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Research Article

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Determination of atazanavir sulfate in pharmaceutical dosage forms by extraction spectrophotometry using acid dyes

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ABSTRACT

Three simple, accurate and sensitive extraction spectrophotometric methods (A, B and C) have been developed for the determination of atazanavir sulfate in its pharmaceutical dosage forms. The methods are based on extraction of colored ion-association complexes formed between Atazanavir sulfate and BTB, BPB and BCG in to chloroform in potassium hydrogen phthalate buffer (pH-2.4).The complexes show λ_{max} at 414nm, 410nm and 417nm respectively. Beer's law is obeyed in the concentration range 2-14μgmL^{-1} of atazanavir sulfate for methods A and B and in the range 2-12 μgmL^{-1} for method C. Results of analysis for all the methods are validated statistically and by recovery studies. The proposed methods are economical, sensitive and can be used for routine analysis of atazanavir sulfate in bulk and pharmaceutical formulations.

Key words: UV-Visible Spectrophotometry; Atazanavir sulfate; BromoThymol Blue (BTB); Bromo Phenol Blue (BPB); BromoCresol Green (BCG); Potassium hydrogen phthalate buffer, Chloroform.

INTRODUCTION

Atazanavir sulfate has the chemical name (3S,8S,9S,12S)-3,12-Bis(1,1-dimethylethyl)-8hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl)phenyl]methyl]-2,5,6,10,13penta aza tetra decanedioic acid dimethyl ester, sulfate (1:1). It is a white to pale yellow crystalline powder with a molecular formula of $C_{38}H_{52}N_6O_7 \cdot H_2SO_4$ and a molecular weight of 802.9. The free base molecular weight is 704.9.The basic structure of the drug is shown in Fig.1.

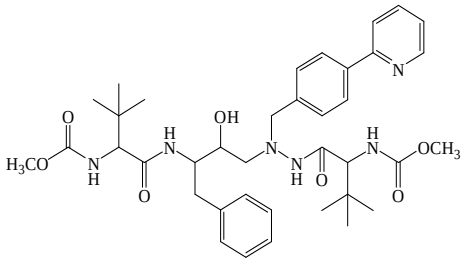


Fig.1. Structure formula of atazanavir

Atazanavir sulfate is an antiretroviral protease inhibitor used in the treatment of human immunodeficiency virus (HIV) Type-II and AIDS. [1-2]. Literature survey indicated that few HPLC [3-12] methods have been reported for

its estimation from blood plasma, cerebrospinal fluid and blood serum. Few UV-Visible spectrophotometric [13-17] methods also have been reported for the estimation of atazanavir sulfate in bulk and pharmaceutical dosage forms. The present study describes simple, rapid, sensitive and economical spectrophotometric methods A, B and C for the determination of atazanavir sulfate in pharmaceutical dosage forms. Atazanavir forms yellow coloured ion-association complexes with acid dyes, BromoThymol Blue (BTB), Bromo Phenol Blue (BPB) and BromoCresol Green (BCG) in phthalate buffer (pH-2.4). The resultant yellow coloured solutions show λ_{max} at 414nm, 410nm and 417nm respectively. The coloured solutions formed one highly sensitive and fairly stable

EXPERIMENTAL SECTION

Instrument: Shimadzu UV-1700 Pharmaspec with 1cm matched quartz cell was used for spectral measurements. Digi Sun Digital pH-meter was used for pH measurements.

Reagents: All the chemicals used were of analytical grade reagents.
1. The acid dye solutions were prepared by dissolving 200mg of BTB or BPB or BCG in a mixture of 8.0mL NaOH (0.02N) and 25.0mL ethanol (95%). The solution was then made up to 100mL with distilled water.
2. Potassium hydrogen phthalate buffer (pH-2.4): 4.1gm of potassium hydrogen phthalate was dissolved in 100mL of distilled water. To 25mL of this solution, 21.0mL of 0.2M HCl were added and diluted to 100mL with distilled water to obtain a buffer solution of pH-2.4.

Experimental
A standard stock solution of the drug was prepared by dissolving 10mg of atazanavir sulfate in 10mL of distilled water to get a concentration of 1000 μgmL^{-1}. This was further diluted with distilled water to get the working standard solutions of 100 μgmL^{-1}.

Assay procedure:
In a set of different 25mL separatory funnels, variable volumes (0.2 to 1.5mL) of atazanavir sulfate (100 μgmL^{-1}), 2.0mL of suitable acid dye (0.2%w/v) and 4.0mL of potassium hydrogen phthalate buffer (pH-2.4) were mixed. To this 10mL of distilled chloroform were added and the contents were equilibrated for 5 minutes and allowed for the separation of two layers. The yellow colored organic layer was then removed and dried by adding small amount of anhydrous sodium sulphate. The chloroform layers were finally transferred quantitatively in to 10mL volumetric flasks and made up to the mark with chloroform. The absorbance of the made up solutions was then measured at their respective λ_{max} values and plotted against the amount of atazanavir sulfate.

The methods were extended for the determination of atazanavir sulfate in capsule formulations (REYATAZ®300mg). Ten Capsules of Reyataz® (300mg- Bristol Myers Squibb) were procured and their drug content (powder) was separated. The capsule powder equivalent to 100mg of atazanavir was accurately weighed and mixed with 50mL of distilled water. The resultant mixture was sonicated for 15 mins, filtered and washed with distilled water. The filtrate and the washings were combined and the final volume was made up to 100mL with distilled water. 5mL of the above solution was diluted to 50mL with distilled water to get the working sample solution (100μgmL^{-1}). The solution was analyzed as given under the assay procedure for bulk samples. The analysis procedure was repeated three times with capsules formulations.

RESULTS AND DISCUSSION

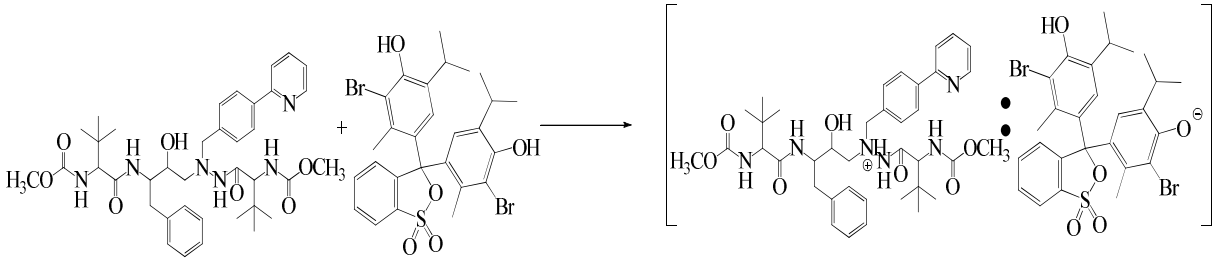
In the present study all the three acid dyes bromo thymol blue (BTB), bromo phenol blue (BPB) and bromo cresol green (BCG) form yellow colored soluble species with atazanavir in potassium hydrogen phthalate buffer solution of pH 2.4, which are quantitatively extractable in to chloroform. The extracted species showed absorption maxima in the wave length range410-420nm. The effect of pH on the color formation was studied in the range 2.0 to 4.0. It was noticed that all the colored species exhibited maximum absorbance at pH 2.4 which was chosen for analytical studies. The stabilities of the yellow colored species were evaluated by measuring the absorbance of the experimental solutions for 8 hours with 15 minutes time interval. It was observed that the color was stable up to 5 hours and then gradually decreased.

The acid dyes, BTB, BPB and BCG are acid-base indicators and at low pH they undergo deprotonation forming $H^+$and In^- species. The drug, atazanavir possesses di substituted secondary nitrogen atom which may be undergoing

protonation in acid buffer solution converting in to positively charged species as shown in the structure. Thus, the cationic drug species and anionic acid dye combine forming ion-association complexes, the potential analytical species, whose absorbance is measured at 414nm, 410nm and417nm for BTB, BPB and BCG respectively (Fig.2).
The measured absorbance values were found to be linear giving straight lines in the concentration range 2.0-14.0 μgmL^{-1}, 2.0-14.0 μgmL^{-1} and 2.0-12.0 μgmL^{-1} of the drug with BTB, BPB and BCG respectively. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and sandell's sensitivity are presented in Table-1. The regression analysis using the method of least squares was made and the slope (m), intercept(c) and the correlation coefficient of the regression plots are summarized in Table-1. The reproducibility and precision of the methods are very good as shown by the values of RSD. The mean percentage recovery value of 99.99% for Method A, 99.00% for Method B and 100.00% for Method C, indicate non-interference from the excipients present in the powder for injection. All the validated parameters are summarized in Table-2

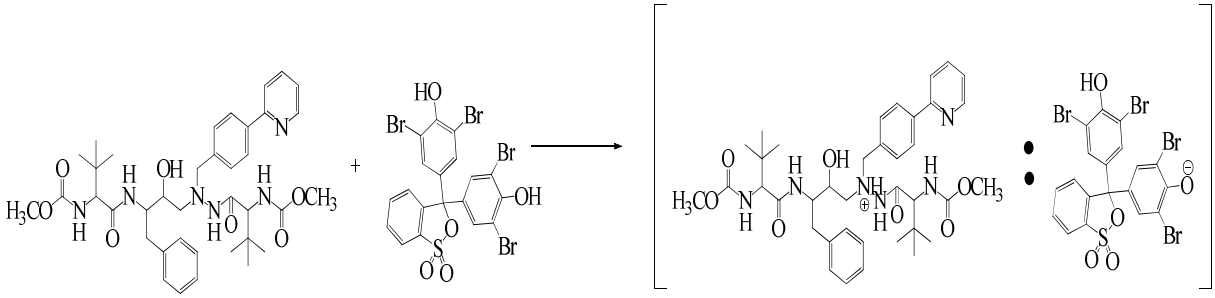
CHEMISTRY OF COLORED SPECIES

Method A



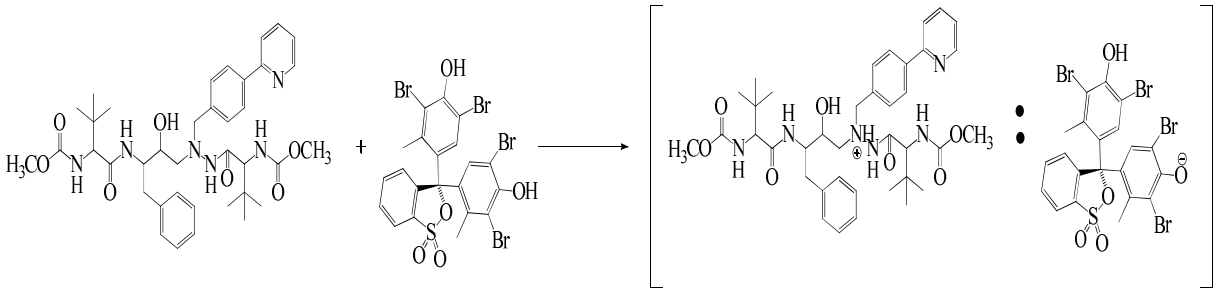
Atazanavir Bromo Thymol Blue Ion-association complex

Method B



Atazanavir Bromo Phenol Blue Ion-association complex

Method C



Atazanavir Bromo Cresol Green Ion-association complex

Fig.2. Formation of Ion-Association complexes between Atazanavir and Acid dyes

Table-1: Optical characteristic and precision data

Parameters	Method A	Method B	Method C
λ_{max} (nm)	414	410	417
Beer's law limits(μgmL^{-1})	2.0-14.0	2.0-14.0	2.0-12.0
Molar Absorptivity(Lt $mole^{-1}cm^{-1}$)	$4.849x10^4$	$4.581x10^4$	$5.180x10^4$
Sandell's Sensitivity ($\mu g\ cm^{-2}$ /0.001 absorbance unit)	0.0145	0.0153	0.0136
Regression Equation* (Y= mx +c) Slope(m) Intercept(c)	 0.067 0.006	 0.065 -0.004	 0.072 0.001
Correlation Coefficient (r)	0.999	0.999	0.999
Precision(% RSD)	0.340	0.480	0.290

Table-2: Assay of atazanavir sulfate in tablet formulations

S.No	Labeled Amount(mg)	*Amount obtained by proposed method (mg)			** % Recovery by the proposed method		
		Method A	Method B	Method C	Method A	Method B	Method C
1	300	299.4	299.2	299.5	100.15	98.91	99.93
2	300	299.8	299.9	299.9	99.89	99.85	99.89
3	300	299.7	299.4	300.1	99.95	98.25	100.2

** Average three determinations.* *** After spiking the sample.*

CONCLUSION

The proposed methods are simple, sensitive, accurate and economical for routine analysis of atazanavir sulfate in bulk and its parenteral formulations. Based on molar absorptivity data and Beer's law range, it may be concluded that among the proposed methods, method C is more sensitive than method A which in turn is more sensitive than the method B.

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