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FORCED DEGRADATION STUDIES ON ATAZANAVIR AND COBICISTAT BY RP-HPLC METHOD

PRATIK K. VORA*, SURESH C AMETA, MRUNAL K SHIRSAT

Research Scholar, Faculty of Science, Pacific Academy of Higher Education and Research University, Udaipur.

ABSTRACT

The objective of the current study was to conduct forced degradation studies of Atazanavir and Cobicistat drug substances in order to identify whether the developed HPLC method is capable to accurately estimate the drug content and its degradation products in higher stress conditions. Forced degradation study was carried out to evaluate the degradation pattern, intrinsic stability of the molecule and to verify that the analytical procedures used for Assay and Related Substances are stability indicative.These studies provide information and discrimination between process related, degradant related and degradants from drug-excipient combination. If the drug product does not show any degradation when it was exposed to stress conditions, further stress study is unnecessary.Atazanavir and Cobicistat drug substance samples were subjected to forced degradation under different stress conditions such as Acid degradation (0.1N HCl, 1 mL at 70°C for 3 hours), Base degradation (0.1N NaOH, 1 mL at 70°C for 3 hours), Oxidation (3% H_2O_2, 2 mL at 70°C for 3 hours), Photo Degradation (For 4 days) and Thermal degradation (1 mL at 110°C for 3.5 hours). The stressed samples were analyzed using a reversed-phase HPLC method.

KEYWORDS: RP-HPLC Method, forced degradation, Atazanavir,Cobicistat

PRATIK K. VORA
Research Scholar, Faculty of Science, Pacific Academy of Higher Education and Research University, Udaipur.

***Corresponding Author**

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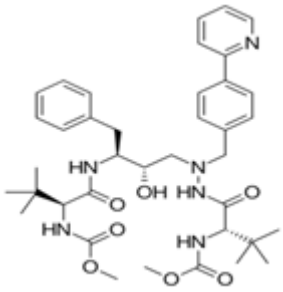
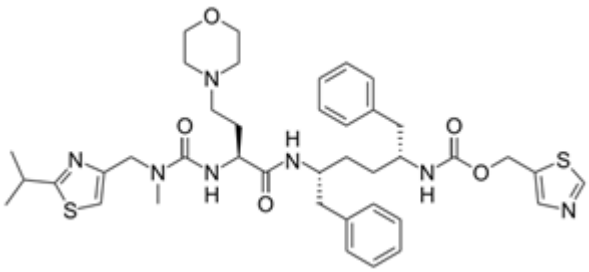
INTRODUCTION

In this present study an attempt was made to prove that the HPLC method is capable to accurately estimate the drug content in presence of degradant. i.e. stability indicating nature of the method. Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. Forced degradation studies provide data to support identification of possible degradants; degradation pathways and intrinsic stability of the drug molecule and validation of stability indicating analytical procedures.[1] Atazanavir is an antiretroviral drug of the protease inhibitor (PI) class. It is used to treat infection of human immunodeficiency virus (HIV). Chemical name is methyl N[(1S)-1-{[(2S,3S)-3-hydroxy-4-[(2S)-2-[(methoxycarbonyl) amino]-3,3-dimethylN'{[-4-(pyridine-2-yl)phenyl]methyl}butanehydrazido]-1-phenylbutan-2-yl]carbamoyl}-2,2-dimethylpropyl] carbamate. Its chemical formula Is $C_{38}H_{52}N_6O_7$ and Molecular Weight is 704.856 g/mol.[2-3] Cobicistat is a CYP3A inhibitor. It increases the systemic exposure of atazanavir or darunavir in combination with other antiretroviral agents in the treatment of HIV-1 infection. Chemical name is Thiazol-5-ylmethylN-[1-benzyl-4-[[2-[[(2-isopropylthiazol-4-yl)methyl methylcarbamoyl] amino]-4-morpholinobutanoyl] amino]5-phenylpentyl] carbamate. It has a molecular formula of $C_{40}H_{53}N_7O_5S_2$ and a molecular weight of 776.023 g/mol. [4-5]



Atazanavir



Cobicistat

To develop a simple, accurate, sensitive and rugged analytical method for related estimation of Atazanavir and Cobicistat in combined dosage form by HPLC and to validate the same as per ICH guidelines.By the Purpose of this Study it is possible to perform Stability indicating RP-HPLC Method for the Simultaneous Estimation of Atazanavir and Cobicistat in Combined Dosage form.Combination drugs needs a stability indicating analytical method for individual estimation of drug substances. Official pharmacopoeial methods for both individual and combined dosage form for estimation of Atazanavir and Cobicistat are not available. Literature review revealed that very few analytical methods appeared in the literature for the determination of Atazanavir and Cobicistat available in Combined Dosage form.[6-16] Since Atazanavir and Cobicistat drug substances having different physicochemical properties[17], polarities, and wide difference, it's a challenge task to develop accurate, sensitive and rugged analytical method.Reversed-phase high-performance liquid chromatography (RP-HPLC) has been the most widely used method for pharmaceutical analysis, as it ensures accurate quantification of drugs without interference from any of the excipients that are normally present in pharmaceutical dosage forms. RP-HPLC method is capable of producing accurate, specific, and reproducible results. Forced degradation testing plays an important role in the development of a stability-indicating analytical method, as it helps to determine the degradation pathways and degradation products of the Active pharmaceutical ingredients (APIs) that could form during storage and facilitate formulation development, manufacturing, and packaging.[17-18] Degradation products or compounds are formed due to change in the active ingredient as a result of processing or storage (eg, oxidation and hydrolysis) or reaction of the active ingredient with an excipient or container.[19-20] It is recommended that forced degradation studies should be performed to evaluate the effect of temperature, humidity, photo stability, oxidation, and hydrolytic degradation.

MATERIALS AND METHODS

Instrumentation

Component	Brand / Model / Software
HPLC	Shimadzu LC-20 AT
HPLC Column	C18 (25cm x 0.46 cm) Hypersil BDS
Detector	SPD-20A

Instrumentation

Chromatographic Parameters

Parameters	:	Chromatographic Condition
Mode of elution	:	Isocratic
Mobile Phase	:	Buffer (Potassium Phosphate, pH 5.0) : Acetonitrile (70:30)
Column	:	C18 (25cm x 0.46 cm) Hypersil BDS
Flow rate	:	1mL/min
Runtime	:	10 min
Injection volume	:	20 µL
Detection wavelength:		235 nm

Preparation of standard solution of mixtures of Atazanavir (20 ppm) and Cobicistat (10 ppm)

Atazanavir standard stock solution: (200 µg/mL)

Transfer an accurately weighed quantity of about 20 mg of Atazanavir into a 100 mL volumetric flask. Dissolve and dilute the volume with mobile phase and mix well.

Cobicistat standard stock solution: (100 µg/mL)

Transfer an accurately weighed quantity of about 10 mg of Cobicistat into a 100 mL volumetric flask. Dissolve and dilute the volume with mobile phase and mix well.

Preparation of standard solution of binary mixtures of Atazanavir (20 µg/mL) and Cobicistat(10 µg/mL)

Transfer ~~an~~accurately 1 mL of Atazanavir stock solution and 1mL of Cobicistat stock solution into a 10 mL volumetric flask and dilute the volume with mobile phase and mix well.

Sample Stock Solution (Cobicistat 100 µg/mL, and Atazanavir 200 µg/mL)

Transfer an accurately weighed quantity of finely powdered tablets equivalent to 10 mg of Cobicistat, and 20 mg of Atazanavir into a 100 mL volumetric flask. Add exact 60 mL mobile phase. Sonicate for 15 minutes with intermediate shaking (During sonication necessary care has been taken to control the temperature between 20 to 25°C using cold water). Dilute the volume with mobile phase and mix well. Filter through 0.45µ nylon filter. Discard few mL of the filtrate.

Working Sample Preparation (Cobicistat 10 µg/mL, and Atazanavir 20 µg/mL)

Take 1 mL from standard stock solution and transfer~~red~~ to 10 mL volumetric flask and make up volume up to the mark with the mobile phase.

Procedure for all degradation conditions

Inject single injection of each of the above stressed diluents, placebo preparations and sample preparations into the liquid chromatographic system equipped with detector and analyze the sample as per test method.Demonstrate peak purity for the Cobicistat and Atazanavir peaks in all degraded samples using detector.If spectral purity is not achieved due to high concentration, dilute the solution to obtain an optimum peak area. Use the suitably diluted solutions to obtain peak purity.Note: If degradation is not achieved further acceptable drastic or mild degradation conditions should be performed.

Acceptance criteria

- No peak should be detected at the retention time of Cobicistat and Atazanavir and its impurities in the chromatograms of diluents and placebo preparations.
- All impurities should be well separated from the Cobicistat and Atazanavir and from each other.
- Cobicistat and Atazanavir peaks in all degraded sample preparations should be spectrally pure

Formula for Calculating Mathematical /Statistical Parameters

The relative standard deviation (RSD)

$$S = \sqrt{\frac{\sum_{i=1}^{n} (\bar{X}-Xi)^2}{n-1}}$$

Where, $(\%)\ \mathrm{RSD} = 100\ \frac{S}{\bar{X}}$

x_i = Value of discrete observations indexed by i

$\bar{x}$ = Mean value of discrete observations

n = Number of discrete observations

S = Standard deviation

Tailing factor

$$T = \frac{W_{0.05}}{2f}.$$

where,
$W_{0.05}$ = width of peak at 5% height.
f = distance from the peak maximum to the leading edge of the peak ,the distance being measured at a point 5% of the peak height from the baseline.

Theoretical plates, N: (By tangent method)

$$N = 16\left(\frac{t}{W}\right)^2$$

Where;
t is the retention time of the substance
W is the width of the peak at its base, obtained by extrapolating the relatively straight sides of the peak to the baseline.

RESULTS AND DISCUSSION

Acid degradation

Acid decomposition studies were performed by refluxing 1mL of stock solution was transferred in to 10 mL of volumetric flask. Two mL of 0.1 N Hydrochloride solutions was added. Sonicate for 30 minutes with intermediate shaking (During sonication necessary care has been taken to control the temperature between 20 to 25°C using cold water). Expose the flask at 70°C for 3 hours in water bath. After time period the content was cooled to room temperature. Then the volume was adjusted with diluent to get 20 µg/mL for Atazanavir and 10 µg/mL for Cobicistat.

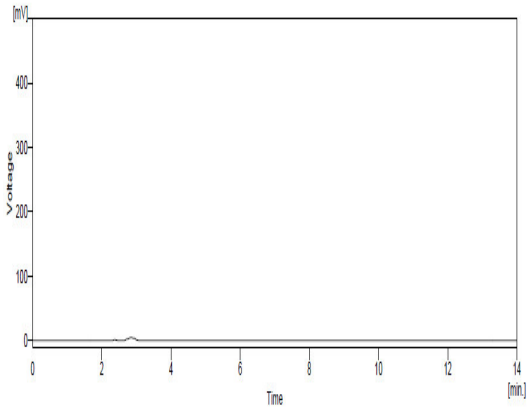


Figure 1
Atazanavir and Cobicistat Acid Degradation Blank

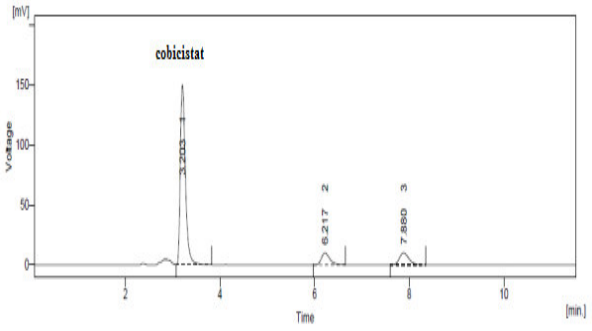


Figure 2
Cobicistat Acid Degradation Standard

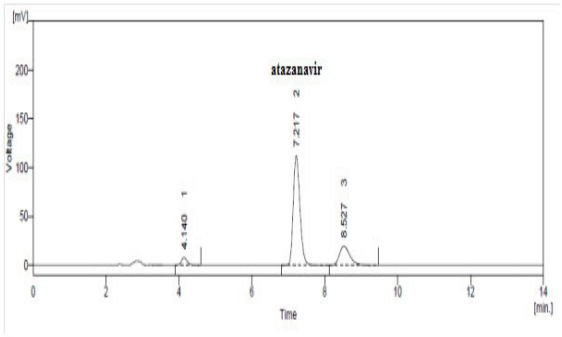


Figure 3
Atazanavir Acid Degradation Standard

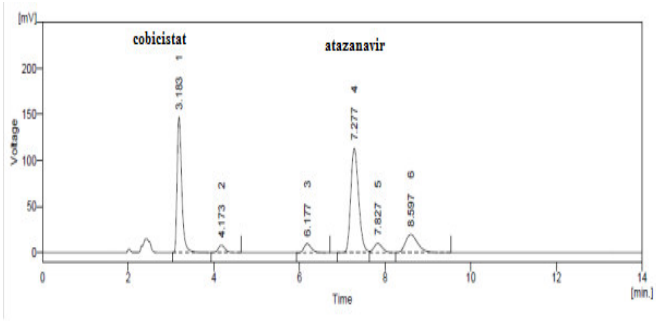


Figure 4
Atazanavir and Cobicistat Acid Degradation Sample
Base degradation

Basic decomposition studies were performed by refluxing. 1 mL of stock solution was transferred in to 10 mL ~~of~~ volumetric flask. Two mL of 0.1 N NaOH solutions was added. Sonicate for 30 minutes with intermediate shaking. (During sonication necessary care has been taken to control the temperature between 20 to 25°C using cold water). Expose the flask at 70°C for 3 hours in water bath. After time period the content was cooled to room temperature. Then the volume was adjusted with diluent to get 20 µg/mL for Atazanavir and 10 µg/mL for Cobicistat.

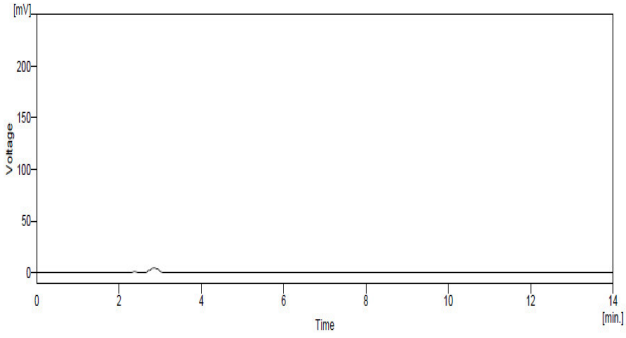


Figure 5
Atazanavir and Cobicistat Base Degradation Blank

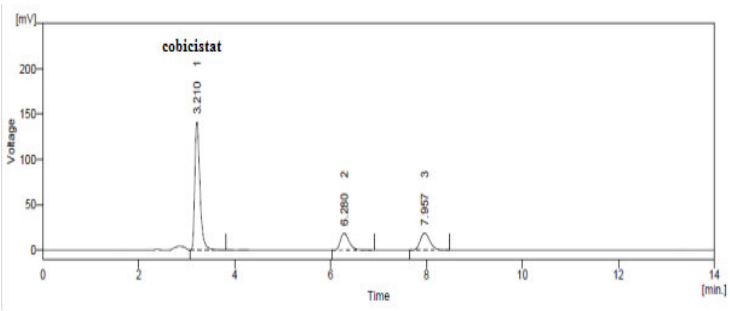


Figure 6
Cobicistat Base Degradation

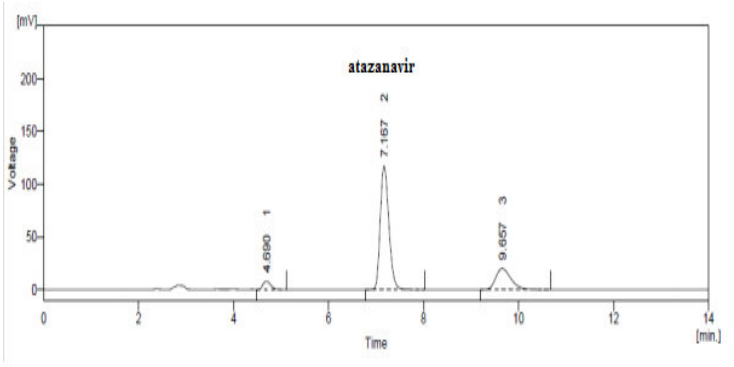


Figure 7
Atazanavir Base Degradation

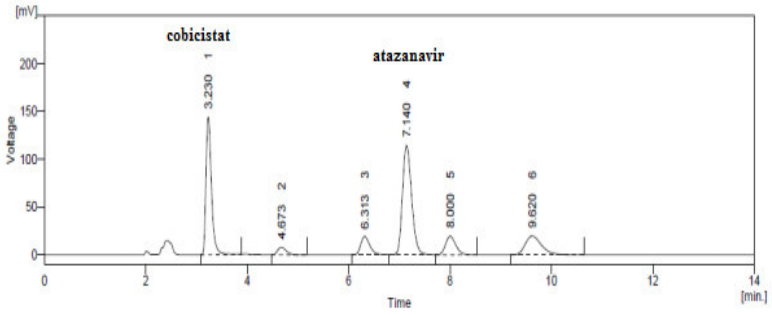


Figure 8
Atazanavir and Cobicistat Base Degradation Sample
Oxidative degradation

Oxidative decomposition studies were performed by refluxing.1mL of stock solution was transferred into 10 mL ~~of~~ volumetric flask. Add exact 2 mL of 3% v/v H_2O_2 in to the flask. Expose the flask at 70°C for 3 hours in water bath. After the stipulated time period remove the flask from water bath & cool; Filter through 0.45 μ nylon filter. Discard first few mL of the filtrate. Then the volume was adjusted with diluent to get 20 µg/mL for Atazanavir and 10 µg/mL for Cobicistat.

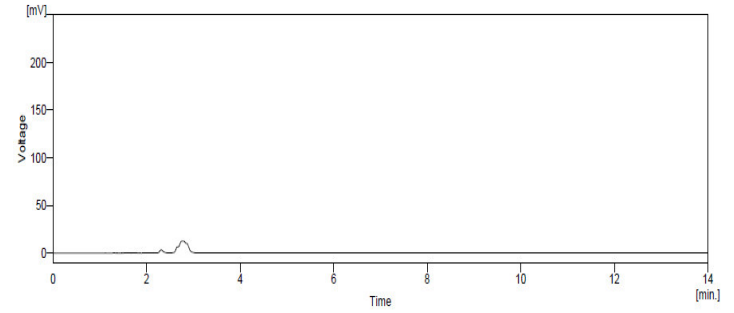


Figure 9
Atazanavir and Cobicistat Oxidation Degradation Blank

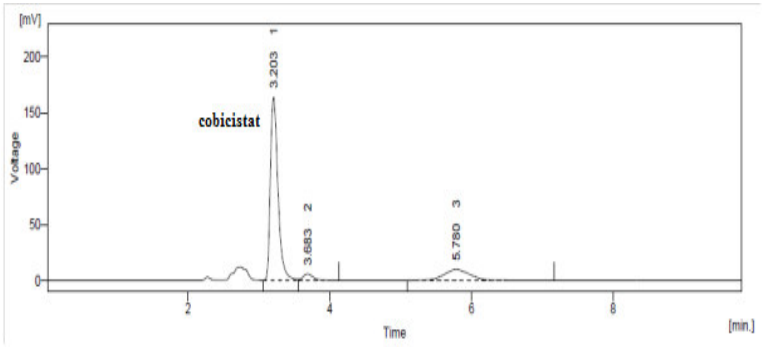
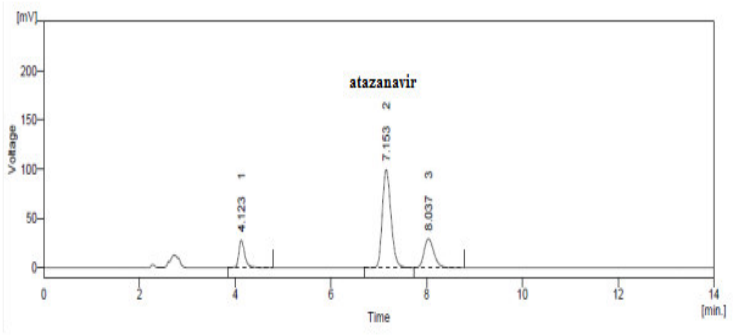


Figure 10
Cobicistat Oxidation Degradation



Fiugre 11
Atazanavir Oxidation Degradation

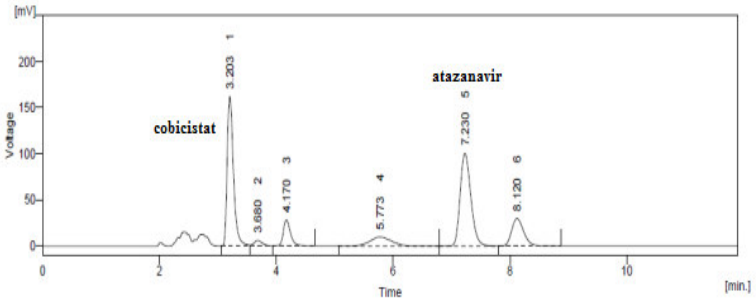


Figure 12
Atazanavir and Cobicistat Oxidation Degradation sample

Photo degradation

Photo degradation studies were performed.1mL of stock solution was transferred in to 10 mL of volumetric flask. The volumetric flask was kept in presence of Sunlight for 4 hrs. Then the volume was adjusted with diluent to get 20 µg/mL for Atazanavir and 10 µg/mL for Cobicistat.

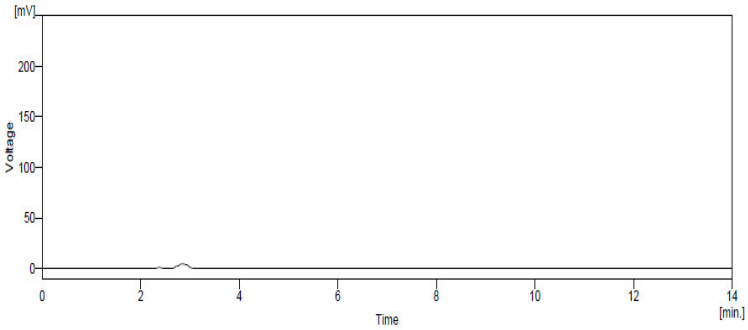


Figure 13
Atazanavir and Cobicistat Photo Degradation Blank

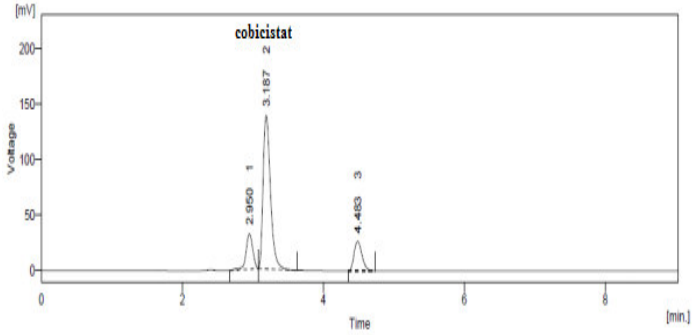


Figure 14
Cobicistat Photo Degradation

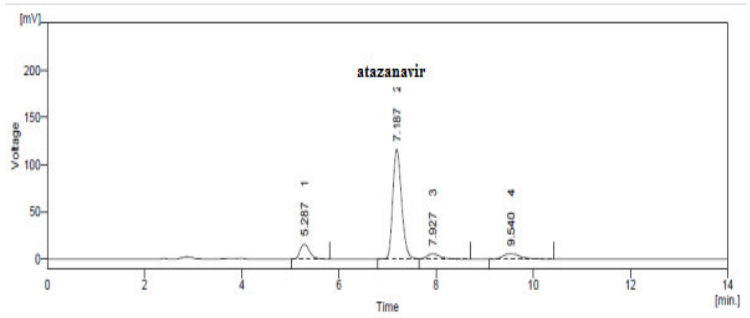


Figure 15
Atazanavir Photo Degradation

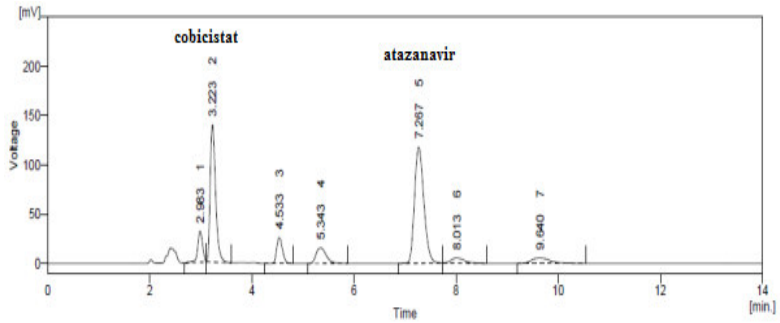


Figure 16
Atazanavir and Cobicistat Photo Degradation sample
Thermal degradation

Thermal degradation studies were performed.1mL of stock solution was transferred into 10 mL volumetric flask. The volumetric flask was stored in hot air oven and exposes the sample at 110°C for 3.5 hrs. Then the volume was adjusted with diluent to get 20 µg/mL for Atazanavir and 10 µg/mL for Cobicistat.

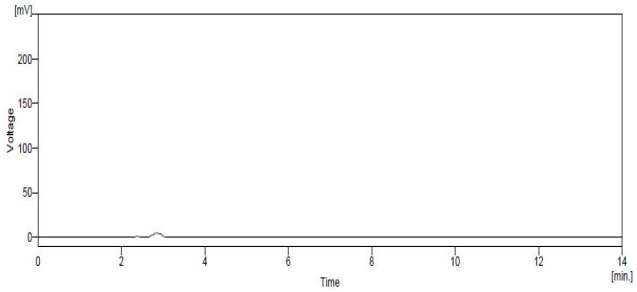


Figure 17
Atazanavir and Cobicistat Thermal Degradation Blank

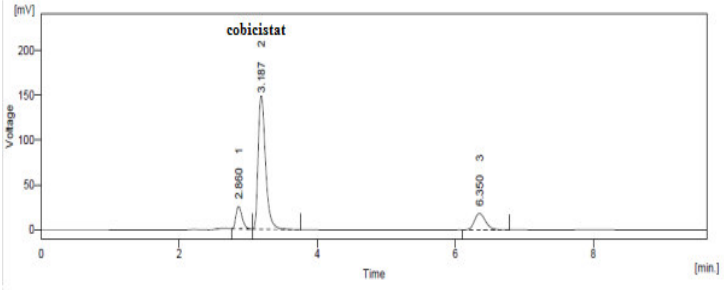


Figure 18
Cobicistat Thermal Degradation

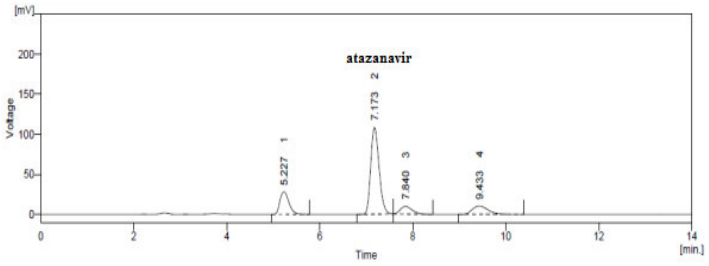


Figure 19
Atazanavir Thermal Degradation

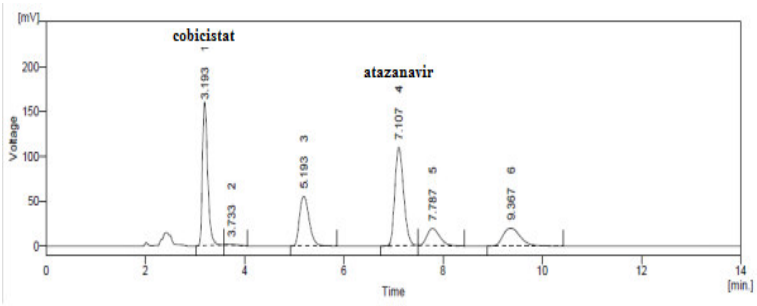


Figure 20
Atazanavir and Cobicistat Thermal Degradation sample

Buffer Preparation

0.05M Phosphate Buffer and 6.8 gm of Potassium dihydrogen Phosphate Preparation

6.8 gm of Phosphate Buffer was taken and transferred to 100ml volumetric flask and 80ml of water was added and shacken for 15min to dissolve it and volume was made up with Water to produce 100ml of 0.05M Phosphate buffer.pH of buffer was adjusted to 5.0 by adding 0.1N NaOH Solution to the above prepared 0.05M Buffer Solution.

Calculation for Stability

Table 1
Atazanavir and Cobicistat STD for stability

Drugs	Peak Purity	Area
Atazanavir	0.999	1615.362
Cobicistat	0.999	1311.663

Table 2
Atazanavir % Degradation

Atazanavir						
Parameter	Standard			Sample		
	Peak Purity	Area	% Degradation	Peak Purity	Area	% Degradation
Acid	0.994	1328.310	17.770	0.994	1348.202	16.539
Base	0.995	1301.164	19.451	0.993	1339.770	17.061
Oxidation	0.995	1253.891	22.377	0.994	1278.633	20.845
Photo	0.993	1372.283	15.048	0.995	1399.346	13.373
Thermal	0.993	1350.146	16.418	0.994	1356.119	16.049

Table 3
Cobicistat % Degradation

Cobicistat						
Parameter	Standard			Sample		
	Peak Purity	Area	% Degradation	Peak Purity	Area	% Degradation
Acid	0.996	1119.279	14.667	0.994	1108.507	15.488
Base	0.994	1055.766	19.509	0.993	1085.737	17.224
Oxidation	0.995	1025.512	21.816	0.994	1011.192	22.908
Photo	0.995	1007.610	23.181	0.996	1026.783	21.719
Thermal	0.993	1107.886	15.536	0.995	1078.054	17.810

CONCLUSION

The present study revealed that no peaks are detected at the retention time of Atazanavir and Cobicistat in the chromatograms of diluents and Placebo preparations. Cobicistat and Atazanavir peaks in all degraded sample preparations are spectrally pure. The drugs found to be stable under acid, neutral, hydrolysis, thermal degradation, and photolytic conditions.The degradation pathway of Atazanavir and Cobicistat was established. From the data presented it is concluded that the HPLC methods adopted are able to identify the degradation occurred to the product at different conditions and hence can be concluded that these methods are stability indicating.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

Conflict of interest declared none.

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