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Der Pharmacia Lettre, 2018, 10 [6]: 1-9
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ISSN 0975-5071
USA CODEN: DPLEB4

New Method Development and Validation for Simultaneous Determination of Atazanavir and Cobicistat in Bulk and Tablet Dosage Form by UPLC

Devilal J[1], Narottam Pal[2]*, Sumalatha K[3], Srinivasa Rao A[4]

[1]*Associate Professor, Department of Pharmaceutical Chemistry, Bhaskar Pharmacy College, Yenkapally (V), Moinabad (M), R.R. District, Hyderabad*

[2]*Assistant Professor, Department of Pharmaceutical Analysis, Bhaskar Pharmacy College, Yenkapally (V), Moinabad (M), R.R. District, Hyderabad*

[3]*Assistant Professor, Department of Pharmacognosy, Bhaskar Pharmacy College, Yenkapally (V), Moinabad (M), R.R. District, Hyderabad*

[4]*Principal and Professor, Bhaskar Pharmacy College, Yenkapally (V), Moinabad (M), R.R. District, Hyderabad*

***Corresponding author:** Narottam Pal, Assistant Professor, Department of Pharmaceutical Analysis, Bhaskar Pharmacy College, Yenkapally (V), Hyderabad. India. Tel: 09989129588; E-mail: narottampal8224@gmail.com*

ABSTRACT

The present analytical work is a unique method development and validation for the simultaneous determination of Atazanavir and Cobicistat by using reverse phase ultra-performance liquid chromatography (UPLC) with isocratic elution technique. Here the stationary phase used was C18 HSS column (2.1 × 100 mm, 1.8 µm) mobile phase was 45% OPA (0.1%) and 55% Acetonitrile. pH of the mobile phase was maintained at 3.0, flow rate 0.2 ml/minute. Eluted material underwent for monitoring at the detector wavelength of 254 nm. Retention time for Atazanavir and Cobicistat was found to be 0.536 minutes and 1.366 minutes, linearity range was 75 µg/ml to 450 µg/ml and 37.5 µg/ml to 225 µg/ml respectively. The new method was evaluated according to ICH guideline and as far as validation results are concern correlation coefficient value was 0.999 for both of the compounds, LOD 0.76 and 0.37, LOQ 0.2.30 and 1.11, percentage recovery 99.74% and 99.34%, repeatability results relative

standard deviation (%RSD) 0.4 and 0.6 for Atazanavir and Cobicistat respectively. The developed UPLC method was found to be a simple and rapid one for regular analysis in professional laboratory.

Keywords: Atazanavir, Cobicistat, Simultaneous, UPLC.

INTRODUCTION

Cobicistat (COBI) is an ideal co therapy along with other antiviral [1,2] compounds as it inhibits cytochrome P450 3A and thereby the exposure of an antiviral is enhanced in the physiological system. Chemically this compound is known as (1,3-thiazol-5-yl)methylN-[(2R,5R)-5-[(2S)-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl})carbamoyl]amino}-4-(morpholin-4-yl)butanamido]-1,6-diphenylhexan-2-yl]carbamate (Figures 1 and 2).

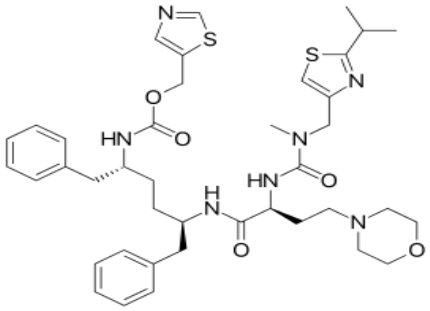

Figure 1: Structure of Cobicistat.

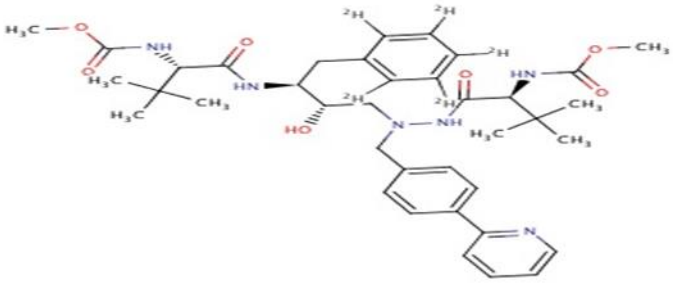

Figure 2: Structure of Atazanavir.

Atazanavir (ATAZ) is an antiviral compound which is able to bind to the active site HIV protease and thereby prevents the virus from cleaving the pro-form of viral protein part into the useful working machinery of the virus. If the enzyme HIV protease does not work, the virus remains no more infectious, and no mature virions will be made. Chemically the compound is known as

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- dimethyl propyl]carbamate.

As per literature survey [3-13] it is learned that there are many methods available for the simultaneous determination for ATAZ and COBI. D. Sindu Priya et al. Uttam Prasad Panigrahy et al. Kommana Balaram Kumar et al. and M. Venkata Siva Sri Nalini et al. developed conventional RP-HPLC method whereas Masthannamma SK et al., B. Valli Purnima et al., Pratik K. Vora et al., innovated stability indicating HPLC method to simultaneously estimate the above mentioned drugs. We observed in all these case that the retention time for the compounds was like around 2 minutes to 3.6 minutes for Cobicistat and around 2.5 minutes to 7.11 minutes for ATAZ. In our present project we have attempted to use a very simple chromatographic condition as well as ensured that the time consumption must be as low it was possible and in fact it is the lowest one as on date.

MATERIALS AND METHODS

Materials

ATAZ and COBI pure drugs were procured from Spectrum Labs Ltd Hyderabad. Combination of ATAZ and COBI tablets (EVOTAZ) was supplied by Medindia Pharma network. Distilled water, acetonitrile, methanol, potassium di-hydrogen ortho phosphate, ortho-phosphoric acid were from Rankem.

Instruments

WATERS UPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.

UV-VIS spectrophotometer: PG Instruments T60, bandwidth of 2 nm, matched quartz cells integrated with UV win 6 Software was used to measure the absorbance.

Experimental procedure

The new method development work [14,15] we started after adequate amount of literature survey and getting adequate idea about the different chromatographic factors which ultimately could deliver a very good method for the simultaneous determination.

Diluent

Based upon the solubility of the drugs, diluent was selected. It was observed that water and acetonitrile at 50:50 ratio was suitable.

Preparation of stock solutions (Standard): Accurately weighed 15mg of COBI, 30mg of ATAZ and transferred to volumetric flasks separately. 3/4th of diluent (as mentioned) was added to both of these flasks and subjected for sonication for 10 minutes. Flask volume till the mark was made up with diluents and labeled as stock solution (standard) 1and 2. (1500 µg/ml of COBI and 3000 µg/ml of ATAZ).

Preparation of Standard working solutions (100% solution): 1 ml solution from each stock preparation was pipetted out and collected into a volumetric flask (10ml) and made up the required volume with diluent. (150 µg/ml of COBI and 300 µg/ml of ATAZ).

Preparation of stock solutions (Sample): 10 tablets were weighed, average weight of single tablet was calculated, then the weight (equivalent) of 1 tablet was collected into a volumetric flask (100 ml), 50 ml of diluents was mixed and sonicated for around 25 minutes. It was then subjected for making the volume with diluent and filtered by UPLC filters (1500 µg/ml of COBI and 3000 µg/ml of ATAZ)

Preparation of working sample solutions (100% solution): 1 ml volume of filtered sample solution was transferred to volumetric flask (10ml) and the volume was made up with same diluent. (150 µg/ml of COBI and 300 µg/ml of ATAZ)

Preparation of buffer

0.1% OPA: 1 ml of ortho phosphoric acid was diluted to 1000 ml with UPLC grade water.

Validation parameters

The method was evaluated as per protocol of ICH [16]. The evaluation parameters took into consideration were precision accuracy, intermediate precision, linearity, limit of quantification, limit of detection, robustness studies etc.

Accuracy

The accuracy for the present UPLC methods was examined by calculating the extant of recoveries of ATAZ and COBI by the method called standard addition. Correct amount of solutions (standard) of ATAZ and COBI (each 1, 50%, 100%, and 50%) were added and injected to pre-quantified solution of sample. The quantity of each substances recovered were determined.

Precision

The experimental repeatability as well as intermediate precision was examined by repeatedly applying six injections containing ATAZ (300 ppm) and COBI (150 ppm) at two subsequent days. Number of theoretical plates, retention time, peaks resolution, peak symmetry etc. was the subject of observation.

Linearity

Following concentration for both the compound were designed to conduct linearity test.

ATAZ

75 ppm, 150 ppm, 225 ppm, 300 ppm, 375 ppm, 450 ppm.

COBI

37.5 ppm, 75 ppm, 112.5 ppm, 150 ppm, 187.5 ppm, 225 ppm. To build up calibration curve Concentration and area were considered at X and Y axis respectively.

LOD and LOQ: Calculation for Limit of detection as well as Limit of quantification had been done by using standard Equations. LOD = 3.3 × σ/S, LOQ = 10 × σ/S. Here σ denotes for standard deviation of intercepts of regression lines, S denotes for slope.

Robustness

Evaluation for robustness had been conducted by making alteration in different chromatographic parameters. These parameters included flow rate, temperature, mobile phase composition etc.

Assay of marketed formulation: The formulation (Tablet-Evotaz) was procured from Medindia Pharma network. Ten tablets had been taken, weighed and collected in a mortar. Tablets were triturated to powder form and collected an equivalent quantity of ATAZ 300 mg and COBI 150 mg in a volumetric flask (50 ml). Powders were treated with diluent and subjected for sonication. The volume was made with diluent. 1 ml of the solution was pipetted out into a volumetric flask (10 ml) and the volume was made with diluent. 10 µl of resultant solution was injected to the Chromatographic system and result was studied as compared to standard. Peak area response was taken into consideration.

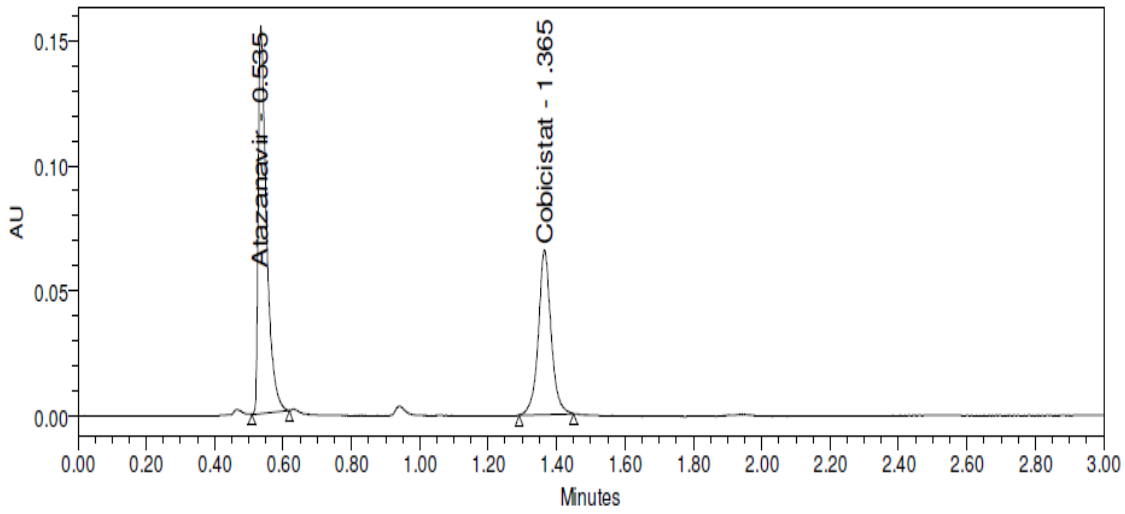

Figure 3: Optimized chromatogram of Cobicistat and Atazanavir.

RESULTS AND DISCUSSION

System suitability parameters

The optimized chromatographic method as developed resulted in the simultaneous solution of ATAZ and COBI at 0.535 minute and 1.365 minutes. Figure 3 is the representative chromatogram of standard ATAZ and COBI. System suitability results were

evaluated taking six replicates of standard at 300 µg/ml and 150 µg/ml for both the compounds respectively. Table 1 narrates about the results of system suitability parameters.

Table 1: System suitability.

Compounds	Rt (min)	Area	USP plate count	Tailing factor	Resolution
Atazanavir	0.536	257508	2424	1.90	---
Cobicistat	1.371	166035	7144	1.02	14.9

Accuracy results

Recovery of ATAZ and COBI standard 50%, 100% and 150% were 100.49%, 100.57%, 100.66% and 99.90%, 99.35%, 99.04% respectively. Table 2 contains all the results of accuracy studies.

Table 2: Accuracy results.

Quantity of Sample (µg /ml)		Quantity of Standard (µg /ml)		Amount recovered (µg /ml)		% Recovered	
Atazana	Coboci	Atazana	Coboci	Atazana	Coboci	Atazana	Coboci
300	75	150	75	150.73	74.93	100.49	99.90
300	75	300	150	301.72	149.02	100.57	99.35
300	75	450	225	453.01	222.85	100.66	99.04
Note: N=3							

Precision results

Result of intraday precision as mean area of peak and %RSD for ATAZ standard injections were –257506 and 0.45. For ATAZ sample injection results were 260499 and 0.40. For COBI standard injection results were 166038 and 0.49, for sample injection results were 168616 and 0.60. Results of inter day precision study in terms of average area of peak and %RSD for ATAZ sample injections were 259850 and 0.40. Results of intraday precision study in terms of average peak area and %RSD for COBI sample injections were 171842 and 0.60. Table 3 narrates precision results in details.

Table 3: Precision results.

Compounds	Result	Standard area	Sample (Intraday)	Sample(Interday)
Atazanavir	Mean	257506	260499	259850
	SD	1100.2	1026.6	1099.0
	% RSD	0.45	0.40	0.40
Cobicistat	Mean	166038	168616	171842
	SD	995.50	965.9	1054.4
	% RSD	0.49	0.60	0.60

Regression analysis

Results of linearity test revealed that mean Y intercept value, slope value, and value of correlation coefficient for ATAZ was 5718.0, 2173, 0.999 at the concentration range of 75 µg/ml to 450 µg/ml. Mean Y intercept value, slope value, and value of correlation coefficient for COBI was 2173, 1080.0 and 0.999 at concentration range of 37.5 µg/ml to 225 µg/ml. Table 4 explains about sensitivity and results of regression analysis.

Table 4: Regression analysis and sensitivity test results.

Parameters	Atazanavir	Cobicistat
Linearity (µgm/ml)	75 µg/ml to 450 µg/ml	37.5 µg/ml to 225 µg/ml
Correlation Coefficient.(r)	0.999	0.999
Regression slope	847.1	1080.0
SD of Slope	0.134	0.201
Regression Intercept (mean)	5718.0	2173.0
%RSD of Intercept	0.323	0.118
LOD	0.76	0.37
LOQ	2.30	1.11

Robustness results

This evaluation had been done by bringing variation in certain chromatographic parameters such as increasing and reducing flow rate, variation in the ratio of aqueous phase and organic phase, temperature of column etc. Retention time, plate counts, asymmetric or tailing factor etc., was observed with very negligible variation. All the observed values are given in Table 5 as tabular form.

Table 5: Robustness results.

	Chromatographic Condition	Retention Time (minutes)	USP Theoretical Plates	Asymmetric Factor	% Assay
Atazanavir	Flow rate +	0.520	2388	1.88	101.23
	Flow rate -	0.546	2555	1.80	99.22
	OPA:CAN(40:60)	0.521	2390	1.84	100.03
	OPA:CAN(50:50)	0.539	2499	1.84	99.57
	Temperature 25°C	0.537	2498	1.85	99.53
	Temperature 35°C	0.530	2385	1.82	100.05
Cobicistat	Flow rate +	1.355	7002	1.0	98.38
	Flow rate -	1.371	7342	1.0	98.28
	OPA:ACN(40:60)	1.351	6989	1.01	100.64
	OPA:ACN(50:50)	1.373	7410	1.0	99.71
	Temperature 25°C	1.369	7238	1.02	100.37
	Temperature 35°C	1.350	7116	1.0	99.71

CONCLUSION

The present UPLC method for the simultaneous determination of ATAZ and COBI was found to be the least time consuming, simple, highly accurate technique as results of all the validation parameters were with low value of %RSD. It also proved that the innovated technique is precise and robust. Therefore the above mentioned novel analytical technique is suitable for simultaneous evaluation of bulk and combined tablet formulation of ATAZ and COBI in laboratory.

ACKNOWLEDGEMENTS

We are very much thankful to Dr. A. Srinivasa Rao Garu, Professor and Principal Bhaskar Pharmacy College, Hyderabad for his guidance, kind help and constant encouragement at every step during the progress of this research work. We also express our gratitude to Dr. V.B. Rao Garu, CEO, and Mr. J.V. Krishna Rao Garu, secretary, J.B. Group of Educational Institution for providing a healthy working environment which is an essence in research field.

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