Research Article

DEVELOPMENT OF STABILITY INDICATING RP-HPLC METHOD FOR MULTICOMPONENT TABLET FORMULATION CONTAINING ATAZANAVIR AND COBICISTAT

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ABSTRACT

A reverse phase-high performance liquid chromatographic method for simultaneous analysis of Atazanavir (ATA) and Cobicistat (COB) in a tablet dosage form has been developed and validated. The method was performed with Durashell C 18 column (250 X 4.6 mm) 5-micron particle column with 30:20:50 of water, methanol and acetonitrile respectively as mobile phase, pH adjusted to 5.5 with triethylamine, at a flow rate of 1 ml per minute. UV detection at 272 nm. By the proposed method, ATA and COB were eluted with retention times of 2.3 and 7.7 minutes respectively. The method was validated as per the ICH guidelines. The method was simple, rapid. specific, accurate, precise, reliable and reproducible. Calibration curve plots were linear over the concentration ranges of38 to 58 mcg/ml for ATA and 19 to 29 mcg/ml for COB. Limit of detection were 3.3 and 1.41 mcg/ml and Limit of quantitation were 10.0 and 4.28 mcg/ml for COB and ATA respectively. The forced degradation study using acid, base, oxidation with hydrogen peroxide, elevated temperature and photolytic conditions revealed the stability of solution for first and third day.

Keywords: Atazanavir, Cobicistat, Simultaneous estimation, RP-HPLC.

INTRODUCTION

Atazanavir is an antiretroviral drug of the protease inhibitor class.
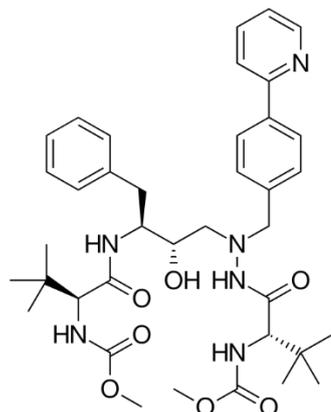
Chemically it is methyl N-[(1S)-1-{[(2S,3S)-3-hydroxy-4-[(2S)-2-[(methoxycarbonyl)amino]-3,3-dimethyl-N'-{[4-(pyridin-2-yl)phenyl]methyl}butanehydrazido]-1-phenylbutan-2-yl]carbamoyl}-2,2-dimethylpropyl]carbamate.

Cobicistat is a licensed drug for use in the treatment of human immunodeficiency virus infection (HIV/AIDS).
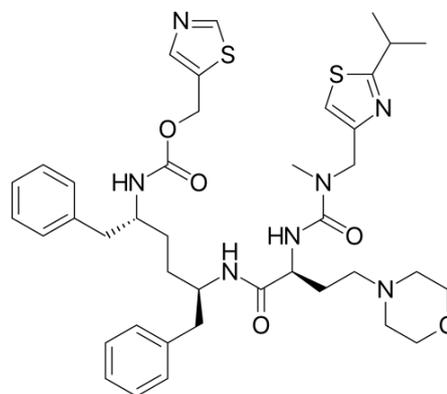
Chemically it is 1,3-thiazol-5-ylmethyl [(2R,5R)-5-{[(2S)-2-[(methyl{[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}carbamoyl)amino]-4-(morpholin-4-yl)butanoyl]amino}-1,6-diphenylhexan-2-yl]carbamate.

Recent research shows that there is a new analytical method for the analysis of reltigravir and elvitegravir[1]. Other methods include, stability indicating RP-HPLC method for simultaneous estimation of Atazanavir and Ritonavir in bulk and their dosage forms[2]. A new RP-HPLC method was developed and validated for the drug Cobicistat in bulk and tablet formulations [3]. There is another method reported for the estimation of Atazanavir sulphate and ritonavir in bulk and formulation[3]. There is another validated RP-HPLC metjod for the determination of atazanavir in pharmaceutical dosage form[4]. Using RP-HPLC method, another method was reported for simultaneous estimation of atazanavir and ritonavir in bulk and pharmaceutical dosage forms[5]. A novel validated RP-HPLC method for the determination of atazanavir sulphate in bulk and dosage forms[6]. Another method was reported

for development and validation of RP-HPLC method for the simultaneous estimation of atazanavir sulphate and ritonavir in bulk and formulations[7]. Development of noval and simple analytical method was reported for the estimation of atazanavir sulphate in pharmaceutical formulations by RP_HPLC[8]. There is a stability indicating HPLC method for the simultaneous estimation of emtricitabine, tenofovir disoproxil fumarate, cobicistat and elvitegravir in pharmaceutical dosage form[9]. Analytical method development and validation for the estimation of atazanavir in bulk and pharmaceutical dosage forms and its degradation studies using spectrophotometric method[10]. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of atazanavir and cobicistat in bulk and tablet formulation is reprted[11-14].

MATERIALS AND METHODS

Pharmaceutical grade working standards Cobicistat and Atazanavir were obtained from Bright labs, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from SD Fine chemicals Limited, Mumbai. HPLC grade water was collected from the (Milli Q academic) Millipore.

Instrumentation

The analysis was carried out on a THERMO SCIENTIFIC HPLC system equipped with ACCELA-1250 pump with autosampler and aphotodiode array detector. Data acquisition, data handling and instrumentation control were performed by CHROM/QUEST software. Column C 18 (250 X 4.6 mm) 5-micron particle column was used to optimize the method.

Optimization of RP-HPLC Method

Durashell C 18 column (250 X 4.6 mm) 5-micron particle column with 30:20:50 of water, methanol and acetonitrile respectively as mobile phase, pH adjusted to 5.5 with triethylamine, at a flow rate of 1 ml per minute. UV detection at 272 nm

Mobile phase preparation and diluent

Mixing of water, HPLC grade methanol and acetonitrile in the ratio of 30:20:50 respectively, and pH was adjusted to 5.5 with triethylamine.

Preparation of standard solution of Cobicistat

Weighed accurately about 30 mg of cobicistat and transferred into a 50 ml volumetric flask, and added about 15 ml of diluent, sonicated for 5 minutes to dissolve and diluted to 50 ml with diluent. Pipetted out 1 ml of the above solution into a 25 ml volumetric flask and diluted up to the mark with diluent. (Figure 1)

Preparation of standard solution of Atazanavir

Weighed accurately about 60 mg of atazanavir and transferred into a 50 ml volumetric flask, and added about 15 ml of diluent, sonicated for 5 minutes to dissolve and diluted to 50 ml with diluent. Pipetted out 1 ml of the above solution into a 25 ml volumetric flask and diluted up to the mark with diluent. (Figure 2)

Sample Preparation

Analysis of tablet dosage form was performed by determining average weight of the tablets using 20 tablets, then the tablets were crushed to a fine powder. The powder equivalent to 60 mg of COB

and 120 mg of ATA was transferred into a 100 ml volumetric flask and added about 30 ml of diluent, sonicated for 5 minutes to dissolve and diluted to 100 ml with diluent. Pipetted out 1 ml of the above solution into a 25 ml volumetric flask and diluted up to the mark with diluent. The final concentration were 24 mcg/ml of COB and 48mcg/ml of ATA. Then injected the above solution into the chromatographic system and analysed quantitatively. The analysis was repeated six times and possibility of interference of excipients from the tablet dosage form was examined. (Figure 3)

Method validation

The proposed analytical method was validated as per the ICH guidelines. The following validation parameters were performed: Linearity, accuracy, precision, system suitability, limit of detection, limit of quantitation and robustness

Linearity

Linearity of the method was studied by injecting the mixed standard solutions with the concentration ranges from 19.07 to 29.30 mcg/ml and 37.53 to 58.35 mcg/ml of COB and ATA respectively. The above concentrations were arrived out of the ranges from 10 to 30 mcg/ml and 30 to 60 mcg/ml for COB and ATA respectively. The experiment was repeated 6 times by injecting constant injection volume and the peak areas were plotted against the concentration to obtain the linearity graphs. (Figure 4 and 5 and Table 1 and 2)

Precision

The precision of optimized method was evaluated by performing six independent assays of test sample. % RSD of the assay values was calculated.

Limit of detection and Limit of quantitation

The LOD and LOQ was determined from the linearity data by using the slope of the line and standard deviation of Y intercept. LOD was expressed by establishing the lowest concentration at which the analyte can be detected. LOQ was considered as the lowest concentration of analyte that can be detected and quantified with acceptable accuracy and precision.

System suitability

The system suitability parameters including tailing factor, theoretical plates, repeatability and resolution between COB and ATA peaks were defined.

Robustness

Robustness was studied by determining the effect of small deliberate variations in the chromatographic conditions. The conditions studied were flow rate was altered by ±0.5 ml/min. and detection wavelength were altered by ± 2 nm. These chromatographic variations are evaluated for resolution between COB and ATA.

Forced degradation study

Forced degradation or stress testing of a drug substance will help to identify the degradation products, which can help to establish the stability of the molecule. The stability indicating study of COB and ATA were undergoes acid, alkali, oxidation degradation, photolysis and thermal degradation.

Accuracy

Accuracy was carried out by spiking the pure drug to the test sample (tablet formulation) in four different levels, that is 100%, 110%, 120% and 130%. Each level was repeated 3 times and the percentage recovery and %RSD was calculated, the results of accuracy is given in Table 4.

Analysis of marketed dosage form

The marketed tablet formulation was assayed by an above description. The peak areas were measured at 272nm and the determination of sample concentrations was using multilevel calibration developed on the same system under the similar conditions using regression analysis, the assay results are given in Table 5.

RESULTS AND DISCUSSIONS

The simultaneous estimation of COB and ATA was done by RP-HPLC method. The optimized method consists of 30:20:50 of water, methanol and acetonitrile respectively as mobile phase, pH adjusted to 5.5 with triethylamine, at a flow rate of 1 ml per minute. UV detection at 272 nm. The retention time of ATA and COB is 2.3 and 7.7 minutes respectively. Linearity of the method was demonstrated over the concentration ranges of 38 to 58 mcg/ml for ATA and 19 to 29 mcg/ml for COB. Limit of detection were 3.3 and 1.41 mcg/ml and Limit of quantitation were 10.0 and 4.28 mcg/ml for COB and ATA respectively. The accuracy was performed in 4 different levels and in all the levels the % RSD is within the limit (<2%) The forced degradation study using acid, base, oxidation with hydrogen peroxide, elevated temperature and photolytic conditions revealed there is no change in the retention time and symmetry for first day and third day (Figure 6 and table 3).

CONCLUSION

The proposed HPLC method for the estimation of ATA and COB is simple, accurate and precise. The method can be utilised for routine analysis to determine ATA and COB in bulk drugs and in formulations without prior separation.
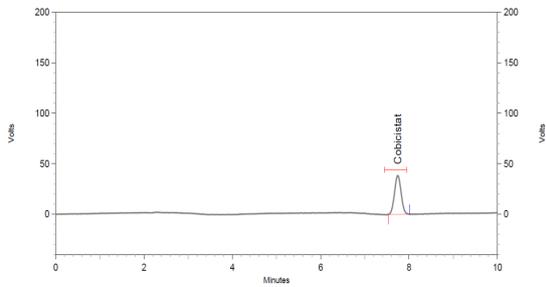
Figure 1: Chromatogram of Standard solution of Cobicistat
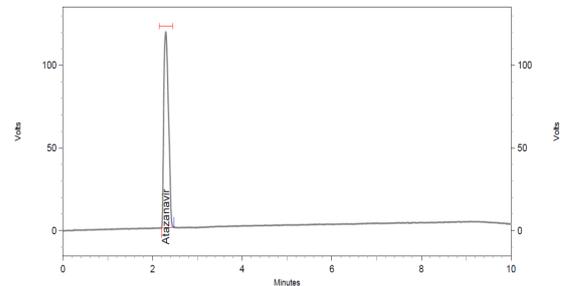
Figure 2: Chromatogram of Standard solution of Atazanavir
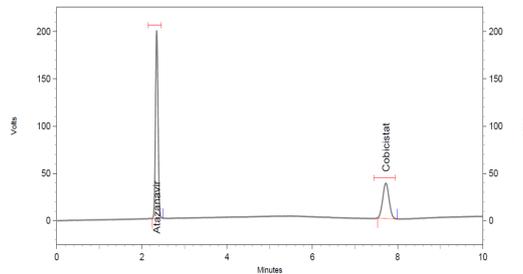
Figure.3: Chromatogram of COB and ATA at 272 nm from Pharmaceutical dosage form (EVOTOZ)
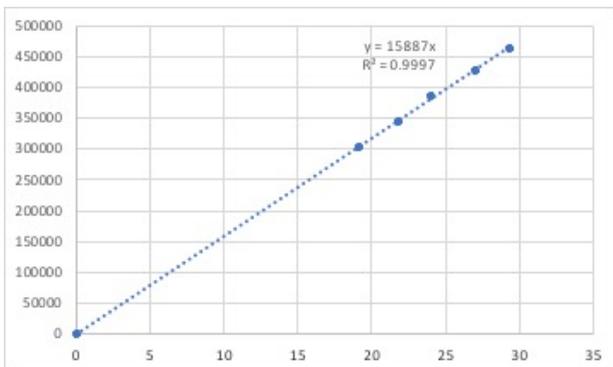
Figure. 4: Linearity graph for Cobicistat
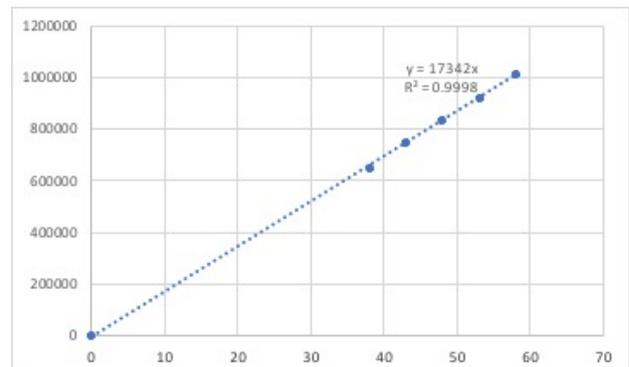
Figure. 5: Linearity graph for Atazanavir
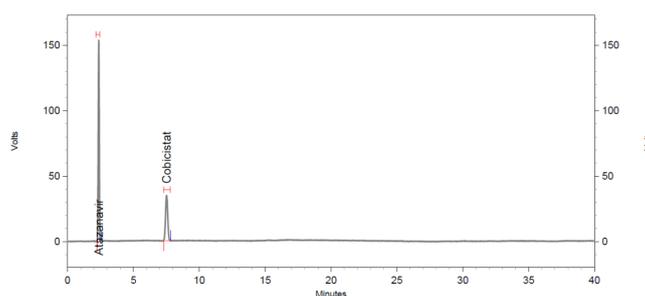
Figure. 6: Forced degradation study at 3ʳᵈ day with alkali

Table 1: Linearity for atazanavir

Sl.no.	Concentration in mcg/ml	AUC
1	38	649056
2	43	746925
3	48	830612
4	53	922703
5	58	1009600

Table 2: Linearity for Cobicistat

Sl.no.	Concentration in mcg/ml	AUC
1	19.1	302198
2	21.8	344960
3	24	387089
4	27	427601
5	29.3	463860

Table 3: Forced degradation study report after 3 days of alkali treatment

Name Of the drug	Retention time (min)	Area	Area %	Theoretical Plate (USP)	Asymmetry	Resolution
Atazanavir	2.37	844150	67.58	4660	0.94	0.00
Cobicistat	7.512	404888	32.42	9354	1.10	22.88
Total		1249038	100			

Table 4: Accuracy

Percentage level	Standard Area	Sample Area*	Theoretical (mg)	Experimental mg/tab*	Percentage*(%)
100	830970	834350	300	298.15	99.38
110	830970	919029	330	328.41	109.47
120	830970	1004286	360	358.88	119.63
130	830970	1089989	390	389.50	129.83

*Ave of 3 determinations

Table 5: Assay

Standard area	Sample area	mg/tab*	Percentage* (%)
386523.4	386080	149.80	99.87

*Ave of 6 determinations

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