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DEVELOPMENT AND VALIDATION OF RP HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TENOFOVIR DISPROXIL FUMERATE AND LAMIUDINE IN COMBINED DOSAGE FORM

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ABSTRACT

A highly sensitive isocratic reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Tenofovir disproxil fumerate and Lamivudine in Bulk drug and Pharmaceutical dosage forms. Separation of Lamivudine and Tenofovir successfully achieved on symmetry C18 (4.6 x 150mm, 5μm, Make: Thermosil) or equivalent utilizing HPLC Methanol and Water in the ratio of 70:30 v/v as mobile phase at a flow rate of 1mL/min and the eluates was monitored at 270 nm. Chromatogram showed peak at a retention time of 2.318 ± 1 min and 3.535 ± 1 min. The method was validated for system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, LOD and LOQ. Recovery of Tenofovir disproxil fumerate and Lamivudine were found to be in the range of 100.4% and 100.5% and showing linearity in the range of 20-60 µg / ml. The S/N for LOD and LOQ for estimation of Tenofovir disproxil fumerate and Lamivudine were found to be 3.04 µg / ml and 9.94µg/ml and 2.97µg/ml and 9.98µg/ml respectively. Proposed method can be successfully applied for the quantitative determination of Tenofovir disproxil fumerate and Lamivudine in Bulk drug and Pharmaceutical dosage form.

KEYWORDS: Lamivudine, Tenofovir disoproxil fumerate, RP-HPLC, methanol and water.

INTRODUCTION

Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTIs). Chemically it is 4 – amino – 1 – [(2R, 5S) – 2 – (hydroxyl methyl) – 1, 3 – oxathiolan – 5 – yl] – 1, 2 – dihydro pyrimidin – 2 – one. It can inhibit both types (I and II) of HIV reverse transcriptase and also the reverse transcriptase of Hepatitis B. Tenofovir disoproxil Fumarate is fumaric acid salt of the bis isopropoxy carbonyl oxy methyl ester derivative of tenofovir. Chemically it is 9-[(R)-2-[[(isopropoxcarbonyl)- oxy] methoxy] phosphinyl] methoxy] propyl] adeninefumarate [1-3]. Fig.1 show the nucleotide reverse transcriptase inhibitors (NtRTIs) used in combination for the treatment of HIV infection. Lamivudine is official in IP [4], BP [5] and USP [6]. Tenofovir disoproxil fumerate is official in IP [7]. Literature survey reveals that Tenofovir disoproxil fumerate is estimated individually by UV [8], derivative-HPLC [9], Plasma RP-HPLC [10-11] and Plasma LC/MS/MS [12-14] methods. Similarly for Lamivudine, HPLC [15], Titrimetry [16-17] and HPLC in plasma

[18-20] were reported. Few RP-HPLC [21-23] methods were reported for estimation of Emtricitabine, Tenofovir and efavirenz in pharmaceutical formulation.

RP-HPLC [24] and LC-MS/MS [25] and HPTLC [26] methods were reported for the simultaneous estimation of Emtricitabine and Tenofovir disoproxil fumerate in human plasma and in formulations. Also UV [27-32], HPLC [33-39], LC – MS [40], HPTLC [41-42] and enzymatic assay [43] methods were reported for the simultaneous estimation of Lamivudine with other antiretero viral drugs. To the best of our knowledge, there is no reported RP-HPLC method for simultaneous estimation of Lamivudine and Tenofovir disoproxil fumerate in pharmaceutical formulations, previous to our work. Thus, efforts were made to develop fast, selective and sensitive analytical method for the estimation of Lamivudine and Tenofovir disoproxil fumerate in their combined dosage form using reverse phase high performance liquid chromatographic method.

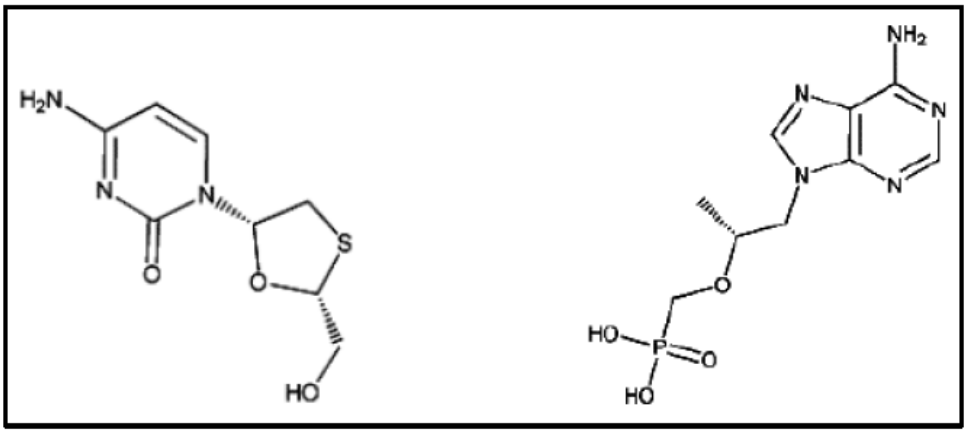
Now the authors report a simple, reliable and reproducible RP-HPLC method which was duly validated by statistical parameters precision, accuracy and recovery. The method has been satisfactorily applied to the simultaneous estimation of Lamivudine and Tenofovir disproxil fumerate in bulk and pharmaceutical dosage forms.

EXPERIMENTAL

Materials

Tenofovir disoproxil fumerate and Lamivudine was obtained as a gift sample from Aurobindo Pharma Ltd, Hyderabad. HPLC grade Methanol (Ramkem), and Milli-Q water was used in mobile phase preparation. Commercially available Lamivudine, and Tenofovir disoproxil fumerate dosage forms (TENVIR-L) (Vireday, Cipla) were purchased from local market.

Figure 1: Chemical structures of Lamivudine and Tenofovir disoproxil fumerate



Instrument/Equipment details

Waters High Performance Liquid Chromatography with auto sampler and UV-visible detector and Auto injector mode was used with EMpower software, Analytical Balance (Lab India), UV Spectrophotometer (Lab India), Sonicator (Lab India), Vaccum pump.

Chromatographic conditions

Chromatographic separations were achieved by symmetry C18 (4.6 x 150mm, 5μm, Make: Thermosil) or equivalent utilizing HPLC Methanol and Water in the ratio of 70:30 v/v as mobile phase at a flow rate of 1mL/min and the eluates was monitored at 270 nm.

Preparation of mobile phase

Mix a mixture of Methanol HPLC 700 mL (70%) and 300 mL of HPLC Water (30%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 μ filter under vacuum filtration. Mobile phase used as Diluent.

Preparation of Standard Stock solution

Accurately 10 mg of Tenofovir disoproxil fumerate and Lamivudine standards were weighed and taken in10 ml volumetric flask. Dissolved by sonication in 7 ml of Diluent (in a ratio of 70:30 Methanol and HPLC water) and then diluted to10 ml with the Diluent to get 1mg/ml standard stock solution.

Preparation of working Standard solution

0.4 ml of the above standard stock solution was taken in 10 ml volumetric flask and made up to 10 ml with diluent to get a concentration of 40μg/ml for Tenofovir disproxil fumerate and Lamivudine.

Preparation of sample solution

10 Tablets of TENVIR-L commercially available tablets (Tenofovir and Lamivudine formulation) were weighed and powdered in glass mortar. The powder equivalent to the 10mg of active ingredient was transferred into a 10 ml volumetric flask, 7 ml of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent and allowed to stand until the residue settles before taking an aliquot for further dilution. 0.4 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 μm filter before injecting into HPLC system.

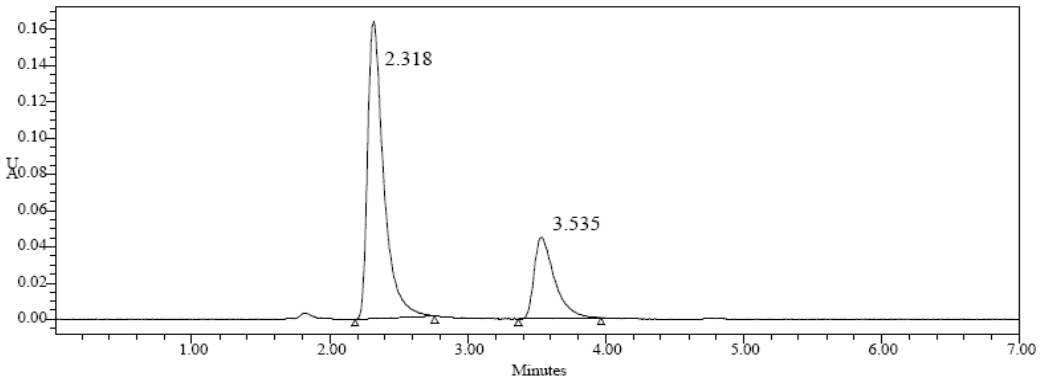


Figure: 2 Typical Chromatogram of Lamivudine (2.318min) and Tenofovir (3.535min

Linearity

Adequate dilutions were made from stock solution to get concentration ranging from 20-60 µg/ml for Lamivudine and Tenofovir disoproxil fumerate respectively. Evaluation was performed with UV-visible detector at 270 nm and Peak area was recorded for all the peaks and a Calibration graph was obtained by plotting peak area versus concentration of Lamivudine**(Fig 2 A)**, and Tenofovir disoproxil fumerate **(Fig 2 B)**. The plot of peak area of each sample against respective concentration was found to be linear in the range of 20-60 µg/ml with correlation coefficient of 0.999 and 0.999

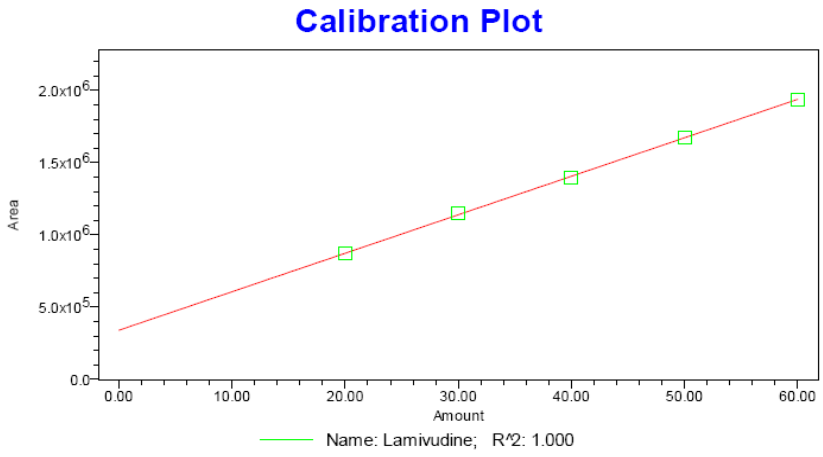


Figure 3 A: Calibration curve of Lamivudine by

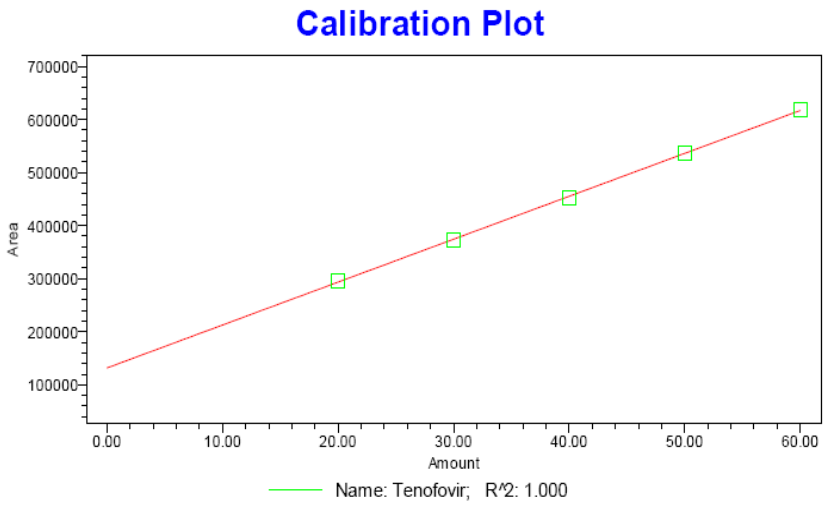


Figure 3 B: Calibration curve of Tenofovir Disproxil by HPLC

RESULTS AND DISCUSSION

As per the USP-XXVI system suitability tests were carried out on freshly prepared standard stock solution ofLamivudine and Tenofovir disoproxil fumerate. Parameters that were studied to evaluate the suitability of the system are given in Table 1. These parameters indicate good sensitivity, more ruggedness and robustness of the method.

From the typical chromatogram of Lamivudine and Tenofovir disoproxil fumerate as shown in fig 2, it was found that the retention times 2.318mins for Lamivudine and 3.535mins for Tenofovir disoproxil fumerate. Methanol and water in a ratio 70:30v/v as mobile phase was found to be most suitable mobile phase combination to obtain well defined peaks with sharp peak shapes, high theoretical plates and less tailing. In the present developed HPLC method, the standard and sample preparation involve very simple extraction procedure and required very less time. A good linear relationship (r=0.999) was observed for Lamivudine and Tenofovir Disoproxil fumerate in the concentration range of 20-60 µg/ml. The percentage assay was found to be 99.8% for Lamivudine and 99.9 % for Tenofovir disoproxil fumerate in tablets. Recovery studies shows good extraction and recovery from 50% to 150% of test concentration. It was found percentage recovery was about100.36% for Lamivudine and 100.43 % for Tenofovir disoproxil fumerate indicates good extraction and good recovery and accuracy of the method. There is no additional peaks in the chromatogram at the main peak Retention times indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive, rugged and reproducible.

Table: 1 VALIDATION PARAMETERS OF THE PROPOSED METHOD FOR LAMIVUDINE AND TENOFOVIR

S.NO.	PARAMETERS			RESULT		LIMIT
				Lamivudine	Tenofovir	
1.	System Precision (n=6)	% RSD		1.76	0.64	NMT-2.0%
2.	Method Precision (n=6)	% RSD		1.76	1.78	NMT-2.0%
3.	Assay	% Mean Assay		99.8%	99.9%	95-105%
4.	Accuracy	% Recovery	50 %	100%	99.7%	% Recovery 98-102
			100 %	99.8%	99.9%	
			150 %	101.3%	101.7%	
5.	Robustness	% RSD	Flow -	1.75	1.81	% RSD NMT 2.0.
			Flow +	1.78	1.79	
			Organic -	1.74	1.84	
			Organic+	1.79	1.79	
6.	Ruggedness	Day-1, Analyst-1		0.811	1.09	% RSD NMT 2.0.
		Day-2, Analyst-2		0.811	1.09	
7.	S/N for LOD			2.97	3.04	Shall be 3
8.	S/N for LOQ			9.98	9.94	Shall be 10

CONCLUSION

A method was developed for the simultaneous estimation of Lamivudine and Tenofovir disproxil fumerate in bulk and pharmaceutical dosage forms which is simple, quick, reliable, inexpensive and simple. The results indicate that the described method can be used for quantitative analysis of the compound.

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