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VALIDATED HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF LAMIVUDINE AND ABACAVIR SULPHATE IN TABLET DOSAGE FORM

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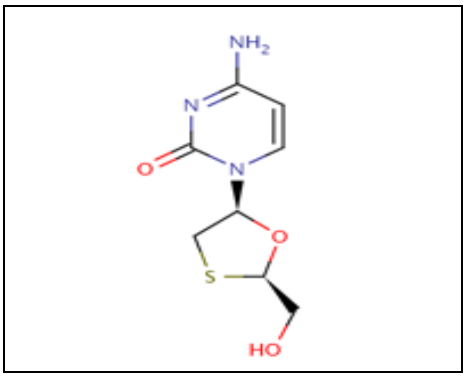
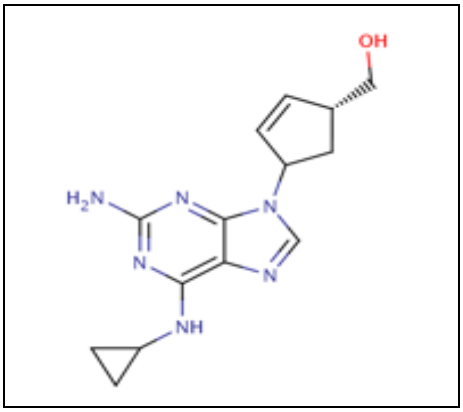
ABSTRACT

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of Lamivudine and Abacavir sulphate in combined dosage forms. The stationary phase was precoated silica gel 60_F254. The mobile phase used was a mixture of (Acetone: chloroform: methanol 4: 4: 2 v/v/v). The detection of spot was carried out at 265nm. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 500 to 3000 ng with regression coefficient of 0.9998. The proposed method can be successfully used to determine the drug content of marketed formulation.

INTRODUCTION: The combination of Lamivudine (LAM) and Abacavir (ABA) has recently been introduced in the market. Chemically Lamivudine [1] is (Lam) 4 amino-1- [2R, 5S) - 2- (hydroxyl methyl) - 1, 3- oxathiolan- 5yl] pyrimidine-2-one. Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) with an activity against human immunodeficiency virus type (1) (HIV) 1 and hepatitis B. Abacavir [2] is (Aba) [1R] - 4- (2 amino-6-cyclopyridine) purine- 9- yl) -1- cyclopent- 2 enyl] methanol. It is a nucleoside reverse transcriptase inhibitor (NRTI) with an activity against human immune deficiency virus Type (1) (HIV1).



LAMIVUDINE



ABACAVIR SULPHATE

The drugs are prescribed invidually, as well as multicomponent dosage forms available in the market. A number of methods have been published for the estimation of above said analytes.

- Spectrophotometric estimation of Abacavir sulphate [3]
- Spectrophotometric estimation of Lamivudine [4]
- Lamivudine in human plasma by RP-HPLC [5]
- Titrimetric and spectrophotometric estimation of Lamivudine [6]

A method was reported for simultaneous analysis of Abacavir and Lamivudine in human plasma by LC/MS/MS [7]. Determination of Abacavir, Lamivudine, Zidovudine in pharmaceutical tablets human serum and in drug dissolution studies by HPLC [8] was also reported in the literature. Literature survey reveals that so, far no HPTLC method has been reported for the simultaneous estimation of ABA and Lam formulation. In the present HPTLC studies investigation, an attempt have been made to develop a rapid, accurate, precise and cost effective HPTLC method for simultaneous estimation of LAM and ABA in combined dosage form.

MATERIALS AND METHODS: LAM and ABA standards were procured as a gift samples from Hetero labs Hyderabad. Silica gel 60_F254TLC plates (4X10cm) were used as stationary phase. All chemicals and reagents used were of analytical grade and obtained from Qualigens. Marketed formulation Abamune-L, manufactured by containing Aba (600mg) and Lam (300mg) was used for the analysis. The instrument used in the present study was Camag Linnomat V- automatic sample applicator, Hamilton syringe (100µl) camag TLC scanner 3, cagmag Wincats soft ware, cagmag Twin through chamber (4x10cm). To carry out HPTLC analysis [9] the TLC plates were prewashed with methanol. Activation of plates was done in an oven at 50°C for 5 minutes. The chromatographic conditions maintained were

precoated silica gel 60_F254 aluminum sheets (4x10cm) as stationary phase, Acetone: chloroform: methanol (4: 4: 2 v/v/v) as mobile phase, chamber and plate saturation time of 30 min, migration distance was 90 mm, wavelength was selected by scanning standard solution of both drugs over 200 nm to 400 nm. LAM showed maximum absorbance at 366 nm and ABA at 254 nm. Both components showed reasonably good response at 265 nm. Therefore photometric measurements were performed at 265 nm absorption mode with cagmag TLC scanner 3 using win CATS soft ware. Stock solutions of LAM and ABA were prepared by separately dissolving 25 mg of Aba and 25 mg of Lam in 25 ml methanol. Further dilution was made by diluting 2.5 ml with mobile phase to obtain 50 µg/ml solutions. Working stock solution were prepared by diluting the stock solution with mobile phase to obtain final concentration of 5, 10, 15, 20, 25 and 30 µg/ml of both drugs (LAM and ABA) were applied separately on the TLC plate.

TLC plate was dried, developed and analyzed photometrically as described earlier. The calibration graph was plotted using peak area against concentration. The procedure was repeated for three times to determine the LOD and LOQ. The marketed formulation Abamune-L contains (300mg of LAM and 600mg of ABA). Twenty tablets were weighed accurately, finely powered and mixed. The average mass per tablet was determined. The powder tablet equivalents to 25 mg of each were accurately weighed and added a minimum quantity of methanol to dissolve the substance the total volume as brought to 25 ml with more methanol (1000 µg/ml) in a volumetric flask. The solutions were sonicated for 10 minutes and then filtered through whatmann filter paper No 41 to separate out the insoluble excipients. Collect the filtrate after rejecting the first portion of the filtrate. From the clear solution, further dilutions were made by diluting 2.5ml to 50ml with mobile phase to obtain 50 µg/ml further dilution was made by diluting 3 ml to 10 ml with mobile phase to obtain 15 µg/ml. 15 micro liters of sample solutions were spotted on to the TLC plate and developed. The analysis was repeated for six times. The content of the drug was calculated from the peak areas recorded. The accuracy of the method was confirmed by recovery studies. The recovery was performed at three different concentrations (20%, 40%, 60%) were added to fixed amount of pre analyzed sample and the amount of each of the drug were determined by the proposed method. Further the precision of the developed method was confirmed by interday and intraday analysis.

RESULTS AND DISCUSSION: A simple, precise, accurate HPTLC method has been developed for the estimation of Lamivudine and Abacavir sulphate in bulk and in tablet formulation. A cagmag HPTLC instrument was used for the analysis. The mobile phase containing acetone: chloroform: methanol (4:4:2 v/v/v). Rf values of 0.09 and 0.34 for LAM and ABA respectively with dense and compact spots desired for quantification of Aba and Lam in pharmaceutical formulations (**fig-1**). The linearity of an analytical method to its ability to elicit test results that are directly or by a well defined mathematical transformation proportional to the concentration of analyte samples with in a given range. The linearity of the method was observed with in the expected range demonstration its suitability for analysis. The linearity concentrations range of 5 to 30µg/ml. The correlation coefficient r^2 value (n=3) for LAM and ABA were found to be 0.9996 and 0.9998 respectively. The value of intercept was less than 2% of the response of 100% of the test concentration in all cases indicates functional linear relationship between the concentration of

analyte and area under the peak area. The proposed method was validated as per ICH guidelines [10, 11].

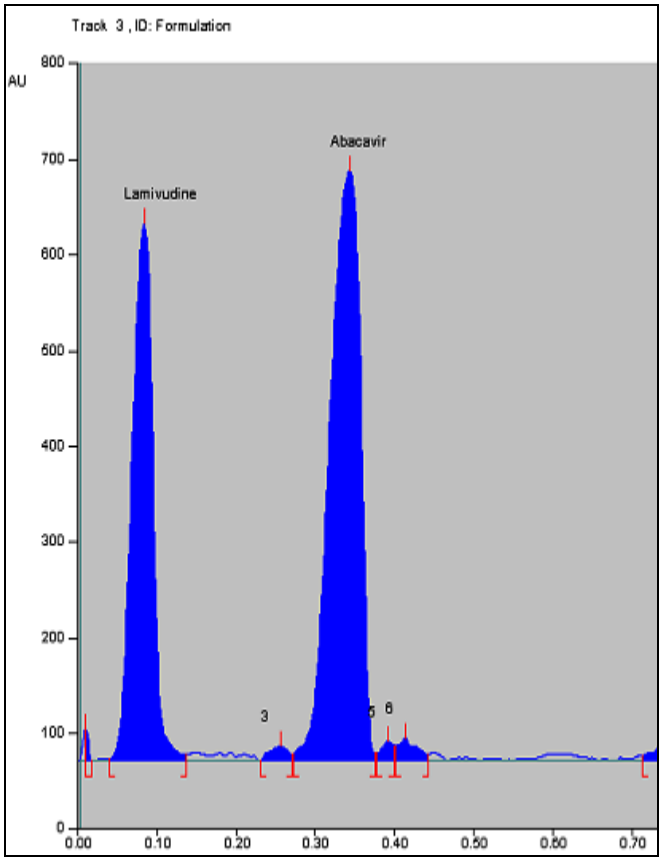


FIG.1: A TYPICAL HPTLC CHROMATOGRAM OF LAM AND ABA

Limit of Quantification was found to be 0.0254 and 0.0105 µg/ml for LAM and ABA respectively. Limit of detection was found to be 0.0083 and 0.0034 µg/ml for LAM and ABA respectively (**Table 1**). Precision is the degree of reproducibility or repeatability of the method under normal operating condition. The method passed the test for repeatability as determined by % RSD [12] of the peak area of six replicate. The % concentration of LAM and ABA were found to be 100.01 ± 0.4300 and 99.50 ± 0.5911 respectively.

TABLE-1: METHOD VALIDATION PARAMETERS

Parameters	Values	
	Lamivudine	Abacavir
Linearity range	5-30µg/ml	5-30µg/ml
Correlation coefficient	0.9996	0.9998
Regression equation	Y=1993.37X+571.05	Y= 3965.88X+279.30
Slope	1993.37	3965.88
Intercept	571.02	279.30
Limit of detection	0.0083	0.0034
Limit of Quantification	0.0254	0.0105

The low % RSD value indicated that the method has good precision. The results of the analysis are shown in **Table 2**. Further the precision of the developed method is confirmed by interday and intraday analysis. The result show good agreement with the label claim of the formulation. To evaluate the accuracy of the method, known amount of pure drug was added to the previously analyzed solution containing pharmaceutical formulation and the mixture was analyzed by the proposed method and the recoveries were calculated.

To evaluate the accuracy of the method, known amount of pure drug was added to the previously analyzed solution containing pharmaceutical formulation and the mixture was analyzed by the proposed method and the recoveries were calculated. The % recovery of LAM and ABA were found in the range of 100.33 ± 99.88 and 99.83 ± 100.41 respectively. The % RSD value for LAM and ABA were found to be 0.2269 and 0.3078 respectively. The low percentage RSD value indicated that there is no interference due to the excipients used in formulation. Hence the accuracy of the method was confirmed (**Table 3**). Specificity is the ability to asses unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix.

TABLE 2: ASSAY OF LAM AND ABA

Formulation	Drugs	Label claim	Amount found	%purity	Average	SD	%RSD
Abamune-L	Lamivudine	300mg	298.642	99.54	100.01	0.4300	0.4299
			301.604	100.53			
			300.114	100.03			
			299.190	99.73			
			299.164	99.72			
			301.604	100.53			
	Abacavir	600mg	599.190	99.86	99.50	0.5911	0.5940
			603.285	100.54			
			594.190	99.03			
			595.266	99.21			
			596.190	99.36			
			594.285	99.04			

TABLE-3: RECOVERY STUDIES OF LAM AND ABA

Label claim	Amount Added	Amount Recovered	% Recovery	Average	SD	%RSD
Lam 300mg	3.00	3.01	100.33	100.12	0.2272	0.2269
	6.00	6.01	100.16			
	9.00	8.99	99.88			
Aba600mg	6.0	5.99	99.83	100.06	0.3080	0.3078
	12.0	12.05	100.41			
	18.0	17.99	99.94			

CONCULSION: The HPTLC method developed for LAM and ABA shows good precision and accuracy. The low % RSD value in the recovery studies indicates that there is no excepients used in the formulation. Hence, it is concluded that the developed method is simple, precise, accurate and rapid for the analysis of LAM and ABA in pure and tablet dosage form. Then the developed method can be adopted for the routine analysis of LAM and ABA in bulk and in tablet dosage form.

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