to get the concentration of 3000µg/ml of Atazanavir Sulphate and 1500µg/ml of Cobicistat further pipette 10ml and transferred into a 100ml volumetric flask and dilute up to the mark with diluent to get the concentration of 300µg/ml of Atazanavir Sulphate and 150µg/ml of Cobicistat.

a.) **Preparation of 50% standard solution**

From the standard stock solution of 300µg/ml of Atazanavir Sulphate and 150µg/ml of Cobicistat further pipette 0.5ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 15µg/ml of Atazanavir Sulphate and 7.5µg/ml of Cobicistat.

b.) **Preparation of 100% standard solution**

From the standard stock solution of 300µg/ml of Atazanavir Sulphate and 150µg/ml of Cobicistat further pipette 1ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 30µg/ml of Atazanavir Sulphate and 15µg/ml of Cobicistat.

c.) **Preparation of 150% standard solution**

From the standard stock solution of 300µg/ml of Atazanavir Sulphate and 120µg/ml of Cobicistat further pipette 1.5ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 45µg/ml of Atazanavir Sulphate and 22.5µg/ml of Cobicistat. Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Precision studies for Atazanavir Sulphate and Cobicistat

Method precision (Repeatability)

Evotaz tablets contains equivalent amount of Atazanavir Sulphate 300mg and Cobicistat 150mg were taken into 100ml clean dry volumetric flask, diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent. Further pipette out

10ml from the above Atazanavir Sulphate and Cobicistat sample stock solution into a 100ml volumetric flask and diluted up to the mark with diluent to get the concentration of 300µg/ml of Atazanavir Sulphate and 150µg/ml of Cobicistat further pipette 1ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 30µg/ml of Atazanavir Sulphate and 15µg/ml of Cobicistat. A homogenous sample of a single batch is analyzed six times and was checked whether the method is giving consistent results. The %RSD for the assay of six replicate injections was calculated as mentioned in Table 5. Acceptance Criteria: The % RSD for the assay of six sample injections should not be more than 2%.

System precision

The system precision was carried out to ensure that the analytical system is working properly. The standard preparation concentration of 30µg/ml of Atazanavir Sulphate and 15µg/ml of Cobicistat was injected six times into the HPLC system and the %RSD for the area of six replicate injections was calculated as mentioned in Table 5. Acceptance Criteria: The % RSD for the peak area of six standard injections should not be more than 2%.

Intermediate precision/ruggedness

The intermediate precision (also known as Ruggedness) of the method was evaluated by performing precision on different laboratories by different analysts and different days. The sample preparation concentration of 30µg/ml of Atazanavir Sulphate and 15µg/ml of Cobicistat was injected six times into the HPLC system and the %RSD for the assay of six replicate injections was calculated as mentioned in Table 5. Acceptance Criteria: The % RSD for the assay of six sample injections should not be more than 2%.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3.3×SD/S and 10×SD/S respectively as per ICH guidelines, Where SD is the standard deviation of the response (Y-intercept) and S is the slope of the calibration curve. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3).

The LOD of Atazanavir Sulphate and Cobicistat was calculated and shown in Table 5. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Atazanavir Sulphate and Cobicistat was calculated and shown in Table 5.

Robustness

As part of the Robustness, deliberate change in the flow rate and mobile phase proportion of ±10% was made to evaluate the impact on the method. The results reveal that the method is robust. The results are summarized in Table 5.

Stability of solution

The %RSD of the assay of Atazanavir Sulphate and Cobicistat from the solution stability and mobile phase stability experiments was within 2%. The results of the solution and mobile phase stability experiments confirm that the sample solutions and mobile phase used during the assays were stable upto 48hours at room temperature was calculated and shown in Table 6.

Table 1: Assay of marketed formulation of Atazanavir Sulphate and Cobicistat

Drug	Evotaz Tablet Label Claim (mg)	Amount Found (mg) (n=6)	% Label Claim ± % RSD (n=6)
Atazanavir Sulphate	300	299.29	99.76± 0.23
Cobicistat	150	150.11	100.08± 0.09

Table 2: System suitability parameters for Atazanavir Sulphate and Cobicistat

Parameter (n=6)	Atazanavir Sulphate	Cobicistat
Retention Time (Mins)	2.243	6.043
Theoretical plates	6201	12778
Tailing factor	1.02	1.1
Resolution		4.806

Table 3: Linearity data for Atazanavir Sulphate and Cobicistat

Linearity of Atazanavir Sulphate		Linearity of Cobicistat	
Concentration (µg/ml)	**Peak Area**	**Concentration (µg/ml)**	**Peak Area**
15	12890285	7.5	12120522
30	24668956	15	23651273
45	36238480	22.5	35612967
60	48508345	30	47151794
75	59490279	37.5	58740635

Table 4: Recovery studies of Atazanavir Sulphate and Cobicistat

Recovery study data of Atazanavir Sulphate				
Sample name	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**%Recovery**	**Statistical Analysis**
S_1:50%	15	15.008	100.05	Mean=100.03% (n=3) S.D=0.07 %RSD=0.07
S_2:50%	15	15.014	100.09	
S_3:50%	15	14.99	99.96	
S_4:100%	30	29.99	99.95	Mean=99.92% (n=3) S.D=0.14 %RSD=0.14
S_5:100%	30	30.01	100.04	
S_6:100%	30	29.93	99.77	
S_7:150%	45	45.01	100.01	Mean=99.97% (n=3) S.D=0.05 %RSD=0.05
S_8:150%	45	44.99	99.97	
S_9:150%	45	44.96	99.92	
Recovery study data of Cobicistat				
Sample name	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**%Recovery**	**Statistical Analysis**
S_1:50%	7.5	7.48	99.75	Mean=99.91% (n=3) S.D=0.15 %RSD=0.15
S_2:50%	7.5	7.49	99.92	
S_3:50%	7.5	7.50	100.04	
S_4:100%	15	15.02	100.16	Mean=100.05% (n=3) S.D=0.1 %RSD=0.1
S_5:100%	15	14.99	99.95	
S_6:100%	15	15.01	100.05	
S_7:150%	22.5	22.52	100.08	Mean=100.01% (n=3) S.D=0.09 %RSD=0.09
S_8:150%	22.5	22.48	99.90	
S_9:150%	22.5	22.51	100.05	

Table 5: Summary of validation parameEter for Atazanavir Sulphate and Cobicistat

Parameters	**HPLC method**			
	Atazanavir Sulphate		**Cobicistat**	
Linearity range (µg/ml)	15-75		7.5-37.5	
Slope	79214		217846	
Intercept	59402		20629	
Correlation coefficient	0.999		0.999	
LOD (µg/ml)	0.6		0.2	
LOQ (µg/ml)	1.8		0.6	
Method Precision (% RSD, n=6)	0.2		0.1	
System precision (% RSD, n=6)	0.83		0.98	
Ruggedness (% RSD, n=24)	Lab-1	Lab-2	Lab-1	Lab-2
	0.19	0.24	0.11	0.17
Reproducibility (% RSD, n=48)	0.22		0.14	
% Accuracy	99.92-100.03		99.91-100.05	
Robustness (% RSD, n=6)	Less Flow rate	More Flow rate	Less Flow rate	More Flow rate
	0.9	0.7	0.8	0.9
	Less Organic phase	More Organic phase	Less Organic phase	More Organic phase
	0.5	0.8	0.12	0.6

Table 6: Summary of solution stability-effect of P^H of mobile phase (0.01M Potassium dihydrogen phosphate buffer and Acetonitrile (30:70, v/v) (P^H adjusted to 3.5 with orthophosphoric acid) for Atazanavir Sulphate and Cobicistat for 48 hours at room temperature.

	Solution stability for Atazanavir Sulphate					
S.No.	**Concentration (µg/ml)**	**Retention time (min)**	**Peak Area**	**%Assay**	**USP Plate Count**	**Asymmetry**
1	30	2.240	24472884	99.98	6201	1.02
2	30	2.243	24418213	99.76	6318	1.01
3	30	2.240	24367397	99.55	6268	1.01
4	30	2.240	24481732	100.02	6201	1.02
5	30	2.243	24389152	99.64	6301	1.03
6	30	2.240	24379308	99.60	6252	1.01
Average		2.241	24418114	99.76	6257	1.02
SD		0.001549	48916.11	0.19985	49.14231	0.008165
%RSD		0.07	0.2	0.2	0.8	0.8
Solution stability for Cobicistat						
S.No.	**Concentration (µg/ml)**	**Retention time (min)**	**Peak Area**	**%Assay**	**USP Plate Count**	**Asymmetry**
1	15	6.040	23292377	100.03	12778	1.1
2	15	6.043	23308438	100.10	12689	1.1
3	15	6.047	23318233	100.14	12667	1.09
4	15	6.040	23293726	100.04	12791	1.08
5	15	6.043	23310382	100.11	12603	1.07
6	15	6.040	23314325	100.13	12336	1.09
Average		6.042	23306247	100.09	12644	1.09
SD		0.002787	10770.04	0.04625	166.5317	0.01169
%RSD		0.05	0.05	0.05	1.3	1.1

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Atazanavir Sulphate and Cobicistat were obtained with a mobile phase containing a mixture of 0.01M Potassium dihydrogen phosphate (pH adjusted to 3.5 with orthophosphoric acid) and Acetonitrile (30:70, v/v) was delivered at a flow rate of 1ml/min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 260nm based on peak area. The retention time of Atazanavir Sulphate and Cobicistat was found to be 2.243min and 6.043min respectively with resolution of 4.806. Linearity was established for Atazanavir Sulphate and Cobicistat in the range of 15-75µg/ml for Atazanavir Sulphate and 7.5-37.5µg/ml for Cobicistat with correlation coefficients (r^2=0.999) and the percentage recoveries were between 99.92-100.03% and 99.91-100.05% for Atazanavir Sulphate and Cobicistat respectively, which indicate accuracy of the proposed method. The % RSD values of accuracy for Atazanavir Sulphate and Cobicistat were found to be < 2 %. The % RSD values of method precision are 0.2% and 0.1% for Atazanavir Sulphate and Cobicistat respectively and % RSD values of system precision are 0.83% and 0.98% for Atazanavir Sulphate and Cobicistat. The % RSD values of reproducibility are 0.22% and 0.14% for Atazanavir Sulphate and Cobicistat respectively, reveal that the proposed method is precise.

LOD values for Atazanavir Sulphate and Cobicistat were found to be 0.6µg/ml and 0.2µg/ml respectively and LOQ values for Atazanavir Sulphate and Cobicistat were found to be 1.8µg/ml and 0.6µg/ml respectively.

The % RSD values of robustness studies were found to be < 2% reveal that the method is robust enough. These data show that the proposed method is specific and sensitive for the determination of Atazanavir Sulphate and Cobicistat.

CONCLUSION

The present RP-HPLC method for simultaneous estimation of Atazanavir Sulphate and Cobicistat in their combine dosage form was established and validated as per the ICH guidelines. Linearity was achieved for Atazanavir Sulphate and Cobicistat in the range of 15-75µg/ml for Atazanavir Sulphate and 7.5-37.5µg/ml for Cobicistat with correlation coefficients (r^2=0.999). The

percentage recoveries of Atazanavir Sulphate and Cobicistat were achieved in the range of 98-102% which was within the acceptance criteria. The percentage RSD was NMT 2 % which proved the precision of the developed method. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust. Hence it can be used for the hyphenated instrumental analysis of Atazanavir Sulphate and Cobicistat in their bulk and combined dosage form.

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