Research Article

A Validated RP-HPLC Method for the Simultaneous Estimation of Lamivudine, Tenofovir Disoproxil Fumarate, and Doravirine in Combined Dosage Forms

Dr. J.N Suresh Kumar, Dr. B.Satya Prasad, E. Siva Parvathi*

Department of Pharmaceutical Analysis, Narasaraopeta Institute of Pharmaceutical Sciences, Kottappakonda Road, Yellamanda (P), Narasaraopet (M), Palnadu (Dt), 522601, AP, India.

***Corresponding author's E-mail:** eedasivaparvathi@gmail.com

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ABSTRACT

A new simple, precise and robust isocratic reverse-phase high performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous determination of lamivudine, tenofovir disoproxil fumarate (TDF), and doravirine in bulk and pharmaceutical dosage form. The validation included specificity, linearity, system suitability, precision, robustness, LOD and LOQ characteristics. The chromatographic separation was achieved on C18 X bridge phenyl column (150 4.6 mm, 3 m particle size) eluted with acetonitrile and methanol (pH 2.5; 50:50, v/v) at a flow rate of 0.8 mL/min and monitored at 243 nm over a run time of 12 min. The retention times of lamivudine, TDF, and doravirine were found to be 2.45, 7.3, and 8.79 min. respectively. The method was linear in the range of 5– 100 g/mL (r2 = 0.999) for lamivudine and TDF and in the range of 1.75– 35 g/mL (r2 = 0.999) for doravirine. The percentage recoveries of three drugs were within the acceptable limits (98– 102%). The method was found to be precise as confirmed by % RSD < 0.6. Forced degradation study was conducted as per ICH guidelines, and the three drugs showed degradation within 21.4–33.8% under acidic, basic, oxidative, photolysis, and hydrolysis conditions. The proposed RP-HPLC method can be used for the quantification of lamivudine, TDF, and doravirine in API and tablets without any interference from excipients.

Keywords: RP-HPLC, Linearity, Accuracy, Retention time, ICH guidelines, forced degradation.

INTRODUCTION

Lamivudine is a synthetic nucleoside analogue used to treat human immunodeficiency virus (HIV-1) infection and hepatitis-B. Chemically, lamivudine is (2R-cis)-4-amino-1 (2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one. Lamivudine is a negative enantiomer of cytidine and exhibits its activity through an active metabolite triphosphate formed by phosphorylation. Triphosphate acts as a competitive inhibitor of viral DNA polymerase and blocks viral replication[1]. Tenofovir disoproxil fumarate (TDF) is an oral pro-drug of bioavailable tenofovir. This nucleoside analogue reverse transcriptase inhibitor is used to treat HIV infection[2]. Chemically, TDF is 9-[(R)-2[[bis- [[(isopropoxycarbonyl)oxy]methoxy]propyl] adenine fumarat. It exhibits its activity by terminating viral DNA chain elongation, acting an adenosine 5-monophos phate analogue. TDF inhibits the activity of viral DNA poly merase and terminates DNA chain elongation by competing with natural substance[3]. Doravirine chemically is 3-chloro-5-({1-[(5-hydroxy-4-methyl-4H-1,2,4-triazol-3-yl) methyl]-2-oxo-4-(trifluoromethyl)-1,2-dihydropyridin-3-yl} oxy) benzonitrile. Doravirine is a pyridinone non-nucleoside reverse transcriptase inhibitor used to treat HIV infections in adult patients with no prior antiretroviral treatment history. Doravirine shows its activity by inhibiting viral replication through non-competitive inhibition of HIV-1 reverse transcriptase[4].

Varieties of methods are in use for the estimation of TDF as single entity [5, 6] and lamivudine as a single component[7– 9]in dosage forms and biological samples. Several methods have also been reported for the combination of TDF and lamivudine along with other drugs[10– 14] in a variety of matrices. However, no methods have been reported till now for the simultaneous determination of Lamivudine, TDF, and doravirine in bulk and pharmaceutical dosage form. In this study, efforts were made to develop a new, simple, precise and robust analytical method for the simultaneous determination of lamivudine, TDF, and doravirine in bulk and pharmaceutical dosage form using reverse phase high performance liquid chromatography (RP-HPLC) technique [15-19].

MATERIALS AND METHODS

Chemicals:

Pharmaceutical grade reference standards of lamivudine, TDF, and doravirine were obtained as gift samples from Laurus Labs, Hyderabad, India. Fixed dosage combination tablet containing 300mg lamivudine, 300 mg TDF, and 100mg doravirine (DELSTRIGO) was obtained as gift sample from Laurus Labs, Hyderabad, India. All chemicals were HPLC grade purchased from S. D. Fine Chem., Mumbai, India. Milli 'Q' water was used throughout the study.

Instrumentation:

Chromatographic analysis was carried out on Waters 2695 separation module (Waters Corporation, USA) equipped with auto sampler and waters 2998 PDA detector, and X-bridge Phenyl (150 4.6 mm, 5.6 m particle size) column.

Preparation of Solutions:

Buffer solution was prepared from accurately weighed (about 2.5 g) hexane-1-sulfonic acid dissolved in water in

1000 mL volumetric flask. The volume was made up to 1000 mL, the solution pH was adjusted to 2.5 with 0.1% orthophosphoric acid, and the solution was filtered through 0.45 m membrane filter.

Stock solution:

About 5 mg of lamivudine and 5 mg of TDF were accurately weighed in 10 mL volumetric flask. About 5 mg doravirine was accurately weighed in another 10 mL volumetric flask, dissolved and made up with an equal mixture of acetonitrile and methanol as diluent. From this solution, 3.5 mL was pipetted into a 10 mL volumetric flask containing TDF and lamivudine, and finally made up to 10 mL with diluent.

Working standard solutions: These were prepared from 1 mL stock solution pipetted into a 10 mL volumetric flask and made up to volume with diluent to get 50 g/mLlamivudine, 50 g/mL TDF, and 17.5 g/mL doravirine. This solution was used as standard and further diluted as required.

Preparation of Tablet Samples:

Twenty tablets were weighed and powdered. An amount of tablet powder equivalent to 50 mg of lamivudine, 50 mg of TDF, and 17.5 mg of doravirine was accurately weighed and transferred to 100 mL volumetric flask containing 70 mL of diluent. This mixture was subjected to sonication for 30 min to ensure complete extraction of drugs, made up to 100 mL with diluents, and filtered. From this solution, 1mL was taken and diluted to 10 mL with diluent to get final concentration of 50 g/mL lamivudine, 50 g/mL TDF, and 17.5 g/mL doravirine. Optimised chromatographic conditions are listed in Table 1. The chromatogram of lamivudine, TDF, and doravirine standard is presented in Fig. 1.

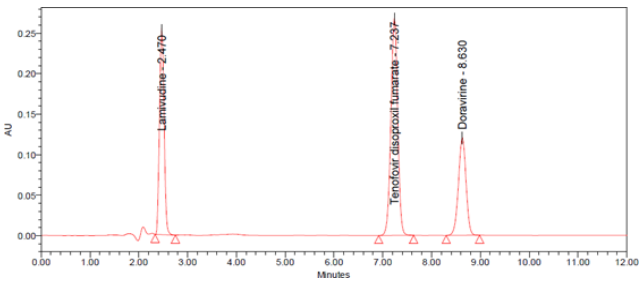

Figure 1: Chromatogram of lamivudine, tenofovir disoproxil fumarate, and doravirine (standard)

Table 1: Optimized Chromatographic Conditions

Parameter	Value
Column	Waters X-bridge phenylC18 column (150x4.6 mm,5.6 µm)
Mobile phase	Acetonitrile and methanol pH 2.5 (50:50,v/v)
Elution mode	Isocratic
Detection wavelength	243nm
Column temperature	25ºC
Volume of injection	0.8µL
Run time	12min.

METHOD VALIDATION

The developed RP-HPLC method was validated for the linearity, precision, system suitability, robustness, LOD and LOQ characteristics as per ICH guidelines[15].

Linearity: Appropriate aliquots were pipetted from the stock solution to a series of 10 mL volumetric flasks and the volume made up with diluent to get the final concentrations within 5– 100 g/mL of lamivudine and TDF and 1.75– 35 g/mL of doravirine. Each solution was injected in triplicate. Calibration curves were plotted as the observed peak areas versus corresponding concentration, followed by determination of regression equations and calculation of correlation coefficients.

Precision:

The intraday precision was studied using the analysis of six different sample solutions prepared using the same working standard solution. Each solution was injected in triplicate, the peak areas obtained were used to calculate the assay, and the % RSD was computed. Intermediate precision was determined in a similar manner on different days.

System Suitability:

System suitability study was carried out with six injections of standard concentration (50 g/mL lamivudine, 50 g/mL TDF, and 17.5 g/mL doravirine) into the HPLC system. Sets of parameters including retention time, number of theoretical plates, tailing factor, and resolution were determined. 3.4. Accuracy Accuracy of the proposed method was estimated from recovery studies. The tablet sample solution was diluted to obtain solutions corresponding to 50, 100, and 150% in triplicate, each solution was injected twice and %recovery of three drugs was calculated.

Robustness:

Robustness of the method was analyzed by altering the chromatographic conditions including the mobile phase composition, detection wavelength, etc. Small deliberate changes in the chromatographic conditions were introduced, and the extent to which the method was robust was determined. Deviations of 0.2 mL/min in the flow rate and ±5mL in the amount of organic solvent in mobile phase composition were tried individually. The sample solution was injected in triplicate for each altered condition and the obtained peak areas were measured to calculate the assay value and %RSD. The system suitability parameters including the tailing factor and number of theoretical plates were monitored during the study.

Limit of Detection (LOD):

LOD is defined as the lowest amount of analyte in the sample which is to be detected and need not be quantified under given experimental conditions. It is determined at a signal/noise ratio of 3:1.

Limit of Quantitation (LOQ):

LOQ is the concentration of analyte in the sample which should be quantified with the precision and accuracy under stated experimental conditions. It is determined at a signal/noise ratio of 10:1.

Specificity:

Specificity of the method is determined by testing standard substances against potential interferences. The method was considered specific when the sample solution was injected and no interferences related to excipients were found. The chromatogram obtained by injecting the tablet solution was compared with the chromatogram obtained by injecting standard solution to study the interferences due to excipients.

RESULTS

In order to develop and validate a chromatographic method for simultaneous quantification of lamivudine, TDF, and doravirine by RP-HPLC, several trials were undertaken. Initially the drug solution was analyzed using a mixture of acetonitrile and 0.1% orthophosphoric acid (pH 3.2; 80:20, v/v) at a flow rate of 1 mL/min, in which case the peak resolution and symmetry were not satisfactory. Several mobile phase compositions including 70:30, 50:50, and 20:80 v/v were tried, but the peak asymmetry and tailing were ob served. Then, the buffer composition was changed and a mixture of acetonitrile with methanol at 50:50 v/v provided good peaks at a flow rate of 0.8 mL/min with detection at 243 nm. The retention times of lamivudine, TDF, and doravirine were found to be 2.45, 7.3, and 8.79 min respectively. The proposed method obeyed linearity (Fig. 2) for lamivudine and TDF in the range of 5–100 g/mL (r2=0.9996, 0.9995) and for doravirine within 1.75– 35 g/mL (r2 = 0.999).

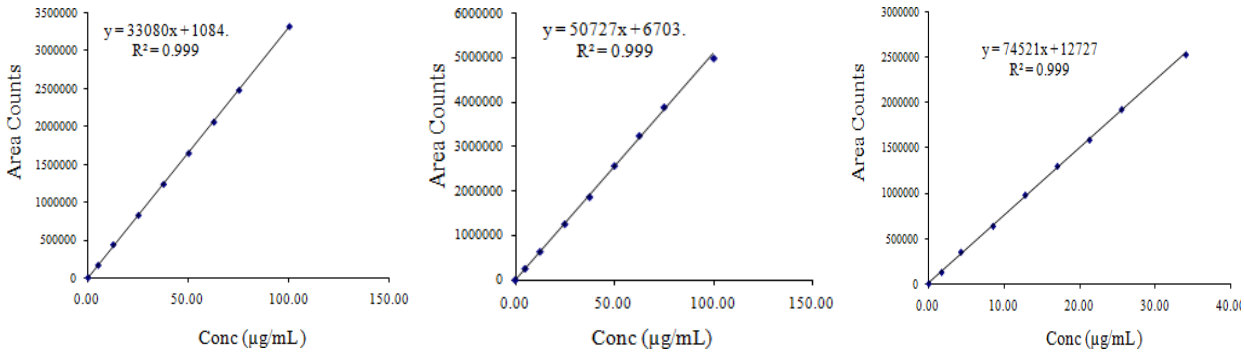

Figure 2: Calibration curves of lamivudine, tenofovir disoproxil fumarate, and doravirine.

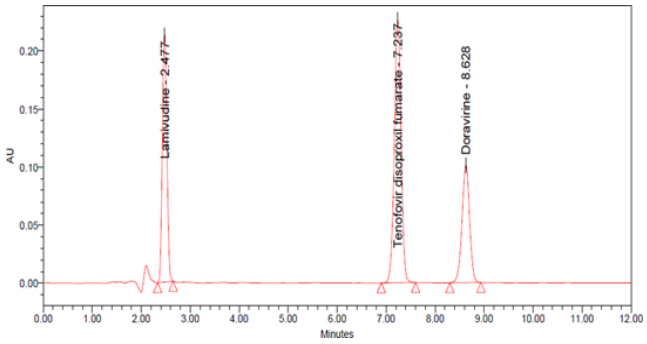

Figure 3: Chromatogram of tablet sample solution

The proposed method was also found to be specific, as the chromatogram (Fig. 3) obtained by injecting tablet solution showed no peaks close to the individual retention times of lamivudine, TFD, and doravirine.

The proposed method gave consistent results indicating its precision as observed from the % RSD data (Table 2).

Accuracy of the method was analyzed using recovery studies for the commercially available formulations. The percentage recovery values (Table 3) and Figure 5 were in the range of 99.6–100.3% for Lamivudine, 99.3–99.7% for TDF, and 99.4–99.7% for doravirine, which indicate that there are no interferences from excipients in formulation.

Table 2: Results of Precision Studies

Sample no.	Sample weight	Lamivudine		Tenofovir disoproxil fumarate		Doravirine	
		Mean peak area counts	*Assay(%w/w) ±SD, % RSD	Mean peak area counts	*Assay(%w/w) ±SD, % RSD	Mean peak area counts	*Assay (%w/w) ±SD, % RSD
1	259	1716598	99.4±1.6, 1.610	2685089	99.2±0.75, 0.760	1325089	99.5±0.85, 0.850
2	270	1789492	99.4±1.1, 1.110	2786873	98.8±0.75, 0.760	1386873	99.9±1.05, 1.050
3	260	1725984	99.6±0.25, 0.250	2692703	99.4±1.29, 1.290	1330703	99.5±0.1, 0.100
4	250	1655549	99.4±0.40, 0.410	2609657	99.9±1.1, 1.100	1289578	100.3±0.56, 0.560
5	240	1589863	99.4±0.95, 0.960	2490107	99.3±1.6, 1.610	1226579	99.3±0.55, 0.550
6	230	1515412	98.8±0.45, 0.460	2389209	98.7±1.41, 1.420	1179209	99.7±0.7, 0.700

Table 3: Recovery Studies

Drug	Level %	Amt. recovered	% Recovery	Mean % Recovery	% RSD
Lamivudine	50	25.15	99.7	99.9	0.050
	100	50.19	99.9		0.450
	150	75.36	100.2		0.260
TDF	50	25.04	99.3	99.5	0.350
	100	50.17	99.7		0.400
	150	74.92	99.6		0.240
Doravirine	50	8.47	99.7	99.5	1.120
	100	17.03	99.4		0.320
	150	25.60	99.5		0.360

Table 4: Study of Robustness

Parameter	Condition	Lamivudine			TDF			Doravirine		
		% RSD	TF	TP	% RSD	TF	TP	% RSD	TF	TP
Flow Rate	0.6	0.9	1.07	3691	0.56	0.98	13872	0.15	0.97	15544
	0.8	0.046	1.06	3064	0.066	0.97	12893	0.42	0.96	14556
	1.0	0.66	1.07	2621	0.15	0.98	11935	0.95	0.96	13939
Mobile Phase composition	45:55	0.53	1.06	3299	0.21	0.95	11528	0.38	0.94	16902
	50:50	0.046	1.06	3064	0.066	0.97	12893	0.42	0.96	14556
	55:45	0.1	1.06	2921	0.31	1.0	10291	0.5	1.0	11859

Table 5: Assay of Tablet Components in Marketed Formulation

Formulation	Lamivudine			TDF			Doravirine		
Delstrigo	Label claim (mg)	Amt. found (mg)	Assay (%)	Label claim (mg)	Amt. found (mg)	Assay (%)	Label claim (mg)	Amt. found (mg)	Assay (%)
	300	298.2	99.4	300	297.2	99.06	100	99.9	99.9

Small deliberate variations in the chromatographic conditions such as flow rate and mobile phase composition did not produce significant effect on the parameters like tailing factor (2000). The % RSD calculated for each modified parameter (Table 4) was less than 2 which indicates proper robustness of the method.

The LOD and LOQ value were found to be 0.05 and 0.5 g/mL for lamivudine and TFD and 0.017 g/mL and 0.17 g/mL for r2 = doravirine, which indicate proper sensitivity of the proposed method. These figures are respectively in Fig 4 & 5.

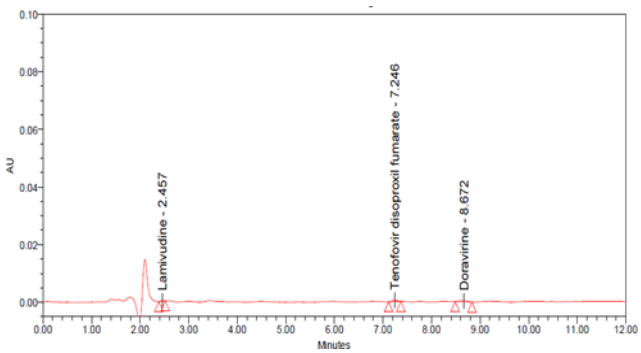

Figure 4: Chromatogram of LOD

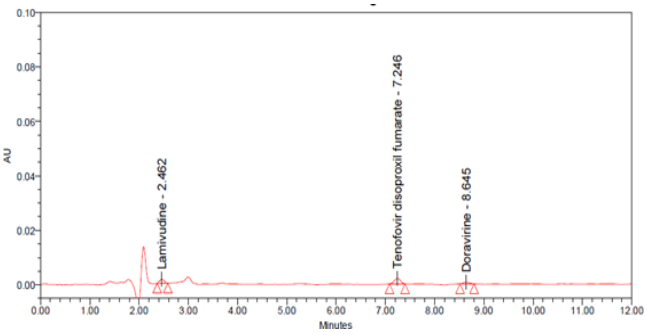

Figure 5: Chromatogram of LOQ

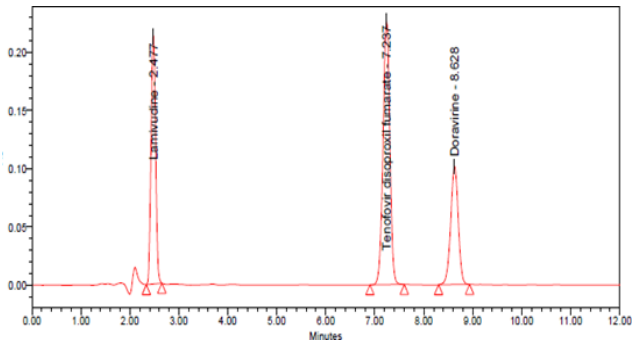

Figure 6: Chromatogram for accuracy

Finally, the proposed method was applied to the quantifi cation of lamivudine, DTF, and doravirine in marketed tablet formulation and assay was found to be 98.7, 98.5 and 99.7% w/w. respectively, in agreement with label claim (Table 5).

DISCUSSION

As there is a developing interest for anti-HIV drugs, it is also necessary to develop a rapid, sensitive and robust analytical method. The statistical investigation of obtained information demonstrated that the proposed RP-HPLC method was accurate, linear, robust and economical. The optimized method is appropriate for determining of pharmaceutical drugs in the marketed formulation with virtually no interferences of excipients. Hence, the method can be effectively applied to the quality control analysis. In conclusion, this study was aimed at the quantification of lamivudine, TDF, and doravirine in bulk and tablets. The developed method was found to be linear, accurate, robust and reliable. An evident advantage is the simplicity of sample preparation and speed of analysis, since the three compounds were eluted within 10 minutes. Hence, the developed method can be used for routine analysis in quality control laboratories and for further research.

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