A SIMPLE AND SENSITIVE HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF ABACAVIR SULPHATE AND LAMIVUDINE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, sensitive, precise and rapid high performance thin layer chromatographic (HPTLC) method has been developed for the quantitative estimation of abacavir sulphate and lamivudine in tablet dosage form. Abacavir sulphate and lamivudine were chromatographed on silica Gel 60 GF-254 TLC plate. The solvent system selected was methanol:acetone:n-butyl acetate in the ratio of 1:1:2 (v/v/v) and scanning was carried out in the absorbance mode at 284 nm. The spots were found compact for abacavir sulphate with Rf value of 0.58 and for lamivudine with Rf value of 0.35. Linearity of abacavir sulphate and lamivudine were in the range of 240-1200 ng/spot and 120-600 ng/spot, respectively with acceptable value of correlation coefficient. The method was validated for accuracy, precision, robustness and recovery studies. The limit of detection and quantification for abacavir sulphate were obtained 0.691 and 2.093 ng/spot, respectively, while for lamivudine 1.114 and 3.376 ng/spot, respectively. The suitability of this HPTLC method for quantitative determination of abacavir sulphate and lamivudine was proved by validation in accordance with the ICH guidelines. The developed method can be successfully applied in the routine analysis of commercial pharmaceutical tablets.

Keywords: *Abacavir Sulphate; Lamivudine; HPTLC; Method development and validation.*

INTRODUCTION

Abacavir sulphate (Figure 1), is chemically (1*S*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol sulphate[1]. Abacavir sulphate is novel nucleoside reverse transcriptase inhibitor (NRTI) and a potent *in vivo* and *in vitro* inhibitor of HIV-1, the causative agent of the acquired immunodeficiency syndrome (AIDS).
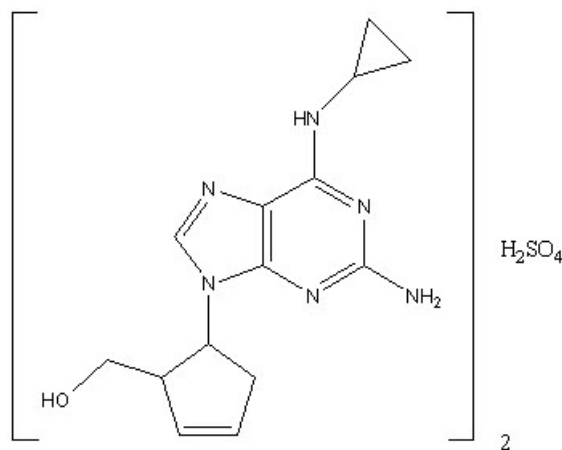

Fig. 1: Structure of abacavir sulphate

Lamivudine (Figure 2), is chemically (-)-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cystosine[2]. Lamivudine is a nucleoside analog having potent *in-vitro* and *in-vivo* inhibitory activity against HIV reverse transcriptase[3]. Literature survey reveals that several HPLC methods with UV detection have been reported to quantify abacavir sulphate[4-14] and lamivudine[4-5],[8-14] along with other anti-HIV drugs in human biological fluids. Several LC-MS-MS methods[15-16] for the simultaneous quantitation of abacavir sulphate and lamivudine with other HIV suppressing drugs have been reported. A simultaneous HPTLC method has been also published for stavudine, lamivudine and nevirapine[17]. No article related to the simultaneous HPTLC determination of abacavir sulphate and lamivudine in tablet dosage form has been reported in literature.

HPTLC methods are useful in the determination of drugs in pharmaceutical formulations especially those containing more than one active component. The aim of the work was to develop a simple HPTLC method for simultaneous quantification of abacavir sulphate and lamivudine in antiretroviral FDCs without the necessity of sample pre-treatment.

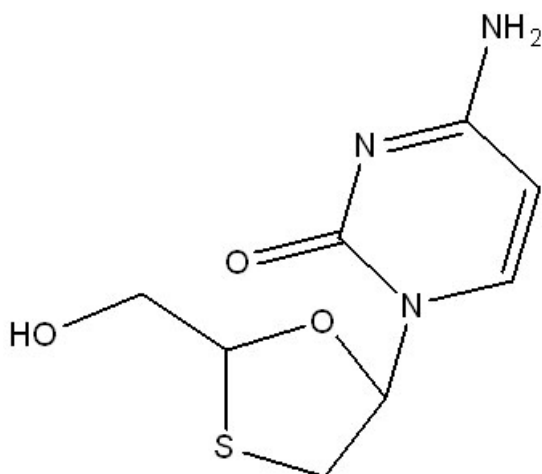***Correspondence :** raman19662002@yahoo.co.in

Fig. 2: Structure of lamivudine

EXPERIMENTAL
Materials and Methods

Standard material of abacavir sulphate and lamivudine was provided by Cipla Ltd, Mumbai. Abacavir and Lamivudine 600/300 (Abacavir sulphate 600 mg + lamivudine 300 mg) tablets manufactured by Cipla Ltd, was purchased from local market. All chemicals and reagents used were of analytical grade. Methanol, acetone, n-butyl acetate were purchased from Merck Chemicals, Mumbai, India.

HPTLC instrumentation

Camag Linomat V applicator was used with Camag TLC scanner III operated with WinCATS software. Twin trough glass chamber (Camag, Muttenz, Switzerland), Camag microlitre syringe and precoated silica gel aluminium plate 60 GF-254, (E. Merck, Darmstadt, Germany) were used.

Standard Preparations

Approximately 24 mg of abacavir sulphate and 12 mg of lamivudine reference substances were accurately weighed and transferred to a 100 mL volumetric flask. The powder was dissolved and diluted up to the mark with methanol, to obtain a solution of abacavir sulphate 240 µg/ml and lamivudine 120 µg/ml.

Sample Preparation

Average weight of 20 tablets were taken and crushed to fine powder. Powder equivalent to about 24 mg abacavir sulphate and 12 mg lamivudine were weighed and extracted with methanol. To ensure complete extraction of the drug it was sonicated for 30 min and volume was made up to 100 ml with methanol (abacavir sulphate 240 µg/ml and lamivudine 120 µg/ml). The solution was filtered through Millipore filter (no.42).

Chromatographic conditions

The plates were washed with methanol and activated at 60°C for 5 min prior to chromatography. The samples were spotted in the form of bands of 6 mm width to the plate using a Camag Linomat V. A constant application rate of 0.1µl/s was employed and space between two bands was set at 5 mm. The slit dimension was kept at 5.00 mm × 0.45 mm and 20 mm/s scanning speed was employed. The monochromator bandwidth was set at 20 nm, each track was scanned thrice and baseline correction was used. The mobile phase consisted of methanol:acetone:n-butyl acetate in the ratio of 1:1:2 (v/v/v) and 10 ml of mobile phase was used per chromatography. Linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber saturated with the mobile phase. After development the TLC plates were dried in a current of air. Densitometric scanning was performed in the reflectance-absorbance mode at 284 nm.

Method validation

The HPTLC method developed was validated with respect to the following parameters as per ICH guidelines[18].

Sensitivity

The sensitivity of the method was determined with respect to LOD, LOQ, linearity range and correlation coefficient. Solution containing 240-1200 ng of abacavir sulphate and 120-600 ng of lamivudine were spotted on TLC plate. The LOD was calculated as 3 times the noise level and LOQ was calculated as 10 times the noise level.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for abacavir sulphate and lamivudine in sample was confirmed by comparing the Rf value and spectra of the spot with that of standard. The peak purity of abacavir sulphate and lamivudine was assessed by comparing the spectra at three different levels, i.e. peak start, peak apex and peak end positions of the spot.

Recovery studies

The recovery studies were carried out by adding known amount of abacavir sulphate and lamivudine standard on pre-analysed sample as 50, 100 and 150% and analyzed by the proposed method, in triplicate. This was done to check the recovery of the drug at different levels in the formulations.

Precision

The intra- and inter-day variation for the determination of abacavir sulphate was carried out at three different concentration levels 240, 720 and 1200 ng/spot and for lamivudine at concentration levels 120, 360 and 600 ng/spot.

Robustness

By introducing small changes in the mobile phase composition and other parameters, the effects on the

results were examined. Mobile phases having different composition like methanol:acetone:n-butyl acetate (1.1:0.9:2, v/v/v), (0.9:1.1:2, v/v/v), (0.9:0.9:2.2, v/v/v), (1.1:1.1:1.8, v/v/v), were tried and chromatograms were run. The plates were prewashed by methanol and activated at 60°C for 2, 5, 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning varied from 0, 20, 40 and 60 min. Robustness of the method was done at three different concentration levels 240, 720 and 1200 ng/spot of abacavir sulphate and 120, 360, 600 ng/spot of lamivudine.

Assay
Standard and sample solutions (3 ml) were separately applied on the TLC plate followed by development and scanning. The amount of abacavir sulphate and lamivudine present in conventional tablets (label claim: 600 mg of abacavir sulphate and 300 mg of lamivudine per tablet), was calculated by comparing the areas measured for the sample with the area measured for the standard solution of abacavir sulphate and lamivudine.

RESULTS AND DISCUSSION
Development of the optimum mobile phase
The composition of the mobile phase for development of chromatographic method was optimized by testing different solvent mixtures of varying polarity. Various mobile phases were evaluated. The addition of n-butyl acetate gave better result. So different concentrations of methanol:acetone:n-butyl acetate were tried. The best result obtained was by using mobile phase consisting of methanol:acetone:n-butyl acetate in the ratio of 1:1:2, v/v/v. This combination of mobile phase offered the optimum migration (Rf values 0.58 for abacavir sulphate and 0.35 for lamivudine) (Fig. 3). Saturation of the TLC chamber with the mobile phase for 30 min assured better reproducibility and better resolution.
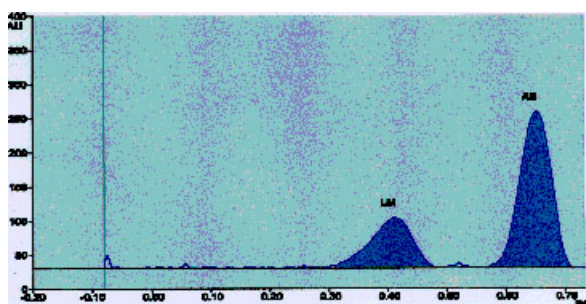

Fig. 3: *HPTLC chromatogram of lamivudine and abacavir sulphate (360&720 ng/spot)*

Calibration curves
The linearity of abacavir sulphate and lamivudine were found in the range of 240-1200 ng/spot and 120-600 ng/spot, respectively, with a correlation coefficient 0.996 for abacavir sulphate and 0.997 for lamivudine.

Sensitivity
The limit of detection was found to be 120 ng/spot for abacavir sulphate and 60 ng/spot for lamivudine. The limit of quantification was found to be 240 ng/spot for abacavir sulphate and 120 ng/spot for lamivudine.

Specificity
The peak purity of abacavir sulphate and lamivudine was assessed by comparing the spectra of standard at peak start, peak apex and peak end positions of the spot i.e., r (start, middle) = 0.9996 and r (middle, end) = 0.9994 for abacavir sulphate and r (start, middle) = 0.9996 and r (middle, end) = 0.9994 for lamivudine were obtained.

Recovery Studies
The proposed method when used for extraction and subsequent estimation of abacavir sulphate and lamivudine from tablet dosage form after spiking with 50, 100 and 150% of additional drug afforded recovery of 98-102% as listed in Table 1.

Table 1. *Recovery studies[a]*

Abacavir sulphate				lamivudine			
Amount of standard added to[c] (in mg)	Amount recovered (mg)	Recovery (%)	% R.S.D	Amount of standard added to[c] (in mg)	Amount recovered (%)	Recovery (%)	% R.S.D
0	198.84	99.42	0.84	0	99.54	99.54	0.75
100	300.12	100.00	0.59	50	151.76	101.1	0.55
200	402.34	100.58	0.43	100	201.76	100.88	0.59
300	503.33	100.66	0.45	150	252.11	100.84	0.74

Precision
The intra-day precision (%RSD) was determined for standard abacavir sulphate (240-1200 ng/spot) and lamivudine (120-600 ng/spot) 3 times on the same day. The inter-day precision (RSD) was calculated for standard abacavir sulphate (240-1200 ng/spot) and lamivudine (120-600 ng/spot) 5 times over a period of one week (Table 2). The %R.S.D. for both drugs was found to be less than 2%. These values indicate that the method is precise.

Table 2. *Intra-day and inter-day precision of abacavir sulphate and lamivudine by HPTLC method[a]*

Drug	Amount (ng/spot)	Intra-day precisbn				Inter-day precision			
		Area	S.D.	% R.S.D.	S.E.[b]	Area	S.D.	%R.S.D.	S.E.
Abacavir Sulphate	240	2323.85	9.65	0.42	3.94	2318.56	11.76	0.51	4.80
	720	6951.43	17.87	0.26	0.14	6954.95	24.87	0.36	0.10
	1200	10154.8	31.87	0.31	0.08	10158.9	35.98	0.35	0.07
Lamivudine	120	875.89	5.65	0.65	2.31	878.76	4.76	0.54	1.94
	360	2623.76	12.87	0.49	0.19	2627.65	12.87	0.49	0.19
	600	4043.65	15.54	0.38	0.16	4039.76	18.76	0.46	0.13

[a] n=3   [b] standard error

Robustness
Statistical analysis showed no significant difference between the results obtained by the developed method and those obtained by variations of some parameters. Thus the method appeared to be robust.

Assay
The content of abacavir sulphate in the tablet varied from 98.50% to 101.45%, while the content of lamivudine varied from 98.15% to 100.51%.

Stability in sample solution

Solutions of two different concentrations (240 and 1200 ng/spot of abacavir sulphate; 120 and 600 ng/spot of lamivudine) were prepared from sample solution and stored at room temperature for 6.0, 12.0, 24.0, 48.0 and 72.0 h, respectively. The solutions were stored in tightly capped volumetric flasks protected from light at normal temperature ($25\pm2^\circ$C). They were then applied on the same TLC plate, after development the chromatogram was evaluated. The % R.S.D. for the samples analyzed at different elapsed assay times was found to be less than 2%. There was no indication of compound instability in the sample solution.

Spot stability

Two dimensional chromatography using same solvent system was used to find out any decomposition occurring during spotting and development. In case, if decomposition occurs during development, peak(s) of decomposition product(s) shall be obtained for the analyte both in the first and second direction of the run. No decomposition was observed during spotting and development.

CONCLUSION

The developed HPTLC technique is precise, specific, accurate and fast. Statistical analysis proves that the method is selective for the analysis of abacavir sulphate and lamivudine in tablet dosage form without any interference from the excipients. Its advantages are use of less quantity of reagents, speed and simplicity of sample treatment than other methods.

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