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SENSITIVE EXTRACTION SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF ATAZANAVIR SULFATE IN BULK AND IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT
Two simple, simple and sensitive extraction spectrophotometric methods for the determination of atazanavir sulfate in bulk and in pharmaceutical formulations has been developed and validated. These methods are based on extraction of this drug into chloroform as ion-pair with azo dyes, Tropaeolineo-oo (TPooo) and alizarine Red S (ARS). The optimum conditions of the reactions for the proposed methods were studied and optimized. Results of the assays were statistically validated and recorded. The proposed methods were applied successfully for the determination of atazanavir in commercial tablets dosage forms and no significant interference was observed from the excipients commonly used as pharmaceutical aids with the assay procedure.
Keywords: Atazanavir, ion-pair complex reactions, Visible Spectrophotometric determination, Validation, Beer's Law.

INTRODUCTION

Atazanavir sulphate[1,2] (Fig.-1),{(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)} is an Protease Inhibitor used in the treatment of human immunodeficiency virus (HIV) Type II infection. Literature survey reported few analytical methods for the determination of atazanavir are based on high performance liquid chromatography (HPLC)[3-12] in biological samples like blood plasma, biological cells, cerebrospinal fluid (CSF) and blood serum. Stress degradation studies were reported analysed by HPLC and ultraviolet spectrophotometry[13,14]. To the best of our knowledge, three spectrophotometric methods[15-18] for the analysis of atazanavir in dosage forms were reported in the literature. As the analytically useful functional groups of atazanavir were not fully exploited and hence, the author had made an attempt to develop two simple and sensitive extractive spectrophotometric methods for the estimation of atazanavir in bulk and in pharmaceutical formulations using some acidic dyes.

EXPERIMENTAL

Instrumentation

All spectral measurements were done using an Elico, UV – Visible spectrophotometer (**SL-159**) with 1.0cm matched quartz cells respectively.

Materials and Reagents

All the chemicals used were of analytical reagent grade and used as received. Double distilled water was used for the preparation of all solutions. All the solutions were prepared fresh daily.
TPooo solution (Fluka;0.2%) was prepared by dissolving 200mg of tropaeoline ooo in 100ml of distilled water. ARS solution (Fluka;0.2%) was prepared by dissolving 200mg of ARS dissolved in 100ml distilled

water. HCl(E.Merck, 0.1M): Prepared by diluting 8.6ml of concentrated hydrochloric acid to 1000ml with distilled water and standardized. Chloroform (Qualigens) of AR grade was used for the present assay.

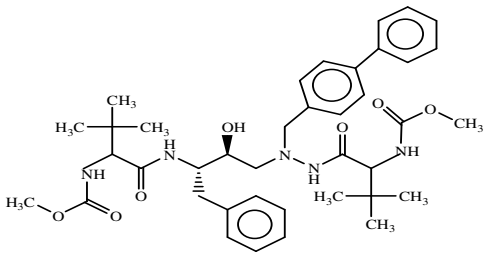

Fig.-1: Structure of atazanavir

Preparation of Stock Solution

Standard stock solution of atazanavir sulphate was prepared by dissolving 10mg of atazanavir sulphate in 10ml of methanol to produce aconcentration of 1000µg/ml. 5ml of this stock solution was taken and then diluted up to 100ml by using methanol to produce a concentration of 50µg/ml which is the standard stock solution.

Table-1: Results of optical characteristics, precision and accuracy of the proposed methods for Atazanavir assay

Parameter	TPooo	ARS
λ_{max} (nm)	480	445
Beer's law limits (µg/ml)	2.5 –12.5	2.5 – 12.5
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	5.126×10^3	4.982×10^3
Sandell's sensitivity (µg.cm^{-2}/0.001 absorbance unit)	0.09524	0.3694
Optimum photometric range (µg/ml)	3.0 – 9.0	2.5 – 10.0
Regression equation (Y=a+bc) ;slope (b)	0.04376	0.0282
Standard deviation on slope (S_b)	2.487×10^{-4}	4.704×10^{-4}
Intercept (a)	5.20×10^{-3}	1.50×10^{-3}
Standard deviation on intercept (S_a)	1.434×10^{-4}	2.714×10^{-4}
Standard error on estimation (S_e)	1.966×10^{-3}	3.719×10^{-3}
Correlation coefficient (r)	0.9995	0.9995
Relative standard deviation (%)*	0.814	1.074
% Range of error (confidence limits)		
0.05 level	0.680	0.836
0.01 level	1.006	1.237

* Average of six determinations considered

Assay of of tablets formulations [ATAZOR -300mg]

A quantity of powder equivalent to 50mg of atazanavir was taken in a 50ml volumetric flask and it was dissolved and diluted upto the mark with methanol. The resultant solution was ultrasonicated for 5 minutes. The solution was then filtered using whatmann filter paper No.40.From the filtrate, appropriate dilutions were made in methanol to obtain the desired concentration (50µg/ml) and was subjected to analysis by the procedure described above.

Proposed Procedures

TPooo

Into a series of 60ml separating funnels, aliquots of standard atazanavir sulfate solution of 0.5-2.5ml containing 50µg/ml, 6.0ml, 0.1M HCl and 2.0ml of 2% acidic dye solution (TPooo) were added successively and the total volume of aqueous phase in each separating funnel was made to 15.0ml with distilled water. To each separating funnel 10ml of chloroform was added and the contents were shaken for

2min. The two phases were allowed to separate and the absorbance of the organic phase (chloroform) was measured at 480nm against a reagent blank similarly prepared. A standard calibration curve was prepared to calculate the amount of the analyte drug in unknown samples 480nm for TPooo against reagent chloroform blank.

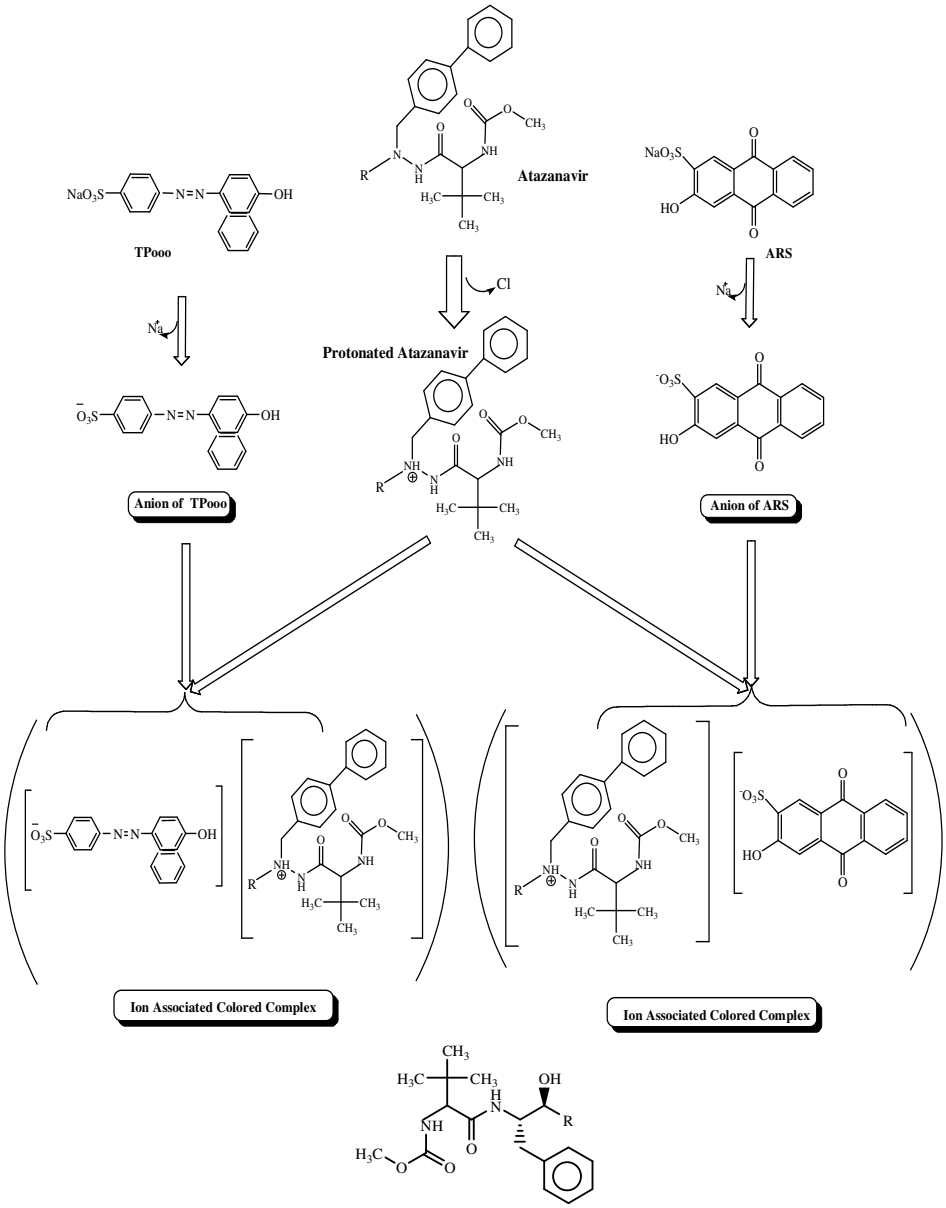

Fig.-2: Reaction scheme of atazanavir with TPooo & ARS

Table- 2:Estimation of Atazanavir in Pharmaceutical formulations(ATAZOR)

Sample	Labelled amount (mg)	Amount obtained (mg) Proposed methods*		Reference method[18]	%Recovery of Proposed methods	
		TPooo	ARS			
ATAZOR (Capsules)	300	299.93±0.49 F=1.353 t=0.915	300.01±0.45 F=1.604 t=1.562	299.96±0.57	99.98	100.01

*Average of six determinations

ARS

Same procedure described above in TPooo was used for the assay of atazanavir sulfate solution of concentration (50µg/ml) using 2.0% ARS dye solution. Absorbance of this final extracted chloroform layer was measured at wavelength maxima 445nm against blank. A calibration curve was plotted between concentration of drug and measured absorbance.

RESULTS AND DISCUSSION

The positively charged secondary nitrogen of atazanavir molecule in acid medium is expected to attract the negatively charged part of the acidic dye TPooo and form an ion pair held together through electrostatic attraction forming orange red colored exhibiting absorption maximum λ_{max} at 480nm(Fig-2). The same ion-association reaction was obtained for atazanavir with ARS dye forming a yellow colored ion association complex which exhibited absorption λ_{max} at 445nm(Fig-2). The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from six replicate samples containing 3/4th of the amount of the upper beer's law limits) were calculated for the proposed methods and the results are summarized in Table-1. Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e), % range of error (0.05 and 0.01 confidence limits) and detection limit were calculated for both methods (Table-1). The proposed methods were applied for the quantification of atazanavir in commercial tablets. Statistical analysis of the results did not detect any significant difference in performance between the proposed method and reference method[18] with respect to accuracy and precision as revealed by the Students t-value and variance ratio F-value. The results of assay are given in Table-2.

CONCLUSION

The two methods developed in the present paper were simple, rapid and were validated in terms of accuracy and precision. The lower values of % RSD (0.8-1.0) (Table-1) in signified the accuracy of the methods for the quantification of atazanavir in formulations confirming the applicability of these methods for the quality control of the formulations of atazanavir in day to day evaluation. Moreover, the instrument and the chemicals used in the developed methods are very easily available in any laboratory, making the proposed visible spectrophotometric methods applicable to small laboratories for routine analysis of atazanavir in bulk and pharmaceutical formulations.

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