



VALIDATED VISIBLE SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF ABACAVIR SULPHATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, accurate and economical spectrophotometric method in visible region was developed for the determination of Abacavir sulphate in bulk and dosage forms have been described. This method is based on the oxidation of MBTH with Fe(III) followed by coupling with mentioned drug forming a highly stable violet colored chromogen measured at 620nm. Common excipients used as additives in pharmaceutical preparations do not interfere in the proposed methods. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method. The results were favorably compared with those obtained by reference UV spectrophotometric method. No interference was observed from common pharmaceutical adjuvants. These two methods were successfully applied to the pharmaceutical formulations.

Keywords: Abacavir sulphate and MBTH.

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INTRODUCTION

Abacavir sulphate, [(1S,4R)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol hemisulfate] is a novel nucleoside reverse transcriptase inhibitor (NRTI) that is potent in vivo and in vitro inhibitor of HIV-1, the causative agent of the acquired immunodeficiency syndrome (AIDS). Literature survey reveals few analytical techniques have been reported for the determination of abacavir sulphate in bulk and dosage forms which includes HPLC³⁻⁵, LC-ESI-MS⁶, and UV spectrophotometry^{7,8}. Existing analytical methods reveal that little attention was paid in developing visible spectrophotometric methods by exploring thoroughly the analytically useful functional groups in abacavir sulphate. The present paper describes the development and validation of three visible spectrophotometric methods based on different chemical reactions of abacavir sulphate with the above mentioned reagents and is extended to bulk and pharmaceutical dosage forms as well.

EXPERIMENTAL

All the spectral measurements were made on ELICO SL-159-Double beam spectrophotometer. All chemicals used were of analytical grade.

MBTH (3-methyl-2-Benzothialinone hydrazone hydrochloride) solution: (Loba; 0.5%, 2.14×10^{-2} M)

Prepared by dissolving 500 mg of MBTH in 100 ml distilled water.

Fe(III)Cl₃ Solution (Sd.fine; 0.25%, 1.53×10^{-3} M)

Prepared by dissolving 250 mg of anhydrous Fe (III) Cl₃ in 100 ml of distilled water.

NaOH solution: (Loba; 0.1M)

Prepared by dissolving 400 mg of NaOH in 100 ml of distilled water and standardized.

HCl solution : (Sd.fine; 1.0 M)

Prepared by dissolving 8.6 ml of Conc. HCl in 100 ml of distilled water and standardized.

Preparation of standard drug solution

Abacavir sulphate (100mg) was accurately weighed, dissolved in water and transferred to standard 100 ml volumetric flask. The final volume was made up to the mark with distilled water. The final concentration was brought upto $100 \mu\text{g} \cdot \text{mL}^{-1}$ respectively.

Procedure for the Assay of Abacavir sulphate in Pharmaceutical dosage forms

Two tablets of the (Zaigen) abacavir sulphate drug were weighed and powdered, and a quantity of the powder equivalent to 100 mg was transferred into a 100 ml volumetric flask, dissolved in 5 ml of methanol, stirred well for 2 minutes. The solution was mixed well by shaking for 10 minutes, and then make up to the mark with acetonitrile. The solution was filtered. The filtrate was quantitatively diluted with methanol to yield concentrations in the linear range of the assay of abacavir sulphate.

Proposed Procedures for the Determination of Abacavir sulphate

Aliquots (0.5-3.0ml, 100 $\mu\text{g}\cdot\text{mL}^{-1}$) of standard abacavir sulphate were transferred into a series of 10 ml calibrated tubes and then 1.0ml of water, 0.5 ml of 0.5% MBTH and 0.5 ml of 0.1N NaOH were added to each tube. The contents were heated for 10 minutes in a water bath at 100°C and cooled for 5 minutes in a water bath at 15°C. Then 0.5 ml of 1N HCl and 2.0 ml of Fe (III)Cl₃ solution were added successively and kept aside for 1hour .The absorbance was measured at 620nm against reagent blank. A calibration curve was prepared by plotting the absorbance versus the concentration and the unknown was read from the calibration curve, or deduced using a regression equation, calculated from Beers law data.

RESULTS AND DISCUSSION

In the proposed method, MBTH loses two electrons and one proton on oxidation, forming the electrophilic intermediate which has been postulated to be the active coupling species. The intermediate reacts with abacavir sulphate to form colored species (Scheme-1) exhibiting λ_{max} at 620 nm. Beer's law was obeyed over the concentration range of 5.0-25.0 $\mu\text{g}\cdot\text{mL}^{-1}$ respectively. The proposed procedure is validated by determining various optical parameters, which are listed in Table -1. The linearity, intercepts and the slope have been calculated using regression equation $Y = a + bC$, where Y represents optical density, 'C', the concentration of the drug in $\mu\text{g}\cdot\text{mL}^{-1}$ and 'a' and 'b' represents intercepts and slope respectively. Precision and accuracy of the proposed methods were tested by carrying out the determination of six replicates of pure and dosage samples of the drug, whose concentration lie within Beer's law range. The values of standard deviation (% R.S.D.) and percent range of error (0.05 level and 0.01 level confidence limits) were calculated for the above method is presented in Table- 1. The values obtained for the determination of abacavir sulphate in different brands of Tablet samples by the proposed are compiled in Table-2. The formation of colored species by the proposed method is given the scheme and is confirmed by H¹NMR[predicted], Fig.-1.

CONCLUSION

The method reported here are found to be simple, sensitive, accurate and precise. The present method involves the formation of highly stable colored species which makes it easier for the determination of abacavir sulphate from pharmaceutical dosage forms in a routine analysis. Further statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. The H¹NMR spectra[predicted] of the developed color species confirm the stability of the proposed method.

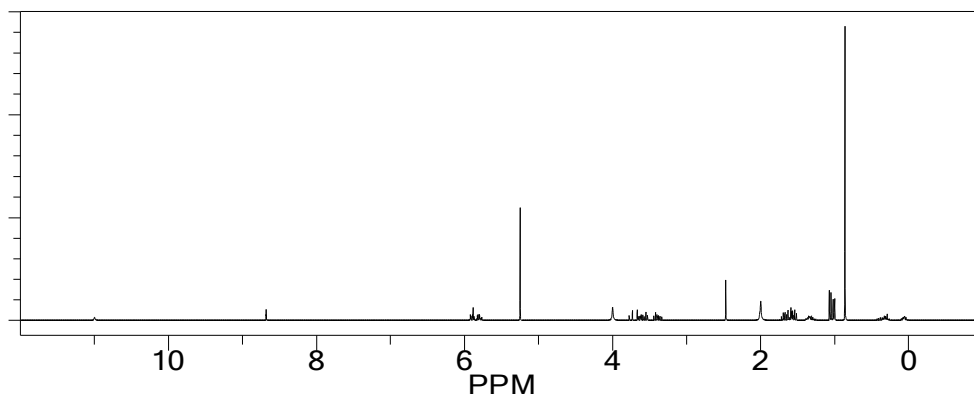
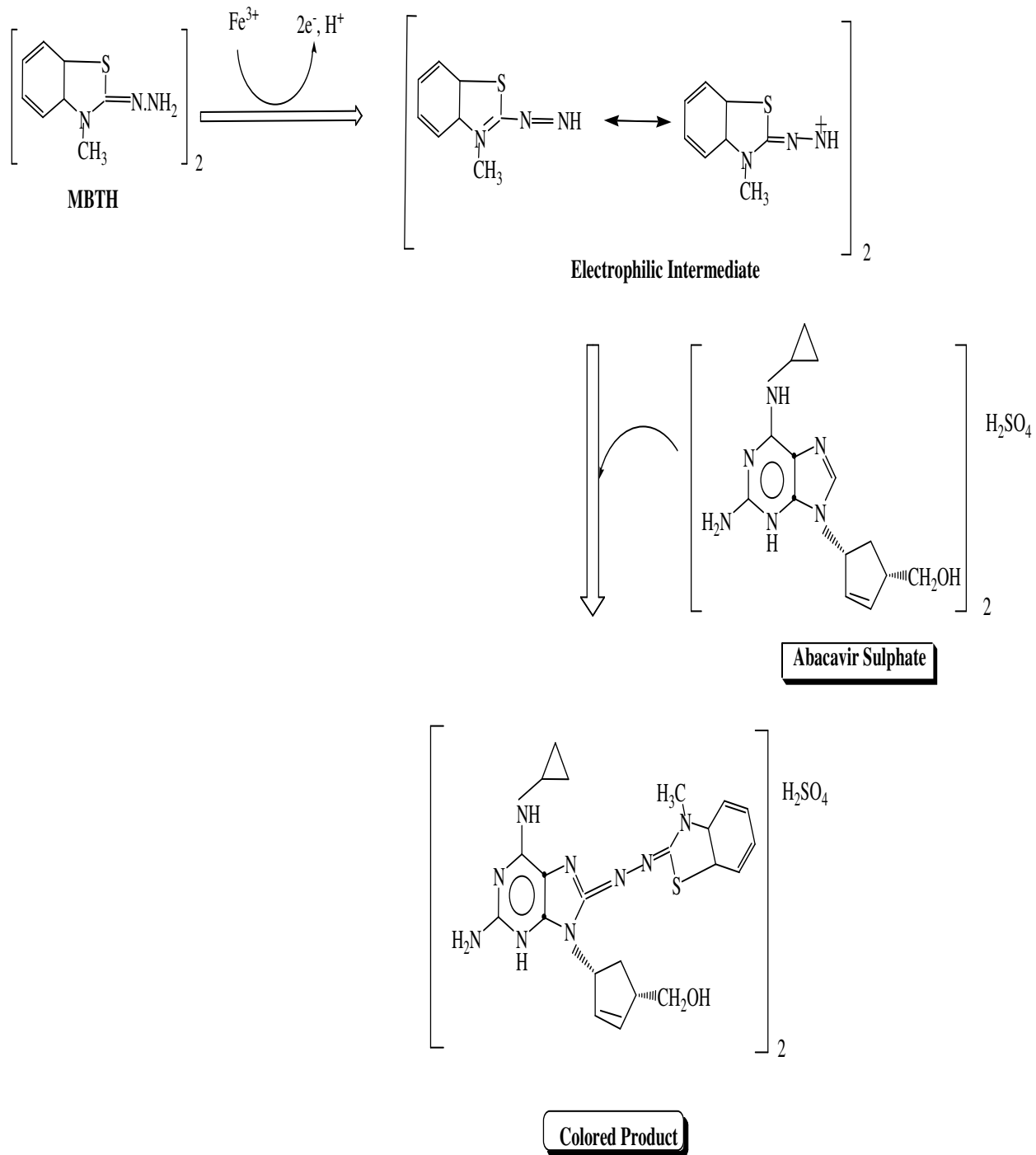


Fig.-1: H¹NMR Spectra of colored product[Predicted]

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Scheme-1

Table-1: Optical Characteristics and Precision Data

Parameter	Results
$\lambda_{\max}(\text{nm})$	620
Beer's law limits, mcg/mL	5.0 - 25.0
Molar absorptivity, L/mol.cm	2.0392×10^4
Sandell's sensitivity, (mcg/cm ² /0.001 absorbance units)	0.03289
Slope (b)	0.1212
Intercept(a)	0.0026
Correlation Coefficient(r^2)	0.9999
%RSD*	0.6205
0.05 level	0.6512
0.01 level	1.0213
Limit of Detection	0.4132

*Average of six determinations

Table-2: Assay and recovery of abacavir sulphate in tablet dosage form.

Tablet formulation	Labeled Amount,mg	Amount obtained by proposed method,mg*	**%recovery by the Proposed method
(Ziagen)	300	298.3	99.4

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