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Development of validated spectrophotometric methods for the assay of Abacavir sulfate in bulk and pharmaceutical formulations

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Abstract: Two simple, accurate, rapid and sensitive methods A and B for the spectrophotometric determination of abacavir sulfate in either the pure form or in pharmaceutical formulations were proposed. These methods are based on the formation of purple color and red-violet colored chromogens obtained when the drug was diazotized with nitrous acid followed by coupling with Phloroglucinol and Resorcinol, exhibiting λ_{max} and ε_{max} at 520nm and $5.50x10^4$l.mol^{-1} cm^{-1} for Method-A and 600nm and $4.19x10^4$ l. mol^{-1} cm^{-1} for Method-B respectively. The resulting colors are well developed within 20–30 min and are stable for at least 10hrs. Results of analyses of pure drugs and their dosage forms by the proposed methods are highly reproducible and have been applied for the analysis of some commercial dosage forms.

Key words: Abacavir sulfate,Chromogen, Spectrophotometry.

INTRODUCTION

Abacavir sulfate [1-2],[(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 very few analytical methods such as high-performance liquid chromatography (HPLC) [2-7] , LC-ESI-MS[8]and spectrophotometric assay[9-10] for the assay of abacavir sulfate in human plasma and in various phramaceutical formulations has been described. The aim of this work was to develop simple and reproducible spectrophotometric procedures for the determination of abacavir sulfate in pure forms and commercial dosage forms through diazo coupling reactions.

MATERIALS AND METHODS

Instrumentation and conditions: All spectral and absorbance measurements were made on ELICO SL-159, UV-visible spectrophotometer with 1cm quartz cells was used.

Reagents and Solvents: All chemicals used were of analytical grade.

Phloroglucinol solution: (Loba; 0.1%, 8.26×10^{-3} M): Prepared by dissolving 100 mg of phloroglucinol in 100 ml distilled water.

Resorcinol Solution (Sd.fine; 0.1%, 9.08×10^{-3}M): Prepared by dissolving 100 mg of resorcinol in 100 ml of distilled water.

NaOH solution (Loba; 4.0%, 1.0M): Prepared by dissolving 400 mg of NaOH in 100 ml of distilled water and standardized.

HCl solution (Sd.fine; 0.25 M): Prepared by dissolving 2.15ml of Conc.HCl in 100 ml of distilled water and standardized.
Sodium nitrite solution (Loba; 0.1%, 1.45×10^{-2} M): Prepared by dissolving 100 mg of Sodium nitrite in 100 ml distilled water.

Preparation of standard drug solution: Abacavir sulfate (100mg) was accurately weighed, dissolved in water and transferred to standard 100 ml volumetric flask. The final volume was made upto the mark with distilled water. The final concentration was brought upto 100µg.mL^{-1} respectively.

Procedure for the Assay of Abacavir sulfate in Pharmaceutical dosage forms: Two tablets of the abacavir sulfate(Zaigen) 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 sulfate**.**

Proposed Procedures for the assay of abacavir sulfate:
Method-A: Aliquots of (0.5-2.5ml) *abacavir sulfate* (0.5ml =100µg) were transferred into a series of 25ml volumetric flasks. To each of the above aliquots, hydrochloric acid (dilute) (1.0ml) and 1.0ml cold aqueous solution of sodium nitrite (0.1% w/v) were added and set aside for 10 min at 0-5^{o}C temperature. Later 1.0ml of Phloroglucinol (0.1% w/v) and 1.5ml of aqueous Sodium hydroxide (4% w/v) were added successively, and then the volume in each tube was made up to 25ml with distilled water. The absorbance was measured at 520nm against reagent blank. The amount of *abacavir sulfate* was computed from calibration curve. The color was found to be stable for more than 10hrs at room temperature. The concentration of *abacavir sulfate* was calculated either from calibration curve or from regression equation.

Method B: Aliquots of (0.5-2.5ml) *abacavir sulfate* (1.0ml =100µg) were transferred into a series of 25ml volumetric flasks. To each of the above aliquots, hydrochloric acid (dilute) (1.0ml) and 1.0ml cold aqueous solution of sodium nitrite (0.1% w/v) were added and set aside for 10 min at 0-5^{o} C temperature. Later 1.0ml of Resorcinol (0.1% w/v) and 1.5ml of aqueous Sodium hydroxide (4% w/v) were added successively, and then the volume in each tube was made up to 25ml with distilled water. The absorbance was measured at 600nm against reagent blank. The amount of *abacavir sulfate* was computed from calibration curve. The color was found to be stable for more than 10 hrs at room temperature. A calibration graph was drawn and the corresponding regression equation was computed to obtain the concentration of *abacavir sulfate*.

Table-1. Quantitative Parameters and Precision Data

Parameter	**Method A**	**Method B**
λmax(nm)	520	600
Beer's law limits, mcg/mL	3.0-8.0	3.0-8.0
Molar absorptivity, L/mol.cm	$5.50\text{x}10^{4}$	$4.19\text{x}10^{4}$
Sandell'ssensitivity, (mcg/cm^2/-0.001absorbance units)	0.03048	0.016
Slope (b)	0.9411	0.0312
Intercept(a)	0.0002	0.001
Correlation Coefficient(r^2)	0.9999	0.9998
%RSD* **	0.5747	0.169
0.05 level	0.6032	1.228
0.01level	0.9460	1.925
Limit of Detection	0.0685	0.0637

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

Tablet formulations	Labeled Amount,mg	Amount Obtained by proposed method,mg*		*%recovery by the Proposed method	
		Method - A	Method -B	Method - A	Method - B
(ziagen)	300	298.3	298.9	99.4	99.6

*Average of six determinations.

RESULTS AND DISCUSSION:

The presence of primary amino group in *abacavir sulfate* enabled the use of diazotization of the drug with nitrous acid in presence of sodium hydroxide solution resulting in the formation of diazonium salt with phloroglucinol producing purple colored chromogen in Method-A with λ_{max} at 520nm and ε_{max} $5.50x10^4 l.mol^{-1}$ cm^{-1} and in Method-B, similar diazotization reaction was observed with resorcinol resulting in the formation of red-violet chromogen exhibiting λ_{max} at 600nm and ε_{max} $4.19x10^4$ $l.mol^{-1}$ cm^{-1}. The calibration curve for *abacavir sulfate* was obtained by plotting the peak area versus concentration. Linearity was found to be in the range of 3.0 - 8.0 $\mu g.ml^{-1}$ for Method –A and 3.0 - 8.0 µg.mL–1 for Method –B and with significantly high value of correlation coefficient $r^2 = 0.9999$[Method –A] and $r^2 = 0.9998$[Method –B], the regression equation was y = 0.9411x + 0.0002for for Method –A and y = 0.0312x + 0.001 for Method –B. The quantitative parameters for assay of *abacavir sulfate* in pharmaceutical dosage forms for the proposed methods [Method –A&B] are listed in Table-1.The values obtained for the determination of a*bacavir sulfate* in various brands of Tablet samples by the proposed are compiled in Table-2 respectively**.**

CONCLUSION

The obtained and statistical parameters for determination of *abacavir sulfate* in pure and commercial dosage forms demonstrate that the proposed visible spectrophotometric methods are simple, accurate, fast and precise. The method showed acceptable linearity and accuracy. The proposed methods are highly sensitive; therefore it could be used easily for the routine analysis of pure drugs and their vial formulations.

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