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New validated RP-HPLC method for simultaneous estimation of lamivudine and Tenofovir disproxil fumarate in tablets

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Abstract

A simple, specific and precise reverse phase high performance liquid chromatographic method was developed and validated for simultaneous estimation of Lamivudine and Tenofovir disproxil fumarate in tablets. Quantification was achieved by using a reverse-phase C18 column (Inertsil ODS 3V, 250 mm x 4.6 mm; 5µ) at 31°C. The mobile phase consisted of a mixture of phosphate buffer and acetonitrile in the ratio of 55:45 v/v at a flow rate of 1.2 mL/min. The retention times of Lamivudine and Tenofovir disproxil fumarate were found to be 2.430 min and 4.550 min respectively. The developed method was validated as per ICH Guidelines for linearity, accuracy, precision, detection limit, quantification limit, ruggedness, robustness, specificity and system suitability. The percentage recoveries for both of the drugs from their tablets were found to be 98.48 % and 98.64 % respectively. The method may successfully be employed for the simultaneous determination of Lamivudine and Tenofovir disproxil fumarate in pharmaceutical tablet dosage forms.

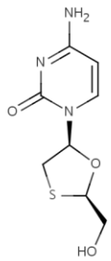
Keywords: Lamivudine; Tenofovir disproxil fumarate; RP-HPLC; tablets.

1. Introduction

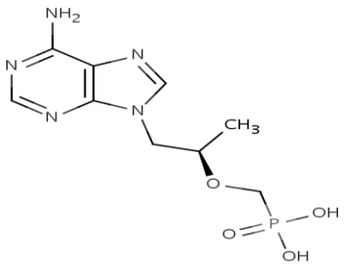
Lamivudine[1]-[3] is potent nucleoside analogue reverse transcriptase inhibitor, chemically [4-amino-1-[(2R, 5S)-2- (hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2- dihydropyrimidin-2-one] [Fig. 1]. The drug is soluble in water and sparingly soluble in methanol. It can inhibit both types (I and II) of HIV reverse transcriptase of Hepatitis B. This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination.

Tenofovir[4]-[9] is 9-[(R)- 2[[(isopropoxcarbonyl)-oxy] methoxy]phosphinyl] methoxy] propyl] adenine fumarate [Fig. 2]. The drug is soluble in water and methanol. Tenofovir is a nucleotide analogue reverse transcriptase inhibitor, which block reverse transcriptase, an enzyme useful in viral production.It is used in the treatment of HIV and Chronic Hepatitis B. The structure of a) Lamivudine and b) Tenofovir disproxil fumarate are shown in figure 1.

Figure 1: Structure of a) Lamivudine and b) Tenofovir disproxil fumarate.



(a)



(b)

The literature survey revealed that very few RP-HPLC[1]-[17] and spectroscopic[18] methods were reported for the simultaneous estimation of Lamivudine and Tenofovir disproxil fumarate in formulations. The authors now propose a new validated, sensitive and reproducible HPLC method for simultaneous determination of Lamivudine and Tenofovir disproxil fumarate. The applicability of this method in determining the drugs in commercial dosage forms were also studied.

2. Experimental

2.1 Materials

Standard Tenofovir disproxil fumarate and Lamivudine were obtained from SD-fine chemicals, Maharashtra. Market formulation TENVIR-L was purchased from Aurobindo pharmacy Ltd, Hyderabad. Each tablet contains 300 mg of Lamivudine and 245mg of Tenofovir disproxil fumarate. Other materials required were HPLC grade water, phosphate buffer, acetonitrile HPLC grade (Finar chemicals Limited. Ahmadabad).

2.2 Instrumentation

A Shimadzu LC 20-AT VP high performance liquid chromatographic instrument with spin chrome software and an inertsil ODS (250 mm x 4.6 mm; 5µ) column was used for separation. The detection was done using an UV – VIS SPD 20A Detector.

2.3 Optimized Chromatographic Conditions

An inertsil ODS, C18 (250 mm x 4.6 mm; 5 µ) column was used for the analytical separation. The mobile phase consisted of mobile phase A: phosphate buffer (20 mM) and mobile phase B: acetonitrile in the ratio of 55:45 v/v (Ph3.5) with isocratic elution program. The flow rate was adjusted to 1.2 mL/min, the injection volume was set at 20 µL with a detection wavelength of 264 nm. The separation of Lamivudine and Tenofovir disproxil fumarate under optimised conditions is shown in figure 2.

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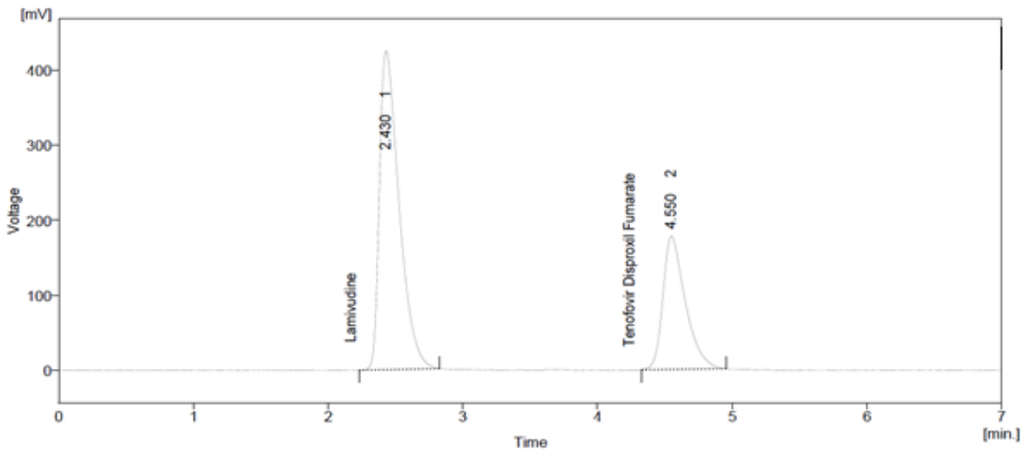
Fig 2: Typical HPLC Chromatogram corresponding to mixed standard solution of Lamivudine and Tenofovir disproxil fumarate



2.4 Preparation of Standard solutions:

2.4.1 Lamivudine stock solution

Lamivudine (60 mg) was accurately weighed and 50mL of diluent is added, allowed to sonication for 15 min. Then volume was made upto the mark with mobile phase in a 50 mL standard flask to get a concentration of 1200 µg/mL solution. 20 µl of this solution was injected and the chromatogram was recorded.

2.4.2 Tenofovir stock solution

Tenofovir (49 mg) was weighed precisely and 50mL of diluent is added, allowed to sonication for 15 min. Then volume was made upto the mark with sufficient volume of mobile phase in a 50 mL standard flask to get a concentration of 980 µg/mL solution. 20 µl of this solution was injected and the chromatogram was recorded.

2.4.3 Preparation of mixed standard stock solution

Lamivudine (60 mg) and Tenofovir (49 mg) were weighed precisely into a 50 mL volumetric flask and dissolved with sufficient mobile phase then sonicated for 15 min and the volume was made up with the same.

2.5 Preparation of mixed sample solution

Lamivudine and Tenofovir (10 tablets) were weighed accurately and crushed to fine powder. Each tablet contains 300 mg and 245 mg. The quantity of powder equivalent to 60 mg Lamivudine was weighed and dissolved by using sufficient amount of mobile phase in a 50 mL volumetric flask and the volume was made up to give a concentration 1200 µg/mL of Lamivudine and 980 µg/mL of Tenofovir. The solution was filtered through 0.45 µ nylon membrane filter paper. Then the resulting solution was suitably diluted to get the working concentrations. 20 µl of one of this solution was injected and the chromatogram recorded. The amount of Lamivudine and Tenofovir present in each tablet formulation was calculated by comparing the peak area of the standard.

The amount of drug in each tablet was calculated separately using the given formula:

$$\%\ \textbf{Assay} = \frac{\textbf{Sample Avg.peak area}}{\textbf{Standard Avg.peak area}} \times \frac{\textbf{Wt.of drug (mg)}}{\textbf{dilution of standard}} \times \frac{\textbf{dilution of tablet solution}}{\textbf{wt.of Sample}} \times \frac{\textbf{\% Purity}}{\textbf{100}} \times \frac{\textbf{Avg.wt}}{\textbf{Lable Claim}} \times \textbf{100}$$

2.6 System suitability parameters

Five replicates of working mixed standard solution were injected and the parameters like theoretical plate number (N), tailing factor (K) and resolution are calculated to check the system suitability. The results are presented in Table1.

Table 1: System suitability test results

Sr. No	Parameters	Lamivudine	Tenofovir
1	Peak area (mV*min)	4590.601	2252.278
2	No. of theoretical plates	4278	3675
3	Retention time (min)	2.430	4.550
4	Asymmetry	1.370	1.892
5	Resolution	7.411	

3. Results and Discussion

The chromatographic conditions were optimised to develop RP-HPLC method for simultaneous determination of Lamivudine and Tenofovir Disproxil Fumarate with adequate resolution and rapid analysis time.

3.1 Method Validation

The analytical method was developed and validated according to ICH guidelines. Analytical variable parameters such as linearity, precision, accuracy, specificity and system suitability were tested using the optimized chromatographic conditions and instruments.

3.1.1 Linearity

Mixed standard stock solution was suitably diluted with the mobile phase to obtain the concentration of 72, 96, 120, 144 and 168 µg/mL of Lamivudine & 58.8, 78.4, 98.0, 117.6 and 137.2 µg/mL of Tenofovir disproxil fumarate. The solution was filtered through 0.45 µ nylon membrane filter paper and 20 µl of each was injected and the chromatogram was recorded. Peak areas were plotted against concentrations and their correlation coefficient (R^2) was calculated from the graph. The results are exhibited in Table 2.

Table 2: Linearity study data for Lamivudine and Tenofovir

Sr. No	Drugs	Slope	Intercept	Correlation coefficient
1	Lamivudine	29.71	1067	0.995
2	Tenofovir	18.01	487.2	0.998

3.1.2 Accuracy

Mixed sample solution was taken in 3 different volumetric flasks and suitably diluted with mobile phase to obtain concentration of 96, 120, 144 µg/mL of Lamivudine & 78.4, 98.0, 117.6 µg/mL of

Tenofovir Disproxil Fumarate that gives 80 %, 100 % and 120 % of the analytical method target concentrations. To that a known amount of standard Lamivudine and Tenofovir Disproxil Fumarate was added. The corresponding peak areas and % recovery were measured. The results are presented in Table 3.

Table 3: Recovery study data for Lamivudine and Tenofovir

Sr. No	Drug	%Recovery	%RSD
1	Lamivudine	98.48	0.0064
2	Tenofovir	98.64	0.017

3.1.3 Precision

System Precision: System precision was established by injecting three replicate preparations of the standard drug solution of Lamivudine and Tenofovir Disproxil Fumarate. The corresponding peak areas and retention times were measured and % RSD calculated as presented in Table 4.

Method Precision*:* The method precision study was performed for three replicate sample preparations of marketed formulation containing Lamivudine and Tenofovir Disproxil Fumarate. The corresponding peak areas were measured and % RSD calculated as exhibited in Table 4.

Table 4: Precision study data for Lamivudine and Tenofovir Disproxil Fumarate

Sr. No	Wt. of Sample (mg)	Peak area of Standard		Peak area of Sample		%Label claim	
		Lamivudine	Tenofovir	LAM	TDF	LAM	TDF
1	208.80	4294.931	2061.368	4266.586	2067.055	99.34	100.27
2	208.95			4265.463	2066.072	99.31	100.22
3	208.84			4266.391	2067.921	99.33	100.31
Mean						99.32667	100.266
S.D						0.015275	0.04502
%RSD						0.0153	0.044

3.1.4 Specificity

The specificity was determined to check whether there is any interference due to presence of excipients, impurities or other components with the retention time of analytical peaks which may affect the specificity of the analytical method. The HPLC chromatograms were recorded for the drug-matrix (mixture of the drug and excipient) which showed almost no interfering peaks within retention time ranges.

3.1.5 Robustness

Robustness of the developed analytical method was tested by evaluating the affect of small variations in analytical method parameters such as change in flow rate of 1.2mL/min by ±0.2 mL/min and change in wavelength of 264 nm by ±2 nm. The results are exhibited in Table 5.

Table 5: Robustness study data for LAM and TDF

System suitability Parameters		% RSD of peak area response (n=3)		Mean tailing factor (n=3)		Mean retention time in min. (n=3)	
Variations		LAM	TDF	LAM	TDF	LAM	TDF
Change in Flow rate (mL/min)	+0.2	0.003	0.006	2.910	5.439	1.595	1.775
	0	0.87	0.52	1.370	1.892	2.430	4.550
	-0.2	0.002	0.004	2.129	3.811	1.260	1.802
Change in Wavelength (nm)	+2	0.017	0.004	2.406	4.617	1.541	1.631
	0	0.87	0.52	1.370	1.892	2.430	4.550
	-2	0.005	0.001	2.467	4.568	1.601	1.797

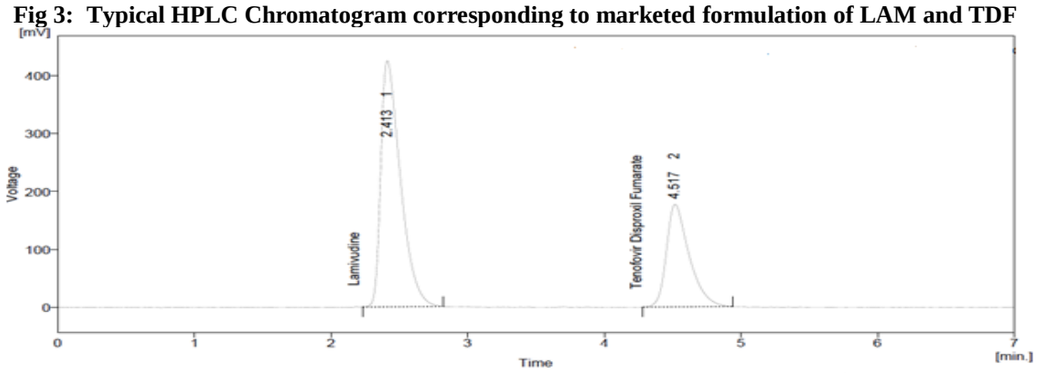
3.2 Application of the method to commercial formulation

Twenty micro litres of each of the standard and sample solutions were injected separately. The chromatograms were recorded and the corresponding peak areas were measured. The procedure was repeated three times, individually weighing the tablet powder each time. The peak areas obtained for standard and sample were used to calculate the amount of each drug in the tablet formulation. The results obtained are shown in table 6. The chromatogram showing separation of Lamivudine and Tenofovir in tablet formulation is shown in figure 3.

Table 6: Results for tablet formulation study

Sr. No	Wt. of std. (mg)		Weight of sample (mg)	Peak area of std		Peak area of sample		%Label claim	
	LAM	TDF		LAM	TDF	LAM	TDF	LAM	TDF
1	60	49	208.80	4281.874	2065.009	4246.145	2065.267	99.16	100.01
2			208.92			4302.386	2075.293	100.48	100.50
3			208.86			4266.586	2062.138	99.64	99.86
Mean								99.76	100.12
S.D								0.669	0.334
%RSD								0.007	0.003

Fig 3: Typical HPLC Chromatogram corresponding to marketed formulation of LAM and TDF



4. Conclusion

The proposed RP-HPLC method is simple, sensitive, reproducible, less time consuming and is applicable for analysis of Lamivudine and Tenofovir in bulk and in tablet dosage forms. The method was duly validated by evaluation of required parameters.

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