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Development and Validation for the Simultaneous Estimation of Lamivudine and Tenofovir Disoproxil Fumarate by RP-HPLC Method

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Abstract

A simple, rapid reverse phase high performance chromatographic method has been developed and validated for the simultaneous estimation of Lamivudine and Tenofovir disoproxil fumarate in pure and in tablet dosage form. The chromatographic separation of Lamivudine and Tenofovir was achieved on symmetry column C_{18} 4.6 x 150mm, 5 µm, Make: Ace with a mixture of methanol and water pH adjusted with 3.2 in the ratio of 70:30 v/v as mobile phase at a flow rate of 0.8 mL/ min and elutes was monitored at 260 nm. The method was validated for linearity, accuracy, precision, robustness and ruggedness as per ICH guidelines. The retention time for Lamivudine and Tenofovir disoproxil fumarate were found to be 2.45 and 3.99 respectively. Recovery of Lamivudine and Tenofovir disoproxil fumarate were found to be the range of 100.12 % and 99.53 % and linearity in the range of 25-125 µg/ml. The proposed method was rapid, economical and suitable for routine control quality analysis

Keywords: Validation, Tenofovir, Lamivudine, Linearity, Chromatographic

Introduction

Lamivudine, chemically 4-amino-1-[(2*R*,5*S*)-2-(hydroxyl methyl)-1,3- oxathiolan-5-yl]- 1,2-dihydropyrimidin-2-one is a nucleoside reverse transcriptase inhibitors (NNRTIs). Reported to be active against HIV-1, HIV-2 and Hepatitis B Virus. This compound belongs to the class of organic compounds known as 3'-thia pyrimidine nucleosides.[1-4] Tenofovir disoproxil fumarate is fumaric acid salt of the bis isopropoxy carbonyl oxy methyl ester derivative ester of Tenofovir. Tenofovir disoproxil fumarate chemically, ({[(2*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl]oxy}methyl) phosphoric acid, belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors *In vivo* Tenofovir disoproxil fumarate is converted to Tenofovir, an acyclic nucleoside phosphonate analog of adenosine 5'- monophosphate.[5]

Both the drugs are marketed as combined dose tablet formulation in the ratio of 300:300 mg of both drugs. Literature survey revealed that various UV[6-11], HPLC[12], HPTLC[13], LC-MS methods and have been reported the estimation of Lamivudine and Tenofovir. The present study illustrates development and validation of a simple, accurate, precise, economical estimation of Lamivudine and Tenofovir in bulk and their combined dosage form.

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Material and Methods

Acetonitrile (HPLC grade) and water (HPLC grade) were obtained from Merck Pvt. Ltd., Mumbai, India. Potassium di hydrogen ortho phosphate (KH_2PO_4) (Sigma-Aldrich) was obtained from ISO. Methanol (HPLC grade) was obtained from Finnar. Pure drugs of Lamivudine and Tenofovir as a gift sample from Hetero Labs India. Tenolam Tablets manufactured from Hetero Pharmaceutical Ltd purchased from local pharmacy. The label claims states that formulation contains 300 mg of Lamivudine and 300 mg of Tenofovir disoproxil fumarate.

sInstrumentation and Chromatographic Parameters

HPLC method and validation were done on Shimadzu liquid chromatography equipped with P-3000-M Reciprocating pump, : UV-3000-M detector and Rheodyne 7725i injection with a 20µL loop. The chromatographic separation were accomplished using mobile phase consisting of Methanol and Phosphate buffer at a ratio of 70:30 filtered through 0.45 µm filter using vacuum pump and degas in ultrasonic water bath. Mobile phase was pumped in isocratic mode at a flow rate of 0.8 ml/min at room temperature.

Preparation of Standard Solution:

Accurately 10 mg of Lamivudine & Tenofovir were weighed and transferred into 10ml volumetric flask, about 7ml of diluent was added and sonicated for 5 minutes to dissolve it. The volume was made up with mobile phase. The solution was filtered through 0.45µm membrane filter (Stock solution). From this 0.75ml of solution was pipette out and transferred into 10ml of volumetric flask and the volume was made up with mobile phase. The solution was filtered through 0.45µm membrane filter. Inject 20 µl of the standard solution into the chromatographic system and measure the area for the Lamivudine & Tenofovir. A typical chromatogram obtained from the analysis of drugs using the developed method is shown in figure 1.

Preparation of Sample Solution for Assay

20 Lamivudine & Tenofovir tablets were weighed and the average weight was calculated. Accurately the sample equivalent to 10mg of Lamivudine & Tenofovir was weighed & transferred into 10ml volumetric flask about 7ml of diluent was added and sonicated for 5 minutes to dissolve it content. The volume was made up with mobile phase. The solution was filtered through 0.45µm membrane filter (stock solution).0.75ml of stock solution was pipetted out and transferred into 10ml of volumetric flask and the volume was made up with mobile phase. The solution was filtered through 0.45µm membrane filter.

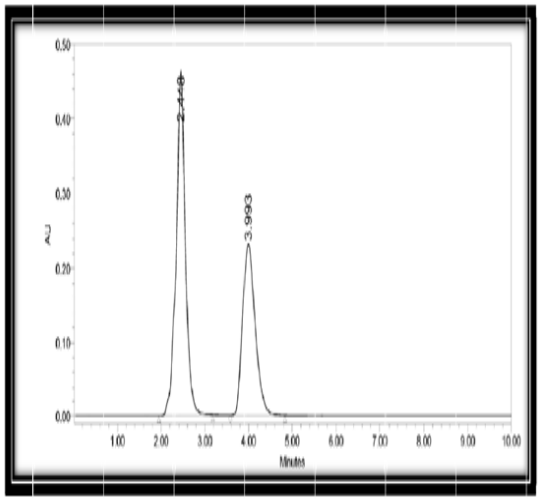

Fig. 1: Chromatogram of Standard Solution (10µg/ml Lamivudine and 10µg/ml Tenofovir)

Results and Discussion

Method Development

For the RP-HPLC, chromatographic conditions were optimized to get best resolution and peak shape. The selection of mobile phase was based on peak parameters like symmetry, theoretical plates and capacity factor. Symmetrical peaks with good separation were obtained with reverse phase column. The mobile phase containing methanol and phosphate buffer pH 3.2 at a ratio of 70:30 at a flow rate of 0.8ml/min. The optimum wavelength for detection and quantification was at 260nm, at which good detector response was obtained for both the results are given in table 1.

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Table 1: Results of method validation for Lamivudine & Tenofovir

S. No	Parameter	Requirement	Results		Acceptance criteria
			Lamivudine	Tenofovir	
1.	System suitability	RT	2.445	3.99	--
2.		Tailing factor	1.1	1.3	NMT 2
3.					
4.		Plate count	2766.24	2853.19	NLT 2000
5.		Assay value	99.56%	99.1	98-102%
6.	Accuracy	% Recovery	98.965	98.84%	98-102%
7.	Precision	%RSD	1.20	1.74	NMT 2%
8.	Specificity	No interference	Pass	pass	No interference
8.	Linearity	Correlation coefficient	0.999	0.999	NLT 0.999
9.	Range	Concentration	25-125µg/ml	25-125µg/ml	Nil
10.	Robustness	%RSD	1.37	1.56	NMT 2%
11.	Ruggedness	%RSD	99.67	99.12	98-102%

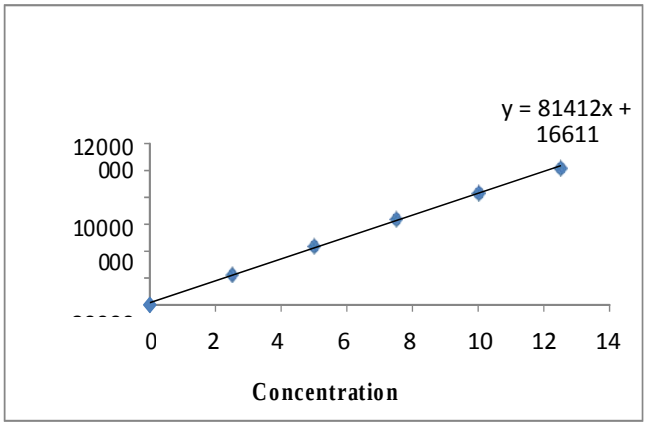

Fig. 2: Linearity graph of Lamivudine

Method Validation

As per ICH guidelines, the method validation parameters checked were linearity, precision, accuracy, robustness and ruggedness.

Linearity and Range:

The linearity was established by least squares regression analysis of the calibration curve. The calibration curve was linear over the concentration range of 25-125 µg/ml for Lamivudine and Tenofovir disoproxil fumarate. Peak areas were plotted versus respective concentrations and linear regression was performed on the resultant curves. Correlation coefficients were found to be 0.999 and 0.9996 for the Lamivudine and Tenofovir disoproxil fumarate respectively (Figure No 2 and 3)

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Table No 2: Robustness results for change in flow rate and mobile phase of LMB & TNF

Flow rate	Inj. Sample	Area	Plate count	Tailing	RT
0.7ml/min	**Lamivudine**	8344338	2671.86	1.1	2.79
	Tenofovir	5675524	2876.79	1.4	4.54
0.9ml/min	**Lamivudine**	6590717	2575.82	1.1	2.58
	Tenofovir	4500085	2800.83	1.5	3.54
Mobile Phase Variation					
35:65	**Lamivudine**	6329717	2652.12	1.2	2.44
	Tenofovir	3990067	2795.62	1.4	3.97
45:55	**Lamivudine**	7408362	2580.74	1.0	2.44
	Tenofovir	5077859	2785.37	1.4	3.34

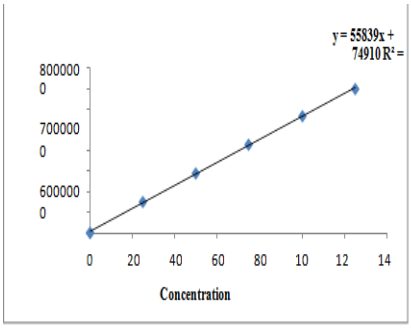

Fig. 3: Linearity graph of Tenofovir

Precision: The precision of the analytical method was studied by multiple sampling of the homogenous sample. The precision was done as system precision and method precision. The % RSD values were found to be calculated and the low RSD values indicate that the method is precise. The results of precision are given in table 1.

Specificity: Specificity was performed to exclude the possibility of interference with the excipients in the region of elution. The specificity of the method was tested under normal conditions and the result of the tests produced that the components other than the drug did not produce a detectable signal at the retention time of Lamivudine and Tenofovir disoproxil fumarate.

Robustness: To evaluate the robustness of the method, the chromatographic conditions were deliberately altered and degree of reproducibility was evaluated. During robustness testing each condition was varied separately, all other conditions being held constant at the optimized values. Robustness of the proposed method was assessed with respect to small alterations in the flow rate and mobile phase variation. The results of the robustness are given in table 2.**Ruggedness:** Ruggedness as the degree of reproducibility of test result obtained by the analysis of the same of the samples under verity of normal test conditions. The results of the ruggedness are given in table 3.

Analysis of the marketed formulation:

The proposed procedures were successfully applied for the simultaneous estimation of Lamivudine and Tenofovir disoproxil fumarate in the formulation and the drug contents in each samle were calculated by comparison with the

Table No 3: Results of Ruggedness analyst of Lamivudine & Tenofovir

S. No	Column Code	Instrument Code	Analyst	Result Obtained (%)	
				Lamivudine	Tenofovir
1.	C-01	W-29	I	99.5%	99.25
2.	C-02	W-30	II	99.85%	99

Table No 4: Quantitative Estimation of Lamivudine & Tenofovir

S. No	Compound name	Assay value
1.	Lamivudine	99.56%
2.	Tenofovir	99.1%

appropriate standard solution of the drug. The results obtained were in agreement with label claim. The results of analysis are given in table 4.

Conclusion

In this study**,** a simple**,** fast and reliable RP-HPLC method was developed in the simultaneous estimation of Lamivudine and Tenofovir disoproxil fumarate. From the results, it can be concluded that the method has been successfully applied for the analysis of marketed tablet and can be used for the routine analysis of formulations containing the combination of the drugs. Since the method was successfully applied for the estimation of selected drugs in bulk as well, therefore this method can be adopted in the study of pharmaceutical release pattern of the drugs while designing new dosage forms.

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