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HPLC-UV Determination of Abacavir Sulphate in Pharmaceutical Dosage Forms

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A new reverse phase high performance liquid chromatographic (RP-HPLC) method with UV detection was developed and used for the estimation of abacavir sulphate (ABS) in bulk and pharmaceutical dosage forms using RPC-18 column using an isocratic HPLC system. The mobile phase consisted of acetonitrile and 0.05 M potassium dihydrogen phosphate (pH 4.2) in the ratio of 50 : 50 at a flow rate of 1 mL/min. The run time was 15 min. Nelfinavir mesylate (NEM) (50 µg/mL) was used as an internal standard. The detection was carried out at 220 nm and the linearity was found to be in the range of 0.5-200 µg/mL. The retention times for drug (ABS) and internal standard (NEM) were 3.558 and 10.725 min, respectively. Recovery studies showed that *ca.* 99.87 % of ABS could be recovered from the preanalyzed samples indicating high accuracy of proposed method. There was no intra-day and inter-day variation found in the method of analysis. The mean drug content in branded ABS tablet dosage forms was quantified and found to be between 99.64 and 100.05%. The method was found to be simple, precise, specific, sensitive and reproducible.

Key Words: HPLC Determination, Abacavir sulphate.

INTRODUCTION

Abacavir sulphate (1592U89 sulphate; CAS Reg. No. 188062-50-2) is ((-)-(1S, 4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-*m*-ethanol) sulphate, is a 2'-deoxyguanosine analogue with potent activity against human immunodeficiency virus (HIV) type 1, classified under nucleoside reverse transcriptase inhibitors category of antiretroviral drugs[1,2]. Some analytical methods for the estimation of abacavir sulphate were reported such as HPLC[3-6], LC-MS[7,8] and SDS-PAGE[9]. The present study is aimed for developing a simple, reproducible and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of ABS in bulk and pharmaceutical dosage forms using nelfinavir mesylate (NEM) as an internal standard (IS).

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EXPERIMENTAL

An isocratic HPLC system (Shimadzu®) consisting of LC-10 AT liquid pump, SPD-10A UV-visible detector, a ODS-18 RP column (4.6 mm I.D. X 25 cm length), 25 µL Hamilton® injecting syringe and MS Windows based Single channel software (Class VP®). Afcoset® electronic balance was used for weighing the materials. Pure samples of abacavir sulphate and nelfinavir mesylate were obtained from Matrix laboratories, Hyderabad, India. Acetonitrile of HPLC grade and potassium dihydrogen phosphate of AR grade were purchased from E. Merck (India) Ltd., Mumbai. Water used was triple distilled prepared by all glass distillation apparatus.

Chromatographic conditions: The optimized chromatographic conditions were as follows:

Chromatograph	Schimadzu HPLC system
Mobile phase	Acetonitrile : 0.05 M potassium dihydrogen phosphate (pH 4.2) (50 : 50)
Column	ODS C-18 RP column (4.6 mm i.d. × 25 cm length)
Flow rate	1 mL/min
Detection	UV set at 220 nm
Injection volume	20 µL
Temperature	Ambient
Retention time	
of Drug	3.558 min
of NEM	10.625 min
Run time	15 min

Stock solutions of ABS and NEM were prepared by dissolving accurately weighed 25 mg of ABS and NEM in 25 mL of acetonitrile : 0.05 M potassium dihydrogen phosphate (50 : 50) to obtain 1 mg/mL solutions. From these solutions 2.5 mL was pipetted out into 25 mL volumetric flask and diluted with the same solvent system to obtain 100 µg/mL solutions. Working standard solutions of ABS each containing internal standard (NEM) solution in the concentration of 50 µg/mL were prepared by taking required aliquots of ABS solutions and then diluted with the same solvent system. The standard solutions prepared above were injected five times into the column at a flow rate of 1mL/min. The ratios of AUC of drug to nelfinavir mesylate were calculated for each of the drug concentrations. The regression equation of drug concentration over the ratio of drug peak is to that of nelfinavir mesylate was obtained. The regression equation was used to estimate the amount of ABS in pharmaceutical tablet dosage forms.

The proposed HPLC method was tested for intra-day and inter-day variations. The recovery studies were carried out by adding known amounts of (10 and 30 µg) of the ABS to the pre-analyzed samples and subjecting them to the proposed HPLC method.

Estimation of abacavir sulphate in its commercial tablet formulations: Contents of ten tablets containing ABS were pooled and powdered. The powder equivalent to 25 mg of ABS was extracted into acetonitrile and the volume was adjusted to 25 mL, mixed and filtered through a 0.45 µ filter. From the filtrate 0.1 mL was pipetted into a 10 mL graduated test tube and spiked with the required aliquot of nelfinavir mesylate solution and then the volume was adjusted to 10 mL with the mobile phase such that the concentration of nelfinavir mesylate in each sample was 50 µg/mL and was injected 5 times into HPLC column. The mean concentration of ABS corresponding to the ratio of AUC of ABS to that of nelfinavir mesylate was calculated from the standard graph. The same procedure was followed for the other brand.

RESULTS AND DISCUSSION

The present study was carried out to develop a specific sensitive, precise and accurate RP-HPLC method using UV detection for the analysis of abacavir sulphate in pharmaceutical tablet dosage forms. The column pressure varied from 165-175 kgf/cm^2. The retention times for ABS and NEM were 3.558 and 10.725 min, respectively. Each of the samples was injected five times and almost the same retention times were observed in all the cases.

TABLE-1
CALIBRATION OF HPLC METHOD FOR ESTIMATION OF ABACAVIR SULPHATE

Concentration of Abacavir sulphate (µg/mL)	Mean ratio of AUC of drug to I.S. (n = 5)	CV (%)
0.5	0.02313	1.88
1	0.07417	1.74
2	0.12506	1.56
5	0.28634	1.69
10	0.53975	2.05
20	1.00286	1.41
40	2.20335	2.89
80	4.06320	1.62
100	4.72220	2.44
160	8.13190	2.15
200	9.81720	1.83

C.V.= coefficient of variation, regression equation (from 0.5 to 200 µg/mL)

The ratio of peak area of ABS to peak area of NEM for different concentrations set up as above were calculated and the average values for five such determinations are shown in Table-1. The peak areas of both drug and internal standard were reproducible as indicated by the low coefficient of variation (< 2.89 %). A good linear relationship (r = 0.9993) was observed

between the concentration of drug and the respective ratio of peak areas. The calibration graph was found to be y = 0.0493x + 0.0434 (where y is the ratio of peak area of drug to that of internal standard and x is the concentration of drug in the range of 0.5 to 200 μg/mL). When ABS solutions containing 10 and 40 μg/mL were analyzed by the proposed HPLC method for finding out intra-day and inter-day variation, a low coefficient of variation was observed (< 2.66%) showing that the method is highly precise (Table-2). About 99.87 % of ABS could be recovered from the preanalyzed samples indicating high accuracy of proposed method as shown in Table-3.

TABLE-2
PRECISION OF THE PROPOSED HPLC METHOD

Abacavir sulphate concentration (μg/mL)	Concentration of Abacavir sulphate (μg/mL) found			
	Intra day		Inter day	
	Mean (n = 5)	CV (%)	Mean (n = 5)	CV (%)
10	10.07	1.36	10.16	1.82
40	40.14	1.09	40.24	2.66

TABLE-3
RECOVERY STUDIES OF ABACAVIR SULPHATE

Amount of drug added (μg)	Mean (± S.D.) amount (μg) found (n = 5)	Mean recovery (%)
10	10.043 (± 0.080)	100.43
30	29.961 (± 0.014)	99.87

The ABS content in branded tablet formulations was quantified using the proposed analytical method and details are shown in Table-4. The absence of additional peaks indicated no interference of the excipients used in the tablets. The tablets were found to contain 99.64 to 100.05 % of the labeled amount. The low per cent of CV (< 1.71 %) indicates the reproducibility of the assay of ABS in the tablet dosage forms. The proposed method was found to be simple, precise, accurate, specific and economical. Hence this method can be employed to estimate ABS in bulk and tablet dosage forms effectively.

TABLE-4
ASSAY OF DIFFERENT BRANDS OF ABACAVIR SULPHATE TABLETS

Brand	Labeled amount of drug (mg)	Mean of labeled amount (n = 5) (%)	CV (%)
X	300	100.05	1.62
Y	300	99.64	1.71

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