Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR DETERMINATION OF ATAZANAVIR SULPHATE IN BULK AND FORMULATION

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Received: 15 Mar 2012, Revised and Accepted: 23 Apr 2012

ABSTRACT

The present study describes a simple, accurate, precise and cost effective UV-Spectrophotometric method for the estimation of Atazanavir sulphate, an Anti-HIV drug, in bulk and pharmaceutical dosage form. The drug was first dissolved in 20% glacial acetic acid and final volume was made up with distilled water. The λ_{max} or the absorption maxima of the drug was found to be 299nm. A linear response was observed in the range of 10-50µg/ml with the regression coefficient of 0.999. The method was validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Atazanavir sulphate in quality control of formulation without interference of the excipients.

Keywords: Atazanavir sulphate, UV-spectrophotometry, 20% glacial acetic acid.

INTRODUCTION

It is a recently introduced azapeptide inhibitor of HIV-1 Protease. It is formulating as 1:1 sulphate salt. The drug was approved by USFDA on June 20, 2003. Literature survey revealed that Atazanavir was quantitatively assayed in biological fluids either individually[1] or in presence of other retroviral drugs using liquid chromatography[2,3], Estimation of Atazanavir in bulk and pharmaceutical dosage forms and its stress degradation studies using UV-VIS spectrophotometric method[4], Determination of atazanavir in pharmaceutical dosage form by a validated RP-HPLC method[5], Simultaneous estimation of atazanavir sulfate and ritonavir in pharmaceutical dosage form[6], Estimation of atazanavir sulfate in pharmaceutical formulations by validated reverse phase high performance liquid chromatography method[7], Simultaneous spectrophotometric estimation of atazanavir sulfate and ritonavir in tablets[8], Development of derivative spectrophotometric estimation of atazanavir sulfate in bulk drug and pharmaceutical dosage forms[9] and Visible spectrophotometric estimation of atazanavir in pharmaceutical formulations[10]. The aim of this work is to develop and validate a simple, accurate and low cost analytical method by using UV spectrophotometry for the estimation of atazanavir sulphate in bulk and pharmaceutical dosage forms.

Chemically,[11] atazanavir sulphate is (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 decanedioic acid dimethyl ester, sulphate (1:1).

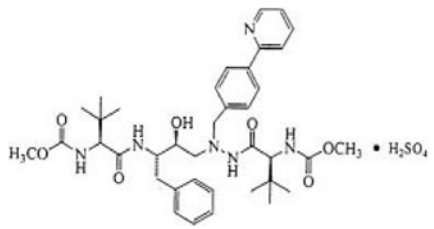

Fig. 1: Structure of atazanavir sulphate

MATERIALS AND METHODS

Pharmaceutical grade atazanavir sulphate was supplied by Hetero Drugs Ltd., Hyderabad, India. The glacial acetic acid was purchased from Fisher scientific and commercially available tablets ATAZOR (equivalent to 300 mg of atazanavir sulphate) one of Hetero drugs Ltd. was purchased from market for analysis.

Shimadzu UV-1800 double beam spectrophotometer with 1 cm path length supported by Shimadzu UV-Probe software, version 2.21 was used for spectral measurements with 1 cm matched quartz cells. Shimadzu balance (BL-220H) was used for all weighing.

Method Development

Solubility Test

Solubility test for the drug atazanavir sulphate was performed by using various solvents, which includes distilled water, ethanol, chloroform, phosphate buffer (pH-4&8), N-N-dimethylformamide, 0.1M NaOH, acetone, 20% glacial acetic acid, 0.1M HCl and ethanol: water (1:9). However, 20% glacial acetic acid and distilled water (1:9) was chosen as solvent for developing the method.

Preparation of Stock Solution

Weigh accurately 25 mg of Atazanavir sulphate and transferred to 25ml volumetric flask. Then add 2.5mL of 20% glacial acetic acid and dissolve the drug by vigorous shaking for 3 to 5 minutes. Then the final volume was made up with distilled water.

Preparation of Working Standard Solution

From stock solution 10 ml was further diluted to 100 ml with distilled water to get the solution having concentration 100 µg/ml.

Determination of λmax

From the above working standard solution, 5 ml was pipette out into a 10 ml volumetric flask and the volume was made up to the mark with distilled water to prepare a concentration of 50 µg/ml. Then the sample was scanned in UV-VIS Spectrophotometer in the range 200-400nm using distilled water as blank and the wavelength corresponding to maximum absorbance (λ_{max}) was found to be 299nm (fig. 1).

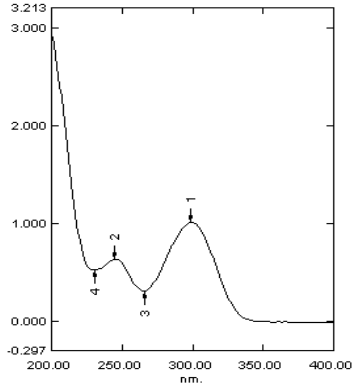

Fig. 1: UV spectrum of atazanavir sulphate (50µg/ml)

Preparation of Calibration Curve

From the working standard solution, pipette out 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml and diluted to 10 ml using distilled water to produce 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50µg/ml solutions respectively. Then measure the absorbance of these solutions at the λ_{max} of 299nm using distilled water as blank. Then, the calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis (in fig.2). The curve shows linearity in the concentration range of 10-50µg/ml. The correlation coefficient (r^2) was found to be 0.9997.

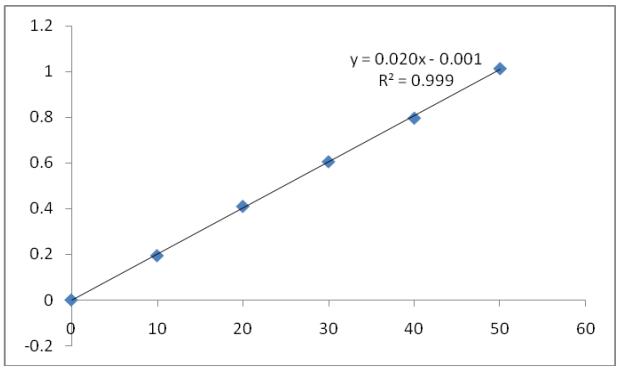

Fig. 2: Calibration curve of Atazanavir sulphate

Assay of Atazanavir sulphate capsules (ATAZOR- 300mg)

A quantity of powder equivalent to 25mg of Atazanavir sulphate was taken in a 25ml volumetric flask and it was first dissolved in 2.5ml of 20% glacial acetic acid by shaking the flask for 3 to 5 minutes and diluted up to the mark with distilled water. Then the solution was filtered using Whatmann filter paper No.40. From this filtrate, appropriate dilutions were made with distilled water to obtain the desired concentration (10, 30 and 50µg/ml). These solutions were analyzed in UV and the result was indicated by % recovery given in Table 8.

Method Validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its pre-determined specifications and quality characteristics.

The method was validated for different parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Linearity

Various aliquots were prepared from the working standard solution (100µg/ml) ranging from 10-50µg/ml. The samples were scanned in UV-VIS Spectrophotometer using distilled water as blank. It was found that the selected drug showed linearity between the 10-50µg/ml (Table 1& 2).

Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations i.e., 50%, 100% and 150% in which the amount of marketed formulation (ATAZOR-300mg) was kept constant (20mg) and the amount of pure drug was varied i.e., 10mg, 20mg and 30mg for 50%, 100% and 150% respectively[12]. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (Table 1 & 4).

Precision

Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study, 6 different solutions of same concentration that is 30µg/ml were prepared and analysed three times in a day i.e. morning, afternoon and evening and the absorbances were noted. The result was indicated by % RSD (table no.1&5). In the inter-day variation study, 6 different solutions of same concentration (30µg/ml) were prepared and analysed three times for three consecutive days and the absorbances were noted. The result was indicated by % RSD (Table No.6).

Robustness

Robustness of the method was determined by carrying out the analysis at five different wavelengths (i.e. 299±0.5). The respective absorbances were noted and the result was indicated by % RSD (Table 1&7).

Ruggedness

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbances were noted. The result was indicated by % RSD (Table No.10).

Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample. The LOD was calculated using the formula involving standard deviation of response and slope of calibration curve (table no.9).

$$LOD = \frac{3.3xSD}{S}$$

Where, SD is the standard deviation of Y-intercept and S is the slop of calibration curve[13].

Limit of Quantification

The LOQ is the concentration that can be quantified reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (Table No.9).

$$LOQ = \frac{10xSD}{S}$$

Where, SD is the standard deviation of Y-intercept and S is the slop of calibration curve.

RESULTS AND DISCUSSION

The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries (99.99% to 101.3%) of the drug were obtained at each added concentration, which indicates that the method was accurate. The LOD and LOQ were found to be in sub-microgram level, which indicates the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values which are less than 2%.

The assay results shows that the amount of drug was in good agreement with the labeled claim of the formulation as indicated by % recovery (101.01%). Summary of validation parameters of proposed spectrophotometric method was shown in Table 1.

Table 1: Summary of validation

Parameter	Result
Linearity indicated by correlation coefficient	0.9997
Precision indicated by %RSD	0.0089
Accuracy indicated by % recovery	98.5-101.95
Limit of detection (LOD), µg mL-1	0.165µg/ml
Limit of quantitation (LOQ), µg mL-1	0.5µg/ml
Linear regression equation	y=0.020x+0.001
Robotness indicated by %RSD	0.00086
Ruggedness indicated by %RSD	0.00895
Assay indicated by % purity	101.01

Table 2: Linearity table to Atazanavir sulphate

S. No.	Concentration (µg/ml)	Absorbance
1.	10	0.194
2.	20	0.410
3.	30	0.606
4.	40	0.797
5.	50	1.014

Table 3: Optical characteristic of Atazanavir sulphate

Optical characteristics	Result
Beer's law limit (µg/ml)	10-50
Molar extinction coefficient	16548.48
Correlation coefficient (r^2)	0.9997
Regression equation	y=0.020x+0.001
Slope (a)	0.020
Intercept (b)	0.001

Table 4: Accuracy studies of Atazanavir sulphate

S. No.	Con. of		% Drug added	Amount found	% Recovery	mean	SD	%RSD
	Tab. (µg/ml)	Pure drug (µg/ml)						
1.	20	10	50	10.04	100.4			
2.	20	10	50	9.85	98.5	100.1	1.47	0.014
3.	20	10	50	10.14	101.4			
4.	20	20	100	19.75	98.75			
5.	20	20	100	20.04	100.24	101.31	1.60	0.015
6.	20	20	100	20.39	101.95			
7.	20	30	150	30.58	101.95			
8.	20	30	150	29.68	98.86	99.99	1.69	0.016
9.	20	30	150	29.75	99.18			

Table 5: Intra-day precision

S. No.	Concentration (µg/ml)	Absorbances			Avg. % RSD
		Morning	A.noon	Evening	
1.	30	0.616	0.610	0.607	
2.	30	0.611	0.605	0.615	
3.	30	0.609	0.609	0.613	
4.	30	0.614	0.617	0.606	
5.	30	0.620	0.616	0.618	
6.	30	0.604	0.604	0.605	
%RSD		0.0091	0.0088	0.0088	0.0089

Table 6: Inter-day precision

S. No.	Concentration (µg/ml)	Absorbances			Avg. % RSD
		Day 1	Day 2	Day 3	
1.	30	0.616	0.613	0.617	
2.	30	0.611	0.618	0.619	
3.	30	0.609	0.606	0.607	
4.	30	0.614	0.611	0.604	
5.	30	0.620	0.607	0.618	
6.	30	0.604	0.610	0.607	
%RSD		0.009	0.0071	0.0109	0.0090

Table 7: Robustness method for Atazanavir sulphate

S. No.	λmax	Absorbance	Statistical analysis
1.	296.6	0.605	
2.	296.7	0.605	
3.	296.8	0.605	Mean =0.60593
4.	296.9	0.606	SD=0.00522
5.	296.9	0.606	%RSD=0.0086
6.	297.0	0.606	
7.	297.1	0.606	
8.	297.2	0.606	
9.	297.3	0.606	
10.	297.4	0.605	
11.	297.5	0.605	
12.	297.6	0.605	

Table 8: Assay of atazanavir sulphate (ATAZOR-300mg)

S. No.	Concentration (µg/ml)	Amount found (µg/ml)	% Recovery	% RSD
1.	10	10.06	100.60	0.019
2.	30	30.25	100.88	0.006
3.	50	50.75	101.51	0.013

Table 9: LOD & LOQ of Atazanavir sulphate

S. No.	LOD	LOQ
1.	0.165µg/ml	0.5µg/ml

Table 10: Ruggedness of method for Atazanavir sulphate

Analyst	Concentration (µg/ml)	Absorbance	Mean	SD	% RSD
	30	0.612			
Analyst 1	30	0.608	0.612	0.005	0.0065
	30	0.617			
	30	0.618			
Analyst 2	30	0.609	0.610	0.007	0.0114
	30	0.604			

CONCLUSION

All the above factors led to a conclusion that the proposed method is accurate, precise, simple, robust and cost effective and can be applied successfully for the estimation of atazanavir sulphate in bulk and pharmaceutical formulation.

ACKNOWLEDGEMENTS

I am very much thankful to Prof. A. Prameela Rani, Principal, University College Of Pharmaceutical Sciences, Acharya Nagarjuna University, Guntur, for providing the necessary chemicals for our work. I am also thankful to Asst. Prof. K. Sujana for giving valuable guidance to me.

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