Stability Indicating RP-HPLC Method Development and Validation for Determination of Abacavir Sulphate in Tablet Dosage Form

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ABSTRACT

The present work describes development and validation of a new simple, accurate, precise and selective stability-indicating reverse phase high performance liquid chromatography (RP-HPLC) method for determination of Abacavir sulphate as bulk drug and in tablet dosage form. As stability testing is key step in new drug as well as formulation development, stress degradation studies were performed according to ICH guidelines. Abacavir sulphate was found susceptible to all the analyzed stress conditions except photolysis. Chromatographic resolution of Abacavir sulphate and its degradation products was accomplished by use of Jasco HPLC system equipped with Grace C_{18} column (150 x 4.6 mm i.d.) as stationary phase and mixture comprising of Potassium dihydrogen phosphate buffer: Acetonitrile (70: 30, v/v) as optimum mobile phase. Densitometric detection was carried out at 286 nm. The retention time was found to be 4.507 ± 0.02 min. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. Results were linear in the range of 10 - 35 µg mL^{-1}. The developed method has been successfully applied for the estimation of drug in tablet dosage form.

INTRODUCTION

Abacavir sulphate1-5 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 [1].

Literature survey revealed that several analytical methods such as spectrophotometry [2-5], High Performance Liquid Chromatography (HPLC) [6-22] have been reported for the determination of Abacavir either as single drug or in combination with other drugs in pharmaceutical formulations.

To best of our knowledge, no reports were found in the literature for determination of Abacavir in tablet dosage form by stability-indicating RP-HPLC method. This paper describes simple, precise, accurate and sensitive RP-HPLC method development and validation as well as stability study (hydrolysis, oxidation, photo-degradation and thermal degradation) as per International Conference on Harmonisation Guidelines [23, 24].

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure Abacavir sulphate was obtained as gift sample from Cipla Pvt. Ltd. (Kurkumbh, India). The pharmaceutical tablet dosage form used in this study was Abamune tablets (Cipla Pvt. Ltd., Patalganga, India) labeled to contain 300 mg of Abacavir was procured from the local market. Acetonitrile (HPLC grade), Potassium dihydrogen phosphate (AR grade) were purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

JASCO HPLC system equipped with Model PU 2080 Plus pump, Rheodyne sample injection port (20 μl), MD 2010 PDA detector and Borwin- PDA software (version 1.5). A chromatographic column Grace C_{18} (150 x 4.6 mm i.d. 3µm) was used. Separation was carried out at flow rate of 1 mL min^{-1} using potassium dihydrogen phosphate buffer: acetonitrile (70: 30 v/v) as mobile phase and detection was carried out at 286 nm.

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Preparation of standard stock solution

Standard stock solution of Abacavir was prepared by dissolving 10 mg drug in 10 mL methanol to get concentration of 1000 µg mL^{-1} which was further diluted with mobile phase to get final concentration 100 µg mL^{-1}.

Tablet formulation analysis

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 10 mg of Abacavir was weighed and transferred to 100 mL volumetric flask containing about 60 mL of methanol and ultrasonicated for 15 min and filtered through Whatman paper No. 41 and volume was made up to the mark with the mobile phase. Three mL of this solution was transferred to 10 mL calibrated volumetric flask and volume was made up to the mark with the methanol to get solution of concentration 30 µg mL^{-1} for Abacavir. After setting the chromatographic conditions, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of drug present per tablet was estimated from the calibration curve.

System suitability

The system suitability was assessed by six replicate injections of the standard Abacavir having concentration 30 µg mL^{-1}. The resolution, peak asymmetry, number of theoretical plates and height equivalent to theoretical plate (HETP) were calculated. The values obtained demonstrated the suitability of the system for the analysis of drug. The results obtained are represented in Table 1.

Table No. 1: System suitability parameters for proposed RP-HPLC method

Sr. No.	Parameters	Abacavir
1	Theoretical plates	4570.41
2	HETP (cm)	0.0054
3	Resolution	3.60
4	Asymmetry factor	1.21

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Stress degradation study

The stability studies were accomplished by subjecting the bulk drug to the physical stress and stability was accessed. The study was carried out at concentration of 100 µg µL^{-1}. The hydrolytic studies were performed by treating the stock solution of drug with 0.1N HCl and 0.1 N NaOH at room temperature for 1 h. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 30 µg µL^{-1}. Neutral hydrolysis study was performed by treating the drug with water at room temperature for 1 h. The oxidative degradation was carried out in 6 % H_2O_2 at room temperature for 1 h and sample was diluted with mobile phase to obtain 30 µg µL^{-1} solution. Thermal stress degradation was performed by keeping drug in oven at 80ºC for period of 3 h. Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt h square meter^{-1} for 3 d. Thermal and photolytic samples were diluted with mobile phase to obtain 30 µg µL^{-1} concentration.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary target in developing this stability indicating HPLC method is to achieve the resolution between Abacavir and its degradation products. To achieve the separation, we used C-18 column as stationary phase and mixture of 10 mM potassium dihydrogen phosphate buffer and acetonitrile in ratio of (70:30, v/v) as mobile phase. The tailing factor obtained was less than two and retention time was 4.507 ± 0.02. The representative chromatogram of the standard drug solution is shown in Figure 1.
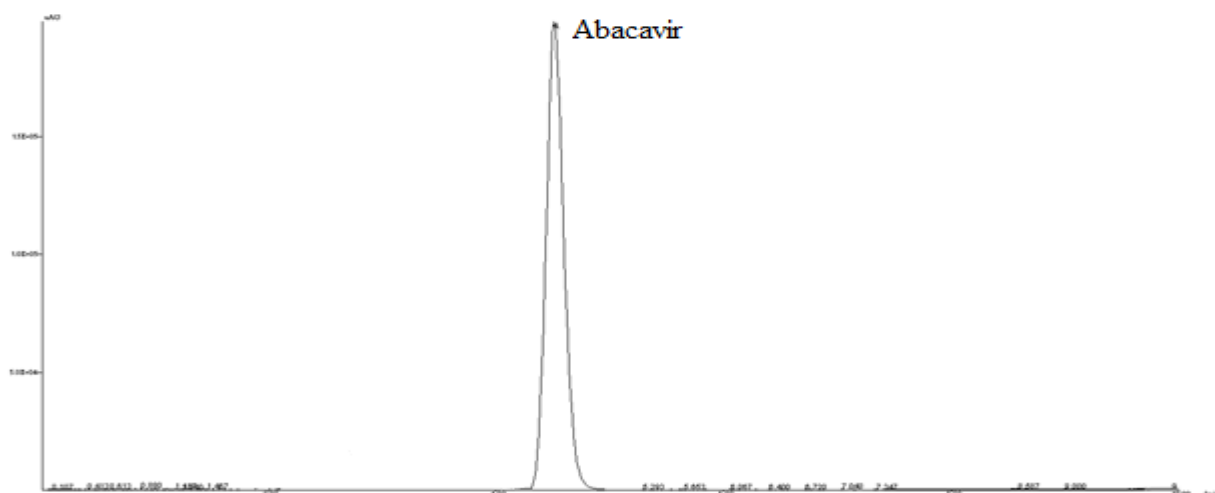
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Figure No. 1: Representative chromatogram of standard drug solution (25 µg mL^{-1}, 4.507 min)

Result of stress degradation studies

The stress degradation outcomes indicated susceptibility of drug to hydrolytic, oxidative and thermal stress conditions and stability to photolytic stress conditions. Stress degradation study demonstrated that the method is highly specific as no degradation products were eluted at retention time of drug. Figures 2-4 symbolizes the chromatograms of acid, alkali and neutral hydrolytic degradation, while Figures 5 and 6 show the chromatograms of oxidative degradation and thermal degradation, respectively. The stress degradation studies data is summarized in Table 2.
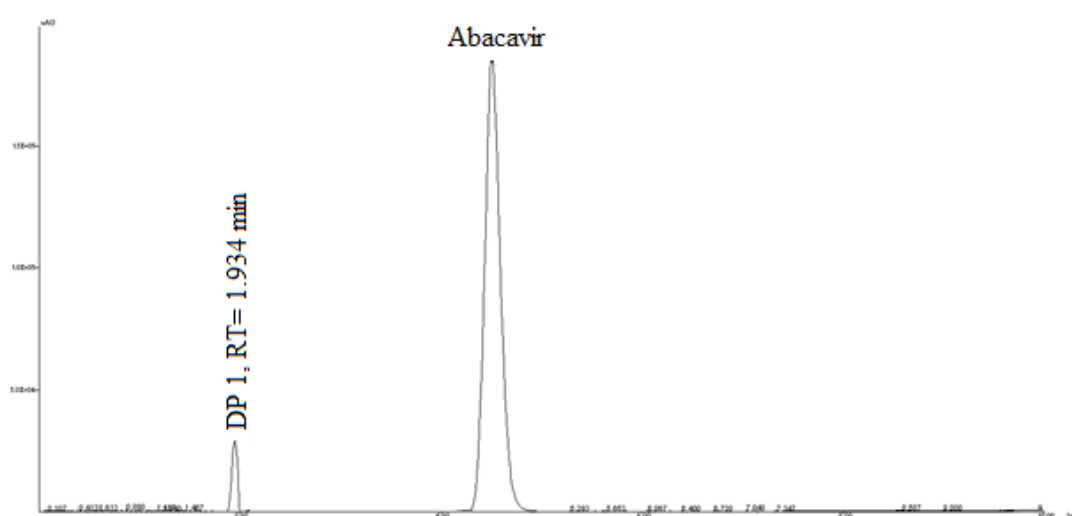

Figure 2: Chromatogram obtained after acid treatment with degradation product (DP1, RT = 1.934 min)
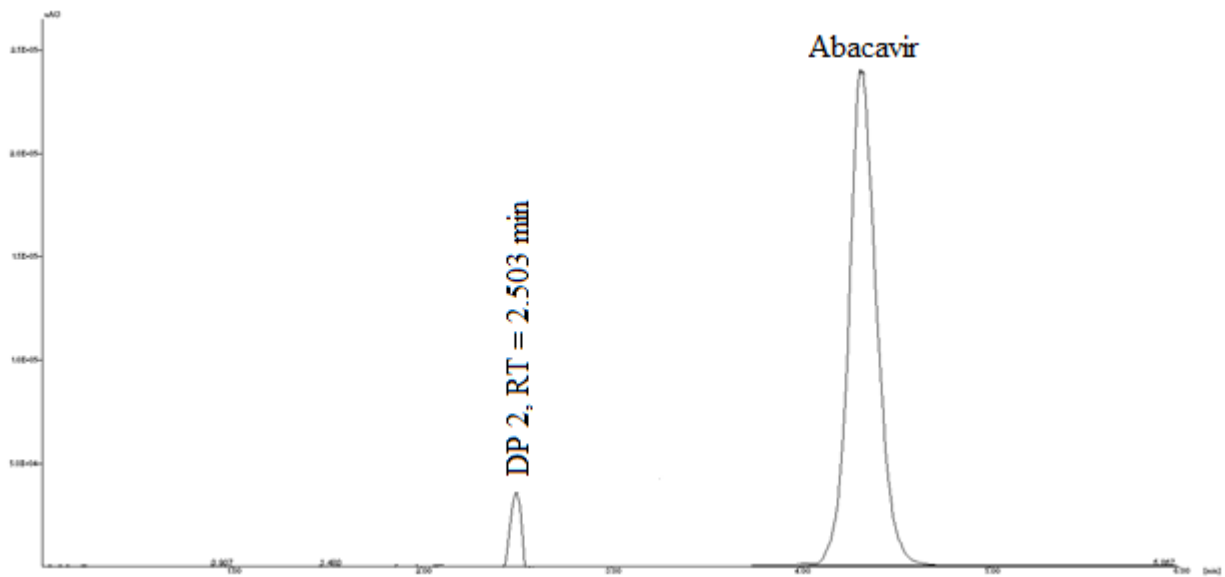
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Figure No. 3: Chromatogram obtained after alkali treatment with degradation product (DP2, RT = 2.503 min)
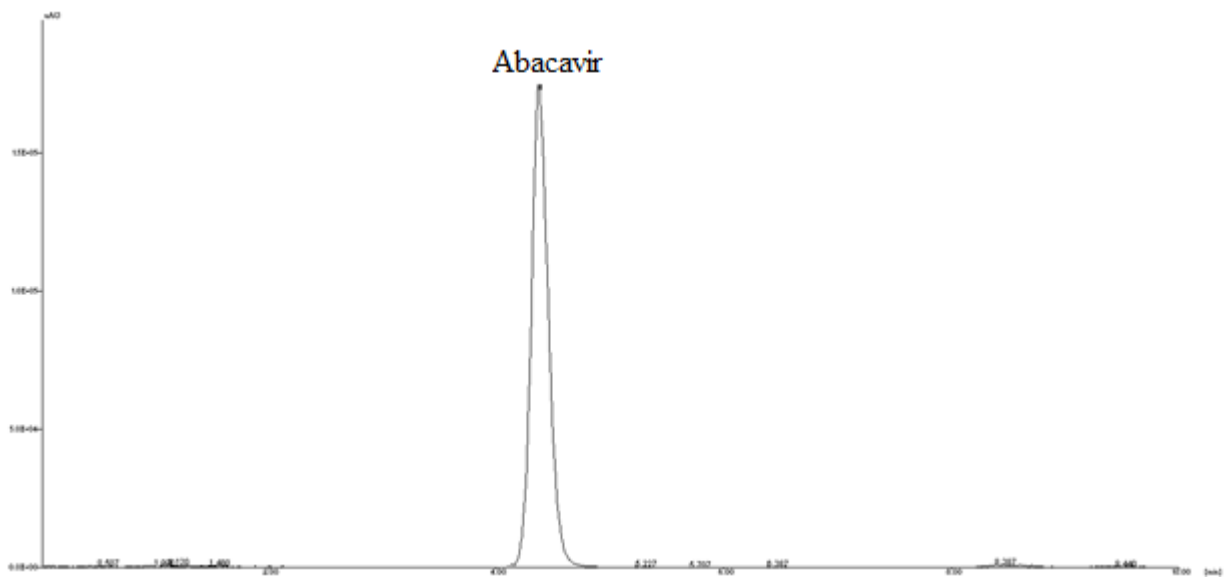
Figure No. 4: Representative chromatogram after neutral hydrolytic degradation

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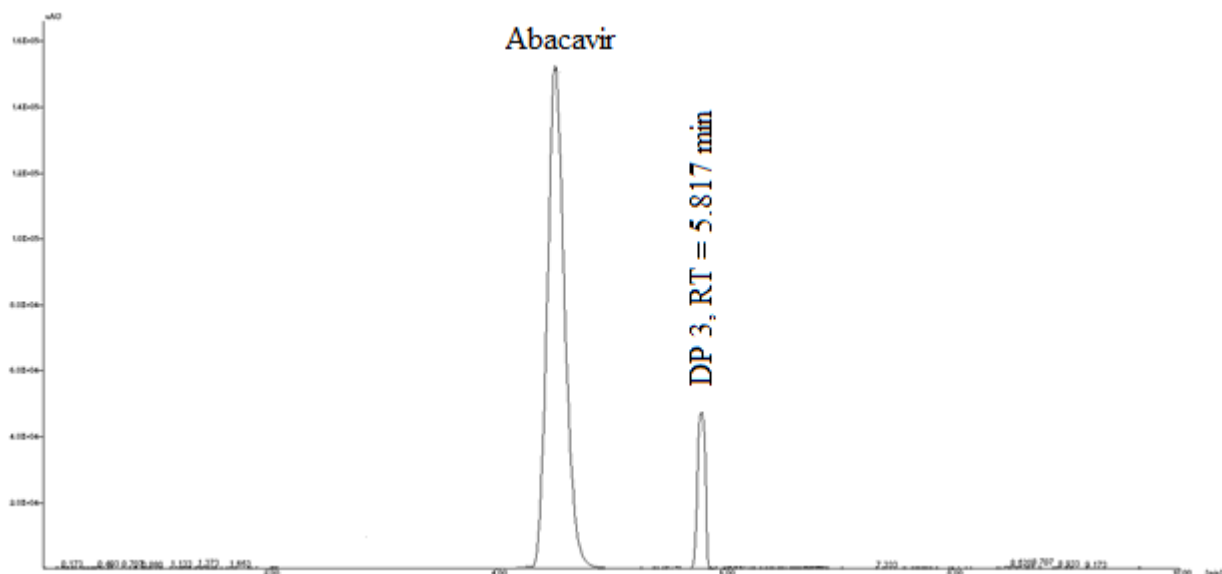
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Figure No. 5: Oxidative degradation chromatogram with degradation product (DP3, RT = 5.817min)
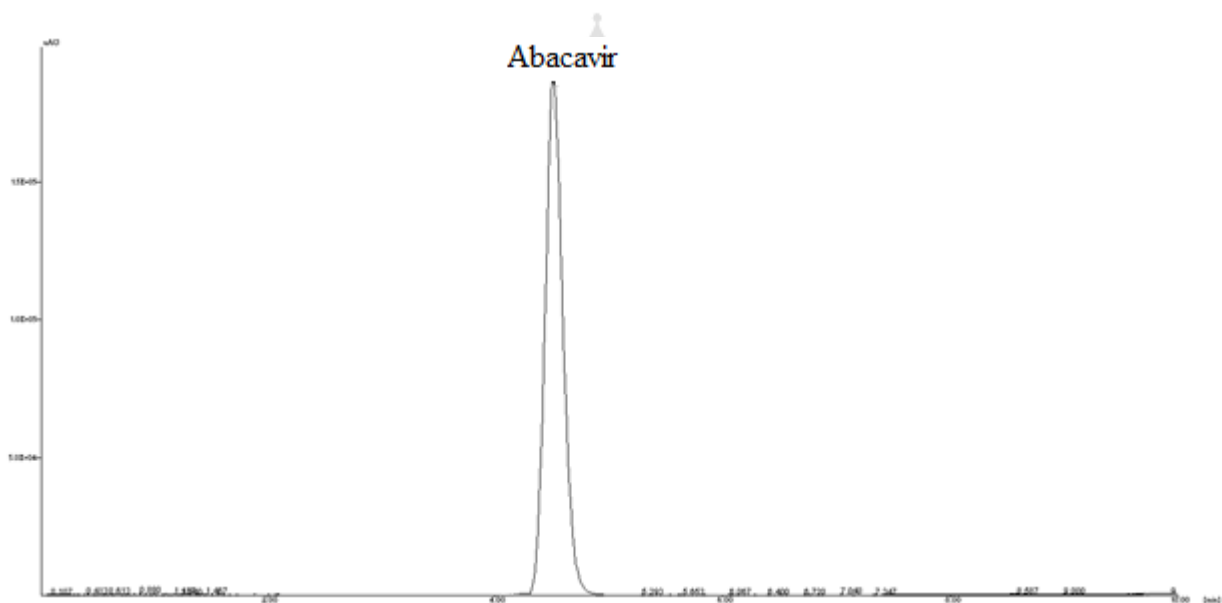
Figure No. 6: Chromatogram obtained after thermal stress

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Table No. 2: Summary of stress degradation studies

Stress conditions/ duration	% Recovered	% Degradation
Acidic / 0.1 N HCl/ Kept at RT for 1 h	85.30	14.69
Alkaline /0.1 N NaOH/ Kept at RT for 1 h	89.84	10.15
Neutral/H_2O/ Kept at RT for 1 h	88.40	11.59
Oxidative /6 % H_2O_2 / Kept at RT for 1 h	83.14	16.85
Dry heat/ 80ºC/ 3 h	91.02	08.97
Photolysis: UV light 200 watt h square meter[-1] 3 d	99.43	----

Method Validation

The method was validated for linearity, accuracy and intra-day and inter-day precision, specificity and robustness, in accordance with ICH guidelines [23].

Linearity

The linearity of the responses of the drug was verified at six concentration levels, ranging from 10-35 µg mL^{-1} for Abacavir. The calibration graph was obtained by plotting peak area versus the concentration and data was treated by least-squares linear regression analysis. The equation of the calibration curve was y = 76549x + 276193. The calibration graph was found to be linear in the plotted concentrations with coefficient of correlation 0.994 for Abacavir. The calibration curve obtained is represented in Figure 7.
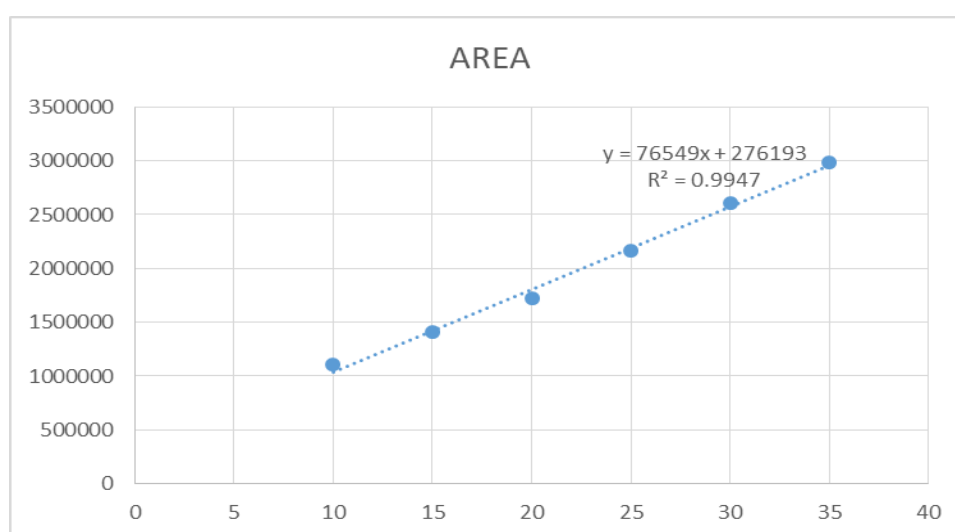

Figure No. 7: Calibration curve for Abacavir

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Precision

One set of three different concentrations of standard solution of Abacavir (10 µg mL^{-1}, 20 µg mL^{-1}, 30 µg mL^{-1}) were prepared. All the solutions were analyzed thrice, in order to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.44 to 1.08. For Inter day variations study three different concentrations of the standard solution in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.49 to 0.91 for Abacavir.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ values were found to be 0.66 µg mL^{-1} and 2.01 µg mL^{-1}, respectively.

Accuracy

Recovery studies were carried out by standard addition method to check the accuracy of the method. It involved addition of standard drug solution to pre-analyzed sample solution at three different levels 80 %, 100 % and 120 %. Basic concentration of sample chosen was 15 µg mL^{-1} from tablet solution. The drug concentrations were calculated from linearity equation. The results of recovery studies indicated the accurateness of the proposed method for estimation of drug in tablet dosage form. The results obtained are shown in Table 3.

Table No. 3: Recovery studies

Drug	Amount taken (µg mL^{-1})	Amount added (µg mL^{-1})	Total amount found (µg mL^{-1})	% Recovery*	% R.S.D.[a]
	15	12	26.65	98.73	1.15
Abacavir	15	15	29.84	99.49	1.58
	15	18	32.68	99.03	1.25

*Average of three determinations, R.S.D. is relative standard deviation

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Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 993, indicating the no interference of any other peak of degradation product, impurity or matrix.

Robustness

The deliberate variations in method parameters were made to check the robustness of the method. The parameters varied were flow rate of the mobile phase ($\pm$ 0.1 mL min^{-1}), mobile phase composition ($\pm$ 2 % acetonitrile) and wavelength ($\pm$ 1 nm) and the effect on the area of drug was noted. It was observed that there were no marked changes in the chromatograms and peak areas of drug, which demonstrated that the developed RP-HPLC method is robust.

CONCLUSION

Stability indicating RP-HPLC method without interference from excipients or degradation products has been developed and validated for determination of Abacavir in tablet dosage form. The developed method is specific, accurate, precise, and robust. As the developed method is stability indicating, it can be used for assessing the stability of Abacavir in bulk drug and in pharmaceutical tablet dosage form.

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