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NEW COLORIMETRIC METHODS FOR THE ESTIMATION OF ABACAVIR SULPHATE IN BULK AND DOSAGE FORMS

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ABSTRACT

Four simple, sensitive, rapid and accurate visible spectrophotometric methods (designated as A, B, C and D) have been developed for the estimation of abacavir sulphate in pharmaceutical formulations. Methods A and B are based on the ion pair complex formation of abacavir sulphate with dyes erichrome black -T and orange–G in acetate buffer followed by their extraction with chloroform. The absorbance of organic layer was measured for the above complexes at 510 nm and 484 nm, respectively. Method C is based on oxidative coupling between abacavir sulphate and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in presence of cerric ammonium sulphate (CAS) to form a colored product with absorption maxima at 630 nm. Method D is based on the oxidation/reduction reaction between the abacavir sulphate and Folin-ciocalteau reagent (F.C. reagent) to form blue color chromogen having absorption maxima at 752 nm. All the variables have been optimized. The concentrations of measurements are reproducible within a relative standard deviation of less than 1% .The linearity was found to be 5 to 60, 2.5 to 15, 10 to 50 and 10 to 70 µg/mL for method A, B, C and D, respectively. The proposed methods were validated statistically. Recovery studies were carried out by standard addition method.

Key words: Colorimetric methods, Abacavir sulphate, Folins–Ciocalteu reagent, 3-Methyl-2-benzothiazolinonehydrazone hydrochloride.

INTRODUCTION

Abacavir sulphate (ABS) is a nucleoside reverse transcriptase inhibitor. This is used in combination with other drugs for the treatment of HIV. Abacavir sulphate is (1S, 4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol sulfate (Fig. 1).

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The drug is metabolized via stepwise phosphorylation to 5'-mono-, di- and triphosphate. The drug does not show any clinically significant drug-drug interaction.

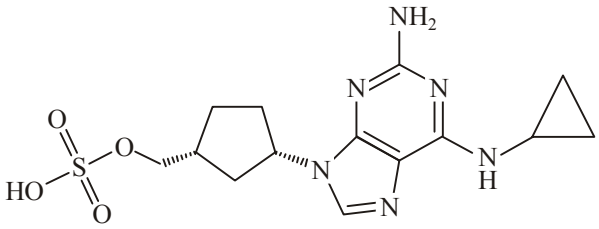

Fig. 1: Structure of abacavir sulphate

Literature survey reveals spectrophotometric and HPLC methods[1-8] for the estimation of abacavir sulphate in pharmaceutical dosage forms. Although spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories, very few colorimetric methods have been reported so far for the determination of ABS. However, no colorimetric method using erichrome black-T, orange-G, F.C. and MBTH reagents are reported so far. Therefore, there is a need for low cost, fast and selective methods especially for routine quality control analysis of pharmaceutical formulations containing ABS.

The author presents four visible spectrophotometric methods for the determination of ABS by making use of the following procedures.

Methods A and B: These have been developed for the estimation ABS by using acidic dyes erichrome black -T and orange-G. These methods are based on the formation of ion-pair complexes of the ABS with erichrome black -T and orange-G in acetate buffer of pH 3.5 followed by their extraction in chloroform. The absorbance of the chloroform layer for each method was measured at its λ max against reagent blank.

Method C: In the present investigation, the authors adopted the procedure for determination of ABS by using oxidant, CAS and MBTH for the first time. Therefore, the method C is based on oxidative coupling between abacavir sulphate and 3-methyl-2-benzothiazolinonehydrazone hydrochloride in presence of cerric ammonium sulphate to form a colored product

Method D: Reduction of heteropolyacid complex was utilized as the basis for the determination of several simple organic compounds particularly phenols, amines and enols, among the various heteropolyacids, phosphomolybdo tungstic acid. The well known F.C. reagent was preferred by a number of workers for determination of drugs containing phenolic or amino groups and also certain other drugs, which contain neither of these groups.

For the first time, the author adopted F.C. reagent, to determine ABS in bulk and pharmaceutical dosage formulations.

EXPERIMENTAL

Instrument

Absorbance measurements were made on Shimadzu UV-1800 double beam UV-Visible spectrophotometer with 10 mm matched quartz cells.

Chemicals and reagents

All the chemicals used were of analytical grade and solutions were prepared with distilled water. Pharmaceutical grade ABS was kindly gifted by M/s Hetero drugs Ltd., Balanagar, Hyderabad, India and certified to contain 99.98% of ABS. ABS tablets were procured from local market.

Orange-G : 0.1% w/v (NR Chem., Bombay): 100 mg of orange-G dissolved in 100 mL of distilled water and washed with chloroform to remove chloroform soluble impurities.

Erichrome black-T : 0.1 % w/v (S.D. Fine Chem. Ltd., Mumbai,): 100 mg of eriochrome black T was dissolved in 100 mL of distilled water and washed with chloroform to remove chloroform soluble impurities

Acetate buffer (pH 3.5): 4 g of anhydrous sodium acetate was added in 840 mL of water and sufficient amount of glacial acetic acid to adjust pH to 3.5 (about 155 mL) and diluted with water to 1000 mL.

Chloroform : (S.D.Fine chemicals Ltd., Mumbai) : It was used as received.

MBTH (0.2 % w/v) : 200 mg of MBTH (S.D. Fine chemicals Ltd., Mumbai) was dissolved in 100 mL of distilled water.

Cerric ammonium sulphate (CAS) : 1 g of CAS (S.D. Fine Chemicals Ltd., Mumbai) was dissolved in 100 mL of 0.1N sulphuric acid.

F.C Reagent : 10 mL of F.C. reagent (Merck Specialties Pvt. Ltd., Mumbai) was diluted to 50 mL with distilled water.

1N Sodium hydroxide : 4 g of sodium hydroxide (S.D Fine Chemicals Ltd., Mumbai) was dissolved in 100 mL of distilled water.

Preparation of standard drug solution

100 mg of ABS was accurately weighed and dissolved in 100 mL distilled water (1 mg/mL) and was used for method A, B, C and D.

Procedure for calibration curve

Methods A and B: Into a series of 60 mL separating funnels, appropriate aliquots of the standard drug solution (Table 1) were pipetted out and made up to 1.0 mL with distilled water. To each separating funnel, acetate buffer solution with pH 3.5 (0.5 mL for method A; 1.0 mL for method B) and dye solution (1.5 mL of 0.1% w/v erichrome black-T solution for method A and 2.0 mL of 0.1% w/v orange-G for method B) were added, respectively. The contents in each separating funnel was mixed thoroughly and repeatedly extracted with chloroform. The combined chloroform extract obtained from each separating funnel was dried over anhydrous sodium sulphate and the each of the color extract is made up to 10 mL with chloroform. The absorbance was measured immediately within 30 min against reagent blank prepared in a similar manner without drug at the absorption maxima 510 nm for method A and 484 nm for method B (Table 1).

Table 1: Optimum conditions and results of the proposed method for the determination of ABS for method A and method B

Reagents	Method A	Method B
Drug solution taken (μg/mL)	5-60	2.5-15
Volume of buffer (mL)	2.0	1.0
pH of the buffer solution	3.5	3.5
Volume of the reagent	3.0	2.0
λ max (nm)	510	484

Method C: Aliquots of standard ABS solution (0.1-0.5 mL) of 1 mg/mL were transferred into a series of 10 mL volumetric flasks. 3.0 mL of MBTH and 2.0 mL cerric ammonium sulphate were added to all the flasks and kept aside for 20 min. at room temperature. The solution in each flask was made up to the mark with distilled water and the absorbance was measured at 630 nm against the reagent blank (Table 2).

Method D: Aliquots of ABS solution (0.1-0.7 mL) of 1 mg/mL were transferred to a series of 10 mL volumetric flasks. Then 1.0 mL of 1N sodium hydroxide solution and 1.0 mL of F. C. reagent were added and kept aside for 5 min at room temperature. The solutions

were made up to the volume with distilled water .The absorbance was measured at 752 nm against reagent blank (Table 2). The blue colored solution was stable for 2 hours .The amount of the drug was computed from Beer's Lambert plot.

Table 2: Optimum conditions and results of the proposed method for the determination of ABS for method C and method D

Reagent	Method C	Method D
Drug solution taken (μg/mL)	10 - 50	10 - 70
Volume of sodium hydroxide (mL)	---	1.0
Volume of CAS (mL)	2.0	----
Volume of reagent employed (mL)	3.0	1.0
λ max (nm)	630	752

Procedure for the assay of ABS in tablets

Twenty tablets were weighed and ground to fine powder. An accurately weighed amount of the tablet powder equivalent to 100 mg of ABS was taken and sample solution was prepared as described for the standard and filtered prior to analysis. An appropriate aliquot was withdrawn and the estimation of drug content was carried out as described under the procedure for calibration curve.

RESULTS AND DISCUSSION

Optimization of parameters

Investigations were carried out to establish the most favorable conditions for the formation of the colored product. In methods A and B, the influence of acetate buffers with pH range 2.5-6.5 on the reaction has been studied. It was observed that absorbance decreased above pH 3.5 and no color was found with phosphate buffer of pH 6.8. Sensitivity with acetate buffer (pH 3.5) is high and hence, acetate buffer of pH 3.5 was used in further studies.

The influence of different amounts of buffer with pH 3.5 on reaction has been studied. It was observed that the absorbance started decreasing above 2 .0 mL for method A and 1 mL for method B. Hence, 2.0 mL buffer for method A and 1.0 mL buffer for method B were used in further studies. The effect of changing the concentrations of erichrome black-T and orange-G over the range of 1.0 to 5.0 mL was examined and it was observed that the

absorbance stated decreasing above 3.0 mL for method A and 2.0 mL of reagent for method B. Hence, 3.0 mL of 1% w/v erichrome black -T and 2.0 mL of 1% w/v of orange-G solution was used in further studies.

The color of the complex was stable up to 30 min. after extraction. However, a decrease in the absorbance was noted there after. Hence, it is recommended that the absorbance be measured within 30 minutes.

In Method C, several oxidants such as chloramines -T, N-bromosuccinamide, cerric ammonium sulphate and iodine was tested. The combinations that give maximum sensitivity for the determination of ABS are cerric ammonium sulphate (Ce IV) with MBTH. In this method, 3.0 mL of MBTH and 2 .0 mL of Ce (IV) were found to be optimum for maximizing color intensity. Reversing the order of addition Ce (IV) and MBTH to the drug, resulted in considerable loss of sensitivity.

In Method D, the effects of various parameters such as nature of alkali (sodium carbonate, sodium hydroxide) and amount of alkali, F. C. reagent, reaction time, and the stability of colored species formed were studied. 0.5 to 4.0 mLof 1N sodium hydroxide and 0.5 to 2.0 mL of F. C. reagent was tried and found that 1.0 mL of sodium hydroxide and 1.0 mL of F. C. reagent were optimal. The reaction time was found to be 5 min. and the color was stable for 3 hours.

The proposed methods are simple, rapid, and precise and do not suffer any interference due to common excipients of tablets like talc, starch, magnesium stearate and lactose.

ABS was found to yield a clear solution of pink colored complex with erichrome black –T and orange colored complex with orange-G, which are conveniently extracted with chloroform and have the absorption maxima of 510 nm and 484 nm, respectively. The colored product is due to the ion pair complex formation of the drug with the dye in acidic medium (pH 3.5 buffer).

In method C, ABS was found to yield green colored complex with MBTH. MBTH on oxidation with Ce (IV) loses two electrons and one proton to give an electrophilic intermediate, which has been postulated to be the active coupling species. One mole of this reacts with drug by electrophillic attack on the most nucleophillic site of drug. The complex formed has absorption maxima at 630 nm.

In method D, ABS reacts with F. C. reagent (orthophosphoric acid combined with acids like periodic, molybdic, vanadic, tungstic or molybdovanadic to form the

corresponding hetero polyacid complexes). Treatment of such complexes with reducing agents results in the formation of the corresponding products, which are in blue color.

Conformity to Beer's law

The optical characteristics such as Beer's law limit, molar absorptivity coefficient, Sandell's sensitivity, and percent relative standard deviation were calculated for all the above methods and the results are summarized in Table 3. Regression analysis using the method of least square was made for the slope (b), intercept (a) and correlation coefficient (r) and percentage range of error (0.05 and 0.01 confidence limits) was calculated for all the methods (Table 3).

Table 3: Regression characteristics of the proposed methods

Parameters	Method			
	A	**B**	**C**	**D**
λ max (nm)	510	484	630	752
Beer's law limits (μg/mL)	5-60	2.5-15	10-50	10-70
Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U)	0.0423	0.0193	0.04665	0.1481
Molar absorptivity ($Lmol^{-1}cm^{-1}$)	0.0016×10^6	0.3495×10^6	0.0014×10^6	0.0453×10^6
Correlation coefficient (r^2)	0.9994	0.9978	0.9987	0.9991
Regression equation (y = b + ac)*				
Slope (a)	0.0227	0.0521	0.0054	0.0271
Intercept (b)	0.0160	-0.0050	-0.0003	-0.1683
Range of errors				
Confidence limit with 0.05 level	0.4155	0.5211	0.6547	0.7435
Confidence limit with 0.01 level	0.6148	0.7710	0.9687	0.5002
% Relative Standard Deviation**	0.4970	0.6232	0.7830	0.8893

**Average of six determinations

*Y = b + ac, where Y is the absorbance, c is concentration in μg/mL

Commercial formulations (tablets) containing ABS were also successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for

formulations were compared statistically by the t test and F test and found not to differ significantly. As an additional demonstration of accuracy, a fixed amount of the drug was added to the pre-analyzed formulations and than recovery experiments were performed. These results are summarized in Tables 4(a) and 4(b).

Table 4(a): Assay and recovery of ABS in pharmaceutical formulations

Pharmaceutical formulations	Labeled amount found (mg)	Amount found in (mg)[a] using proposed methods ± S.D			
		A	B	C	D
Tablet-1	300	300.05 ± 0.67 t=0.0143 F=0.681	300.25 ± 0.66 t=0.7304 F=0.9010	299.98 ± 0.62 t=0.7005 F=0.5066	299.2 ± 0.67 t=0.6132 F=0.6837
Tablet-2	300	299.97 ± 0.74 t=0.8142 F=0.1711	299.83 ± 053 t=0.4836 F=0.3360	299.96 ± 0.83 t=0.8791 F=0.7105	299.90 ± 0.64 t=0.7803 F=0.2371
Tablet-3	300	299.95 ± 0.71 t=0.6349 F=0.7054	299.99 ± 0.84 t=0.4018 F=0.7548	299.97 ± 0.54 t=0.9355 F=0.7941	299.85 ± 0.63 t=0.8113 F=0.9615

Table 4(b): Assay and recovery of ABS in pharmaceutical formulations

Found by reference method[C] ± S.D	% Recovery by proposed methods[b] ± S.D				% Recovery by proposed methods[b] ± S.D			
	A	B	C	D	A	B	C	D
299.89 ±0.79	100.01 ± 0.43	100.07 ± 0.32	99.98 ± 0.25	99.96 ± 0.34	99.93± 0.66	99.7 ± 0.59	100.23 ± 0.54	99.53± 0.66
299.83 ± 0.72	99.94 ± 0.27	99.93 ± 0.45	99.97 ± 0.37	299.95 ± 0.57	99.8 ± 0.57	99.83± 0.66	100.16 ± 0.63	100.03 ± 0.58
300.19 ± 0.23	99.88 ± 0.23	99.98 ± 0.51	99.98 ± 0.31	99.94 ± 0.48	99.6 ± 0.6	99.67± 0.61	99.97± 0.58	99.8 ± 0.59

[a] Average ± Standard deviation of eight determinations, the t and F-values refer to comparison of proposed method with reference method. Theoretical values at 95% confidence limits t = 2.365 and f = 4.88.

[b]Recovery of 10 mg added to the pre-analyzed pharmaceutical formulations (average of three determinations). [c]U.V. method using distilled water as solvent at λ max 287.5 nm

CONCLUSION

All the proposed methods are most economical, simple, more accurate than the existing methods and can be used for routine determination of ABS in bulk as well as in its pharmaceutical preparations.

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