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Original Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF ATAZANAVIR SULPHATE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Two simple, accurate, precise and cost effective UV-Spectrophotometric methods have been developed for estimation of Atazanavir sulphate (ATV), an anti-HIV drug, in bulk and pharmaceutical dosage form. Method A is Absorbance maxima method, which is based on measurement of absorption at maximum wavelength, 247nm. Method B is Area under Curve (AUC), in wavelength range of 240-254nm. The linear responses were observed in the range of 5- 40 µg/ml for both the methods, with the regression coefficient of 0.9996 and 0.9997 respectively. The accuracy of the methods was assessed by recovery studies and was found to be 100.56% and 100.86% respectively. The developed methods were validated for different parameters like linearity, accuracy (recovery), precision and specificity, as per the ICH Q2 R1 (International Conference for Harmonization) guidelines and were found to be satisfactory. These methods can be used for the determination of Atazanavir sulphate in bulk and formulation without interference of the excipients.

Keywords: Atazanavir sulphate, Absorption maxima, Area under Curve, Anti-HIV drug, ICH guidelines.

INTRODUCTION

Atazanavir sulphate (ATV) is a chemically (3*S*,8*S*,9*S*,12*S*)-3,12-Bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-{[4-(2-pyridinyl)phenyl]methyl}-2,5,6,10,13 penta aza tetra decane dioic acid dimethyl ester, sulphate(1:1) (figure1). ATV is an antiretroviral agent for the treatment of HIV infection and consequently it is clinically useful in the treatment of AIDS. Atazanavir sulfate, azapeptide inhibitor of HIV-1 protease, is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection. Atazanavir sulfate is a white to pale yellow powder. It is slightly soluble in water. [2] The drug was approved by the United States Food and Drug Administration (USFDA) in June2003. Structure–activity studies with a series of azadipeptides designed to mimic the transition state of the peptide-cleavage reaction catalyzed by HIV-1 protease identified lead compounds that had either potent antiviral activity against mutant HIV-1 strains or good oral bioavailability, but not both. Atazanavir is given with food by mouth as the sulfate and the usual adult dose is 400 mg once daily. The drug is well absorbed when administered orally with food (bioavailability 68%). The drug is highly bound to plasma proteins (86%) and is metabolized by CYP3A isozyme. It is a moderate inhibitor of CYP3A.Literature survey revealed few RP-HPLC and spectrophotometric methods for estimation of Atazanavir. [1, 2, 3]

The objective of the present work is to develop simple spectrophotometric methods for estimation of Atazanavir sulfate in bulk and formulation with good accuracy, simplicity, precision and economy over other chromatographic methods and which can be used for routine analysis.

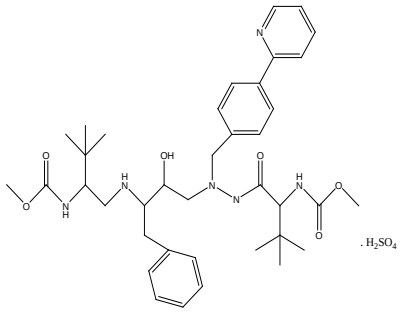

Fig. 1: Structure of ATV

MATERIALS AND METHODS

Chemicals

Pure sample of ATV was received as a gift sample from Ranbaxy lab. The percentage purity of ATV was 99.85% w/w. Formulation containing ATV sulphate 300mg (Atazor Capsule 300mg, Cipla Pharmaceuticals, Hinjewadi, Pune) was used for the analysis. Spectroscopy grade methanol (Thomas Baker) and Distilled water were used. For the studies.

Instrumentation

A Jasco double beam UV–visible spectrophotometer, Model: V-630, with a fixed bandwidth (2nm) and 1-cm quartz cell was used for Spectral and absorbance measurements. In addition, electronic balance, micropipette and sonicator were used in this study.

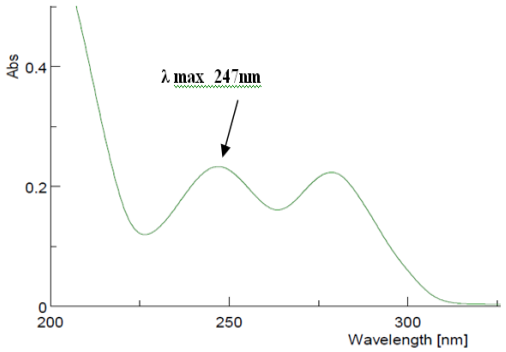

Fig. 2: Spectra of ATV (Conc.10ug/ml)

Preparation of stock solution

Accurately weighed about 100 mg of pure drug and dissolved in 50% methanol and final volume made up to 100 ml with distilled water to give standard stock solution (1000 ug/ml). Aliquots of standard stock solution were pipette out and suitably diluted with distilled water to get the final concentration of 5-40 ug/ml of standard solution for both the method. The solutions were scanned in the spectrum mode from 400 nm to 200 nm wavelength range and the maximum absorbance shown at 247 nm (figure 2) for

Method A and similarly for Method B AUC shown in the range of 240-254 nm (Figure 4).

Selection of wavelength

Standard stock solution of 1000 ug/ml was prepared in methanol (50%) and further aliquots were made using distilled water. The standard solution of concentration 10 ug/ml was prepared in distilled water and scanned between 200-400 nm and λmax was found to be at 247 nm for Method A and similarly for Method B AUC shown in the range of 240-254 nm (Figure 4).

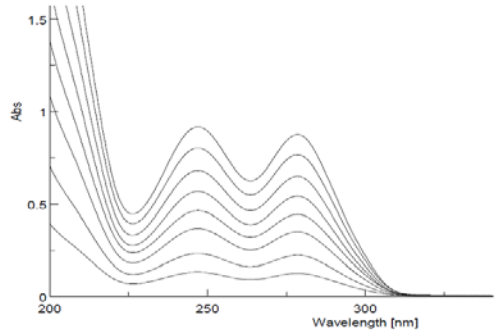

Fig. 3: Absorbance maxima method for ATV (Conc. 5-40ug/ml)

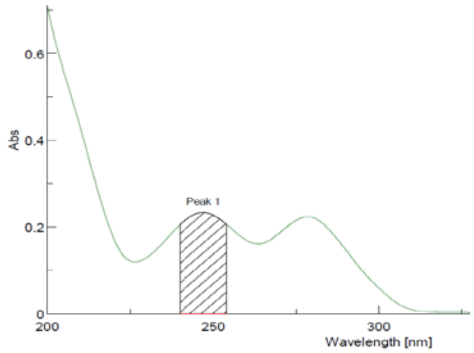

Fig. 4: Area under Curve of ATV

Method A: Absorbance Maxima Method

Aliquots of standard stock solution of concentration 1000 µg/ml were taken and suitably diluted with distilled water to get working standard solutions in the increasing concentration range (5-40ug/ml). These were scanned in the range of 200-400 nm. The absorbance maximum was found to be at 247nm. The calibration curve was plotted with concentration v/s absorbance and regression equation was calculated and it was found to be 0.9996 (Figure 5).

Method B: Area under Curve Method

From the spectra of drug obtained after scanning of standard solution of ATV, area under the curve in the range of 240-254nm was selected for the analysis (Figure 4). The calibration curve was prepared in the concentration range of 5-40 µg/ml at their respective AUC range and regression equation was calculated and it was found to be 0.9997 (Figure 6).

The drug followed the Beer-Lambert's law in the concentration range of 5-40 µg/ml for the both methods. For the both methods (Absorption maxima and Area under Curve) the coefficient of correlation (r), slope (m) and intercept (c) values of this method are given in Table 2.

Analysis of marketed formulation

Twenty capsules (Atazor 300mg, Cipla Pharmaceuticals., Hinjewadi, Pune) were weighed accurately and tablet weight equivalent to 300 mg of ATV was weighed accurately and transferred to 100 ml of mixture of Methanol and Distilled Water (50:50% v/v) to get standard stock solution of 3000 µg/ml and sonicated for 5min. The solution was filtered through filter paper no. 41; 10 ml of this filtrate was further diluted to 100 ml of distilled water. From this solution, further dilutions of ATV were made in the calibration range using distilled water. The solution was scanned in the range of 200-400 nm against blank and maximum absorbance was recorded at wavelength 247 nm for Method A and similarly for Method B AUC was measured in between 240-254 nm.

Validation of uv method

Validation of the UV method was done with respect to following parameters.

Linearity and Range

The standard solutions were prepared by dilution of the stock solution with to reach a concentration range equivalent mixture Methanol and Distilled water. The Absorbance was plotted against the corresponding concentrations to obtain the calibration curves (Figure 5) and (Figure 6) for both the methods.

Accuracy

Recovery studies was carried out by applying the method to drug sample to which known amount of ATV corresponding to 80, 100, 120% of label claim has been added (standard addition method).

Precision

Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies (intra-day) were performed by analysis of ATV respectively on the same day. Intermediate precision (inter-day) of the method was checked by repeating analysis of ATV on a different day. Measurement of peak area for active compound was expressed in terms of % relative standard deviation (%R.S.D.) for both the methods

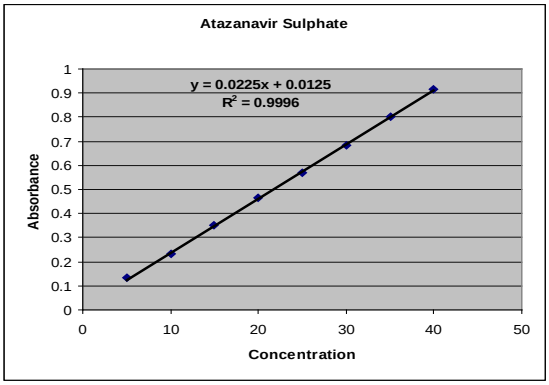

Fig. 5: Linearity of ATV (API), by Method A

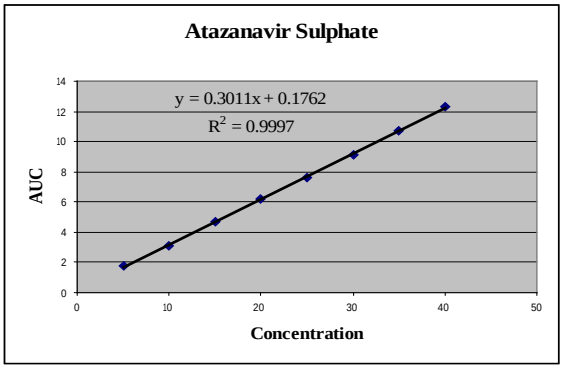

Fig. 6: Linearity of ATV (API), by Method B

Table 2: Table shows Optical characteristics and precision

S. No.	Parameter	Method A	Method B
1	λ max/wavelength range(nm)	247	240-254
2	Beer's law limit (µg/ml)	5-40	5-40
3	Molar absorptivity (L/mol/cm)	18060.47	241787.37
4	Sandell's sensitivity (µg/Sq.cm/0.001)	0.044458	0.03321
5	Correlation coefficient (r)	0.9996	0.9997
6	Slope (m)	0.0225	0.3011
7	Intercept	0.0125	0.1762

Table 3: Table shows Results of Analysis of Tablet Formulation (N^* =6)

Method	Label claim mg	Amount of drug estimated (mg/tab)	%Label claim* ± SD
Method A	300	100.56	100.56±0.001
Method B	300	100.86	100.86±0.018

Table 4: Table shows Result of Recovery studies

Excess drug added to the analyst (%)	% Recovery		%RSD		SE	
	Method A	Method B	Method A	Method B	Method A	Method B
80	99.90	100.22	0.1578	0.1747	0.00106	0.01441
100	100.16	100.51	0.2014	0.1875	0.001306	0.01665
120	100.15	100.13	0.1995	0.2699	0.00040	0.03335

RSD: Relative Standard deviation b) SE: Standard error

Table 5: Table shows result of intra-day and inter-day precision

S. No.	Intra-day precision			Inter-day precision		
	SE	SD	%RSD	SE	SD	%RSD
Method A	0.00220	0.002018	0.5735	0.000486	0.000591	0.1774
Method B	0.01792	0.01856	0.3913	0.006533	0.007949	0.1778

RESULT AND DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for analysis of ATV in its pharmaceutical dosage form. Absorbance maxima of ATV at 247 nm (Method A); Area under Curve of ATV at 240-254 nm (Method B) were selected for the analysis. Linearity for detector response was observed in the concentration range of 5-40 µg/ml for the two methods. Percent label claim for ATV in capsule analysis was found in the range of 100.56% to 100.86% (Table 3).

Standard deviation and coefficient of variance for six determinations of capsule formulation, was found to be less than ± 2.0 indicating the precision of the methods. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery.

Percent recovery for ATV was found in the range of 99.90 % to 100.51 % values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of all the methods (Table 4). Percent RSD for Intra-day assay precision was found to be 0.5735 and 0.3913 for Method A and B (Table 5).

Inter-day assay precision was found to be 0.1774 and 0.1778 for Method A and B (Table 5). Based on the results obtained, it is found that the proposed methods are accurate, precise, reproducible & economical and can be employed for routine quality control of ATV in bulk drug and its pharmaceutical dosage form.

CONFLICT OF INTERESTS

Declared None

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