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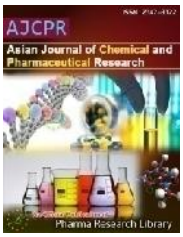

Research Article **Open Access**

Development and Validation of Novel RP-HPLC Method for Simultaneous Estimation of Cobicistat and Atazanavir in Synthetic Mixture

Srinivas Ganta*, Vidyadhara S

Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur - 522018, Andhra Pradesh, India.

A B S T R A C T

A simple accurate and selective Rp-HPLC Assay method was developed for the simultaneous estimation of three drugs Cobicistat (COB) and Atazanavir (ATA) in their tablet formulation. Two drugs were separated on Xterra C_{18} (5µm, 15cm X 4.6mm) with reverse phase elution of the mobile phase compose of 0.05M Phosphate buffer pH 7 adjusted with orthophosphoric acid: MeOH (30:70%v/v) at a flow rate of 0.8 ml/min. The detection was made at 260 nm. The retention times were 2.53 min for COB and 3.36 min for ATA. The linearity ranges for COB and ATA were 10-50 µg/ml and 20 - 100µg/ml respectively with correlation coefficients 0.999. The proposed method statistically validated with respect to system suitability, linearity, precision, accuracy, specificity, robustness, detection and quantitation limits. The method was found to be accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

Keywords: Cobicistat, Atazanavir, tablet, xterra

A R T I C L E I N F O

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***Corresponding Author**
Srinivas Ganta
Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur - 522018, Andhra Pradesh, India.
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1. Introduction

Cobicistat (COB) is a potent inhibitor of cytochrome P450 3A (CYP3A) which acts as a pharmaco-enhancing or "boosting" agent for antiviral drugs used in the treatment of HIV infection. Chemically COB 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. It is adsorbed onto silicon dioxide and is a white to pale

yellow solid powder with a molecular formula of $C_{40}H_{53}N_7O_5S_2$ and a molecular weight of 776.0. COB solubility is 0.1 mg/ml in water at 20 °C[1,2]. Its Chemical structure is given in Figure 1.

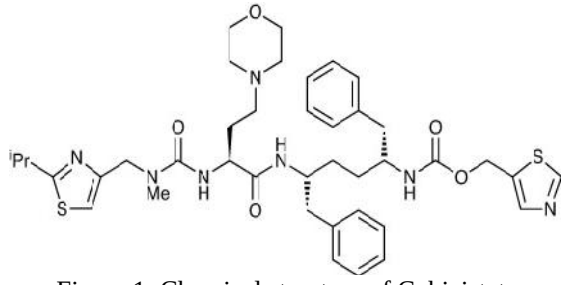

Figure 1: Chemical structure of Cobicistat

Atazanavir (ATA) is chemically known as methyl N-[(1S)-1-{[(2S,3S)-3- hydroxy-4-[(2S)-2-[(methoxycarbonyl) amino] -3,3- dimethyl -N'-{[4- (pyridin-2yl) phenyl] methyl} butanehydrazido] -1-phenyl butan-2-yl] carbamoyl} -2,2-dimethyl propyl] carbamate. Atazanavir sulfate is a white to pale yellow crystalline powder with a solubility of 4 to 5 mg/ml free base equivalents in water at 24°C, the pKa is 4.7. The molecular formula and molecular weight of atazanavir sulphate are $C_{38}H_{52}N_6O_7$ and 802.9416 g/mol respectively. Atazanavir is an antiretroviral drug of the protease inhibitor (PI) class is used to treat infection of human immunodeficiency virus (HIV) [3,4]. Figure 2 shows the chemical structure of ATA.

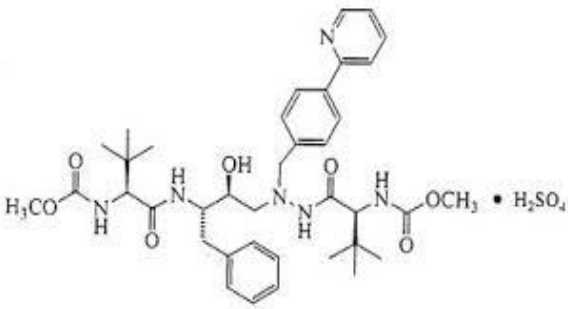

Figure 2: Chemical structure of Atazanavir

Literature survey reveals few UV spectrophotometric methods [5-7] and RP-HPLC methods [8-16] for the determination of atazanavir sulphate alone and simultaneously with other retroviral drugs in formulations and biological fluids. In addition, one HPTLC17 and two LC/MS/MS methods [18-19] were also reported. RP-HPLC methods [20-21] for the analysis of Cobicistat and related impurities in bulk and pharmaceutical dosage forms, Stability indicating HPLC method[22] for simultaneous estimation of emtricitabine, tenofovir disoproxyl fumarate, cobicistat and elvitegravir in pharmaceutical dosage form, A new gradient liquid chromatographic method[23] for simultaneous estimation of Tenofovir, Disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravin in bulk drug and tablet dosage form. The aim of current research work was to develop and validate as per ICH[24, 25] guidelines, a novel Rp-HPLC Assay method for simultaneous estimation of COB and ATA in their tablet formulation.

2. Materials and Methods

The Pharmaceutical grade working standards of ATA and COB were obtained as a gift from Mylan Pharmaceuticals (Hyderabad, India). Tablets ATA and COB was purchased from local market Hyderabad, India. All the chemicals were HPLC grade purchased from SD Fine Chem., Mumbai. MilliQ water was used, prepared in house.

Chromatographic Conditions:

Waters 2695 series HPLC consisting pump, Auto sampler, Auto injector, VWD & photo diode array detector, thermostatic column compartment connected with Empower 2 software connected with a Xterra C_{18} (5µm, 15cm X 4.6mm) column.

Detection wavelength:

10 mg of Cobicistat and Atazanavir was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Cobicistat and Atazanavir. The isobestic point found to be 260 nm, same was taken as detection wavelength. The overlay spectrums are shown in figure 3.

Preparation of mobile phase:

Weighed 0.50 grams of KH_2PO_4 and 0.301 grams of potassium dihydrogen phosphate was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 7 with ortho phosphoric acid. A mixture of pH 7 Phosphate buffer 300 mL (30%), 700 mL of MeoH (70%) were taken and degassed in ultrasonic water bath for 5 minutes. Then this solution was filtered through 0.45 µ filter under vacuum filtration. Mobile phase was used as diluent.

Preparation of Cobicistat standard preparation:

10mg of Cobicistat working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2 ml of (DMF) Di methyl formamide was added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (mobile phase). Further 10.0 ml of the above stock solution was pipette into a 100 ml volumetric flask and diluted upto the mark with diluent.

Preparation of Atazanavir standard preparation:

20 mg of Atazanavir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2 ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (mobile phase). Further 10.0 ml of the above stock solution was pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent.

Preparation of Sample Solution: (Tablet)

Accurately weighed 10 tablets were crushed in mortar and pestle and weight equivalent to 10 mg of Atazanavir and Cobicistat (marketed formulation) was taken into a 10mL clean dry volumetric flask and about 7mL of Diluents was added and sonicated to dissolve it completely and made volume upto the mark with the same solvent (mobile phase) Further 3 ml of above stock solution was pipetted into a 10 ml volumetric flask and diluted upto the mark with diluent.

Procedure:

20 µL of the standard & sample solutions were injected into the chromatographic system and the areas for Atazanavir

and Cobicistat peaks were determined and the %Assay are calculated.

3. Results and discussion

The overlay spectrum of COB and ATA was obtained and the isobestic point of COB and ATA showed absorbance's maxima at 260 nm. The spectrums are shown in Figure 3. Table 1 gives the optimized chromatographic conditions. Figure 4 & 5 represents the standard and sample chromatograms of COB and ATA respectively.

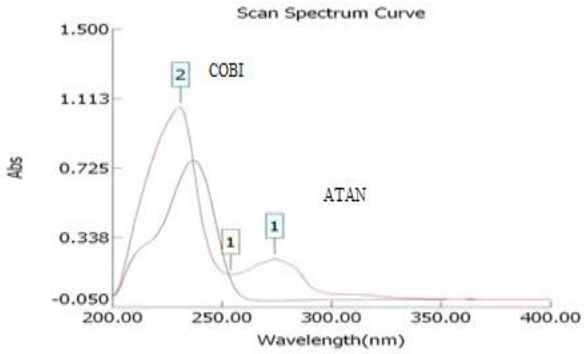

Figure 3: Overlay spectrum of Cobicistat and Atazanavir

Table 1: Optimized chromatographic conditions

S. No	Chromatographic Parameter	Condition
1.	Column	Xterra C_{18} (5µm, 15cm X 4.6mm)
2.	Mobile Phase	0.05M Phosphate buffer and methanol (30:70v/v) pH adjusted to 7 with orthophosphoric Acid.
3.	Flow Rate	0.8 ml/min
4.	Column Temperature	Ambient
5.	Injection Volume	10µl.
6.	Detection Wavelength	260 nm
7.	Rts	2.53 and 3.26 min for Cobicistat, and Elvitegravir respectively
8.	Diluent	Mobile Phase

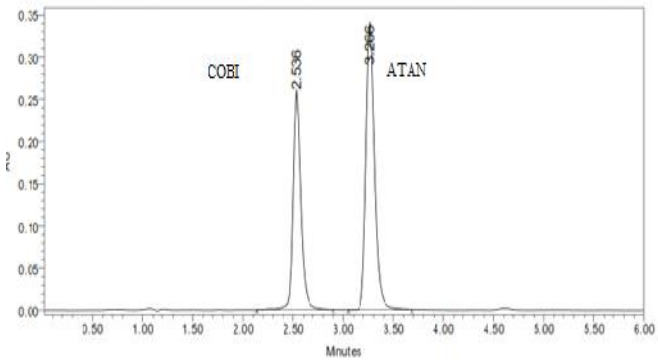

Figure 4: Representative chromatogram of Standard Preparation

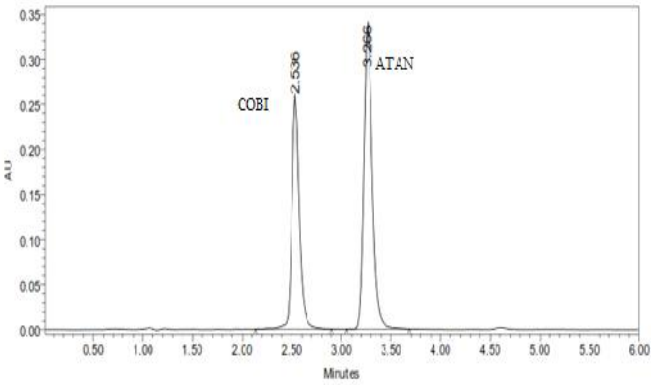

Figure 5: Representative chromatogram of Sample Preparation

Method Validation:

System suitability:

System suitability is an integral part of the method validation to evaluate the parameters like tailing factor, theoretical plates, resolution and %RSD for replicate injections. The results were within the limits and were presented in Table 2. Figure 4 and 5 shows the Standard and sample chromatogram respectively.

Accuracy:

To determine the Accuracy of the proposed method, recovery studies were conducted; known amount of pure drug concentrations was spiked in sample at three different levels, ie, 50%, 100% and 150% and was calculated. Accuracy was calculated as the percentage of recovery. The results were tabulated in Table 3 & 4.

Precision

The precision was evaluated at three levels, repeatability, reproducibility and intermediate precision each level of precision was investigated by six replicate injections of concentrations. The result of precision was expressed as % of RSD and was tabulated in Table 5.

Linearity:

The linearity was evaluated by measuring different concentrations (25% to 150%) of the standard solutions to COB (20-100 µg/ml) and ATA (10-50 µg/ml). The calibration curve was constructed by plotting concentration of standard solutions against mean peak areas and the regression equation was computed. Linearity curves of COB and ATA were shown in figures 6 and 7

Detection limit (DL) and quantitation limit (QL):

Estimation of DL and QL considered the acceptable signal-to-noise ratios 3:1 and 10:1 respectively. The limit of detection and quantitation to be determined as 2.95 and 9.87µg/ml for Cobicistat and 3.04 and 10.30 µg/ml for Elvitegravir respectively.

4. Conclusion

A simple, specific and reliable isocratic HPLC-PDA method was developed for the estimation of Cobicistat and Atazanavir in their pharmaceutical formulation. The Proposed method is specific, sensitive accurate & precise. Hence the developed method can be adapted to regular quality control analysis of COB and ATA tablets.

Table 2: System suitability data

S. No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Cobicistat	3.271	2695932	474830	8068.3	1.2	-
2	Atazanavir	2.544	1561369	302524	6382.1	1.3	1.5

Table 3: Accuracy results of Cobicistat

%Conc. (at specification Level)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	1717685	5	5.0	101.3%	100.0%
100%	3472797	10	9.94	99.4%	
150%	5224472	15	14.8	99.2%	

Table 4: Accuracy results of Atazanavir

% Conc. (at specification Level)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	1724719	5	5.10	101.8%	100.5%
100%	3470411	10	9.99	99.9%	
150%	5413905	15	14.9	99.1%	

Table 5: Precision results of COB & ATA

S.NO	COBICISTAT		ATAZANAVIR	
	AREA	% ASSAY	AREA	% ASSAY
1	2194758	97.9	2194758	97.9
2	1456296	97.8	2195700	97.8
3	1457422	97.7	2196191	97.7
4	1456513	97.6	2195326	97.6
5	1454579	97.5	2200951	97.5
6	1454578	97.4	2200950	97.5
Mean	1451483	97.65	2196585	97.67
Std Dev	2347.6	0.19	2496.0	0.16
%RSD	0.16	0.19	0.11	0.17

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