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Research

Simultaneous estimation of new analytical method development and validation of atazanavir and cobicistat by high performance liquid chromatography

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Abstract

A simple, specific, precise, and efficient method for the Simultaneous estimation of Cobicistat and Atazanavir in pure and pharmaceutical dosage forms by a Reverse Phase-High Performance Liquid Chromatography method is developed and validated. Selected mobile phase were in a combination of ACN, Methanol and Phosphate buffer pH4.6 (10:25:65 v/v). Optimized column is a Develosil C18 (4.6mm×250mm) 5µm particle size and at a flow rate of 1.0mL/min with detection wavelength at 238nm for Cobicistat and Atazanavir. In our study, the validation of analytical method for determination of Cobicistat and Atazanavir in pure and pharmaceutical dosage forms was performed in accordance the parameters including-system suitability, specificity, linearity of response, accuracy, precision (reproducibility & repeatability), robustness (change of wave length±2 nm). The method is validated according to ICH guidelines. The results obtained by RP-HPLC methods are rapid, accurate and precise. Therefore proposed method can be used for routine analysis of Cobicistat and Atazanavir in the pure form as well as in combined pharmaceutical dosage form.

Keywords: Cobicistat and Atazanavir, HPLC, Method Development, Validation.

INTRODUCTION

HPLC

High Performance Liquid Chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography the use of an instrumentation that was originally developed for gas chromatography. High Pressure Liquid Chromatography was developed in the mid-1970 and was improved with the development of column packing material and the additional convenience of on-line detectors. The various components of HPLC are pumps (solvent delivery system), mixing unit, gradient controller and solvent degasser, injector (manual or automatic), guard column, analytical columns, detectors, recorders and/or integrators. Recent models are equipped with computers and software for data acquisition and processing. The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase at a flow rate of 1-5 cm3/min. The mobile phase acts as a carrier for the sample solution. The chemical interactions of the mobile phase and sample with the column determine the degree of migration and separation of components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.

Types of Chromatography

1. Normal-phase chromatography

Mechanism: Retention by interaction with the polar surface of the stationary phase with polar parts of the sample molecules.
Stationary phase: SiO_2, Al_2O_3, $-NH_2$, -CN, -Diol, $-NO_2$, etc.
Mobile phase: Heptane, hexane, cyclohexane, $CHCl_3$, CH_2Cl_2, dioxane, methanol, etc.

2. Reversed-phase chromatography

Mechanism: Retention by interaction of the stationary phase's non-polar hydrocarbon chain with non-polar parts of the sample molecules.
Stationary phase: n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, $(CH_2)_n$-CN, $(CH_2)_n$-diol, etc.
Mobile phase: Methanol, acetonitrile, water, buffer (sometimes with additives of THF or Dioxane), etc.

Advantages of HPLC

1) It provides specific, sensitive and precise method for analysis of the different complicated sample.
2) There is ease of sample preparation and sample introduction.
3) There is speed of analysis.
4) The analysis by HPLC is specific, accurate and precise.

Instrumentation

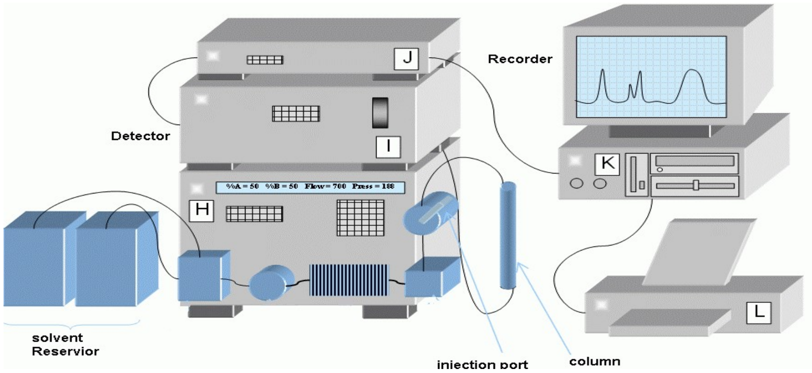

Fig 1: Diagram of HPLC Instrument[3]

The basic components of HPLC are

1. Pumping System
2. Sample Introduction Device
3. Chromatographic Column
4. Detector
5. Data handling Device

MATERIALS AND METHODS

Cobicistat(Pure)-Sura labs, Atazanavir(Pure)-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck

HPLC METHOD DEVELOPMENT: TRAILS

Preparation of standard solution: Accurately weigh and transfer 10 mg of Cobicistat and Atazanavir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1 ml of the above Cobicistat and Atazanavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Orthophosphoric acid and Phosphoric acid (pH 3): Acetonitrile and Methanol: ACN with varying proportions. Finally, the mobile phase was optimized to Buffer: Methanol: ACN in proportion 65:25:10v/v respectively.

Optimization of Column: The method was performed with various columns like C18 column, ODS and Zodiac column. Altima C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used : Waters HPLC with auto sampler and PDAdetector 996 model.
Temperature : 35ºc
Column : Altima C18 (4.6×150mm, 5µ)
Buffer : Phosphate buffer (pH-4.6)-Dissolve 0.9g of anhydrous dihydrogen phosphate and 1.298g of Citric acid mono hydrate in sufficient water to produce 1000mL. Adjust the pH 4.6 by using ortho phosphoric acid.
pH : 4.6
Mobile phase : Buffer: Methanol: ACN (65:25:10v/v)
Flow rate : 1ml/min
Wavelength : 245 nm
Injection volume : 10 µl
Run time : 14 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Phosphate buffer (pH-4.6)

Dissolve 0.9g of anhydrous di hydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000mL. Adjust the p H 4.6 by using ortho phosphoric acid.

Preparation of mobile phase: Accurately measured 650 ml (65%) of Buffer and 250 ml of Methanol (25%) and 100ml (10%) of Acetonitrile were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Buffer: Methanol: ACN (65:25:10v/v)
Column : Altima C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 265 nm
Column temp : 38ºC
Injection Volume : 10 µl
Run time : 14 minutes

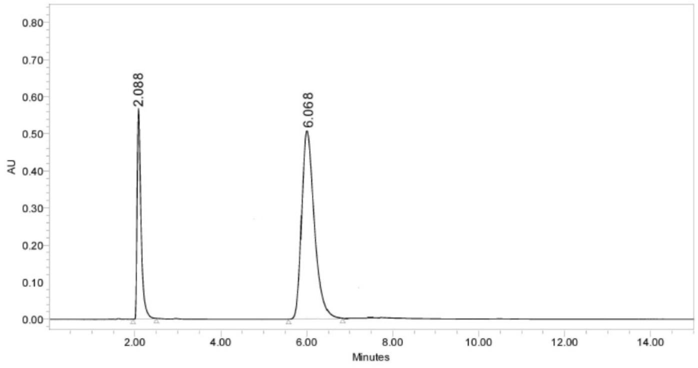

Fig 2: Optimized Chromatogram

Table 1: peak results for trail 4

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Cobicistat	2.088	3425413	567933		1.0	5565.5
2	Atazanavir	6.068	1629854	517733	2.5	1.1	5355.2

From the above chromatogram it was observed that the Cobicistat and Atazanavir peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

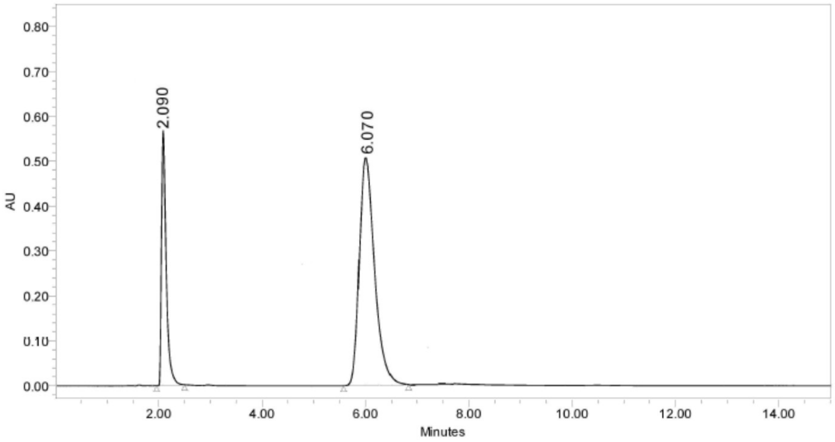

Fig 3: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Cobicistat	2.090	3468547	567933		1.0	5565.5
2	Atazanavir	6.070	16289441	517733	2.5	1.1	5355.2

- *Resolution between two drugs must be not less than 2*
- *Theoretical plates must be not less than 2000*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

System suitability

Table 3: Results of system suitability for Cobicistat

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Cobicistat	2.080	3569412	567917	5568.0	1.0
2	Cobicistat	2.080	3465125	517719	6359.2	1.1
3	Cobicistat	2.080	3598154	567933	5565.5	1.0
4	Cobicistat	2.081	3586491	517733	5355.2	1.1
5	Cobicistat	2.081	3582694	567917	6348.0	1.0
Mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of method precession for Atazanavir

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Atazanavir	2.080	3582264	567917	5568.0	1.0	2.5
2	Atazanavir	2.080	3586491	517719	5359.2	1.1	2.5
3	Atazanavir	2.080	3598154	567933	5565.5	1.0	2.5
4	Atazanavir	2.081	3564125	517733	5355.2	1.1	2.5
5	Atazanavir	2.081	3569412	562173	5568.0	1.0	2.5
Mean			3580089				
Std. Dev			13609.81				
% RSD			0.380153				

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Assay (Standard)

Table 5: Peak results for assay standard

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Cobicistat	2.087	3425681	567917		1.0	5568.0	1
2	Atazanavir	6.067	16235984	517719	2.5	1.1	5359.2	1
3	Cobicistat	2.088	3425413	567933		1.0	5565.5	2
4	Atazanavir	6.068	16298543	517733	2.5	1.1	5355.2	2
5	Cobicistat	2.088	3465423	567933		1.0	5545.5	3
6	Atazanavir	6.068	16265213	517733	2.5	1.1	5352.1	3

Assay (Sample)

Table 6: Peak results for Assay sample

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Cobicistat	2.089	3469821	567917		1.0	6568.0	1
2	Atazanavir	6.069	16259845	517719	2.5	1.1	5359.2	1
3	Cobicistat	2.090	3468547	567933		1.0	5565.5	2
4	Atazanavir	6.070	16287531	517733	2.5	1.1	5355.2	2
5	Cobicistat	2.090	3468143	567813		1.0	5391.1	3
6	Atazanavir	6.070	16282431	517623	2.5	1.1	5564.0	3

$$\%\text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

==16276602 / 16266580 × 10/60 × 60/0.0136 × 99.6/100 × 0.4102/300 × 100
= 100.1%
The % purity of Cobicistat and Atazanavir in pharmaceutical dosage form was found to be 100.1 %.

LINEARITY: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY
Cobicistat

Concentration Level (%)	Concentration μg/ml	Average Peak Area
33.3	10	1010252
66.6	20	2049374
100	30	3072706

133.3	40	3921068
166.6	50	4952813

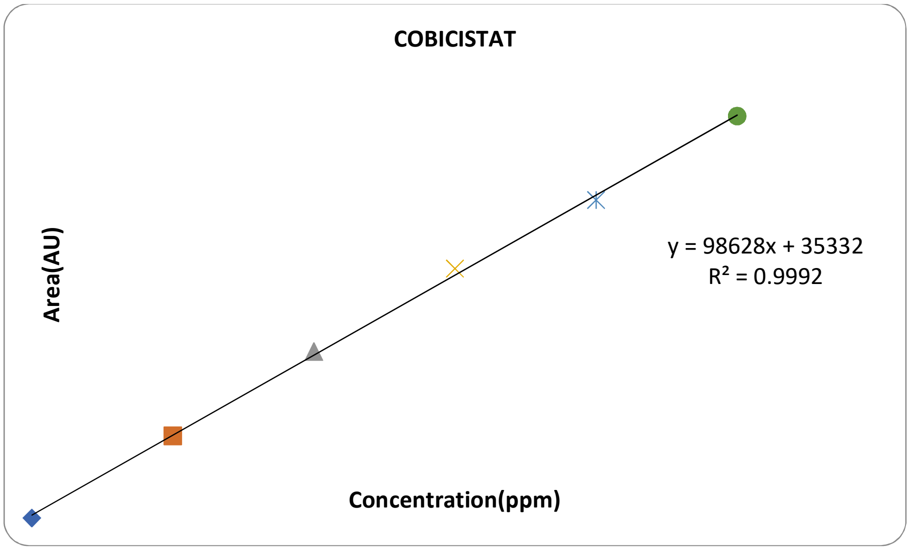

Fig : calibration graph for Cobicistat

Atazanavir

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	20	8040807
66	40	14318417
100	60	21087985
133	80	27913928
166	100	34584741

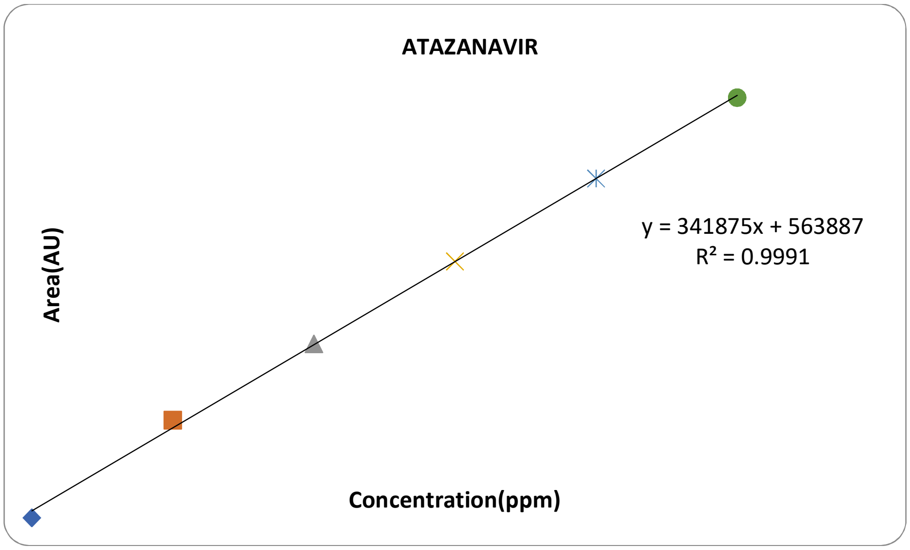

Fig : calibration graph for Atazanavir

REPEATABILITY

Table 7: Results of repeatability for Cobicistat

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Cobicistat	2.084	3569412	567917	5568.0	1.0
2	Cobicistat	2.083	3465125	517719	5359.2	1.1
3	Cobicistat	2.082	3598154	567933	5565.5	1.0
4	Cobicistat	2.081	3586491	517733	5355.2	1.1
5	Cobicistat	2.080	3582694	567917	5568.0	1.0
Mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 8: Results of method precession for Atazanavir

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Atazanavir	6.056	1582264	567917	5568.0	1.0	2.5
2	Atazanavir	6.057	1586491	517719	5359.2	1.1	2.5
3	Atazanavir	6.058	1598154	567933	5565.5	1.0	2.5
4	Atazanavir	6.059	1564125	517733	5355.2	1.1	2.5
5	Atazanavir	6.060	1569412	562173	5568.0	1.0	2.5
Mean			1580089				
Std. Dev			13609.81				
% RSD			0.861332				

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision

Table 9: Results of Intermediate precision for Cobicistat

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Cobicistat	2.081	3481579	567917	5568.0	1.0
2	Cobicistat	2.082	3458121	517719	5359.2	1.1
3	Cobicistat	2.083	3426581	567933	5565.5	1.0
4	Cobicistat	2.084	3465712	517733	5355.2	1.1
5	Cobicistat	2.085	3451476	567917	5568.0	1.0
6	Cobicistat	2.085	3452106	567514	5359.2	1.1
Mean			3455929			
Std. Dev			18188.92			
% RSD			0.5			

- *%RSD of five different sample solutions should not more than 2*

Table 10: Results of Intermediate precision for Atazanavir

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Atazanavir	6.061	15481579	567917	5568.0	1.0	2.5
2	Atazanavir	6.062	15369852	517719	5359.2	1.1	2.5
3	Atazanavir	6.063	15248454	567933	5565.5	1.0	2.5
4	Atazanavir	6.064	15874692	517733	5355.2	1.1	2.5
5	Atazanavir	6.064	15236547	567933	5568.0	1.0	2.5
6	Atazanavir	6.064	15217547	567133	5359.2	1.1	2.5

Mean	15404779
Std. Dev	251289.4
% RSD	1.6

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Table 11: Results of Intermediate precision Day 2 for Cobicistat

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Cobicistat	2.081	3481579	567917	5568.0	1.0
2	Cobicistat	2.082	3458121	517719	5359.2	1.1
3	Cobicistat	2.083	3426581	567933	5565.5	1.0
4	Cobicistat	2.084	3465712	517733	5355.2	1.1
5	Cobicistat	2.085	3451476	567917	5568.0	1.0
6	Cobicistat	2.085	3452106	567514	5359.2	1.1
Mean			3455929			
Std. Dev			18188.92			
% RSD			0.5			

- *%RSD of five different sample solutions should not more than 2*

Table 12: Results of Intermediate precision for Atazanavir

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Atazanavir	6.061	15481579	567917	5568.0	1.0	2.5
2	Atazanavir	6.062	15369852	517719	5359.2	1.1	2.5
3	Atazanavir	6.063	15248454	567933	5565.5	1.0	2.5
4	Atazanavir	6.064	15874692	517733	5355.2	1.1	2.5
5	Atazanavir	6.064	15236547	567933	5568.0	1.0	2.5
6	Atazanavir	6.064	15217547	567133	5359.2	1.1	2.5
Mean			15404779				
Std. Dev			251289.4				
% RSD			1.6				

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

ACCURACY

The accuracy results for Cobicistat

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1543793	15	15.2	101.9	100.9%
100%	3035883	30	30.4	101.4	
150%	4451005	45	44.7	99.4	

The accuracy results for Atazanavir

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1084420	30	30.07	100.2	99.6%
100%	2096069	60	59.6	99.4	
150%	3112684	90	89.3	99.3	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 13: Results for Robustness Cobicistat

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	3425413	2.088	5568.2	1.0
Flow rate of 0.9 mL/min	3425282	3.111	5922.2	1.2
Flow rate of 1.1 mL/min	3517879	1.880	5868.8	1.2
Less aqueous phase	3175485	3.101	5836.2	1.2
More aqueous phase	3365431	1.881	5282.6	1.1

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Atazanavir

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	2029854	6.068	5359.2	1.1
Flow rate of 0.9 mL/min	1738319	7.101	5999.1	1.2
Flow rate of 1.1 mL/min	1638304	5.007	5989.2	1.1
Less aqueous phase	1973724	7.108	5387.2	1.1
More aqueous phase	2102838	5.008	5938.1	1.1

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Cobicistat and Atazanavir in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Cobicistat and Atazanavir was freely soluble in ethanol, methanol and sparingly soluble in water. ACN, Methanol and Phosphate buffer pH4.6 (10:25:65 v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Cobicistat and Atazanavir in bulk drug and in Pharmaceutical dosage forms.

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