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# A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR ABACAVIR SULPHATE

**D. GOWRISANKAR[a], PRAKASH S. SARSAMBI[*] and S. A. RAJU**

[a]University College of Pharmaceutical Sciences, Andhra University,
VISAKHAPATNAM - 530 003 (A.P.) INDIA
H.K.E. Society's College of Pharmacy, GULBARGA - 585 105 (K. S.) INDIA

## ABSTRACT

A simple, rapid and reproducible high performance reversed phase liquid chromatographic method has been developed for the estimation of abacavir sulphate in bulk drug sample and pharmaceutical dosage forms using RPC-18 column. The mobile phase consists of buffer solution and acetonitrile in the ratio of 85 : 15, respectively, and was pumped at 1.0 mL/ min at 41ºC. The detection was carried out at 285 nm and the calibration curve was linear in the range of 0.2µg/ mL to 20 µg/mL. The method was statistically validated for its linearity, precision and accuracy. The intra and interday variation was found to be less than 1% showing high precision of the assay method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining abacavir sulphate in bulk drug samples or in pharmaceutical formulations.

**Key words**: Abacavir sulphate, RP-HPLC.

## INTRODUCTION

Abacavir sulphate[1-5] is chemically (cis, 4R)-4-(2-amino-6-(cyclopropyl]-amino]-9H purin-ayl]-(cyclopent-2-enyl) methanol sulphate. Abacavir sulphate is the most powerful nucleoside analog reverse transcriptase inhibitor (NART) used to treat HIV and AIDS. Literature survey reveals that few methods like determination of abacavir in human plasma using isocratic reverse phase HPLC with ultraviolet detection and few UV methods[6-8] have been reported. Literature survey reveals that so far no methods have been reported for the estimation of abacavir sulphate in bulk drug and pharmaceutical dosage forms. The aim of this study is to develop a simple, rapid, precise and accurate reverse-phase HPLC method for the determination of abacavir sulphate in bulk drug samples or in pharmaceutical dosage forms.

[*] Author for correspondence

# EXPERIMENTAL

## Instrumentation

Quantitative HPLC was performed on a gradient high pressure liquid chromatograph (Shimadzu HPLC class VP series) with two LC-10 AT VP pumps, variable wavelength programmable UV/ Vis detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu), a disposable guard column LC-18 (Pelliguard)$^{TM}$, LC-18, 2 cm, Supelco Inc., Bellefonte, PA and RPC-18 column (4.6 mm x 100 mm I.D., particle size 5 μm) was used. The HPLC system was equipped with the software Class-VP series version 6.01 (Shimadzu).

## Chemicals and reagents

Pure sample of abacavir sulphate was obtained as gift sample from Genix Laboratories, Hyderabad, India. Acetonitrile (HPLC grade), potassium phosphate monobasic (AR grade) and sodium hydroxide (AR grade) were purchased from Merck Limited (Mumbai, India), water (HPLC grade, Qualigens). The commercially available tablets each containing 300 mg of drug were procured from local market.

## Chromatographic conditions

The contents of the mobile phase were buffer solution and acetonitrile in the ratio of 85 : 15. Buffer was prepared by dissolving 6.8 g of potassium phosphate monobasic in 1000 mL of water and pH was adjusted to 7.0 with 0.1M sodium hydroxide. The contents of the mobile phase were filtered before use through 0.45 μm membrane filter, degassed with helium spurge for 15 min and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 mL/ min, which yielded a column back pressure 138-140 Kg/cm². The run time was set at 30 minutes and the column temperature was maintained at 41ºC. The volume of the injection loop was 20 μL. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. The elements were monitored at 285 nm and the data were acquired, stored and analyzed with the software Class VP series version 6.01 (Shimadzu).

## Procedure

About 100 mg of abacavir sulphate was accurately weighed and dissolved in acetonitrile so as to give 1 mg/mL solution. Subsequent dilutions of this solution were made with mobile phase to get concentration of 0.2 to 20 μg/mL of abacavir sulphate. The standard solutions prepared as above were injected five times into the column at a flow rate

of 1.0 mL/ min. The peak areas of the drug concentration were calculated. The regression of the drug concentration over the peak areas was obtained. This regression equation was used to estimate the amount of abacavir sulphate in tablet dosage forms.

Abacavir sulphate solutions containing 8 µg/mL, 12 µg/mL and 20 µg/mL were subjected to the proposed HPLC analysis for finding out intra and inter-day variations. The recovery studies were carried out by adding known amount of abacavir sulphate to the pre-analyzed and subjecting them to the proposed HPLC method.

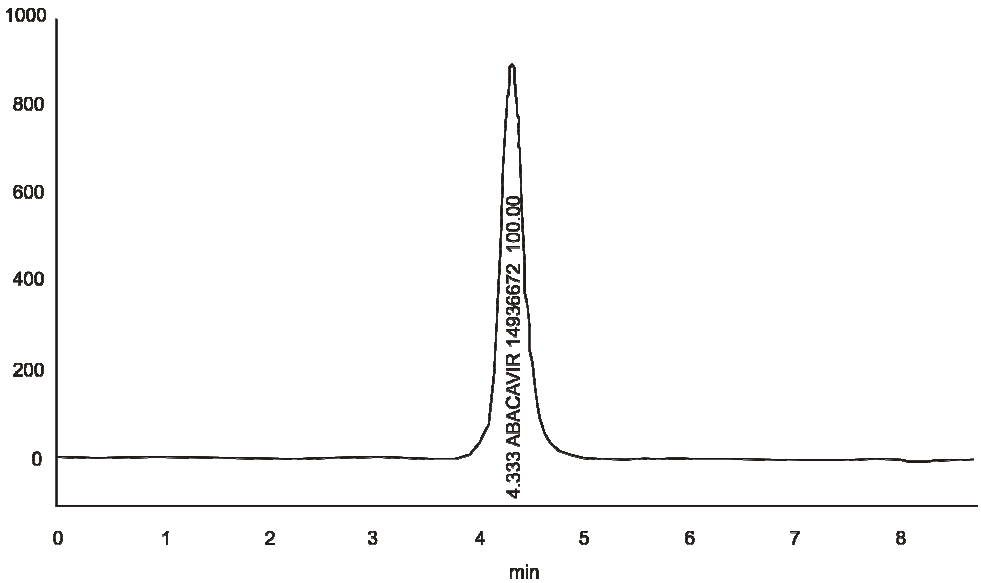


**Fig. 1: Typical chromatogram for abacavir**

## Assay

Ten tablets each containing 300 mg were weighed and powdered. An accurately weighed portion of the powder equivalent to 100 mg of abacavir sulphate was transferred to a 100 mL volumetric flask containing 50 mL of mobile phase. The contents of the flask were sonicated for 15 minutes to dissolve abacavir sulphate and made up to volume with mobile phase and the resulting mixture was filtered through a 0.45 µm filter. One milliliter of this solution was added to a 100 mL volumetric flask and made up to the volume with mobile phase. This solution (20 µL) was injected six times into the column. The mean values of peak areas of six such determinations were calculated and the drug content in the tablet was quantified using the regression equation obtained above. The same procedure

was followed for the estimation of abacavir sulphate in other commercially available tablet dosage forms.

## RESULTS AND DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of abacavir sulphate in bulk sample or pharmaceutical dosage forms. The column pressure varied from 138 to 140 Kg/cm². The retention time for abacavir sulphate was 4.333 min for a run period of 30 min. Each of the samples was injected 5 times and the same retention times were observed in all cases. The peak area of different concentrations set up as above were observed in all cases. The peak area of different concentrations set up as above were calculated and the average values for 5 such determinations are shown in Table 1.

**Table 1. Calibration of the HPLC method for the estimation of abacavir sulphate**

| Concentration of abacavir sulfate (μg/mL) | Peak area (n = 6) |
|---|---|
| 0.2 | 298735 |
| 0.4 | 597467 |
| 0.6 | 746834 |
| 0.8 | 896198 |
| 1.0 | 1493666 |
| 2.0 | 2987332 |
| 4.0 | 5974664 |
| 6.0 | 7468331 |
| 8.0 | 8961996 |
| 10.0 | 14936672 |
| 20.0 | 29873344 |
| Regression equation (from 0.2 to 10 μg/mL): $y = -0.002139 + 0.52301$ ($r = 0.9998$) | |

The peak area for drug solution was reproducible as indicated by low coefficient of variation (1.96%). A good linear relationship ($r = 0.9991$) was observed between the

concentrations of abacavir sulphate and the respective peak areas. The calibration graph was found to be y = –0.002139 + 0.52301x, where y is the peak area and x is the concentration of abacavir sulphate in the range of 0.2 to 20 μg/mL. When abacavir sulphate solution containing 8 μg/mL and 12 μg/mL were analyzed by the proposed reversed phase HPLC method for finding out the intra and inter-day variations, a low coefficient of variation was observed (Table 2).

**Table 2: Inter and intra-day precision for abacavir sulphate assay in pharmaceutical dosage forms by the proposed HPLC method**

| Concentration of abacavir sulphate (μg/mL) | Observed concentration of abacavir sulphate | | | |
|---|---|---|---|---|
| | Intra-day | | Inter-day | |
| | Mean (n = 6) | % CV | Mean (n = 6) | % CV |
| 8 | 7.98 | 0.32 | 8.02 | 0.72 |
| 10 | 10.02 | 0.21 | 10.03 | 0.14 |
| 12 | 12.04 | 0.73 | 12.02 | 0.34 |

This shows that the present HPLC method is highly precise. The amount of abacavir from the preanalyzed sample containing known amounts of the drug are shown in Table 3. About 100.21% abacavir sulphate could be recovered from the preanalyzed sample indicating the high accuracy of the proposed HPLC method.

**Table 3: Experimental values obtained in the recovery test for abacavir sulphate tablets by proposed HPLC method**

| Amount of drug added (μg) to drug solution/ powdered tablet formulation | Recovery from drug solution | | Recovery from powdered tablet formulation | |
|---|---|---|---|---|
| | Mean ( ± SD) amount (μg) found (n = 6) | Mean ( ± SD) % recovery (n = 6) | Mean ( ± SD) amount (μg) found (n = 6) | Mean ( ± SD) % recovery (n = 6) |
| 4 | 3.991 ± 0.25 | 99.72 ± 0.04 | 4.03 ± 0.01 | 100.21 ± 0.03 |
| 8 | 8.14 ± 0.03 | 99.42 ± 0.03 | 4.01 ± 0.02 | 100.32 ± 0.02 |
| 12 | 12.06 ± 0.02 | 100.06 ± 0.02 | 3.981 ± 0.01 | 99.93 ± 0.01 |

The drug content in the tablets was quantified using the proposed analytical method. The mean content of abacavir sulfate in two different brands of tablet dosage forms is shown in table 4.

**Table 4. Mean ( ± SD) amount of abacavir sulphate in tablet dosage forms by the proposed HPLC methods**

| Brand of the tablet | Labelled amount of drug (mg) | Mean ( ± SD) amount found (mg) by the proposed method (n = 6) | Mean ( ± SD)% labelled amount (n = 6) |
|---|---|---|---|
| $T_1$ | 300 | 300.25 ± 0.03 | 100.31 ± 0.02 |
| $T_2$ | 300 | 299.91 ± 0.04 | 99.91 ± 0.03 |
| $T_1$ = Abamune 300 mg (Cipla) | | $T_2$ = Abavir 300 mg (Genix) | |

The absence of additional peaks indicates no interference of the excipients used in the tablet. The tablets were found to contain 99.98 to 100.31 % of the labelled amount. The low 1% CV indicates the reproducibility of the assay of abacavir sulphate in the tablet dosage form. The proposed reversed phase HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

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