



A Simple Spectrophotometric Determination of Abacavir Sulphate in Pharmaceutical Formulations

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ABSTRACT

Two simple, sensitive and economical spectrophotometric methods have been developed for the determination of abacavir in commercial dosage forms. The method A was based on the formation of chloroform extractable complex of abacavir sulphate with wool fast blue. The absorbance of the extractable ion pair complex is measured at the wavelength of maximum absorbance 590 nm against the reagent blank. Method B was based on the charge transfer reactions of abacavir sulphate as n-electron donor with acceptor, 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone. The absorbance of the highly intensive coloured solution was measured at 450 nm against reagent blank treated similarly. Statistical analysis proves that the proposed methods are reproducible and selective for the estimation of abacavir sulphate in bulk drug and in its tablet dosage form.

INTRODUCTION

Abacavir sulphate¹ is chemically {(1S, 4R)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol}. It is a nucleoside reverse transcriptase inhibitor with antiretroviral activity against HIV. It is administered alone or in combination therapy with other anti retrovirals. The present study describes simple, sensitive, accurate, rapid and economical spectrophotometric methods for the estimation of abacavir sulphate in bulk & its tablet dosage forms. Literature survey reveals that, several spectrophotometric[1-4] methods have been reported for the estimation of Abacavir sulphate in pharmaceutical formulations. Few analytical methods were reported in literature for the determination of abacavir sulphate and Lamivudine in combinations which includes spectrophotometric method [5], HPTLC[6] and RP-HPLC[7].

Spectrophotometry is the technique of choice even today in the laboratories of research, hospitals and pharmaceutical industries due to its low cost and inherent simplicity. This paper describes two rapid, simple, sensitive and economical spectrophotometric methods for the determination of abacavir sulphate in commercial dosage forms. Method A is based on the formation of chloroform extractable complex of abacavir sulphate with wool fast blue. The ion association complex is a special form of molecular complex resulting from two components extractable into organic solvents from aqueous phase at suitable pH. One component is a chromogen (wool fast blue) processing charge (Cationic or anionic in nature) & so insoluble in organic solvents. The other is colorless, processing opposite

charge to that of chromogen. Method B utilizes the charge transfer reactions of abacavir sulphate as n-electron donor with acceptor, 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone. The molecular interactions between electron donors and electron acceptors are generally associated with the formation of intensely colored charge-transfer complexes, which absorb radiation in the visible region. The charge-transfer reaction has not been reported yet for abacavir sulphate, therefore the aim of the present study was directed to investigate this reaction.

MATERIALS AND METHODS

All absorbance measurements were made on a Spectronic 1001 plus spectrophotometer (Milton Roy Company, USA) with 1cm matched quartz cells. All the solutions were freshly prepared. All solvents and other chemicals used through this study were of analytical grade. Wool fast blue solution (0.2%) was prepared in distilled water, freshly prepared. 2,3-dichloro 5,6-dicyano-p-benzoquinone(DDQ; Merck, Schuchardt, Munich, Germany) solution(0.1%) was prepared in methanol and it was prepared afresh daily. Buffer solutions of required pH were prepared by mixing appropriate volumes of glycine, sodium chloride and 0.1M Hydrochloric acid.

Preparation of standard solution

A standard stock solution containing 1 mg/ml was prepared by dissolving 50 mg of abacavir sulphate in 50 ml of distilled water for method A and B. From this, a working standard solution containing 100 µg/ml was prepared for both method A and B.

Assay procedures

Method A

Aliquots of standard drug solution of abacavir sulphate 0.5 – 2.5 ml were taken and transferred into a series of 125 ml of separating funnels. To each funnel 2 ml of 0.2% wool fast blue was added. Reaction mixture was shaken gently for 5 min. Then 10 ml of chloroform was added to each of them. The contents are shaken thoroughly for 5 min and allowed to stand, so as to separate the aqueous and chloroform layer. Colored chloroform layer was separated out and absorbance was measured at 590 nm against reagent blank. Calibration curve was prepared from absorbance values so obtained.

Method B

Various aliquots of standard solution of abacavir sulphate ranging from 0.2-1.0 ml were transferred into 10 ml calibrated flasks. To each flask 1.0 ml hydrochloric acid and 1.0 ml of the acceptor solution was added, and the reaction was allowed to proceed at room temperature (25±5°C). The reaction was achieved instantaneously. The solutions were diluted to volume with distilled water. The absorbance of the resulting solutions was measured at the wavelength of maximum absorption 450 nm against reagent blank treated similarly. The amount of drug present in sample is read from the calibration graph.

Pharmaceutical preparations

A total number of twenty tablets of abacavir sulphate accurately weighed and powdered by a mortar and pestle. Tablet powder equivalent to 50 mg of abacavir sulphate was accurately weighed and transferred to 50 ml volumetric flask. Weighed tablet powder is dissolved in 25 ml distilled water and vortexed for 15 minutes. Then the volume diluted to 50 ml with distilled water and mix well. The solution was filtered through Whatmann filter paper no 42, suitably diluted with distilled water and analyzed as given under the assay procedures for bulk samples. The results are represented in Table No.2.

RESULTS AND DISCUSSION

The optimum conditions were established by varying one parameter at a time and keeping the others fixed and observing the effect on absorbance of chromogen for method A and method B. In the present work method A and B have been developed for the estimation of abacavir sulphate from tablet formulations. The developed method A is based on formation of chloroform extractable colored complexes with wool fast blue. Method B is

based on the reaction of abacavir sulphate as n-electron donor with acceptor, 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone. Statistical analysis was carried out and the results were found to be satisfactory. Recovery studies were close to 100 % that indicates the accuracy and precision of the proposed methods. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table No.1. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and results are summarized. The percent relative standard deviation, standard deviation and student's't' test values calculated from the five measurements of abacavir sulphate are presented in Table No.3. Relative standard deviation values and standard deviation were low that indicates the reproducibility of the proposed methods. In the student's't' tests, no significant differences were found between the calculated and theoretical values of both the proposed methods at 95% confidence level. This indicated similar precision and accuracy in the analysis of abacavir sulphate in its tablets.

Table No 1: Optical characteristics of proposed method.

Statistical parameters	Method A	Method B
λ_{\max} nm	590	450
Beer's limits, mcg/ml	50-250	20-100
Sandell's, sensitivity, ($\mu\text{g cm}^{-2}$)	0.167	0.280
Molar absorptivity, ($\text{L mol}^{-1} \text{cm}^{-1}$)	1.9×10^3	2.8×10^3
Regression equation, Y*		
Correlation coefficient, (r)	0.999	0.999
Intercept (a)	0.004	0.005
Slope (b)	0.006	0.010

*Y = a+bX, where Y is the absorbance and X concentration in $\mu\text{g} / \text{ml}$
 a= Intercept
 b= Slope

Table No.2: Assay and recovery of abacavir sulphate in tablet formulations

Tablet formulation	Labeled amount (mg/tab)	* Amount found by proposed method		% Recovery by proposed method	
		Method A	Method B	Method A	Method B
Tablet 1	300	299.91	300.02	99.95	100.08
Tablet 2	300	300.04	300.07	100.1	100.2
Tablet 3	300	300.08	299.93	101.0	99.98

*Average of five determination based on label claim

Table No. 3: Results of statistical analysis of the proposed methods

Tablet formulation	*Standard deviation		% Relative standard deviation*		*t value	
	Method A	Method B	Method A	Method B	Method A	Method B
Tablet 1	0.347	0.1680	0.1140	0.0559	0.5232	0.2663
Tablet 2	0.313	0.2625	0.1043	0.0874	0.2859	0.5967
Tablet 3	0.0963	0.3542	0.0321	0.1180	1.860	0.4419

*Average of five determination based on label claim

CONCLUSION

The proposed methods can be used for determination of abacavir sulphate in tablets. The methods are rapid, simple and have great sensitivity and accuracy. Proposed methods make use of simple reagents, which an ordinary analytical laboratory can afford. The proposed method is suitable for routine determination of abacavir sulphate in its formulations. The commonly used additives such as starch, lactose, titanium dioxide, and magnesium stearate do not interfere with the assay procedures.

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