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DETERMINATION OF ABACAVIR SULPHATE BY ION-ASSOCIATION COMPLEX AND OXIDATIVE COUPLING REACTIONS IN BULK SAMPLE AND DOSAGE FORMS

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Two simple and sensitive visible spectrophotometric methods ($M_1$and M_2) have been developed for the assay of abacavir sulphate in bulk form and in pharmaceutical formulations. Method M_1 is based on the formation of ion-association complex involving the secondary amino group in between cyclopropyl and heterocyclic moieties of abacavir sulphate and the acidic dye bromophenol blue (λ_{max} = 440) to form colored species. Method M_2 is based on the formation of color species between the drug and brucine/periodate (λ_{max} = 520) by means of oxidative coupling reaction. All the variables have been optimized. Methods M_1 and M_2 obeyed Beer' law in the range of 2-10 µg mL^{-1} and 6-18 µg mL^{-1} respectively. The proposed methods are simple, economical and sensitive for the quantitative determination of abacavir sulfate.

INTRODUCTION

Abacavir is a carbocyclic synthetic nucleoside analogue with inhibitory activity against HIV. The chemical name of Abacavir sulphate (AVS) is : [(1S, 4R)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol sulphate (salt) (2 : 1)]. The drug is cited in Martindale [1] and PDR [2]. In literature, a number of analytical methods have been described for estimation of AVS including Alkalimetric titration methods [3], Electrochemical determination [4], HPLC [5-9], LC [10, 11] and LC-MS [12-14], UHPLC [15], UV [16, 17] and X-Ray powder diffraction method [18] . But, relatively little attention has been paid in developing visible spectrophotometric methods [19-30]. To develop sensitive and flexible visible spectrophotometric methods there is a need to exploit functional groups present in AVS. So authors made an attempt in this direction and succeeded in developing visible spectrophotometric methods using BPB (M_1), and Brucine/IO_4 (M_2). The results are statistically validated.

EXPERIMENTAL

Instrumentation: A UNICAM UV 500 spectrophotometer (Thermo Electron Corporation) and Elico SL-177 model visible spectrophotometer with 1 cm matched glass

cells were used for all spectral and absorbance measurements. All pH measurements were made on an Elico LI 120 digital pH meter.

Table 1. Optical characteristics, precision, accuracy of the proposed methods M_1 & M_2

Optical characteristics	BPB	Brucine-IO_4^-
	M_1	M_2
λ_{max} (nm)	440	520
Beer's Law limits (µg mL^{-1})	2 – 10	6 – 18
Limit of detection (µg mL^{-1})	5.68×10^{-1}	8.63×10^{-2}
Limit of quantification (µg mL^{-1})	1.72×10^{-1}	2.88×10^{-3}
Molar absorptivity (l mol^{-1} cm^{-1})	5.58×10^{4}	1.76×10^{4}
Sandell's sensitivity (µg/cm^2/0.001Absorbance unit)	1.20×10^{-2}	3.80×10^{-2}
Regression equation (y = a + bC)*		
Slope (b)	8.32×10^{-2}	2.64×10^{-2}
Standard deviation on slope (SD_b)	2.16×10^{-4}	5.44×10^{-5}
Intercept (a)	4.0×10^{-4}	-4.0×10^{-4}
Standard deviation on Intercept (SD_a)	1.43×10^{-3}	6.93×10^{-4}
Correlation coefficient (r)	0.9999	0.9999
Relative Standard Deviation**	0.37	0.82
% range of error (confidence limit)		
0.05 level	0.39	0.86
0.01 level	0.61	1.35

y = a + bC where C is the concentration of analyte in µg/ml and *y* is the absorbance unit

** Calculated from six determinations (n = 6)

Reagents:

All the reagents were of analytical grade and aqueous solutions of Bromophenol blue (BPB) (Loba; 0.08%, 1.19×10^{-3} M), buffer solution (pH = 2.5), for method M_1; Brucine (Loba; 0.2%, 5.06×10^{-3} M in minimum amount of 0.16 M H_2SO_4, $NaIO_4$ (BDH; 0.2%, 9.35×10^{-3} M), H_2SO_4 (Qualigens; 2.3 N) were prepared in double distilled water.

Standard drug solution:

1.0 mg mL^{-1} solution was freshly prepared by dissolving 100 mg of pure AVS in 100 mL of distilled water and this stock solution was diluted step-wise with distilled water to obtain the working standard solution of 40 µg mL^{-1} (method M_1) and 150 µg mL^{-1} (method M_2).

Recommended procedures

Method M_1 :

Into a series of 125.0 mL separating funnels containing aliquots of standard drug solution [AVS : 0.5 – 2.5 mL , 40 µg mL^{-1}], 5.0 mL of pH 2.5 buffer solution and 5.0 mL of 1.19×10^{-3} M BPB solution were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0 mL with distilled water. To each separating funnel 10.0 mL of chloroform was added and the contents were shaken for 2 min. The two phases

were allowed to separate and the absorbance of the separated chloroform layer was measured at 440 nm against a similar reagent blank. The colored species were stable for 50 min. The amount of drug was deduced from the calibration curve.

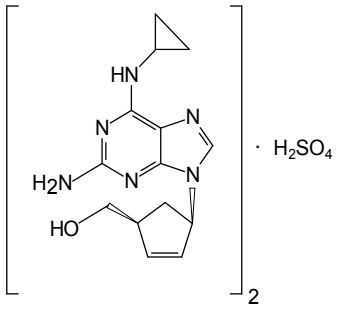
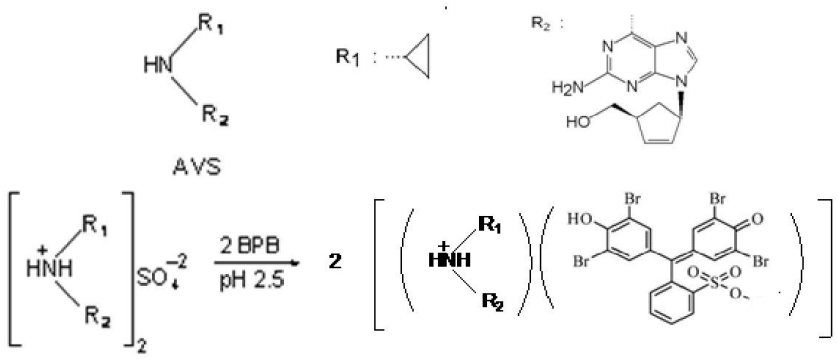


Fig. 1. Structure of abacavir sulphate



Scheme–1. Ion-association complex of AVS with BPB Method (M_1)

Method M_2:

Aliquots of standard drug solution [AVS: 1.0 – 3.0 mL , 150 µg mL^{-1}], 3.0 mL of 5.067×10^{-3} M brucine, 1.5 mL of 9.35×10^{-3} M $NaIO_4$ solution and 2.0 mL of 2.3 N sulphuric acid were added successively into series of calibrated tubes. The volume was brought up to 10.0 mL with distilled water and kept in boiling water bath for 20 min. The solutions were cooled to room temperature and the volume was made up to 25 mL with distilled water. The absorbances were measured at 520 nm against a similar reagent blank within 10 min. The stability of colored species was found to be 40 min. The amount of drug was computed from the calibration curve.

Pharmaceutical Formulations:

Since only one formulation is available (Tablets), four different batches of this formulation were collected and analyzed as 4 sets to verify the proposed methods and the results were compared with the results of bulk drug. Accurately weighed quantity of tablet powder equivalent to 100 mg of AVS was extracted with methanol (3 × 25.0 mL portions) and filtered. The volume of combined extract was brought to 100 mL with methanol to get stock solution (mg/ml). Fifty milliliters of this stock solution (mg/ml) was taken and methanol portion was evaporated to dryness. After cooling, the residue was dissolved and diluted stepwise with the distilled water to obtain the working standard solution of, 40 µg mL^{-1} (for method M_1) and 150 µg mL^{-1} (for method M_2). The UV spectrophotometric method which

was suggested for the identification of AVS has been moulded for its assay and chosen as the reference method for ascertaining the accuracy of the proposed methods.

Table 2. Assay of AVS in pharmaceutical formulations

Formu-lations[a]	Labelled Amount (mg)	Amount found by proposed methods (mg)[b,c]		Reference Method	% Recovery by proposed methods[d]	
		BPB (M_1)	Brucine/IO_4 (M_2)		BPB (M_1)	Brucine/IO_4^- (M_2)
Batch I	300	298.2±1.8 F = 2.08 t =0.05	300.3±2.0 F = 1.98 t =0.56	298.9±2.8	99.40±0.6	100.1±0.7
Batch II	300	298.7±2.8 F = 2.57 t = 0.86	300.5±3.3 F =3.59 t =0.05	300.6±1.8	98.72±1.1	100.2±1.1
Batch III	300	300.9±1.1 F = 1.66 t =1.95	298.7±1.1 F =1.77 t =0.45	299.4±1.5	100.3±0.4	99.6±0.36
Batch IV	300	300.7±2.3 F = 4.48 t =1.09	299.1±2.5 F =1.26 t =0. 29	299.7±2.2	100.2±0.8	99.68±0.84

[a]Four different samples of tablets

[b,c] Mean ± SD(n = 6). Theoretical values of 95% confidence limit, F = 5.05, t = 2.57.

[d]Mean ± SD (n = 3), Average of 3 determinations

Results and discussion

The optimum conditions for each method were established by varying the parameters one at a time [31] keeping the others fixed and observing the effect produced on the absorbance of the colored species.

Optimum conditions

Method M_1

Method M_1 is based on the formation of ion-association complex between drug (AVS) with acidic dye (BPB). The type of buffer solution, concentration of the dye, organic solvent used for extraction, ratio of organic phase to aqueous phase during extraction, stability period was studied. The optimum conditions developed for color development are as follows : 4.5-5.5 mL of BPB solution, 4.0 mL of buffer solution (pH 2.5), 2-5 min. of shaking time and a temperature of (28.0 ± 3°C) were found to be optimum. 5.0 mL of 1.19×10^{-3} M BPB, 5.0 mL buffer solution and 3 min. shaking period for obtaining constant absorbance values was preferred for further investigations. The other water immiscible solvents tested for the extraction of the colored complex into organic phase include chlorobenzene, dichloromethane, carbon tetrachloride, n-butanol, benzene and $CHCl_3$. Chloroform was preferred for its selective extraction of the complex from the aqueous phase. The ratio of aqueous to organic phase on

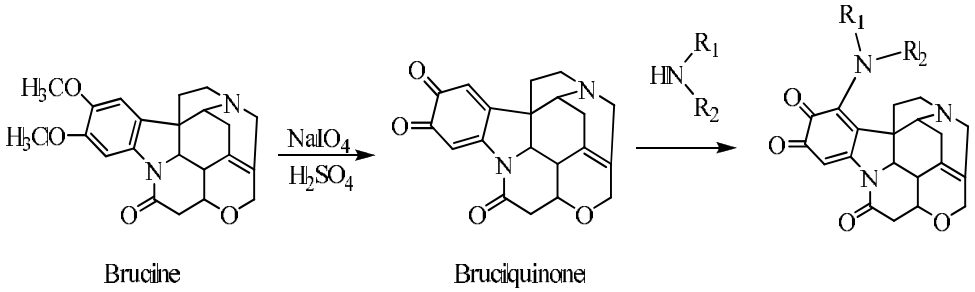
extraction was taken as 3:2. The colored species after separation from organic layer was stable for 50 min. The λ_{max} and ε_{max} values were found to be 440 nm, 5.586×10^4 L mol^{-1} cm^{-1}.

Method M_2

Method M_2 is based on the formation of oxidative coupling reaction of drug (AVS) with brucine (BCN) in the presence of sodium meta periodate (IO_4^-). The nature of oxidant, volume of oxidant, volume of brucine, volume of acid, the time and temperature required for color development, order of addition of reagents, solvent for final dilution and stability period were studied. The optimum conditions developed for color development are as follows: 2.5-3.5 mL of brucine solution, 1.0-2.0 mL of $NaIO_4$ solution, 1.5-2.5 mL. of 2.3 N H_2SO_4, 15-30 min. time on boiling water bath, order of addition is that drug, brucine and oxidant, water for final dilution and stability period up to 40 min. were found to be optimum. In the procedure 3.0 mL of 5.067×10^{-3} M brucine, 1.5 mL of $NaIO_4$ solution, 2.0 mL of H_2SO_4 solution, 20 min. heating time on water bath, with order of addition as drug, brucine, oxidant, water for final dilution, and 10 min. stability period for obtaining constant absorbance values were preferred for further investigations. The λ_{max} and ε_{max} values were found to be 520 nm and 1.76×10^4 L mol^{-1} cm^{-1}.

Mechanism of Reaction

In method M_1, the secondary amino group in between isopropyl and hetero cyclic moieties involves in ion-association complex with BPB (Scheme 1). Brucine-periodate reagent was used for the spectrophotometric determination of **sulphur compounds and** tryptophan [32]. In method M_2, the bruciquinone (formed from brucine and periodate) undergoes nucleophilic attack on the most electron-rich portion of coupler (-NH-) in AVS, to give 1-mono substituted bruciquinone derivative (Scheme 2).



Brucine Bruciquinone

Scheme-2 Oxidative coupling reaction of AVS with brucine / IO_4^- Method (M2)

Method of validation:

The developed method was validated as per ICH guidelines [33] for its linearity, precision, accuracy, limit of detection and limit of quantification. Regression analysis using the method of least square was made to evaluate the slope (b), intercept (a), and correlation coefficient(R) obtained from different concentrations of drug. The result of slope (8.32×10^{-2} and 2.64×10^{-2}) and intercept (4.0×10^{-4} and -4.0×10^{-4}) of drug by the proposed method was given in Table 1. Linearity was found in the concentration range 2 – 10 and 6 – 18 µg mL^{-1} and Beer's law plots (n = 6) were linear with a correlation coefficient value 0.9999 for methods A, and B respectively (Table-1). Limit of detection (LOD) and limit of quantification (LOQ) were established according to ICH guidelines and determined by using the formula LOD = K.SDa/b where K = 3.3 for LOD and 10 for LOQ. SDa is the standard deviation of the intercept and b is the slope of the calibration line. LOD values were found to

be as low as 5.68×10^{-1}, and 8.63×10^{-2} μg mL^{-1} and LOQ values were found to be 1.72×10^{-1} and 2.88×10^{-3} μg mL^{-1} respectively (Table I).

The repeatability of the proposed method was studied by repeating the method six times (n = 6).To study intra-day precision, the method was repeated six times a day. Similarly, the method was repeated on six consecutive days to determine inter-day precision. RSD values for the methods A and B were found to be 0.37 and 0.82 respectively (Table -1).The accuracy of the method was determined in terms of % recovery of AVS standard. Recovery studies were carried out by addition of standard drug solution at three different levels (8, 10, 12 μg mL^{-1}) to previously analyzed sample (tablet) solution. Values of recovery ± SD were found to be in the range of 98.72 $\pm$ 1.1 – 100.2 ± 1.1 (n = 3) for methods M_1 and M_2 respectively (Table II). This indicates that the proposed methods are accurate for the analysis of the drug.

Table 3. Comparison of proposed method with reported methods for Abacavir sulphate

Reagents used	λ_{max} (nm)	Beer's Law Limit (μg ml^{-1})	Correlation coefficient (r)	Molar absorptivity (l mol^{-1} cm^{-1})	LOD μg mL^{-1}	LOQ μg mL^{-1}	Reference
F.C	752	25-150	0.9977	1.47×10^3	NA	NA	21
MBTH/Fe(III)	665	50-300	0.9999	0.455×10^3	NA	NA	21
PDAC	463	25-150	0.9999	1.76×10^3	NA	NA	21
CTC	620	10-50	0.9999	9.58×10^3	3.07×10^{-1}	9.3×10^{-1}	28
BPB	440	2 – 10	0.9999	5.58×10^4	5.68×10^{-1}	1.72×10^{-1}	Present Paper
Brucine/IO_4^-	520	6 – 18	0.9999	1.76×10^4	8.63×10^{-2}	2.88×10^{-3}	Present Paper

FC: Folin Ciocalteu reagent, MBTH:3-methyl-2-benzothiazolinone hydrazone,

PDAC: p-Dimethylamino cinnamaldehyde, CTC: Cobalt thiocyanate: BPB:Bromo phenol blue

Application of the proposed method

The application of the proposed method for the assay of pharmaceutical formulations was examined for tablets and the results were statistically compared with those obtained by UV reference method. The results obtained by the proposed and UV reference method for the formulations were compared by means of Student's *t*-test and *F*- test and it was found that the proposed method do not differ significantly in precision and accuracy. The results are summarized in Table-2. The results obtained by the proposed method are compared with reported methods [16, 23] and found to be more sensitive in the range of 2-18 μg mL^{-1} with λ_{max} value 9.58×10^3 L $mol^{-1}cm^{-1}$.

Conclusion

The proposed methods exploit the various functional groups in AVS**.** The ingredients usually present in pharmaceutical formulations did not interfere in the color development by proposed methods. All the proposed methods are simple, economical and does not require much instrumentation over the literature methods and hence useful for the determination of AVS in pure and pharmaceutical formulations.

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