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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF COBICISTAT AND ATAZANAVIR BY RP HPLC IN PHARMACEUTICAL FORMULATION

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ABSTRACT

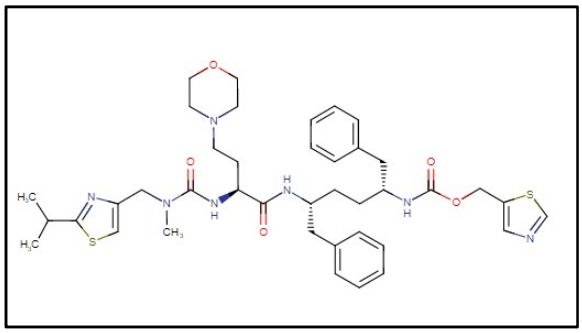
A Specific, Precise, Accurate, Linear and Robust method was developed for the simultaneous estimation of the Cobicistat and Atazanavir in tablet dosage form and Validated done as per ICH Validation guidelines. Method was optimized by Zorbax CyanoColumn (150*4.6mm &5µm) column at a flow rate of 1.0ml/min, Mobile phase was 0.1% Orthophosphoricacid: Methanol (75:25). The Column Oven temperature was maintained at 25°C and working wave length was selected at 234nm. The retention times of Cobicistat and Atazanavir were found to be 2.853min and 4.090min respectively. % RSD of the Cobicistat and Atazanavir were and found to be 0.48% and 0.62% respectively. In Method precision Parameter, % Assay was found 95.0 to 105.0% and % Recovery were obtained as 99.8% and 100.3% for Cobicistat and Atazanavir respectively. Linearity was obtained as 0.9996 and 0.9995 for Cobicistat and Atazanavir.Analytical Range was found from the linearity and accuracy for Azelnidipine was 4µg/mL to 12µg/mL and Telmisartan was 40µg/mL to 80µg/mL.

Keywords: Cobicistat and Atazanavir and Zorbax CN Column

INTRODUCTION

Cobicistat is a mechanism-based inhibitor of cytochrome P450 3A (CYP3A) isoforms. Inhibition of CYP3A-mediated metabolism by cobicistat increases the systemic exposure of CYP3A substrates atazanavir and darunavir and therefore enables increased anti-viral activity at a lower dosage. Cobicistat does not have any anti-HIV activity on its own.

Cobicistat, marketed under the name Tybost (formerly GS-9350), indicated for treating infection with human immunodeficiency virus (HIV). Although it does not have any anti-HIV activity, cobicistat acts as a pharmacokinetic enhancer by inhibiting cytochrome P450 3A isoforms (CYP3A) and therefore increases the systemic exposure of coadministered agents that are metabolized by CYP3A enzymes. More specifically, cobicistat is indicated to increase systemic exposure of atazanavir or darunavir (once daily dosing regimen) in combination with other antiretroviral agents in the treatment of HIV-1 infection. Increasing systemic exposure of anti-retrovirals (ARVs) without increasing dosage allows for better treatment outcomes and a decreased side effect profile **[1-3]**.

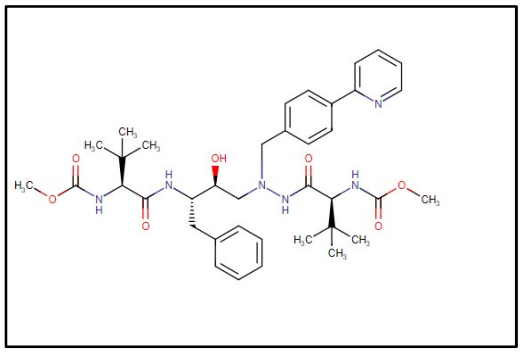


Structure of Cobicistat [1-3]

Atazanavir selectively inhibits the virus-specific processing of viral Gag and Gag-Pol polyproteins in HIV-1 infected cells by binding to the active site of HIV-1 protease, thus preventing the formation of mature virions. Atazanavir is not active against HIV-2.

Atazanavir (ATV) is an azapeptide HIV-1 protease inhibitor (PI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). HIV-1 protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1. Atazanavir binds to the protease active site and inhibits the activity of the enzyme. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Protease inhibitors are almost always used in combination with at least two other anti-HIV drugs. Atazanivir is pharmacologically related but structurally

different from other protease inhibitors and other currently available antiretrovirals **[1-3]**.



Structure of Atazanavir [1-3]

Importance of this Study

This study describes Simultaneous Estimation of Cobicistat and Atazanavir by HPLC in pharmaceutical formulation, this method is stability indicating method so degradants can be separated from the main analyte, % Assay will be get accurately. As well as in this method, mobile phase contains less organic solvents compared to previous research publications so definitely this method is will be reduce cost of the analysis. In this method mobile phase used as a diluent, this diluent had good extraction capacity, marketed drug shown % Assay between 98.0 to 102.0% although recovery point view shown accurate results in this diluent. The bottom line is this method is suitable for quality control purpose for the Simultaneous Estimation of Cobicistat and Atazanavir.

Instruments Used

HPLC Equipped with PDA Detector (Make: Shimadzu make with Lab solution software, UV Spectrophotometer (Shimadzu Make with Vision pro software), pH meter (Make Thermo), Ultra sonicator

Standard, Chemicals and Reagents

Atazanavir and cobicistat standards were provided by Chandralabs PVT Limited., Orthophosphoricacid (AR Grade, SRL Make) , Methanol(HPLC Grade, Merck Make) were used during the present study. Milli-Q water used and Evotaz tablets purchased from Pharmacy (contain 300 mg ofatazanavir and 150 mg of Cobicistat)

Methodology of Analysis

Selection of Wavelength

5mg of each drug was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 2ml to 10ml with methanol individually, The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs individually methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. Isobestic point was found to be 234 nm for atazanavir and Cobicistat.

Method Development:

After Several trials by changing the column, Buffers and Mobile phase Ratios, below mentioned conditions were optimized, In Optimized condition, Blank and Placebo interference were checked and %Assay also checked. Finally these Diluent, mobile phase and Chromatographic conditions were taken as a optimized conditions.

Mobile Phase Preparation:

Accurately Taken 1mL of Orthophosphoric acid and Transferred in to 1000mL of water. Mixed well, filtered through 0.45µm membrane filter.

Mixed 750mL of 0.1% Orthophosphoric acid and 250mL of Methanol, degassed by sonication.

Chromatographic conditions

Column	Zorbax CN Column(150*4.6mm &5µm)
Mobile Phase and Composition	0.1% Orthophosphoric acid :Methanol (75:25)
Flow rate	0.6mL/min
Column oven Temperature	35°C
Injection volume	10µL
Detection wavelength	234nm
Auto sampler Temperature	25°C
Retention Times	2.853 of Cobicistat and 4.090 of atazanavir

Preparation of standard solution

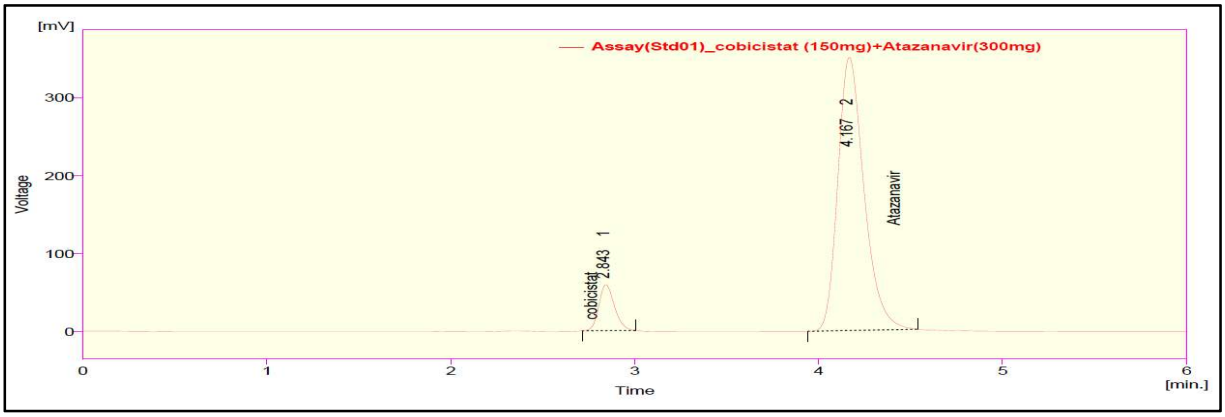
Weigh accurately 150 mg of COBICISTAT and 300 mg of ATAZANAVIR in 100 ml of volumetric flask and dissolve in 70ml of mobile phase and make up the volume with mobile phase From above stock solution 150µg/ml of COBICISTAT and 300µg/ml of ATAZANAVIR is prepared by diluting 5ml to 50ml with mobile phase respectively.

Preparation of sample solution:

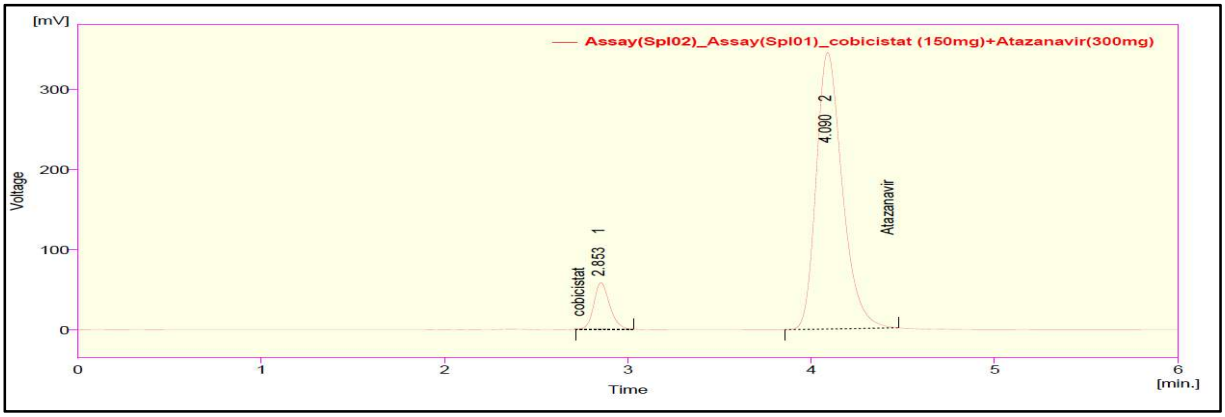
5tablets (each tablet contains 150 mg of Cobicistat and 300mg of Atazanavir) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Weighed crushed powder equivalent to 150 mg of Cobicistat and 300mg of Atazanavir in 100 ml of volumetric flask and dissolve in 70ml of mobile phase by 30min of sonication and make up the volume with mobile phase. Centrifuged sample at 5000rpm for 10min.

From above stock solution 150µg/ml of Cobicistat and 300µg/ml of Atazanavir is prepared by diluting 5ml to 50ml with mobile phase respectively.

Chromatogram of Standard



Chromatogram of Sample



Method Validation

By using Optimized condition Analytical Method of Assay carried out by ICH Guidelined Q2B **[4-5]**: The objective of **validation** of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

System Suitability and System Precision

According to ICH guidelines **[4-5]** System suitability checking out is an integral a part of many analytical procedures.

The tests are based on the idea that the equipment, analytical operations and samples to be analyzed represent an integral system that can be evaluated as such. System suitability check parameters parameters to be established for a particular procedure depend on the type of procedure being validated.

According to ICH Specifications: Theoretical Plates should not be less than 2000, Tailing factor should not be 0.95 to 2.0 and Resolution should not be less than 2.0 between two analytes.

System Precision Specification: % RSD for Area and Retention time for the six replicate injections should not be more than 2.0 of each Analyte.

Results of System suitability

S. No.	Cobicistat			Atazanavir			Resolution
	Rt in min	Plate count	Tailing Factor	Rt in min	Plate count	Tailing Factor	Resolution
1	2.983	5277	1.33	4.457	4483	1.36	6.84
2	2.967	5218	1.33	4.443	4652	1.40	6.95
3	2.943	5100	1.34	4.423	4235	1.37	6.96
4	2.947	5522	1.30	4.413	4590	1.40	6.99
5	2.953	5547	1.30	4.387	4534	1.37	6.83
6	2.947	5148	1.34	4.407	4370	1.40	6.40

Observation: Theoretical Plates, Tailing factor and Resolution was within the acceptance criteria.

Results of System Precision

S. No.	Cobicistat		Atazanavir	
S. No	Rt in min	Area	Rt in min	Area
1	2.983	356.042	4.457	3536.116
2	2.967	342.146	4.443	3520.52
3	2.943	349.990	4.423	3497.845
4	2.947	349.699	4.413	3502.252
5	2.953	347.482	4.387	3497.106
6	2.947	342.859	4.407	3473.103
AVG	2.957	348.036	4.422	3504.490
%RSD	0.52	0.48	0.57	0.62

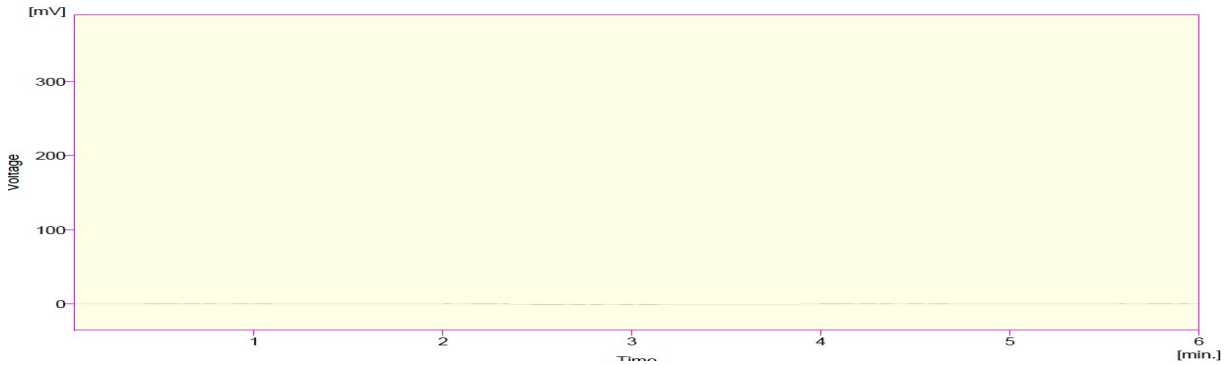
Observation: %Relative standard deviation of Six Replications of Area and Retention time was less than 2.0%

SPECIFICITY

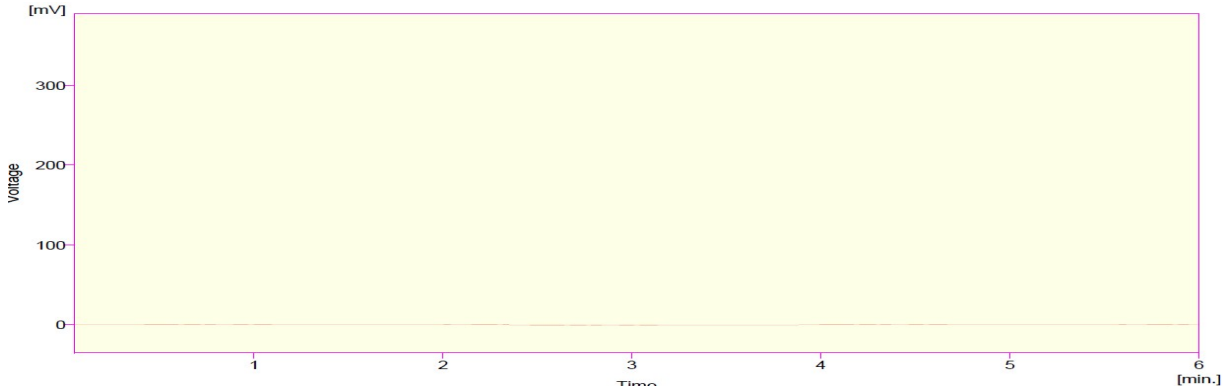
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc **[4-5]**.

Acceptance Criteria:

Blank solution and Placebo solution should not be interfered at the retention time of the three main Analyte peaks.



Blank Chromatogram



Placebo Chromatogram

Observation: No interference was observed at theretention times of the three main Analyte peaks retention time of the three main Analyte peaks.

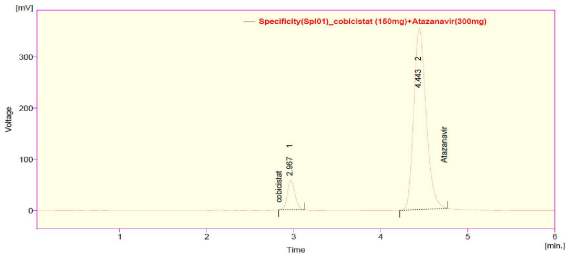
FORCED DEAGRADATION STUDY

Forced Degradation study also as a part of Specificity Parameter, It is a process in which different stress conditions are applied over drug Substance or drug product and it will converted in to different degradant. These studies are mainly used for the determination of stability of molecule under accelerated conditions. Samples were treated with Acid (5N Hydrochloric acid/4Hrs/50°C), Base (5N Sodium Hydroxide/4hrs/50°C), Peroxide (10% Hydrogen Peroxide for 4Hrs at Bench top), Thermal (60°C/48Hrs) and Photolytic condition. In base Sample treatment cobicistat was degraded and degradation was occurred 4.5% and In peroxide Sample treatment cobicistat was degraded and degradation was occurred 7.9%, In remaining all conditions Remaining all conditions, treated sample has shown less degradation (below 5%). All main analytes were Peak purity index shown within the specification. (Specification: All Main Analytes peak purity index should be more than Single point threshold value).

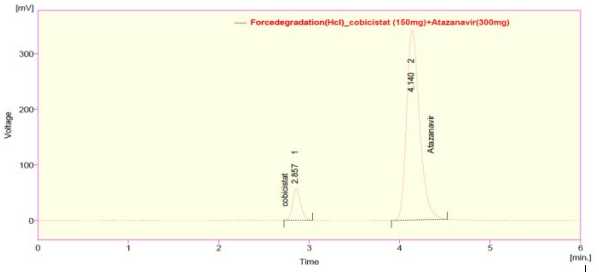
Degradation of Sample

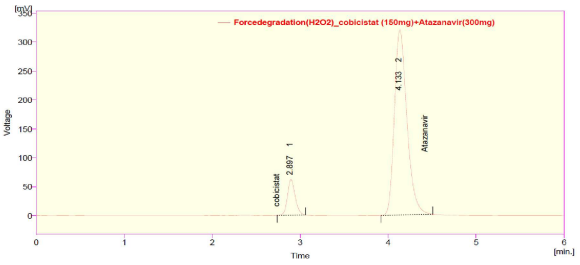
Degradation Condition	%Assay after Degradation	
	Cobicistat	Atazanavir
Control Sample	99.5	99.7
Acid 5N HCl/50°C/4Hrs	100.1	98.6
Base 5N 5NNaOH/50°C/4Hrs	94.5	100.1
Peroxide(4Hrs/10%Hydrogen Peroxide/Bench top)	92.1	99.4
Photolytic(1.2 million lx h)	99.5	99.2
Thermal(60°C/48Hrs)	99.3	100.2

Control Sample

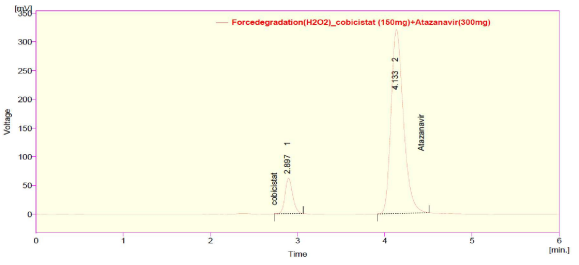


Acid 5N HCl/50°C/4Hrs

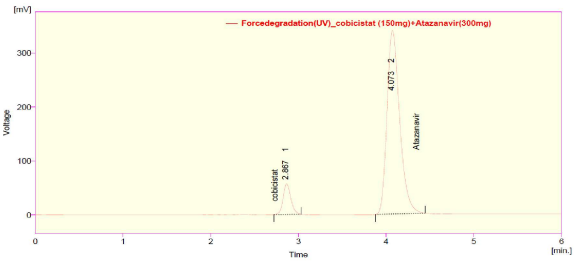




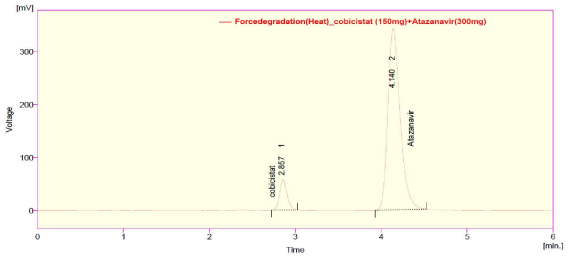
Base Treated Sample(5N NaOH/4Hrs/50°C)



Peroxide Treated Sample(10% H2O2/4Hrs Bench top)



Photolytic Treated Sample



Thermal Treated Sample

METHOD PRECISION

Closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample **[4-5]**.

Sample solutions were six prepared individually and each injected in to HPLC System, Calculated % Assay by using average area of Six Standards.

Name of the Sample	%Assay of Cobicistat	%Assay of Atazanavir
Method Precsion-01	**99.2**	**99.6**
Method Precsion-02	**100.1**	**100.2**
Method Precsion-03	**100.2**	**99.6**
Method Precsion-04	**99.4**	**98.7**
Method Precsion-05	**99.6**	**98.7**
Method Precsion-06	**99.2**	**98.1**
Average	**99.6**	**99.2**
%RSD	**0.4**	**0.8**

Observation: Average and individual %Assay was obtained between 95 to 105% individual preparation and %RSD for %Assay of six replicate preparations was obtained below 2.0

ACCURACY AND RECOVERY STUDIES

Expresses the closeness of agreement between the value which is accepted either as a conventional true value and the value found **[4-5]**.

Three levels (50%, 100% and 150%) of accuracy sample were prepared in triplicate by Standard API addition method to the Placebo, at each level API taken 50%, 100% and 150% respectively in the presence of Placebo.

Name of the Level	Cobicistat	Atazanavir
50% Accuracy	99.5	100.3
100% Accuracy	100.3	100.5
150% Accuracy	99.6	100.1
Mean	99.8	100.3
%RSD	0.2	0.1

Observation: each level of Accuracy and %Recovery mean was obtained 99.8% for Cobicistat, 100.3% for Atazanavir and the %RSD also within in the limit(Less than 2.0%)

LINEARIT AND RANGE:

Five linearity solutions (50%, 80% 100%, 120% and 150%) were prepared from standard stock solution i.e., 75µg/mL to

225µg/mL for Cobicistat, 150µg/mL to 450µg/mL for Atazanavir

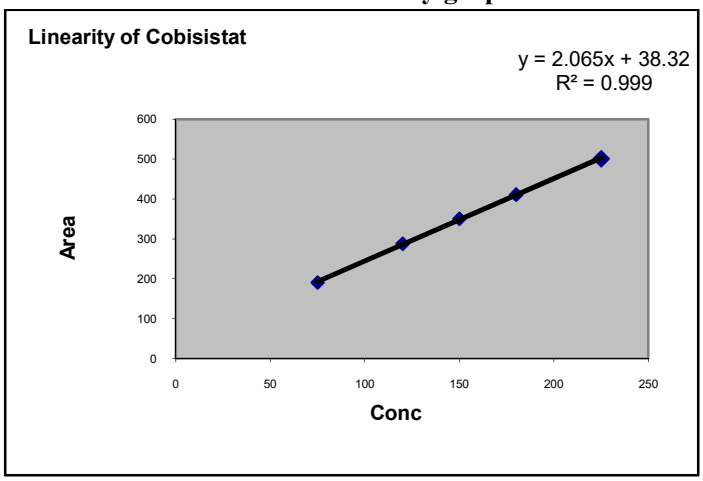
Acceptance criteria

The relationship between the concentration of Cobicistat and Atazanavir and area of Cobicistat and Atazanavir should be linear in the specified range and the correlation should not be less than 0.99.

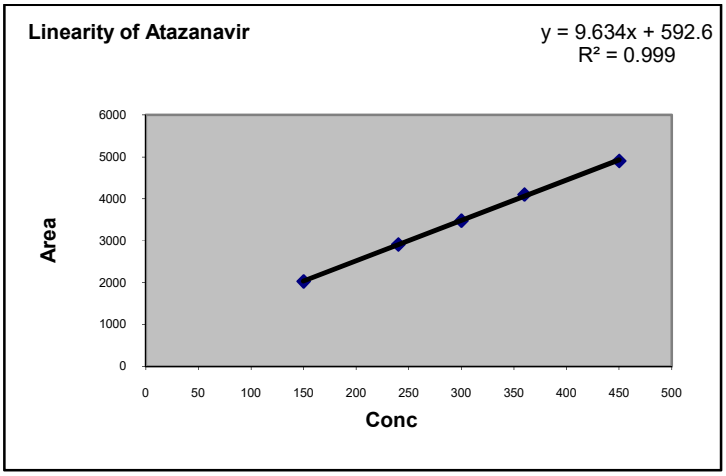
Observation: The Correlation coefficient was found 0.9996 for Cobicistat, and 0.9995 for Atazanavir

Cobicistat		Atazanavir	
Conc. in µg/mL	**Area**	**Conc. in µg/mL**	**Area**
75	**190.719**	**150**	**2027.509**
120	**287.79**	**240**	**2907.075**
150	**350.536**	**300**	**3475.878**
180	**410.874**	**360**	**4101.172**
225	**500.892**	**450**	**4902.489**
Correlation coefficient	**0.9996**	**Correlation coefficient**	**0.9995**

Cobicistat Linearity graph



Atazanavir Linearity graph



ROBUSTNESS

Robustness conditions like flow (1.0mL/min±0.2mL) and Wavelength (234nm±5nm) was maintained in the HPLC System and injected six standards replicate injections in each condition. system suitability parameters were within the acceptance criteria, %RSD for Area of six standard injections for within the limit.

S. No.	Condition	%RSD for Cobicistat	%RSD for Atazanavir
01	Flow Rate_0.8mL/min	0.33	0.21
02	Flow Rate_1.2mL/min	0.14	0.31
03	Wavelength (229nm)	0.25	0.22
04	Wavelength (239nm)	0.31	0.18

CONCLUSION

A Specific, Accurate, Precise and Robust stability indicating Assay method was developed for the simultaneous estimation of the Cobicistat and Atazanavir in tablet dosage form and Validated done as per ICH Validation guidelines. For the Optimized conditions of Assay method was validated by Using ICH Q2B guidelines. Method was shown precise, Specific, accurate, linear and robust results. % RSD of the Cobicistat and Atazanavir were and found to be 0.48 and 0.62% respectively in system precision. In method precision sample has shown precise %Assay a result that was 95.0 to 105.0% and % Recovery was obtained between 98 to 102% for Cobicistat and Atazanavir. Linearity was obtained as 0.9996, 0.9995for Cobicistat and Atazanavirrespectively. So this method very use full to Routine analysis like in Quality control to reduce the time and cost.

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