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VALIDATED STABILITY INDICATING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC DETERMINATION OF ABACAVIR SULPHATE IN TABLET DOSAGE FORM

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ABSTRACT
A simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of Abacavir Sulphate has been developed and validated as bulk drug and in tablet dosage form. As stability testing is major step in the development of new drug as well as formulation, stress degradation studies were carried out 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 achieved by using precoated silica gel 60 F_{254} aluminium plates as stationary phase and Chloroform : Methanol (9.2: 0.8, v/v) as mobile phase. Densitometric detection was carried out at 286 nm. The retention factor was found to be 0.25 ± 0.02. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. Results found to be linear in the concentration range of 200-1200 ng band$^{-1.}$ The developed method has been successfully applied for the estimation of drug in capsule dosage form. The proposed method can be used for routine analysis of drug in quality control laboratories and can also be helpful for monitoring the potency during shelf life.

KEYWORDS: Abacavir Sulphate, HPTLC, Validation, Forced degradation, Tablet dosage form.

INTRODUCTION
Abacavir sulphate, 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] has 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 HPTLC method. This paper describes simple, precise, accurate and sensitive HPTLC 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 working standard was obtained as gift sample from Cipla Pvt. Ltd. (Kurkumbh, India). The pharmaceutical dosage form used in this study was Abamune tablets labeled to contain 100 mg of Abacavir sulphate was procured from the local pharmacy. Chloroform, Methanol (all AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions
Chromatographic separation of drug was performed on precoated silica gel 60 F_{254} (10 cm ×10 cm with 250 µm layer thickness) Merck TLC plates as stationary phase using a Camag Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) with the use of solvent mixture comprising of chloroform: methanol (9.2: 0.8, v/v) as mobile phase.

The mobile phase was saturated in chamber for 15 min. After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on Camag thin layer chromatography scanner III at 286 nm for all developments operated by winCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving 10 mg of drug in 10 mL of methanol to get solution of concentration 1000 ng μL^{-1} which was further diluted to 10 mL to furnish final concentration 100 ng μL^{-1}.

Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 286 nm. So, 286 nm was selected as the wavelength for detection.

Estimation of the drug in tablet dosage form

Commercial brand of tablets namely Abamune containing 300 mg of drug was used to estimate the amount of Abacavir sulphate in available tablet formulation. For this, 20 tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 100 mg was transferred to 100 mL volumetric flask containing 50 mL of methanol and the contents were sonicated for 15 min. The solution was filtered using Whatman paper No. 41 and the volume was made up to the mark with methanol to obtain the final concentration of 1000 ng μL^{-1}. The above solution was diluted further with methanol to get final concentration 100 ng μL^{-1}. Four µL volume of this solution was applied on TLC plate to obtain final sample concentration of 400 ng $band^{-1}$. After chromatographic development peak areas of the bands were measured at 286 nm and the amount of drug present in sample was estimated from the calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Forced degradation study

The stability studies were performed by subjecting the bulk drug to the physical stress (hydrolysis, peroxide, heat and light) and stability was accessed. The study was carried out at concentration of 1000 ng μL^{-1}. The hydrolytic studies were performed by treatment of stock solution of drug separately with 0.1N HCl, 0.1 N NaOH and water at room temperature for period of 1 h. The acid and alkali stressed samples were neutralized with NaOH and HCl, respectively to furnish the final concentration of 800 ng $band^{-1}$. The oxidative degradation was carried out in 6 % H_2O_2 at room temperature for 1 h and sample was diluted with methanol to obtain 800 ng $band^{-1}$ solution. Thermal stress degradation was performed by keeping drug in oven at 50ºC for period of 48 h. Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt h square $meter^{-1}$ for 2 d. Thermal and photolytic samples were diluted with methanol to get concentration of 800 ng $band^{-1}$.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The current research work was undertaken with aim to develop stability indicating HPLTC method which would be capable to give the satisfactory resolution between Abacavir sulphate and its degradation products. Diverse solvent systems containing various ratios of benzene, ethyl acetate, glacial acetic acid, chloroform, toluene, ethanol and methanol were examined (data not shown) to separate and resolve spot of Abacavir sulphate from its impurities and other excipients present in formulation. Finally, the mobile phase comprising of Chloroform: Methanol (9.2: 0.8, v/v) gave the best separation of drug with sharp symmetric peak. Densitometric evaluation was carried out at 286 nm. After the developed method had been fully optimized, compact, sharp, and symmetric peak was obtained for Abacavir sulphate with retention factor value 0.25 ± 0.02 as presented in the densitogram in Figure 1.

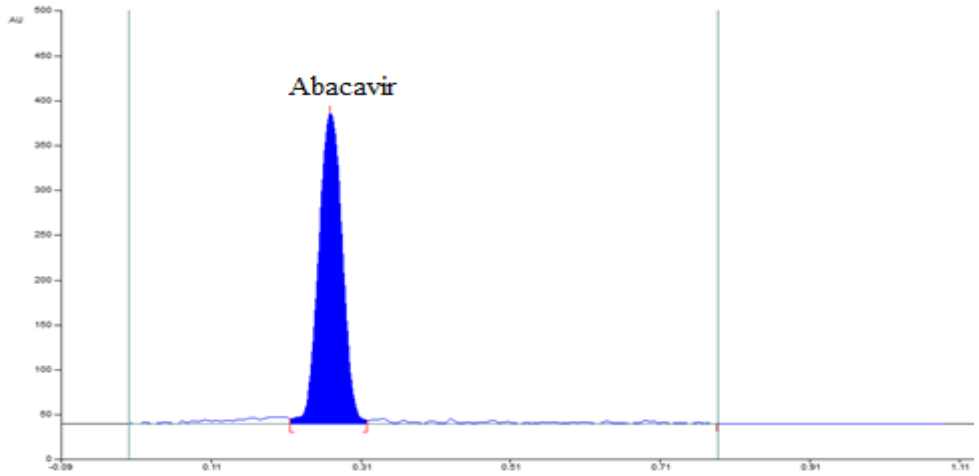

Figure 1: Representative densitogram of standard solution of Abacavir sulphate. (600 ng $band^{-1}$, Rf= 0.25 ± 0.02)

The forced degradation results indicated susceptibility of drug to hydrolytic, oxidative, thermal stress conditions and stability under photolytic stress conditions. Significant degradation product peaks were observed in acidic, basic and oxidative conditions. The degradation products observed after stress degradation were not interfering with the active drug indicating the specificity of the developed procedure. Figures 2-5 represents the densitograms of acid, alkali, neutral and oxidative degradation, while Figure 6 depicts the densitogram of dry heat degradation degradation. The findings of degradation studies are represented in Table 1.

Table 1: Summary of forced degradation studies.

Stress conditions/ duration	%Degradation	% Recovered
Acidic / 0.1 N HCl/ Kept at RT for 1 h	13.31	86.68
Basic/0.1 N NaOH/ Kept at RT for 1 h	10.49	89.50
Neutral/H_2O/ Kept at RT for 1 h	12.37	87.62
Oxidative /6 % H_2O_2 / Kept at RT for 30 min	15.39	84.60
Dry heat/ 50ºC/ 48 h	11.73	88.26
Photolysis: UV light 200 watt h square meter^{-1} 2 d	stable	100.14

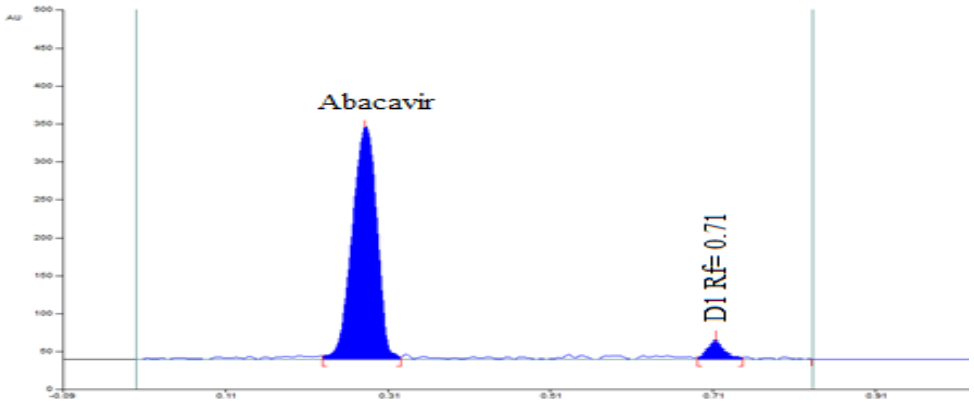

Figure 2: Densitogram after acid hydrolysis with degradation product. (D1, Rf = 0.71)

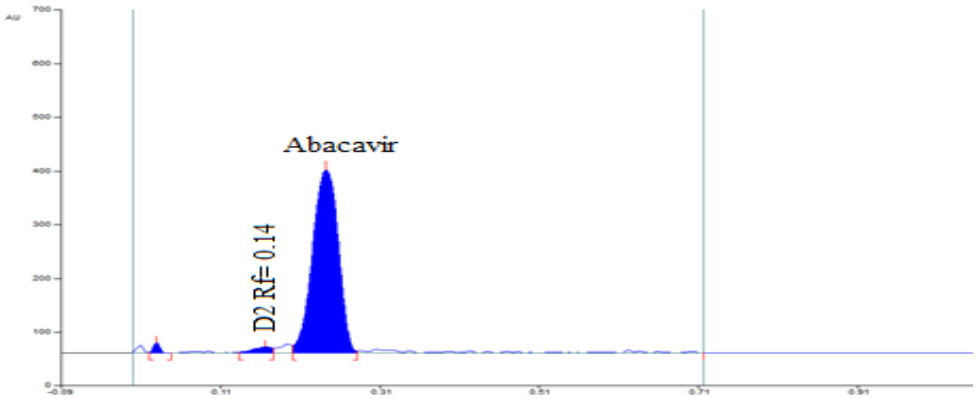

Figure 3: Densitogram after alkali hydrolysis with degradation peak. (D2, Rf = 0.14)

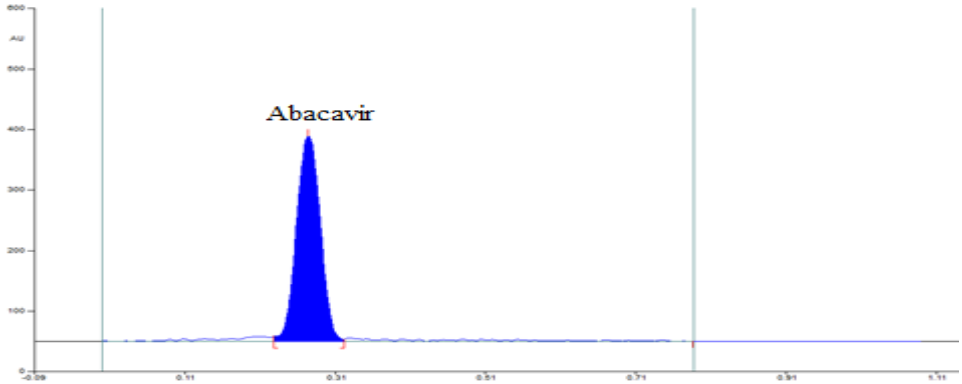

Figure 4: Densitogram after neutral hydrolytic degradation.

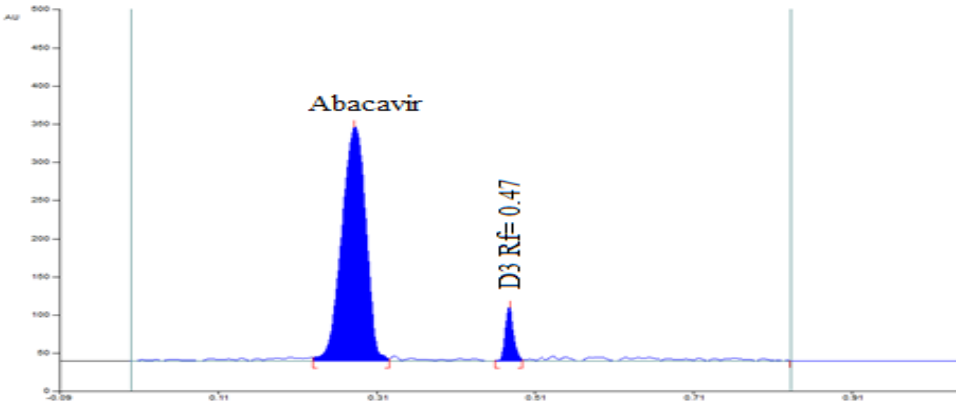

Figure 5: Densitogram after oxidative degradation with degradation product. (D3, Rf = 0.47)

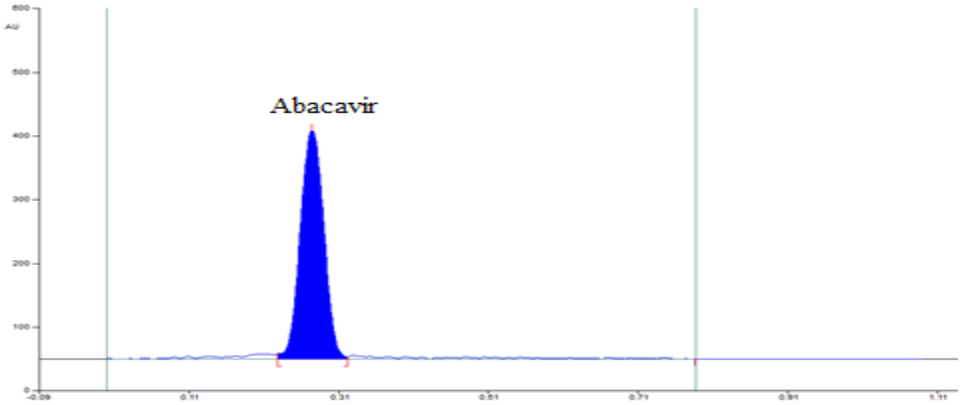

Figure 6: Densitogram obtained after dry heat degradation.

Method validation

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness, in accordance with ICH guidelines.

Preparation of calibration curve

For preparation of a calibration plot, volumes 2, 4, 6, 8, 10 and 12 µL of standard solution of Abacavir sulphate (100 ng μL^{-1}) were spotted onto the TLC plates, developed and scanned under optimized chromatographic conditions. The developed method was found to be linear in the concentration range 200-1200 ng $band^{-1}$ with high correlation coefficient. The linear regression equation was found to be y = 7.5668x + 2016.7 having correlation coefficient 0.994. The calibration curve obtained by plotting concentration vs peak area is represented in Figure 7.

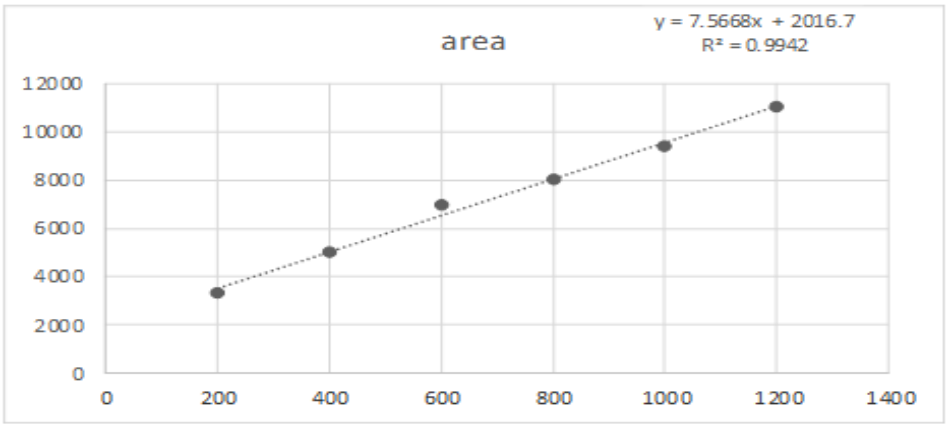

Figure 7: Calibration curve for Abacavir sulphate. (200-1200 ng $band^{-1}$)

Precision

Set of three different concentrations (400, 800, 1200 ng $band^{-1}$) in three replicates of standard solutions of Abacavir sulphate were prepared. All the solutions were analyzed on the same day in order to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.70 to 1.42. For Inter day variation study, three different concentrations of the

standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.47 to 1.70. The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

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 were found to be 39.42 ng band^{-1} and 119.46 ng band^{-1}, respectively.

Recovery studies

Accuracy of developed method was carried out by recovery studies by standard addition method which involved addition of known quantity of standard drug to pre-analysed sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 400 ng band^{-1} from tablet solution. The drug concentrations were calculated from linearity equation. The results of the recovery studies indicated the accuracy of method for estimation of drug in tablet dosage form. The results obtained are represented in Table 2.

Table 2: Recovery studies.

Drug	Amount taken (ng band^{-1})	Amount added (ng band^{-1})	Amount recovered (ng band^{-1})	% Recovery±R.S.D.*
Abacavir sulphate	400	320	727.04	100.97±1.51
	400	400	797.90	99.73±1.08
	400	480	879.45	99.93±0.75

*Average of three determinations

Robustness

Robustness of the method was determined by making intentional variations in method parameters during which mobile phase composition (± 2 % methanol), wavelength (± 1 nm) was altered and the effect on the area of drug was noted. The areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

CONCLUSION

Stability-indicating HPTLC-densitometric method without interference from the excipients or from degradation products has been developed and validated for the determination of Abacavir sulphate as bulk drug and in tablet dosage form. The developed method is simple, precise, accurate, reproducible and specific. The developed method can be used for quantitative analysis of drug in pharmaceutical dosage form. As the method is stability indicating one it may be extended to study the degradation kinetics of drug.

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