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DEVELOPMENT AND VALIDATION OF REVERSE PHASE HPLC METHOD FOR DETERMINATION OF LAMIVUDINE AND TENOFOVIR IN BINARY MIXTURE

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ABSTRACT

A Novel, Selective and Rapid, Isocratic Reversed Phase High Performance Liquid Chromatographic (RP-HPLC) method for the analysis of Lamivudine and Tenofovir in binary mixture has been developed and validated. The Liquid Chromatographic system consisting of LC -10AT VP series model chromatograph, the separation was achieved from Symmetry C8 (4.6x150 mm, 5 µm) column using mobile phase, TEA buffer (pH 5.0): Acetonitrile: methanol (30: 40: 30%, v/v/v). The samples were monitored at 260 nm for detection at a flow rate of 1.0 mL/min and the retention time of Lamivudine and Tenofovir was found to be 2.49 and 3.97 mins. Calibration curve was plotted over the concentration range 75-450 µg/mL for Lamivudine and Tenofovir respectively. The proposed method is accurate in the range of 99.98% - 102.00% recovery and precise (%RSD of intraday variation and %RSD of inter day variation were found to be within the acceptable criteria).

Keywords: Lamivudine and Tenofovir, Novel RP-HPLC and Isocratic.

INTRODUCTION

Lamivudine is chemically known as 4-amino-1-[(2*R*, 5*S*)-2-(hydroxyl methyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one.
Tenofovir is chemically known as({[(2*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2l]oxy}methyl) phosphoric acid. Based on the literature review only one HPLC was reported in combination with Lamivudine and Tenofovir. Anandakumar Karunakaran[4 et] *al.,* proposed a HPLC Method.

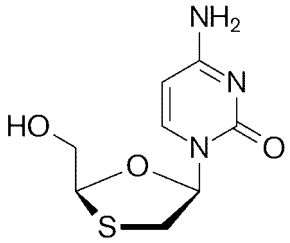

Fig-1: Lamivudine

A few other Spectroscopic[8] and HPLC methods have also been developed and reported for Lamivudine in combination with Efavirenz and Tenofovir[5]. Some other LC-MS/MS[6] methods have been reported for Lamivudine and Tenofovir in combination with Zidovudine, Abacavir, Emtricitabine and Nevirapine. Hence the author attempted to develop a method using economical and eco-friendly solvents to enhance the column life and validated as per ICH norms.

Fig 2: Tenofovir

MATERIALS AND METHODS:

Instrumentation:

The liquid chromatographic system consisting of the following components was used for analysis. LC -10AT VP series model chromatograph equipped with symmetry C8 (4.6 x 150mm, 5 µm) was employed for the study. Sample injection was done and the output signal was monitored and integrated by Spinchrome software.

Drugs and chemicals: The reference standard Lamivudine and Tenofovir were obtained from Hetero Drugs Ltd. Methanol, orthophosphoric acid, and triethylamine was of HPLC grade, (Merck Ltd. Mumbai, India) milli-Q water was used for the analysis.

Preparation of TEA (Triethylamine) buffer pH 3.0: 1 mL of TEA (Triethylamine) was transferred into 250 mL milli-Q water and pH of the solution was adjusted to 3.0 with orthophosphoric acid.

Preparation of mobile phase: 400 mL buffer pH-3.0 was mixed with 600 mL of methanol and sonicated for 5 mins to degas and filtered through 0.45 µm filter under vacuum filtration.

Preparation of Standard Solution: 30 mg of Lamivudine & Tenofovir were weighed and transferred into 100 mL volumetric flask, about 40 mL of diluent was added and sonicated for 5 minutes to dissolve it. The volume was made up with mobile phase, filtered through 0.45 µm membrane filter.

Preparation of Sample Solution: Weigh and transfer 5 tablets were weighed and transfer into 100 mL volumetric flask, about 35 mL of diluent was added, sonicated for about 30 mins, volume was made up with diluent and mix well. 2 mL of above solution was pipette and transferred into 100 mL volumetric flask and diluted with diluent up to 100 mL. Centrifuge about 10 mL of the sample solution at 4000rpm for 10 minutes, the supernatant solution was collected and now the sample of 20 µL was injected and chromatogram was recorded.

Procedure: The column was equilibrated for at least 30 minutes with mobile phase flowing through the system with flow rate of 0.8 mL/minute. Detection was set at a wavelength of 260 nm, with the optimized chromatographic conditions a steady base line was recorded. Separately inject appropriate aliquots (20 µL) of diluent standard preparations and sample preparations into the chromatograph, the chromatograms were recorded and measured the peak area responses for the major peak. The chromatogram of Lamivudine and Tenofovir blank, placebo and standard were shown in Figure: 3 and 4.

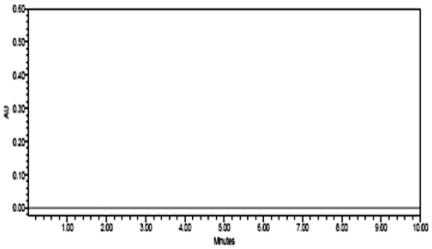

Figure: 3 A Typical Chromatogram of Lamivudine and Tenofovir Blank.

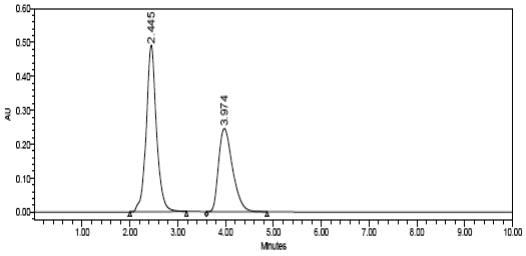

Figure: 4 - A Typical Chromatogram of Lamivudine and Tenofovir Standard.

PRECISION: Precision was studied to find out intra and inter day variations of the proposed method at three different levels (150, 300 and 450 µg/mL for Lamivudine and Tenofovir) on the same and on three different days respectively and the results are tabulated in the Table: 1.

ACCURACY : The accuracy of the HPLC method was confirmed by recovery studies by spiking 50, 100 & 150% of pure drugs (150 µg/mL 300 µg/mL and 450 µg/mL for Lamivudine and Tenofovir) to the pre analyzed samples and the samples after dilution injected into the system (n=3). The peak area of each drug was measured and the recovery values for Lamivudine and Tenofovir were given in the Table: 2

Table: 1 Intraday and Inter Day Precision

Conc. (µg/mL)	Inter-day			Intra-day			Over all %RSD
	Mean Amount Found (µg/mL)	±SD	%RSD	Mean Amount Found (µg/mL)	±SD	%RSD	
Lamivudine (n=3)							
150	149.970	0.020	0.013	149.993	0.015	0.010	0.011
300	300.007	0.015	0.005	299.983	0.006	0.002	0.006
450	449.997	0.021	0.005	449.977	0.023	0.005	0.003
Tenofovir (n=3)							
150	149.997	0.012	0.008	149.983	0.006	0.004	0.007
300	299.997	0.021	0.007	299.993	0.015	0.005	0.001
450	450.007	0.015	0.003	449.990	0.020	0.004	0.003

Table: 2 Accuracy of Lamivudine and Tenofovir

Amount added (µg/mL)	Accuracy of Lamivudine				Accuracy of Tenofovir			
	Amount found (µg/mL)	% Recovery	Statistical Analysis of % recovery		Amount found (µg/mL)	% Recovery	Statistical Analysis of % recovery	
150	150.01	100.007	MEAN	150.01	150.01	100.007	MEAN	100.002
	149.99	99.993	SD	150.02	150.02	100.013	SD	0.014
	149.98	99.987	%RSD	149.98	149.98	99.987	%RSD	0.014
300	300.01	100.003	MEAN	300.01	300.01	100.003	MEAN	99.996
	299.99	99.997	SD	299.98	299.98	99.993	SD	0.007
	300.01	100.003	%RSD	299.97	299.97	99.990	%RSD	0.007
450	449.990	99.998	MEAN	449.99	449.99	99.998	MEAN	100.001
	449.970	99.993	SD	450.01	450.01	100.002	SD	0.003
	449.980	99.996	%RSD	450.02	450.02	100.004	%RSD	0.003

Linearity & Range

A linear response of peak area was observed over the concentration range 75-450 µg/mL for Lamivudine and Tenofovir respectively. The calibration curves of Lamivudine and Tenofovir are presented in Figure: 5 and 6. The regression data was summarized in the Table: 3 and 4 for Lamivudine and Tenofovir respectively

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Table: 3 Linearity for Lamivudine

Linearity Conc. (µg/mL)	Average Area	SD	%RSD
75	166047	1739.066	1.047
150	330529	279.136	0.084
225	495534	291.232	0.059
300	659681	911.107	0.138
375	824088	592.698	0.072
450	989543	103.326	0.010

Figure:5 Linearity graph for Lamivudine

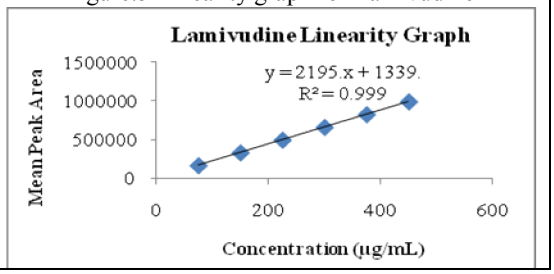

Table: 4 Linearity for Tenofovir

Linearity Conc. (µg/mL)	Average Area	SD	%RSD
75	115378	415.038	0.360
150	228448	1240.827	0.543
225	344566	396.777	0.115
300	459526	676.315	0.147
375	574085	736.919	0.128
450	689673	151.040	0.022

Figure:5 Linearity graph for Tenofovir

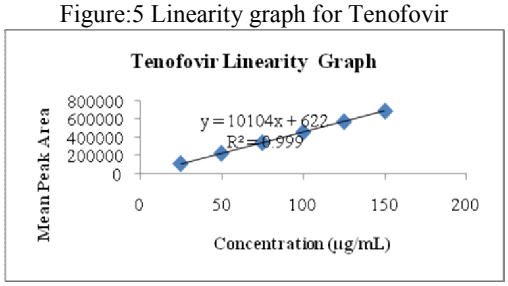

Table: 5 Recovery of Lamivudine and Tenofovir Tablets

Drug	Label claim (mg/tablet) *N=3	*Amount found (mg/tablet) ± SD	% Recovery	% RSD
Lamivudine Tenofovir	300	300.007 ± 0.057	100.002	0.0153

RESULTS AND DISCUSSION:

By applying the proposed method, the retention times of Lamivudine and Tenofovir were found to be 2.44 mins and 3.97 mins respectively. Quantitative linearity was obeyed in the concentration range of 75-450 µg/mL for Lamivudine and Tenofovir respectively. The regression equations of concentration of Lamivudine and Tenofovir over their peak areas were found to be y=2195.x+1339 (R^2=0.999) and y=10104.x+622 (R^2 = 0.999) respectively, The numbers of theoretical plates obtained were 2687 and 3823 for Lamivudine and Tenofovir respectively, which indicates the efficiency of the column.The limit of detection and limit of quantitation were found to be 0.0256, 0.0852 and 0.0845, 0.0281 µg/mL for Lamivudine and Tenofovir respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

CONCLUSION:

A simple, specific, accurate, precise, reverse phase High Performance Liquid Chromatography method has been developed which can be used for accurately quantitative estimation of Lamivudine and Tenofovir for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R1) so it can be used by pharmaceutical industries.

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