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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF ATAZANAVIR SULFATE AND COBICISTAT IN TABLET DOSAGE FORMS

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ABSTRACT

A stability indicating method was developed for the simultaneous estimation of Atazanavir sulfate and Cobicistat in pharmaceutical dosage form by using Reverse Phase High Performance Liquid Chromatography (RP-HPLC). The separation was done on isocratic mode with Discovery C18 (250mm x 4.6 mm, 5µ) column and 0.01N Potassium dihydrogen phosphate and acetonitrile (40:60%v/v) as mobile phase at a flow rate of 1.0ml/min and at room temperature. The detection was done at a wavelength of 235nm. The retention time for Atazanavir was found to be 3.15min and for Cobicistat was found to be 2.32min. A good linearity was observed in the concentration range of 75µg/mL - 450µg/mL for Atazanavir and 37.5µg/mL to 225µg/mL for Cobicistat, with a correlation coefficient of 0.999 for both the drugs. The method was validated according to the ICH guidelines. The developed method was found to be accurate, precise, specific, rugged and robust. The drug was found to be stable at forced degradation conditions and the net degradation was found to be within the limits. The developed method can be used for the quality control of Atazanavir sulfate and Cobicistat in pharmaceutical dosage form.

KEYWORDS: Atazanavir sulfate, Cobicistat, RP-HPLC, stability indicating method, method development, validation.

INTRODUCTION

Atazanavir sulfate (Fig. 1A) is chemically methyl N-[(2S)-1-[2-[(2S,3S)-2-hydroxy-3-[[(2S)-2-(methoxycarbonylamino)-3,3-dimethylbutanoyl]amino]-4-phenylbutyl]-2-[(4-pyridin-2-ylphenyl)methyl]hydrazinyl]-3,3-dimethyl-1-oxobutan-2-yl]carbamate;sulfuric acid. It is a white to pale yellow crystalline powder with slight solubility in water belongs to antiviral category. It has pKa values of 4.33 and 5.12. It is used for the treatment of HIV infection and AIDS.[1] Cobicistat (Fig. 1B) is chemically 1,3-thiazol-5-ylmethyl [(2*R*,5*R*)-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. It is a white to pale yellow solid soluble in water, belongs to antiviral category. It has pKa values of 14.18 and 6.69. It is used for the treatment of HIV infection and AIDS.[2] According to literature survey, it reveals that there are few methods such as RP-HPLC methods[3-7] for the simultaneous estimation of Atazanavir and Cobicistat. The aim of the present study is to develop a stability indicating RP-HPLC method for the simultaneous estimation of Atazanavir and Cobicistat in their pharmaceutical dosage forms.

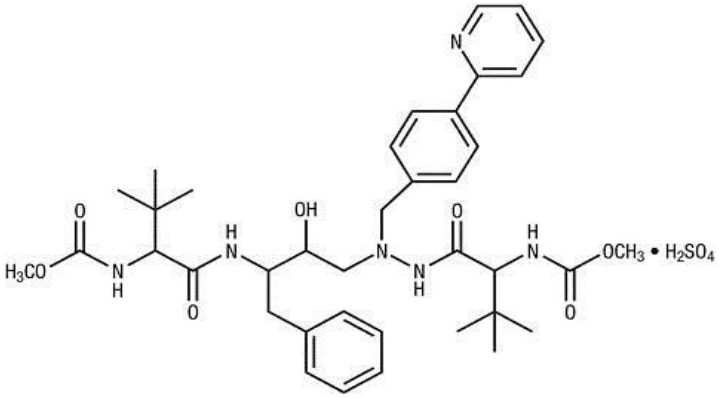

Fig. 1A Chemical structure of Atazanavir sulfate.

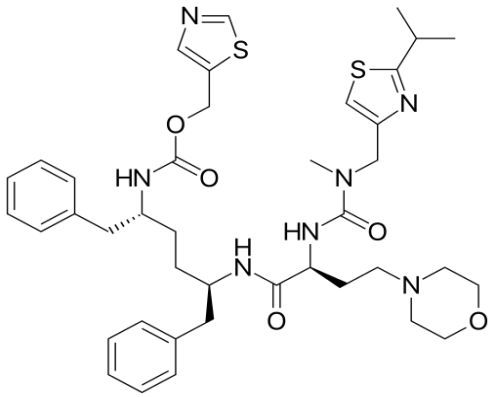

Fig. 1B Chemical structure of Cobicistat.

MATERIALS AND METHODS

Reagents and chemicals

Atazanavir sulfate standard and Cobicistat standard were procured from Spectrum lab, Hyderabad (India) as gift samples. The Atazanavir and Cobicistat tablets (Evotaz) were purchased from local pharmacy. All the solvents used were of HPLC grade and purchased from Merck, Mumbai, India. All the chemicals used for developing method were of AR grade and purchased from sigma Aldrich.

Instrument and analytical conditions

The chromatographic system used is of Water company running with empower 2 software and with PDA detection mode. Discovery C18 (250mm x 4.6 mm, 5µ) column was used to separate the drugs using 0.01N potassium dihydrogen phosphate and acetonitrile (40:60%v/v) as mobile phase on isocratic mode at a flow rate 1.0ml/min. The detection was done at 235nm. The other instruments used were pH meter (EI), Digital Balance (Infra Instruments), Ultrasonic Bath (Wadegati), Hot air oven (Cisco).

Preparation of Diluent

Mixture of Water and Acetonitrile in the ratio 50:50 (v/v) respectively was used as diluent.

Preparation of standard and sample solutions

Dissolve 30mg of Atazanavir sulfate standard and 15mg of Cobicistat standard in 10mL volumetric flask with diluent. Dilute 1mL of above stock solution with diluent to 10mL.

20 Tablets (Evotaz) were weighed accurately and the average weight was calculated. Dissolve an amount equivalent to 30mg of Atazanavir in 10mL volumetric flask with diluent and sonicated for 30min with intermediate shaking. The final volume was made up with diluent. The above solution was filtered and 1mL of the solution was diluted with diluent in 10mL volumetric flask.

Method validation[8]

System suitability

Inject standard solution into the chromatographic system and calculate the parameters such as % relative standard deviation (RSD), tailing factor and plate count.

Linearity

Serial dilutions of standard Atazanavir and Cobicistat in the range of 75µg/mL - 450µg/mL and 37.5µg/mL - 225µg/mL respectively were prepared and injected into the HPLC. A linearity graph was plotted between concentration and peak areas.

Accuracy

The solutions were prepared in three different concentration levels of 50%, 100% and 150%, injected into HPLC and % recoveries were calculated.

Precision

The precision of the method was determined by Intra and Inter-day precision studies. The standard solution was injected six times on the same day (intra-day) as well as on different day (inter-day) and the % RSD was calculated.

Specificity

The specificity of the method was determined by injecting the placebo solution and comparing with standard solution for the interference with Atazanavir and Cobicistat peak.

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

LOD and LOQ are determined by standard deviation (SD) and slope of the calibration curve. The limiting values are calculated as per the following equations: LOD = (3.3 × SD)/ Slope and LOQ = (10 × SD)/ Slope.

Robustness

Robustness of the method was determined by varying the optimum chromatographic conditions such as mobile phase ratio (±10%), flow rate (±0.2mL/min) and column oven temperature (±5ºC). The system suitability parameters were calculated and recorded.

Forced degradation studies

The drugs solution was subjected to the various stress conditions such as acidic (2N Hydrochloric acid, 60ºC for 30 mins), basic (2N sodium hydroxide, 60ºC for 30 mins), oxidative (20% hydrogen peroxide, 60ºC for 30 mins), neutral (refluxing the drugs in water for 6hrs at a temperature of 60ºC), photolytic (exposing the drugs solution to UV light by keeping the beaker in UV Chamber for 7 days or 200Watt hours/m^2 in photo stability chamber) and thermal (drugs solution was placed in an oven at 105ºC for 6 hours) conditions.

RESULTS AND DISCUSSION

The main aim of the study was to develop a stability indicating RP-HPLC method for the simultaneous estimation of Atazanavir and Cobicistat in tablet dosage form and to validate the method. Initially various mobile phase compositions were tried to elute the drug. Mobile phase ratio and flow rate were selected based on peak parameters and retention time. Standard solutions of 10µg/mL was prepared and scanned in the range of 200 – 400nm for detecting the maximum absorption wavelength and from the overlay detection wavelength was found to be 235nm (Fig. 2).

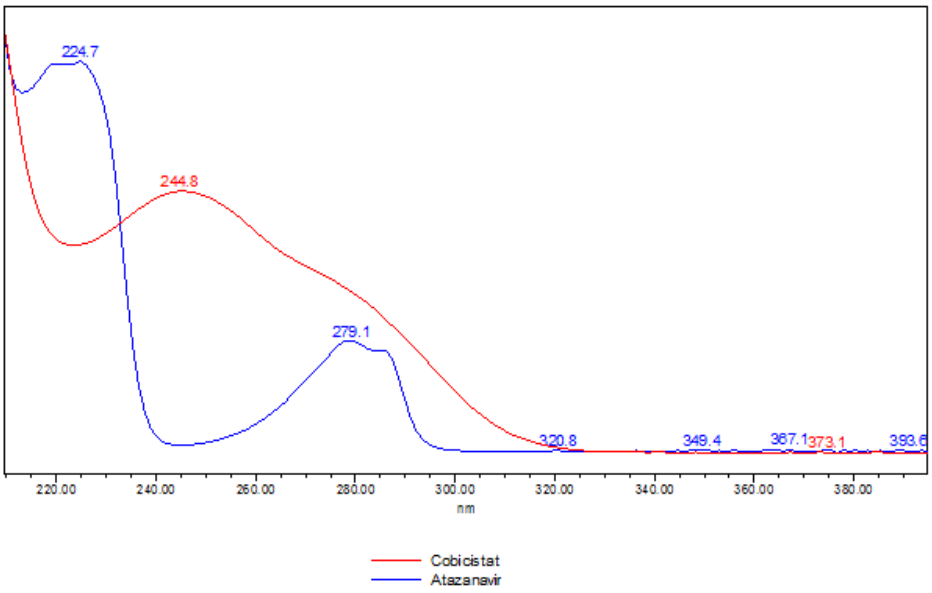

Fig. 2 Overlay UV spectrum of Atazanavir and Cobicistat.

After considering the entire system suitability parameters mobile phase 0.01N Potassium dihydrogen phosphate and acetonitrile (40:60%v/v) run in isocratic mode and flow rate 1.0ml/min was selected. The retention time of Atazanavir was found to be 3.15min and that of Cobicistat was found to be 2.32min. The system suitability parameters are calculated from standard chromatogram (Table 1 and Fig. 3). The sample solution and blank solution were shown in Fig. 4 and Fig. 5.

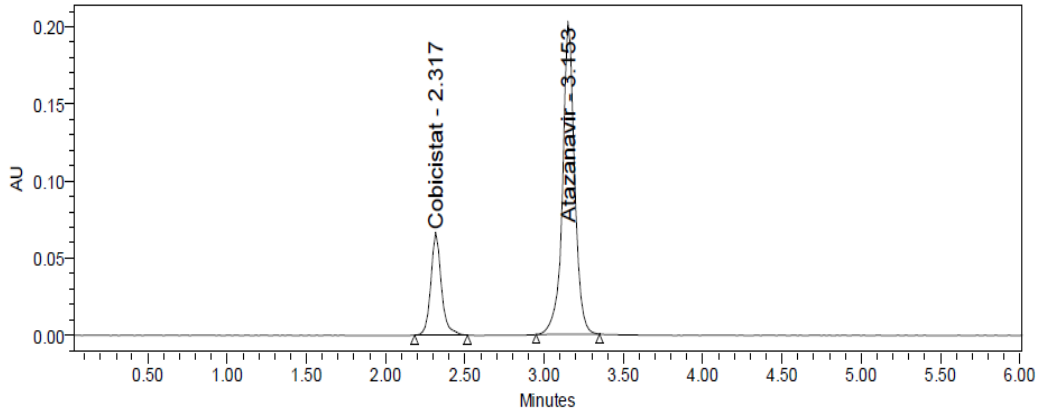

Fig. 3 Standard chromatogram.

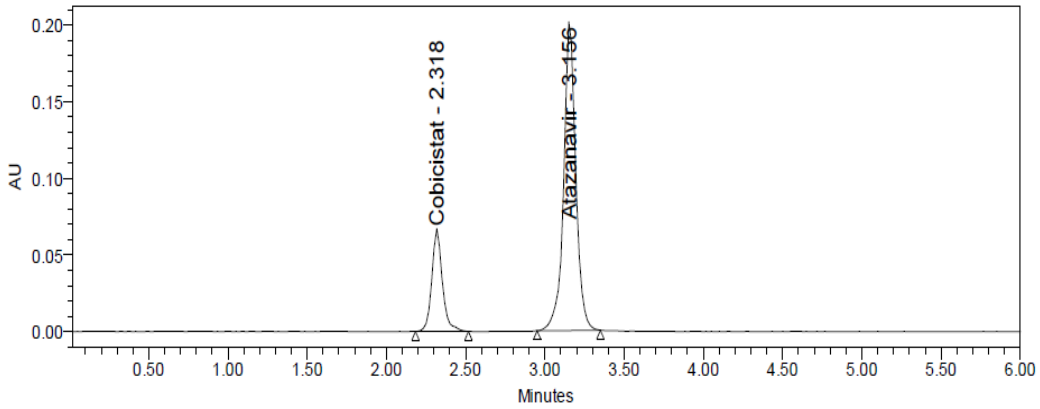

Fig. 4 Sample chromatogram.

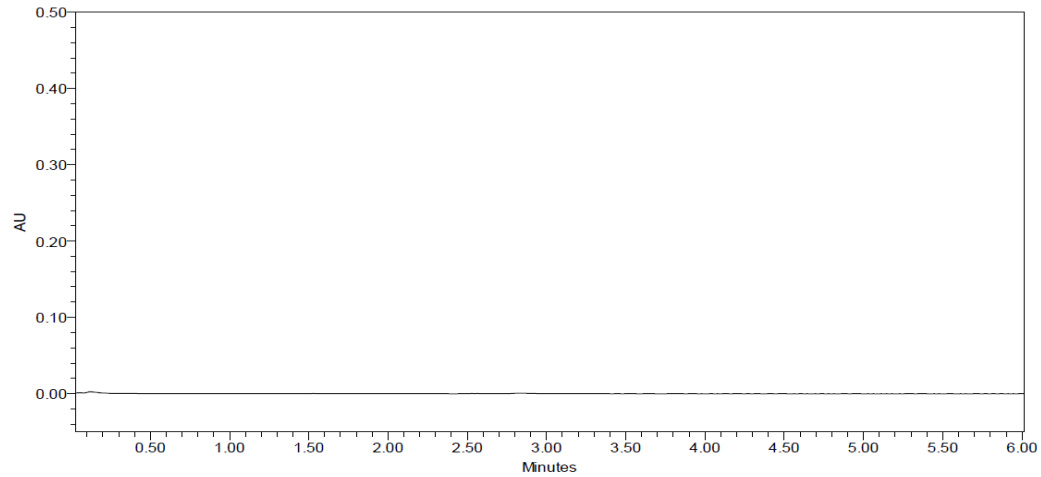

Fig. 5 Blank chromatogram.

For the estimation of linearity of the method, serial dilutions of standard solution were prepared in the concentration range of 75-450µg/ml for Atazanavir and 37.5-225µg/ml for Cobicistat. A linearity graph was plotted by taking concentration on x-axis and peak area on y-axis. Correlation coefficient was found to be 0.9998 for Atazanavir and 0.9996 for

Cobicistat, indicates that the drugs obeys Beer's law. The linearity plots were shown in Fig. 6A and Fig. 6B.

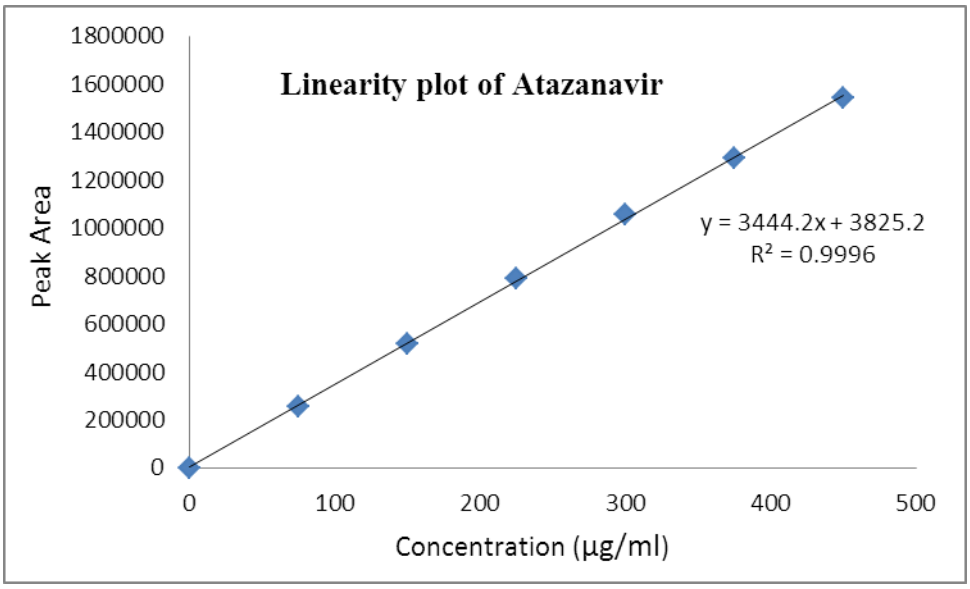

Fig. 6A Linearity plot of Atazanavir.

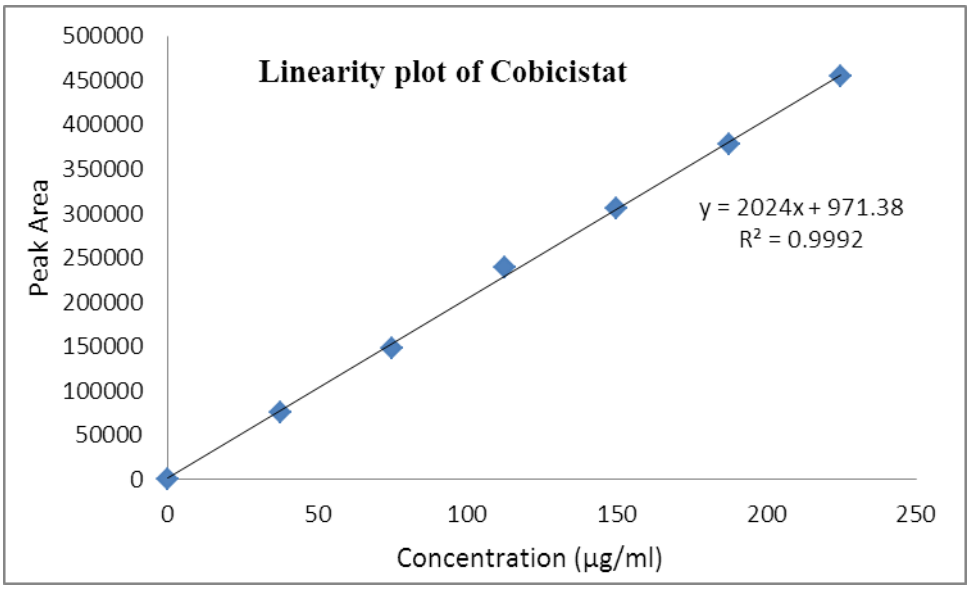

Fig. 6B Linearity plot of Cobicistat.

The % recovery of Atazanavir and Cobicistat was found to be 99.61% - 100.10% and 99.42% -99.90% respectively and % RSD for Atazanavir and Cobicistat was found to be 0.9 and 0.8 respectively. As the results were found to be within the limits, indicates that the method was accurate and precise. The method was found to be robust and stable up to 24hrs. The method was found to be specific, as there is no interference of placebo peaks with the retention time of Atazanavir and Cobicistat as shown in Fig. 7.

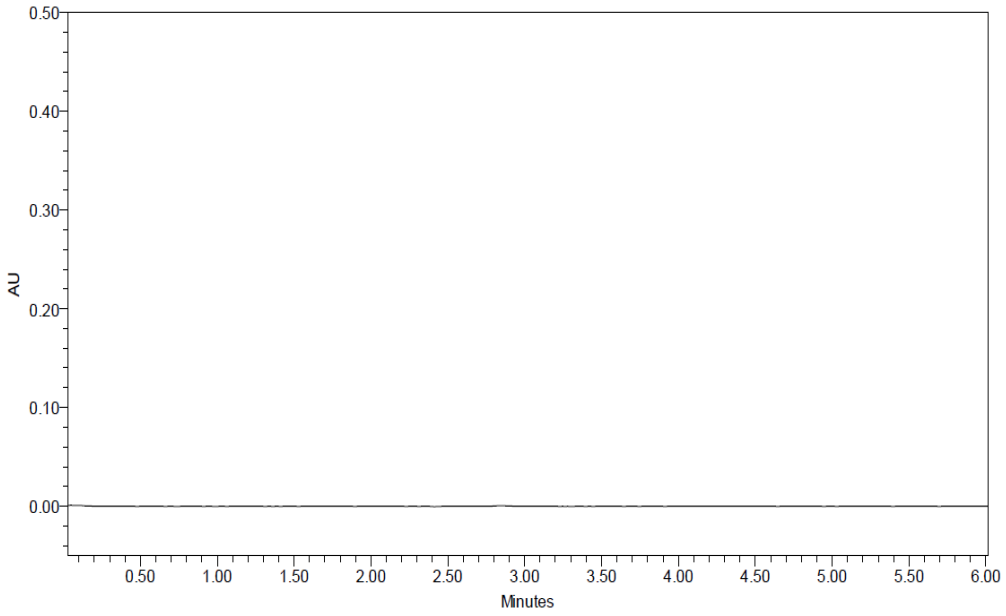

Fig. 7 Placebo chromatogram.

Table 1: System suitability and validation parameters.

Parameter	Atazanavir	Cobicistat
Specificity	Specific, No interference	Specific, No interference
Intra-day Precision (% RSD)	0.9	0.8
Inter-day Precision (% RSD)	0.4	0.5
Accuracy (% Recovery)	99.61% - 100.10%	99.42% - 99.90%
Linearity range (µg/mL)	75 – 450	37.5 – 225
Correlation coefficient, r	0.9998	0.9996
Limit of Detection (µg/mL)	0.47	0.05
Limit of Quantitation (µg/mL)	1.44	0.14
Robustness	Robust	Robust
Stability	Stable	Stable
USP Plate Count	8982	6131
USP Tailing factor	0.97	1.17
USP Resolution	5.7	

The stability of an analytical method was determined by forced degradation studies, in which the stability of the method was carried out by performing Acid stress study, Base stress study, Peroxide stress study, Water stress study, UV light exposure study and Dry heat stress study. The net degradation was found to be within the limits. The results and chromatograms were summarized in Table 2 and Fig. 8.

Table 2: Forced degradation studies results.

S.No.	Stress condition	Atazanavir			Cobicistat			% area of degradation peak
		% Assay	Peak purity Angle	Peak purity threshold	% Assay	Peak purity angle	Peak purity threshold	
1	2N HCL for 30mins at 60°C	95.32	0.110	0.318	95.41	0.484	0.551	0.73
2	2N NaOH for 30mins at 60°C	97.08	0.110	0.359	97.41	0.484	0.571	0.62
3	20% H_2O_2 for 30mins at 60^0C	98.06	0.102	0.317	98.17	0.245	0.372	-
4	Water for 6hrs at60^0C	99.03	0.300	0.325	99.30	0.203	0.380	-
5	UV light 200wts/hr or 7 days	99.35	0.290	0.328	99.50	0.213	0.379	-
6	105^0C for 6hrs	99.44	0.305	0.325	99.17	0.200	0.368	-

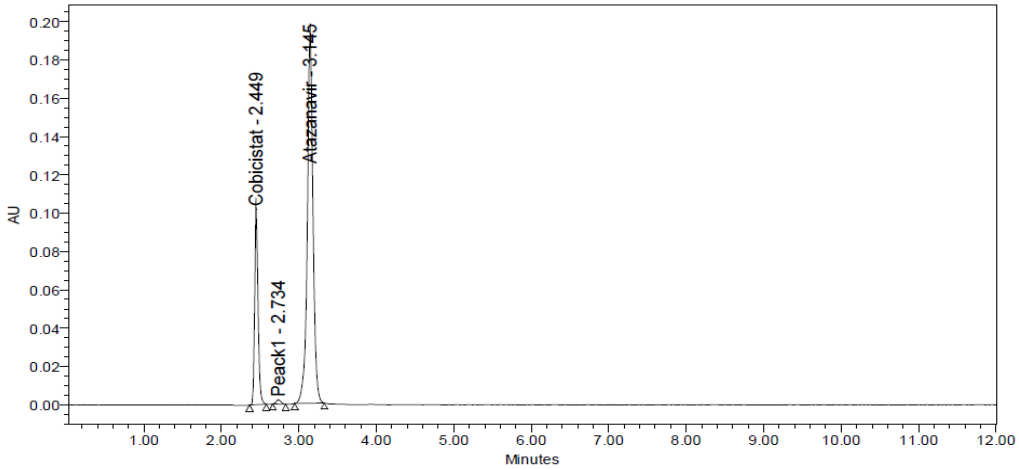

Fig. 8A Acid degradation chromatogram.

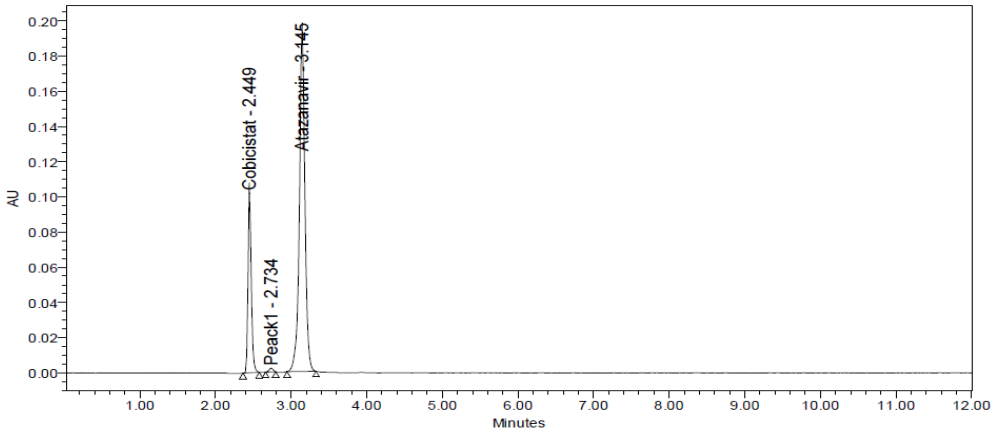

Fig. 8B Base degradation chromatogram.

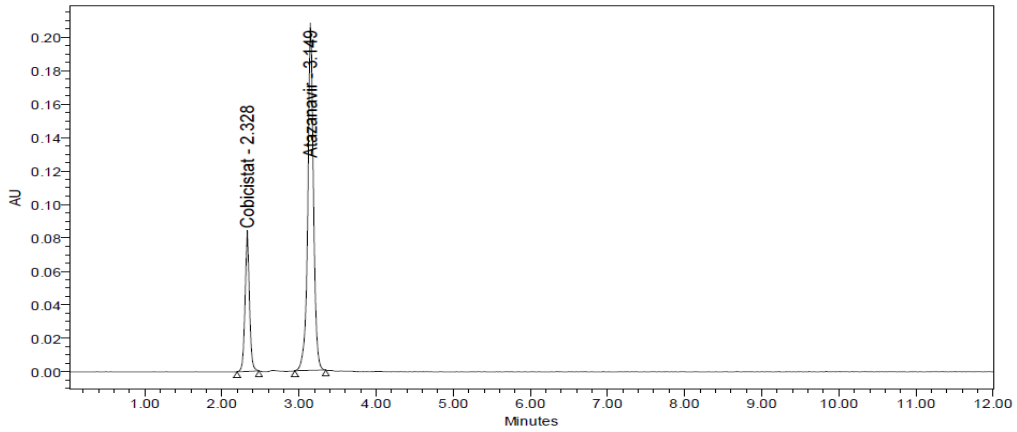

Fig. 8C Peroxide degradation chromatogram.

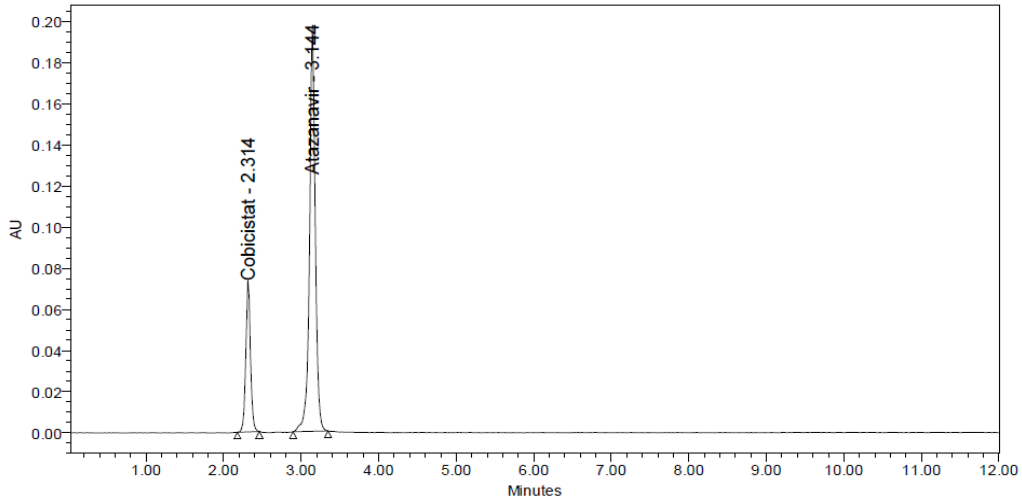

Fig. 8D Water stress study chromatogram.

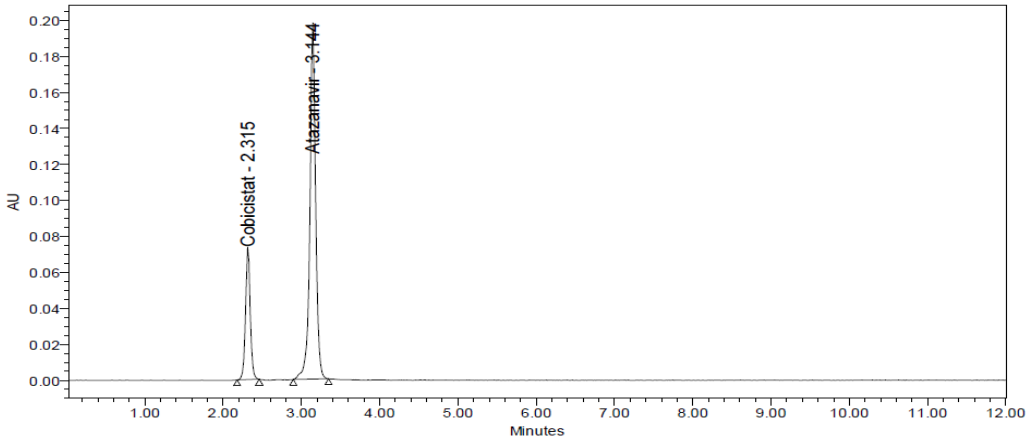

Fig. 8E Photo stability degradation chromatogram.

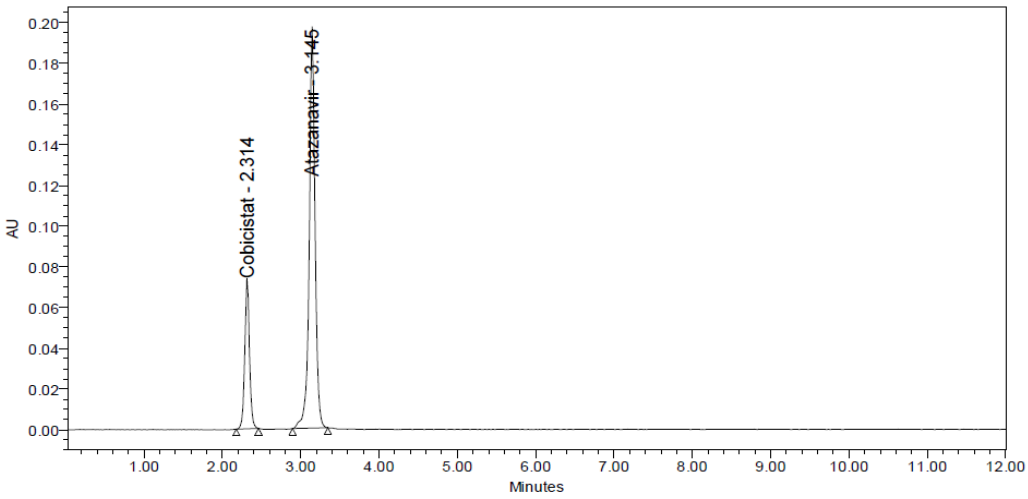

Fig. 8F Dry heat study chromatogram.

CONCLUSION

A specific, accurate stability indicating method was developed for the simultaneous estimation of Atazanavir and Cobicistat in pharmaceutical dosage form using HPLC. The method was validated by using various validation parameters and the method was found to be linear, precise, accurate, specific and robust. From the degradation studies conducted it is concluded that Atazanavir and Cobicistat were more stable at more concentrations of acid, base, peroxide, thermal, UV and water stress study conditions. The run time was 6min which enables rapid quantitation of many samples in routine and quality control analysis of tablet formulations.

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