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**Research Article**

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**DEVELOPMENT AND VALIDATION OF STABILITY INDICATING  
RP- HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF  
ATAZANAVIR AND COBICISTAT IN TABLETS**

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**Abstract**

The objective of the current study was to develop and validate a rapid, precise, specific reverse phase HPLC method for the stability indicating analysis of atazanavir and cobicistat in tablets in its dosage form. The determination is done for the active pharmaceutical ingredient in its pharmaceutical dosage form. The dosage was subjected to analytical studies as per international conference on harmonization (ICH) prescribed. It was found that atazanavir and cobicistat is very sensitive to different conditions. The chromatographic conditions were optimized using the samples. Regression analysis shows an r value (correlation coefficient) 0.999 & 0.997 respectively for drugs. The satisfactory chromatographic separation, with good peak shapes were achieved on Zorbax X DBC-8; 150X4.6mm, 5 $\mu$ m. The method employed an isocratic elution and the detection wave-length was set at 235nm nm. With mobile phase pH 4.2 Buffer: ACN (70:30) with a flow rate of 1.0 ml/min. The developed RP-LC method was validated with respect to linearity, accuracy, precision, robustness and the degradations at different selected parameters.

**Keywords:** Atazanavir and Cobicistat, RP-HPLC method.

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**Introduction**

EVOTOZ is a fixed dose combination of two anti HIV drugs atazanavir (300mg) and cobicistat (150mg) which is used to treat patients suffering from AIDS. This is a combination where administration convenience and better compliance are put together. A dosage with combination is always better than single dosage in terms of cost and patient compliance. Foreseeing the need of different analytical methods for the estimation of ingredients of EVOTOZ, the ultimate goal of the work was to develop a validated HPLC method selective for the two components of the tablet EVOTOZ. Developing a single method for the

combination is a difficult task due to formation of drug-drug and drug-excipients interactions. Extensive literature survey did not reveal any simple, sensitive analytical method for the simultaneous determination of this combination of the drugs in EVOTOZ. Here, is an attempt to develop new, sensitive RP-HPLC method for the stability indicating studies of EVOTOZ.

A novel RP-HPLC method development and validation of Cobicistat in bulk drug and tablet dosage form.<sup>1</sup> Another such method Development and Validation of Stability Indicating RP-HPLC

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Method for Simultaneous Estimation of Atazanavir and Ritonavir in Bulk and Its Pharmaceutical Formulations.<sup>2</sup> There was another method reported development and validation of RP-HPLC method for the simultaneous estimation of atazanavir sulphate and ritonavir in bulk and formulations.<sup>3</sup> There is a stability indicating HPLC method for simultaneous estimation of emtricitabine, tenofovir disoproxyl fumarate, cobicistat and elvitegravir in pharmaceutical dosage form.<sup>4</sup> Recent research on analytical methods of analysis of raltegravir and elvitegravir.<sup>5</sup> Another development of novel and simple analytical method for the estimation of atazanavir sulphate in pharmaceutical formulation by RP-HPLC.<sup>6</sup> Method development and validation for the estimation of atazanavir in bulk and pharmaceutical dosage forms and its stress degradation studies using UV-Vis spectrophotometric method.<sup>7</sup> New validated RP-HPLC method for the determination of atazanavir sulphate in bulk and dosage form.<sup>8</sup> Analytical method development and validation of simultaneous estimation of atazanavir and ritonavir in bulk and pharmaceutical dosage forms by using RP-HPLC.<sup>9</sup> There is another validated RP-HPLC method for the determination of atazanavir in pharmaceutical dosage form.<sup>10</sup>

## Materials and methods

### Instrumentation

Waters LC system equipped with 2695 pump and 2996 photodiode array detector was used. The output signals were monitored and integrated using waters Empower 2.0 software. Analytical balance (Model: AB 204S, Make: Mettler Toledo) and Micro Balance (Model: XP 6, Make: Mettler Toledo) were used for weighing. Systronics digital pH meter 361 was used to adjust the pH of the buffer. Degassing of the mobile phase was done by sonication using Spinco Biotech Ultra Sonicator). Filtration was done by using millipore vacuum filter.

### Drugs and chemicals

Pure standards of Atazanavir and Cobicistat standards were kindly gifted from Hetero drugs Ltd., Hyderabad, India. The HPLC grade methanol, potassium di-hydrogen phosphate, ortho phosphoric acid were purchased from Merck.

### Preparation of solutions

#### Preparations of buffer

Weighed about 1.36gms of sodium acetate into a 1000ml beaker and dissolved and diluted to 1000ml with milli-Q water. Adjusted the pH to 4.2 with acetic acid and filtered through 0.45µm membrane filter.

#### Preparation of Mobile phase

**Mobile phase A:** Methanol

**Mobile phase B:** pH 4.2 buffer

#### Preparation of diluent

Methanol and buffer were mixed in the ratio 70:30v/v and sonicated for 10 minutes.

#### Preparation of solutions for peak identification

##### Preparation of Atazanavir standard solution for peak identification

Weighed accurately 10mg of atazanavir standard into a 25ml volumetric flask and added about 10ml of diluent, sonicated for 10 minutes to dissolve and diluted upto the mark with diluent.

##### Preparation of Cobicistat standard solution for peak identification

Weighed accurately 150mg of Cobicistat standard into a 100ml volumetric flask and added about 10ml of diluent, sonicated for 10 minutes to dissolve and diluted upto the mark with diluent.

#### Preparation of standard solution

Accurately weighed and transferred 150mg of cobicistat and 300mg of atazanavir working standards into a 100ml clean dry volumetric flask, added about 30ml of diluent and sonicated to dissolve it completely and made volume up to the mark with the same diluent.

#### Preparation of placebo solution

Weighed accurately 75.21mg of placebo powder into 100ml volumetric flask, added 30ml of the diluent and sonicated for 20min and diluted to the volume with diluent.

#### Test preparation

Accurately weighed and finely powdered 20 tablets of EVOTOZ and transferred an amount of the powder equivalent to 150mg of cobicistat and 300mg of atazanavir into a 100ml of volumetric flask, added 30ml of the diluent and sonicated for 20min and diluted to the volume with diluent.

**Optimized chromatographic conditions**

After systematic and detailed study of the various parameters involved in the method, the following conditions were employed.

<b>Column</b>	:	Zorbax X DBC-8; 150X4.6mm,5µm
<b>Flow rate</b>	:	1.0 ml per min
<b>Wavelength</b>	:	235 nm
<b>Injection volume</b>	:	10 µL
<b>Column oven Temperature</b>	:	Ambient
<b>Run time</b>	:	20min.

**Procedure**

Column was equilibrated for at least 60 minutes with the mobile phase flowing through the system at a rate of 1.0ml/min. Detector was set at a wavelength of 235nm. Separately injected 20µL of diluent, placebo, peak identification solutions,

standard solution, test solutions into the chromatograph and the chromatograms were recorded. The percent assay values of the atazanavir and cobicistat were calculated by using the following formulae.

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

Where:

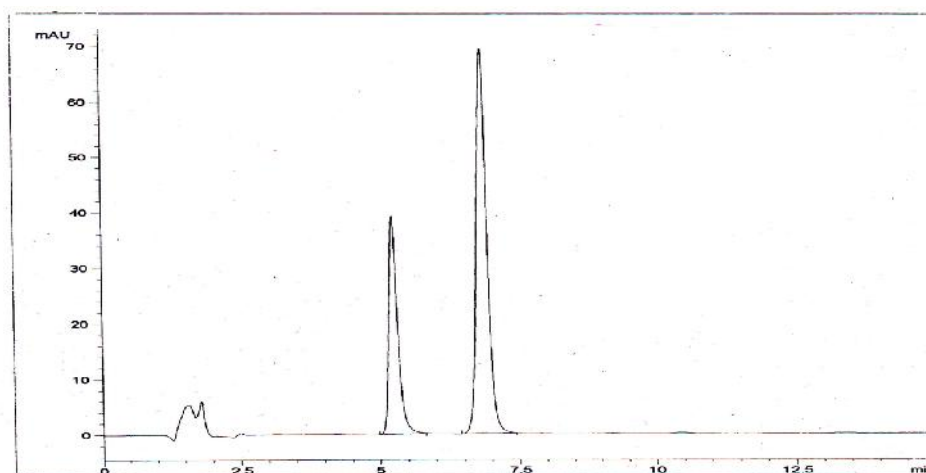
- AT = Peak Area obtained with test preparation
- AS = Peak Area obtained with standard preparation
- WS = Weight of working standard taken in mg
- WT = Weight of sample taken in mg
- DS = Dilution of Standard solution
- DT = Dilution of sample solution
- P = Percentage purity of working standard.



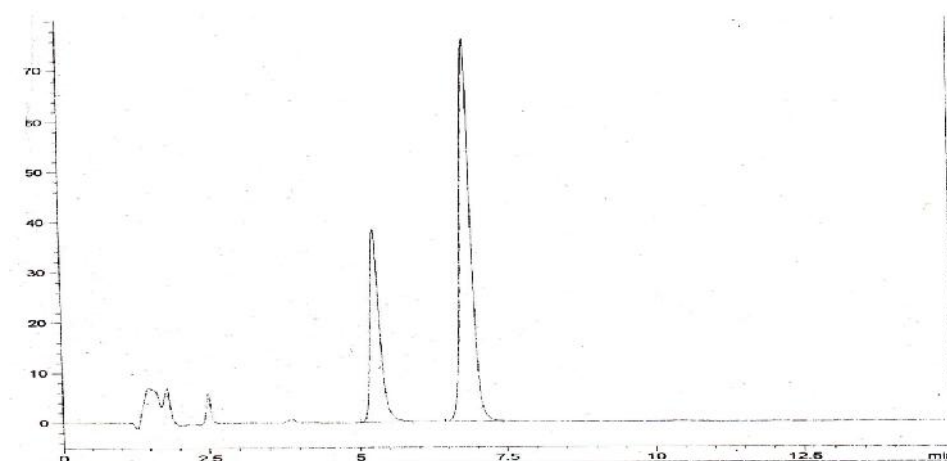
**Fig. No. 01: Representative Model Chromatogram of Blank Solution.**



**Fig. No. 02: Representative Model Chromatogram of Placebo**



**Fig. No. 03: Representative Model Chromatogram of Standard Solution**



**Fig. No. 04: Representative Model Chromatogram of Sample Solution**

### **Analytical method Validation**

#### **System suitability**

According to the USP 33 System suitability is the integral part of the chromatographic method. This test was conducted to verify that the reproducibility and effectiveness of the system is adequate for the analysis.

To ascertain its effectiveness 20 $\mu$ L of freshly prepared standard solution containing 45 $\mu$ g/ml of atazanavir and, 232 $\mu$ g/ml cobicistat was injected 6 times into the HPLC system by using optimized chromatographic conditions and System suitability results were calculated. The %RSD for the peak areas and retention times of both the drugs were found to be less than 2.0%. The theoretical plates were more than 2000 for both the drugs. Tailing factor was found to be less than 2.0. The resolution between the adjacent peaks was found to be more

than 6.0. All the results were tabulated in the below tables.

#### **Specificity**

##### **Blank and placebo interference**

A study to establish the interference of blank and placebo was conducted. Analysis was performed on placebo preparation described previously in triplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of blank and placebo solutions show no peaks at the retention times of atazanavir and cobicista. This indicates that the excipients used in the formulation did not interfere in the estimation. The chromatograms of blank and placebo using the proposed method were shown in figures 1 and 2.

**Table No. 01: System suitability of Atazanavir**

S.No	Retention time	Peak area	Theoretical plates	Tailing
1	5.282	428946	6524	1.2
2	5.277	429023	6421	1.3
3	5.281	424524	6852	1.5
4	5.266	423556	6425	1.4
5	5.259	428924	6423	1.1
6	5.282	422325	6425	1.5
7	5.272	427825	6426	1.2
8	5.275	429026	6426	1.5
9	5.272	422721	6523	1.4
10	5.269	426762	6452	1.1
<b>AVERAGE</b>	5.2775	425503		
<b>SD</b>	0.0059	924.9		
<b>%RSD</b>	0.10	0.3		

**Table No. 02: System suitability of Cobicistat**

S.No	Retention time	Peak area	Theoretical plates	Tailing	Resolution
1	6.873	907842	8564	1.4	6.5
2	6.786	905658	8648	1.2	6.7
3	6.635	903456	8324	1.1	6.6
4	6.789	9078986	8612	1.3	6.8
5	6.869	903244	8645	1.4	6.5
6	6.807	903788	8654	1.3	6.4
7	6.726	905689	8659	1.2	6.6
8	6.808	905478	8546	1.1	6.4
9	6.847	905682	8654	1.3	6.6
10	6.780	905647	8721	1.1	6.5
<b>AVERAGE</b>	6.698	905760			
<b>SD</b>	0.053	935.7			
<b>%RSD</b>	0.734	0.1			

**Interference from degradation products****Preparation of degradation samples****Preparation of sample for Acid degradation**

EVOTOZ sample was refluxed with the 1M HCl at 60°C for 1hour and then neutralized with 1N NaOH. The sample was prepared as per the test method and then further diluted upto the required concentration with the diluent.

**Preparation of sample for Alkaline degradation**

EVOTOZ sample was refluxed with the 1M NaOH. at 60°C for 1hour and then neutralized with 1M HCl The sample was prepared as per the test method and then further diluted upto the required concentration with the diluent.

**Preparation of sample for Oxidative degradation**

EVOTOZ sample was refluxed with the 10% $H_2O_2$  by heating on water bath at 60°C for 1 hour. The sample was prepared as per the test method and then further diluted upto the required concentration with the diluent.

**Preparation of sample for Photolytic degradation**

EVOTOZ sample was exposed to UV (200watt-hr/m<sup>2</sup>) and visible (1.2 million lux hrs).The sample was prepared as per the test method and then further diluted upto the required concentration with the diluent.

**Preparation of sample for Thermal degradation**

EVOTOZ sample was exposed to temperature at 105°C for 24hrs. The sample was prepared as per the test method and then further diluted upto the required concentration with the diluent.

**Preparation of sample for Humidity degradation**

EVOTOZ sample was exposed to 85% humidity for 24hrs .The sample was prepared as per the test method and then further diluted up to the required concentration with the diluent.

All the stressed samples were injected into the HPLC system by using optimized chromatographic conditions and the chromatographs were recorded.

The chromatograms of the stressed samples were evaluated for peak purity of the two drugs using PDA detector and Empower software. In all forced degradation samples the two drugs passed the peak purity (purity angle is less than purity threshold).

All the degradant peaks were well separated from the two drugs. Thus the method can be used for simultaneous estimation of atazanovir and cobicistat in bulk and pharmaceutical formulations and also the method is stability indicating.

**Table No. 03: Degradation Table for Atazanovir**

Stress Condition	Purity Angle	Purity Threshold	% Assay	%Degradation
Acid degradation	0.15	0.18	92.2	7.6
Alkali degradation	0.17	0.23	91.6	8.5
Thermal degradation	0.28	0.33	91.5	9.2
Humidity degradation	0.21	0.25	95.9	4.7
Photolytic degradation	0.15	0.21	92.2	8.7
Peroxide degradation	0.21	0.24	90.1	10.5

**Table No. 04: Degradation Table for Cobicistat**

Stress Condition	Purity Angle	Purity Threshold	% Assay	%Degradation
Acid degradation	0.14	0.18	91.8	7.7
Alkali degradation	0.21	0.24	92.4	7.2
Thermal degradation	0.34	0.38	89.1	10.1
Humidity degradation	0.16	0.23	96.9	2.6
Photolytic degradation	0.20	0.22	98.6	0.9
Peroxide degradation	0.21	0.26	88.5	11.2

#### Method precision

Precision of the method was conducted by performing the assay of EVOTOZ tablets 6 times. The samples were prepared six times according to the test preparation mentioned earlier and analyzed

by using the test method. The % Assay values were calculated for both the drugs and found to be in between 98.0% - 102.0%. The %RSD values were found to be less than 2.0%.

**Table No. 05: Method precision for Cobicistat & Atazanovir**

S.NO.	%ASSAY	
	Cobicistat	Atazanovir
1	99.9	100.1
2	98.1	99.9
3	98.7	100.0
4	100.4	100.2
5	101.2	99.1
6	98.5	100.0
Average	98.8	100.1
SD	0.91	0.93
% RSD	0.92	0.92

#### Limit of Detection and Limit of Quantification

A study to establish the Limit of Detection and Limit of Quantification of atazanovir and Cobicistat was conducted. Limit of detection and Limit and quantification were established based on signal to noise ratio. A series of dilutions of the test

solution were injected. Limit of detection was established by identifying the concentration which gives signal to noise ratio of about 3. Limit of Quantification was established by identifying the concentration which gives signal to noise ratio of about 10.

**Table No. 06: LOQ and LOD values for Atazanovir and Cobicistat**

Component name	Limit of Detection	Limit of Quantification		
	Concentration ( $\mu\text{g/ml}$ )	Concentration ( $\mu\text{g/ml}$ )	%Mean recovery	%RSD
Cobicistat	1.3	2.41	101.6	1.4
Atazanovir	0.5	1.11	101.8	1.2

**Accuracy**

Accuracy for atazanovir and cobicistat was conducted by spiking these three drugs to the placebo powder at three different levels of the target concentration (i.e. 50%, 75%, 100%, 125% and 150%) six times at 50% and 150% level and

three times at remaining levels. The mean %Recovery and %RSD values were calculated. The %Recovery values for both the drugs were found to be between 98.0% to 102.0% and %RSD values were found to be less than 2.0%.

**Table No. 07: Accuracy for Cobicistat**

S.No.	%Spike level	Amount added(mg)	Amount found(mg)	%Recovery	Statistical parameters
1		124.2	123.9	99.8	
2		125.9	124.7	99.8	
3	50%	126.8	125.6	99.8	Mean=99.9 SD=0.18 %RSD=0.18
4		123.5	122.8	100.2	
5		125.2	123.9	99.8	
6		123.7	122.6	99.9	
7		182.9	181.6	99.8	
8	75%	183.1	182.7	99.8	Mean=99.8 SD=0.03 %RSD=0.03
9		182.7	181.4	99.8	
10		245.8	244.30	99.8	
11	100%	243.5	242.3	99.9	Mean=99.8 SD=0.08 %RSD=0.08
12		244.3	243.7	99.8	
13		313.7	312.1	99.8	
14	125%	312.6	311.1	99.8	Mean=99.8 SD=0.02 %RSD=0.02
15		310.9	309.3	99.8	
16		376.8	375.6	99.9	
17		373.6	372.3	99.9	
18	150%	375.1	374.9	99.9	Mean=99.9 SD=0.22 %RSD=0.22
19		370.7	369.5	99.9	
20		373.8	372.4	99.9	
21		371.7	370.5	99.9	

**Table No. 08: Accuracy for Atazanovir**

S.no.	%Spike level	Amount added(mg)	Amount found(mg)	%Recovery	Statistical parameters
1		49.6	49.4	100.2	
2		49.2	49.0	99.6	
3	50%	49.5	49.3	99.6	Mean=99.8 SD=0.30 %RSD=0.30
4		49.5	49.	100.2	
5		49.7	49.6	99.8	
6		49.9	49.6	99.6	
7		74.5	74.3	99.7	
8	75%	74.8	74.6	99.7	Mean=99.7 SD=0.08 %RSD=0.08
9		75.2	74.9	99.6	
10		99.8	99.70	99.9	
11	100%	99.90	99.6	99.7	Mean=99.9 SD=0.20 %RSD=0.20
12		99.7	99.8	100.1	
13		124.8	124.6	99.8	
14	125%	124.6	124.4	99.8	Mean=99.8 SD=0.05 %RSD=0.05
15		124.9	124.8	99.9	
16		149.6	149.6	100.0	
17		149.9	149.7	99.9	
18	150%	149.7	149.5	99.9	Mean=99.9 SD=0.05 %RSD=0.05
19		150.1	149.9	99.9	
20		150.3	150.1	99.9	
21		149.9	149.7	99.9	

**Linearity and range**

Linearity of the detector response was established by plotting a graph of concentration versus peak area. A series of solutions of standard were prepared by appropriate dilutions of Linearity standard stock solution.

**Preparation of Linearity stock solution**

Weighed accurately and transferred 37.5mg atazanavir WS and 25 mg cobicistat WS, into 100ml volumetric flask, added 30ml diluent of the diluent and sonicated for 20min and diluted to the volume with diluent, filtered through 0.45µm filter.

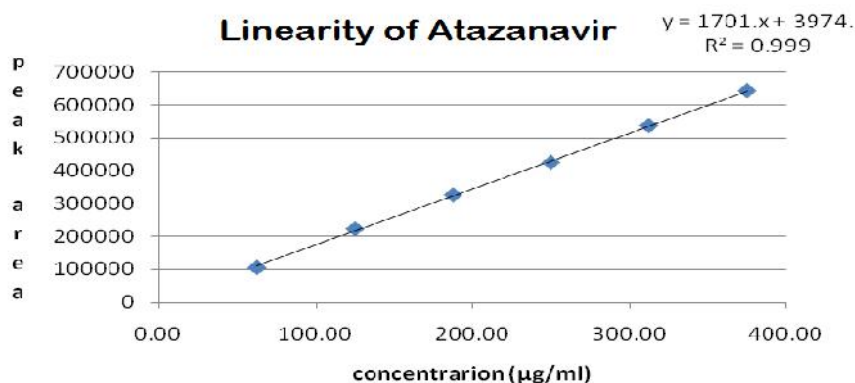
**Preparation of Linearity solutions**

Series of solutions in the range of 25% to 150% of target concentration were prepared by transferring 2.5ml, 5.0ml, 7.5ml, 10.0ml, 12.5mL, 15.0ml of linearity stock solution into separate 50.0mL volumetric flasks and making the volume up to the mark with the diluent.

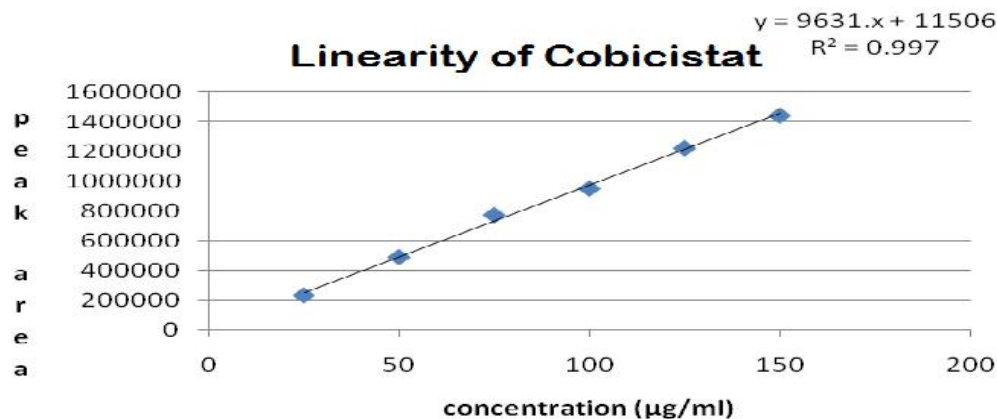
The detector response was found to be linear in the range of 25.0 to 150.0 $\mu$ g/ml for cobicistat and 62.5 to 375.0 $\mu$ g/ml for atazanavir. The correlation coefficient values were found to be within the limits.

**Table No. 09: Linearity for Atazanavir**

S.No.	Linearity level	Concentration( $\mu$ g/ml)	Peak area
1	25	62.50	105456
2	50	125.00	223244
3	75	187.50	325684
4	100	250.00	424396
5	125	312.50	536254
6	150	375.00	642456

**Graph 01: Graphical Representation of Linearity of Atazanavir****Table No. 10: Linearity for Cobicistat**

S.NO.	Linearity level	Concentration ( $\mu$ g/ml)	Peak area
1	25	25	234873
2	50	50	492064
3	75	75	775246
4	100	100	953565
5	125	125	1225019
6	150	150	1445019

**Graph 02: Graphical Representation of Linearity for Cobicistat**



**Table No. 11: Regression Data of the Proposed Method**

S. No.	Parameters	Atazanovir	Cobicistat
1	Linearity ( $\mu\text{g/ml}$ )	62.50 – 375.00	25 – 150
2	Regression( $mx+c$ )	1701x+3974	9631x+11506
3	Slope(m)	1701	9631
4	Intercept(c)	3974	11506
5	Correlation coefficient ( $r^2$ )	0.999	0.997

**Ruggedness**

A study to establish ruggedness of the method was conducted by preparing and analyzing the standard and test preparation on two different days by two different analysts on two different columns and two

different HPLC systems. The system suitability parameters and the %Assay values of all the three drugs were calculated and the differences between the two analysts were evaluated and the method was found to rugged.

**Table No. 12: Ruggedness for Atazanavir**

S.No	Atazanavir		Overall results
	Analyst-1	Analyst -2	
1	98.7	99.7	<b>Mean : 99</b> <b>SD: 0.9</b> <b>%RSD: 0.9</b>
2	99.7	98.4	
3	99.1	100.4	
4	98.1	99.2	
5	98.9	99.4	
6	101.1	100.1	
Average	99.3	99.5	
SD	1.0	0.71	
% RSD	1.0	0.71	

**Table No. 13: Ruggedness for Cobicistat**

S.No	Cobicistat		Overall results	
	Analyst-1	Analyst-2		
1	98.5	98.7	<b>Mean</b> 99.8 <b>SD</b> 1.01 <b>%RSD</b> 1.01	
2	99.4	99.5		
3	99.7	101.5		
4	100.5	100.8		
5	99.7	99.8		
6	98.4	101.1		
Average	99.4	100.2		
SD	0.8	1.07		
% RSD	0.8	1.07		

**Table No. 14: Robustness for the proposed method**

Optimum Conditions	Modifications	Retention Time		Asymmetric factor		Theoretical plates		Resolution
		AT	CO	AT	CO	AT	CO	
<b>Mobile phase composition (Buffer:ACN) (70:30 v/v)</b> <b>pH (4.2)</b> <b>Column</b> <b>temperature (30°C)</b> <b>Flow rate (1.0 mL/min)</b> <b>Wave length (235nm)</b>	60:40	4.818	6.316	0.9	0.9	6631	8521	6.1
	80:20	5.681	7.146	1.2	1.2	6721	8568	6.8
	4.1	5.161	6.741	1.3	1.3	6531	8471	6.3
	4.3	5.371	6.951	1.1	1.1	6836	8582	6.4
	25	5.848	7.241	1.2	1.2	6532	8541	6.5
	35	5.171	6.751	1.1	1.1	6781	8522	6.5
	0.9	5.772	7.454	1.1	1.1	6581	8612	6.8
	1.1	4.781	6.251	1.2	1.2	6812	8712	6.3
	233	5.268	6.839	1.2	1.2	6631	8572	6.4
	237	5.271	6.841	1.2	1.2	6529	8498	6.8

### Robustness

A study to establish the effect of variation in flow rate, column temperature, pH of the buffer in the mobile phase was conducted. Standard and test

solutions prepared as per the proposed method and were injected into the HPLC system. The system suitability parameters, and the %Assay values were evaluated and the method was found to be robust.

### Results and Discussions

The drug solution was scanned from 200-400 nm, it was observed that the drugs show appreciable absorbance at 235nm, hence detection was set at 235nm for method development purpose. Attempts were made to get good separation of the drug by varying parameters like, flow rate, pH, buffer molarity, buffer components, type of organic modifier, gradient times, and buffer: organic modifier ratio and could get good elution time in isocratic mode. To achieve this, experiments were conducted by changing the columns and mobile phases but unsuccessful in getting good peaks with less run time. Then method was optimized to separate the main peak. The satisfactory chromatographic separation, with good peak shapes were achieved on Zorbax X DBC-8; 150X4.6mm, 5 $\mu$ m with mobile phase pH 4.2 Buffer : ACN (70:30) with a flow rate of 1.0 ml/min. All the System Suitability parameters are within the acceptance limits. The calibration curve for atazanovir and cobicistat was obtained by plotting the respective peak areas against their concentration. The graph was found to be linear over the range 62.50-375.00  $\mu$ g/ml for atazanovir and 25-150  $\mu$ g/ml for cobicistat with the correlation coefficient 0.999 & 0.997 respectively. The drug which shows that the good correlation exists between peak area and concentration of the drug. The ruggedness was performed and the % RSD was less than 2, hence, method was rugged. The high % recovery values obtained for the drug show that the method is accurate. The LOD value of atazanovir & cobicistat was found to be 1.2 $\mu$ g/ml and 2.51 $\mu$ g/ml. The LOQ was 0.7 $\mu$ g/ml and 1.10 $\mu$ g/ml respectively. The low values of LOD and LOQ show that the method is sensitive and can estimate at micro gram level. The absence of additional peaks indicates the method is specific and the drugs were stable in the diluents for 8 hours which is sufficient to complete the work. The stability indicating studies were performed for the above mentioned drug viz... acid, alkali, thermal, humidity, photolytic, peroxide and the percentage degradation was 7.6%, 8.5%, 9.2%, 4.7%, 8.7%, 10.5%, and 7.7%, 7.2%, 10.1%, 2.6%, 0.9%, 11.2% for atazanovir & cobicistat respectively.

### Conclusion

The proposed RP-High performance liquid chromatographic method has been evaluated for the accuracy, precision and linearity. The method was found to be precise, accurate and linear over the linear concentration range. In this method, there was no interference from matrix sources. Moreover, the lower solvent consumption along with the short analytical run time of 12.5 minutes that allows the analysis of a large number of samples in a short period of time. Therefore, this RP-HPLC method can be used as a routine analysis of these drugs in bulk, pharmaceutical formulations and also for stability studies.

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