DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DORAVIRINE, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE IN TABLET DOSAGE FORM

Venkata Padmini . M[1*] and Dr. A.Krishnamanjari Pawar[2]

Research Scholar, A.U.College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530016, Andhra Pradesh, India Email id- padmajapriya39@gmail.com, Phone Number: 9515616299

Associate Professor, Research Guide, A.U.College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530016, Andhra Pradesh, India Email id- akmpawar@andhrauniversity.edu.in

[*]**Corresponding author: padmajapriya39@gmail.com**

ABSTRACT

A simple, economic, accurate reverse phase RP-HPLC method was established for the simultaneous estimation of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate in tablet dosage form. The estimation was done by using Phenomenex C18 (150×4.6mm,5µm) column with the flow rate of 1mL/min, at λmax 240nm. The retention time obtained for Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate was 2.200 min, 3.234 min, and 3.819 min, respectively. Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate their combination drug product were exposed to acidic, alkali, thermal, photolytic, and oxidative stress conditions. The current method was validated according to the ICH guidelines for accuracy, precision, linearity, specificity, and sensitivity.

Keywords: Doravirine, Lamivudine, Tenofovir Disoproxil Fumarate, RP-HPLC, Validation

INTRODUCTION

Doravirine is an antiretroviral drug in the treatment of HIV drug [1]. It is chemically 3-Chloro-5-({1-[(4-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-3yl)methyl]-2-oxo-4-(trifluoromethyl)-1,2-dihydro-3-pyridinyl}oxy)benzonitrile.Chemical structure is shown in Figure 1.

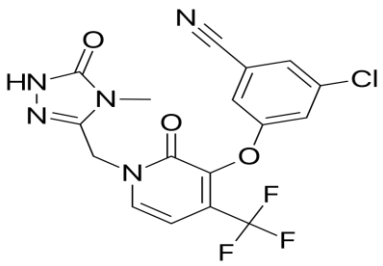

Figure 1: Chemical structure of Doravirine

Lamivudine is an Nucleoside Reverse Transcriptase Inhibitor [2, 3]. It is chemically 4-amino-1-[(2R,5S)-2-(hydroxyl methyl)-1,3-oxathiolan-5-yl] 1, 2- Dihydropyrimindin-2-one. Chemical structure is shown in Figure 2.

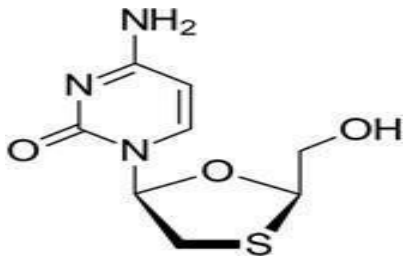

Figure 2: Chemical structure of Lamivudine

Tenofovir Disoproxil Fumarate is an HIV Reverse Transcriptase Inhibitor [4]. It is chemically [(2R)-1-(6-aminopurin-9-yl) propan-2-yl] oxy methyl-(propan-2-yl oxycarbonyloxymethoxy) phosphoryl] oxymethylpropan-2-yl carbonate [5]. Chemical structure is shown in Figure 3.

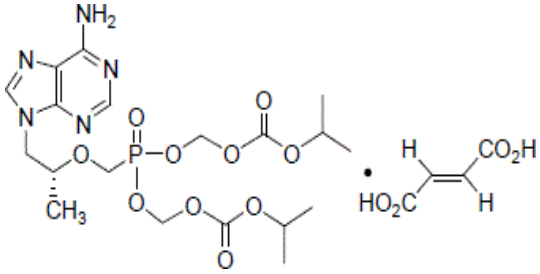

Figure 3: Chemical structure of Tenofovir Disoproxil Fumarate

Literature review reveals that there are few analytical methods have been reported for Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate.

MATERIAL AND METHODS

Chemicals and Solvents:

Solvents of HPLC grade like Water, Methanol and Acetonitrile was purchased from Ramkem, Haryana, India. Ortho- Phosphoric Acid, Potassium dihydrogen ortho phosphate was purchased from Fischer Scientific, Mumbai, India.

Instrumentation and Chromatographic Conditions:

HPLC method assay was carried out on Waters HPLC system 2695 equipped with 2996 photo diode array detector, auto sample injector and column Phenomenex C18 (150×4.6mm, 5µm) respectively. The output signal was monitored and integrated using Waters Empower 2

software. Electronic balance ELB 300 was used for weighing the materials, pH meter (Metsar Technologies Pvt. Ltd. Hyderabad, India) was used for all pH measurements, Ultrasonic bath (Labman Scientific Instruments Pvt. Ltd. Chennai, India) and Hot air oven was used to carry out forced degradation studies.

Preparation of Working Standard Solution:

Working Standards 25mg of Doravirine, 75mg of Lamivudine and 75mg of Tenofovir Disoproxil Fumarate were accurately weighed and transferred into 25mL volumetric flask and 10 mL of diluent was added and sonicated for 10 minutes. The final volume was made up to the mark with the diluent to get the concentration 1000 µg/mL of Doravirine, 3000 µg/mL of Lamivudine, 3000 µg/mL of Tenofovir Disoproxil Fumarate.1mL of above stock solution was pipetted out into 10mL volumetric flask and the volume made up to the mark with diluent to get the final concentration 100 µg/mL, 300 µg/mL, 300 µg/mL of Doravirine, Lamivudine, Tenofovir Disoproxil Fumarate respectively.

Preparation of Sample Solution:

10 tablets were weighed and crushed with a mortar and pestle. Average weight was calculated. Accurately weighed and equivalent amount of one tablet was transferred into 25mL volumetric flask, and 10 mL of diluent was added and the mixture was sonicated for 25 minutes, finally the volume was made up to the mark with diluent and filtered to get the concentrations 1000 µg/mL of Doravirine, 3000 µg/mL of Lamivudine, 3000 µg/mL of Tenofovir Disoproxil Fumarate. 1mL of filtered sample stock solution was transferred into 10mL volumetric flask and made up to the volume with diluent to get the final concentration 100 µg/mL, 300 µg/mL, 300 µg/mL of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate respectively.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT:

The proposed methods reported or developed and validate RP-HPLC method for simultaneous estimation of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate. The method developed was proceeding within wavelength selection as 240nm.In order to get the optimized RP-HPLC method various mobile phases and columns were used to get better resolution. Finally the analysis was performed by using KH_2PO_4 and Acetonitrile in the ratio 60:40 at a flow rate 1mL/min at an injection volume of 10µL and separation was carried out

by using Phenomenex C18, (250×4.6mm,5µm) column. The data was collected by Empower 2 software. The chromatogram trails were performed are given below in Figure 4.

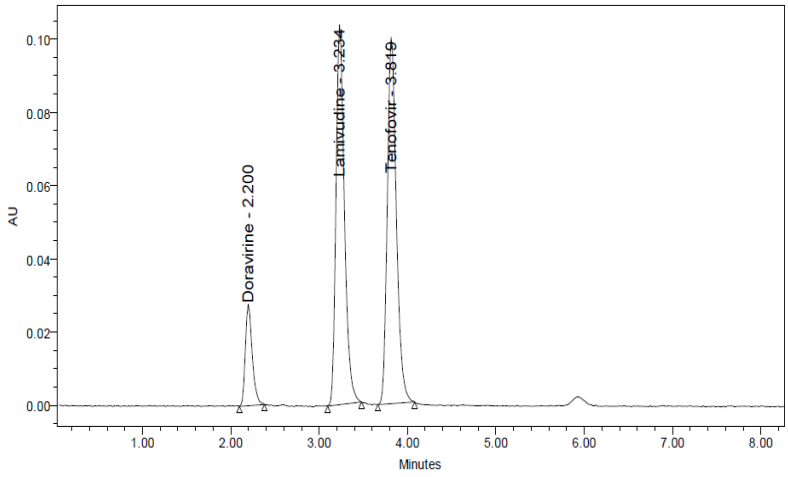

Figure 4: Chromatogram of standard solution

ANALYTICAL METHOD VALIDATION

The optimized method was validated according to the ICH guidelines and validation parameters were studied for Specificity, Linearity, Accuracy, Precision, Robustness, System suitability, Limit of Detection, Limit of Quantification[11, 12]

System suitability:

Six replicates of the working standard solution were prepared and injected 10µL to carry out the system suitability parameters like Retention time, Peak area, Plate count, USP Resolution and Tailing. The system suitability parameters values are given in table 1. The theoretical plates were found to be not less than 3000 for all three drugs. The tailing factor was found to be not more than 2.0 and resolution was less than 1.5.

Table 1: System suitability test parameter

S.No	Peak Name	Retention Time (min)	Peak Area	USP Plate Count	USP Resolution	USP Tailing
1.	Doravirine	2.200	149804	3684.4	-	1.3
2.	Lamivudine	3.234	683835	5670.7	3.4	1.3
3.	Tenofovir	3.819	711811	6723.1	3.2	1.2

Specificity:

10µL of working standard and formulation solution was injected into the chromatographic system and chromatograms were recorded.

Linearity:

Aliquots of 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5mL were taken from stock solution of concentration 25µg/mL of Doravirine, 75µg/mL of Lamivudine and 75µg/mL of Tenofovir Disoproxil Fumarate and these diluted up to mark with diluent. Such that the final concentrations were in the range 12.5-75µg/mL of Doravirine, 37.5-225µg/mL of Lamivudine and 37.5-225µg/mL of Tenofovir Disoproxil Fumarate. The calibration standard solutions of these three drugs were injected using a 10µL injector into the chromatographic system and the chromatograms were recorded at 240nm. Calibration curve was constructed by plotting the peak areas versus drug concentration of these drugs are shown in figures 5 to7. The linearity data of the proposed method are presented in table 2.

Table 2: Linearity data of Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate

Doravirine		**Lamivudine**		**Tenofovir Disoproxil Fumarate**	
Concentration (µg/mL)	**Peak Area**	**Concentration\ (µg/mL)**	**Peak Area**	**Concentration (µg/mL)**	**Peak Area**
12.5	44696	37.5	216262	37.5	203877
25	77619	75	378215	75	356258
37.5	122801	112.5	584913	112.5	570193
50	158095	150	744032	150	729539
67.5	201132	187.5	953050	187.5	928065
75	241944	225	1147568	225	1103577
Regression equation y= 3181.9x + 1841 R^2= 0.9987		**Regression equation y = 4855.8x +11258 R^2= 0.9987**		**Regression equation y = 4983.x +16689 R^2= 0.9996**	

*= Average peak area of 3 replicate injections for each concentration

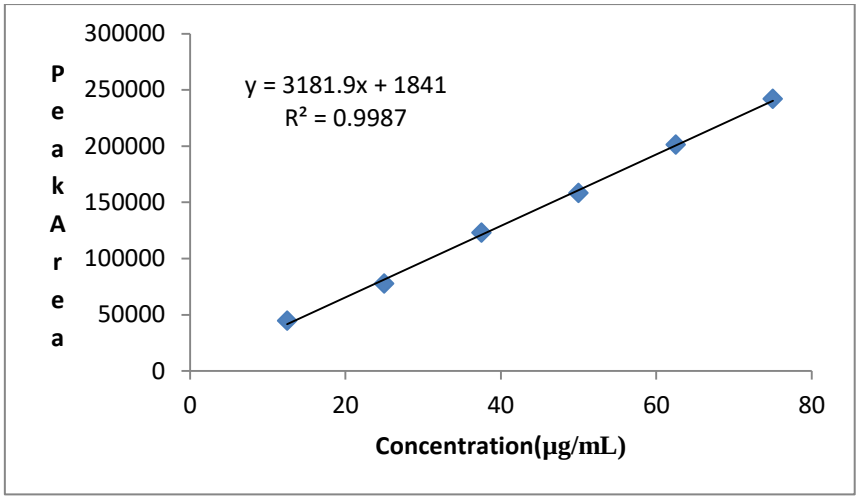

Figure 5: Standard calibration graph of Doravirine

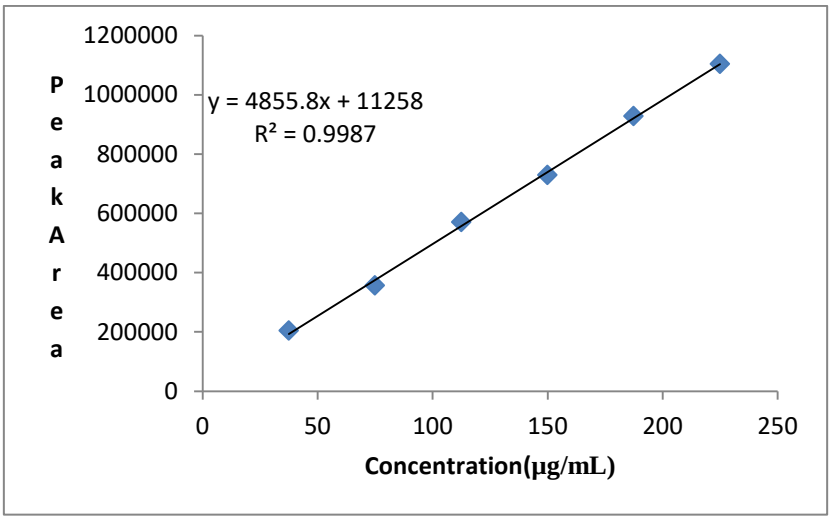

Figure 6: Standard calibration graph of Lamivudine

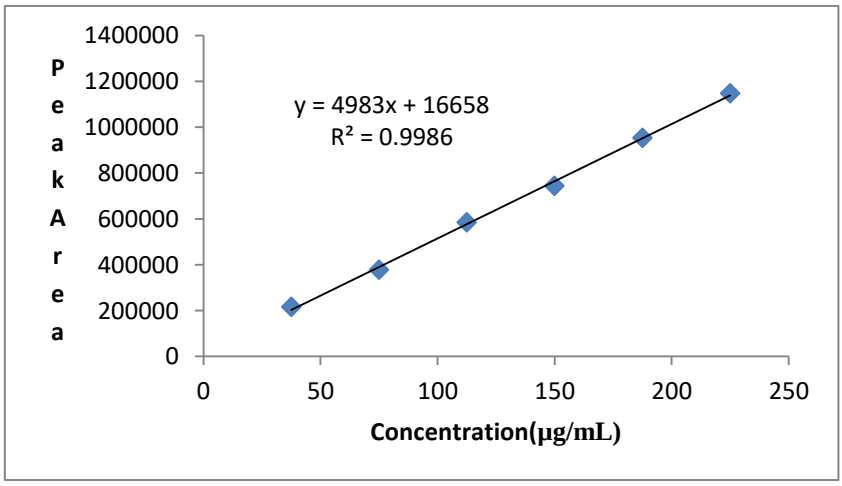

Figure 7: Standard calibration graph of Tenofovir Disoproxil Fumarate

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of Detection and Limit of Quantification of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate were determined by calibration curve method. .The sensitivity results are shown in table 3. Chromatograms of LOD and LOQ were given in figures 8 to 9.

LOD =3.3×σ/S and LOQ = 10×σ/S

Where σ = the standard deviation of the response and S= slope of the calibration curve

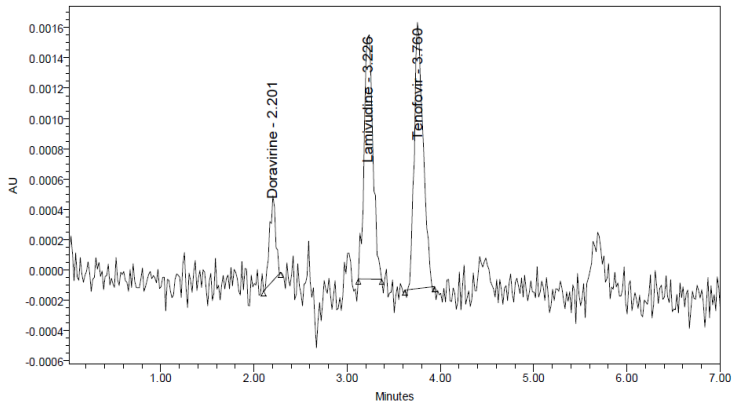

Figure 8: Chromatogram of LOD

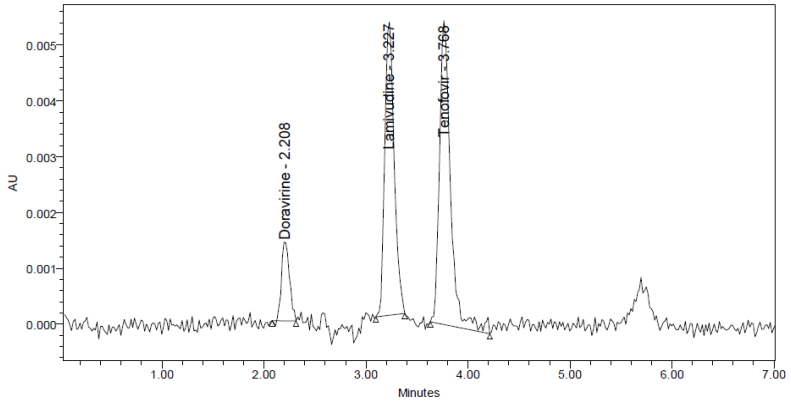

Figure 9: Chromatogram of LOQ

Table 3: Limit of Detection and Limit of Quantification data

S.No	Parameter	Measured values (µg/mL)		
		Doravirine	Lamivudine	Tenofovir Disoproxil Fumarate
1	LOD	0.70	1.11	0.96
2	LOQ	2.13	3.36	2.90

LOD and LOQ values were obtained by the proposed method indicate that the method is sensitive.

Method Precision:

10µL of working sample solution was injected six times into the HPLC system and chromatograms were recorded. % RSD value of the peak area was calculated. Method precision data are given in table 4.

Table 4: Method precision data of Doravirine, Lamivudine, and Tenofovir Disoproxil Fumarate

Sample No	Peak area response of drugs		
	Doravirine	Lamivudine	Tenofovir Disoproxil Fumarate
1	149917	697418	713988
2	149232	700754	721132
3	147872	695023	714314
4	149274	702177	715711
5	151096	695353	725921
6	148605	697074	714622
Mean	149333	697967	717885
Std dev	1107.0	2901.4	4799.2
% RSD	0.7	0.4	0.7

*= Average assay of 3 replicates injection at each time interval

%RSD values should be less than 2 and this method is said to be quite precise

Accuracy:

A known amount of standard drugs at each three concentration levels 50%, 100% and 150% were added to a pre-analyzed sample solution and injected in triplicate into the HPLC system and the chromatograms were recorded. The mean % recovery and percentage RSD at each level was calculated. Results are presented in tables 5 to 7.

Table 5: Data of accuracy studies of Doravirine

Spiked level	Amount Spiked (µg/mL)	Amount Recovery (µg/mL)	% Recovery	Mean% Recovery + RSD
50%	25	25.145	100.57	99.99%+0.58
	25	25.140	100.57	
	25	25.208	100.83	
100%	50	49.987	99.97	
	50	50.048	100.10	
	50	49.885	99.77	
150%	75	74.401	99.20	
	75	74.502	99.34	
	75	74.648	99.53	

Table 6: Data of accuracy studies of Lamivudine

Spiked level	Amount Spiked(µg/mL)	Amount Recovery(µg/mL)	% Recovery	Mean% Recovery + RSD
50%	75	75.504	100.67	100.32%+0.6
	75	75.504	100.67	
	75	74.820	99.76	
100%	150	150.654	100.44	
	150	151.70	100.78	
	150	151.378	100.92	
150%	225	225.277	100.12	
	225	226.132	100.50	
	225	222.835	99.04	

Table 7: Data of accuracy studies of Tenofovir Disoproxil Fumarate

Spiked level	Amount Spiked(µg/mL)	Amount Recovery(µg/mL)	% Recovery	Mean% Recovery + RSD
50%	75	75.827	101.16	100.32%+0.62
	75	74.981	99.98	
	75	74.981	99.98	
100%	150	151.337	100.89	
	150	151.330	100.87	
	150	151.231	100.82	
150%	225	224.092	99.60	
	225	225.436	100.19	
	225	223.820	99.48	

*= Average results of 3 replicate injections

The individual recoveries values at each level were within the acceptable limits. This indicates that the method is accurate

Robustness:

10µL of the prepared working standard solution as per test method was injected into the chromatograph at varied conditions of flow rate at ±0.1mL/min, organic phase composition by ±10% and column temperature by ± 5 $^{\circ}$C. The results of robustness study are shown in table 8 to 10.

Table 8: Robustness study results of Doravirine

Parameter	Optimized Conditions	Used Condition	Peak Area	Retention Time	Plate Count	Tailing Factor
Flow rate ±0.1mL/min	1.0mL/min	0.9mL/min	172919	2.415	3946	1.29
		1.1mL/min	150998	2.040	3843	1.30
Column temperature(30^0C)	30^0C	25^0C	156446	2.197	3945	1.32
		35^0C	156446	2.197	3925	1.32
Organic Phase composition(5%v/v)	60:40	65:35	152326	2.191	4061	1.33
		55:45	149672	2.233	3732	1.28

Table 9: Robustness study results of Lamivudine

Parameter	Optimized Conditions	Used Condition	Peak Area	Retention Time	Plate Count	Tailing Factor
Flow rate ±0.1mL/min	1.0mL/min	0.9mL/min	773133	3.524	5525	1.30
		1.1mL/min	654079	2.983	5382	1.30
Column temperature (30^0C)	30^0C	25^0C	716590	3.196	5586	1.28
		35^0C	716590	3.196	5586	1.28
Organic Phase composition (5%v/v)	60:40	65:35	686488	3.173	5355	1.29
		55:45	694034	3.298	5705	1.26

Table 10: Robustness study results of Tenofovir Disoproxil Fumarate

Parameter	Optimized Conditions	Used Condition	Peak Area	Retention Time	Plate Count	Tailing Factor
Flow rate ±0.1mL/min	1.0mL/min	0.9mL/min	799641	4.100	6734	1.23
		1.1mL/min	676169	3.480	6649	1.25
Column temperature (30^0C)	30^0C	25^0C	745351	3.677	6886	1.24
		35^0C	750321	3.783	6763	1.25
Organic Phase composition (5%v/v)	60:40	65:35	722253	3.602	6688	1.24
		55:45	722618	3.968	6869	1.24

Forced Degradation Studies:

A stress study was conducted to demonstrate the effective separation of degradation from the main analyte peaks of the sample when exposed to the following stress conditions. All the stressed samples were suitability diluted to required concentration with diluents and injected twice into the UPLC system by using optimized chromatographic conditions and the chromatograms were recorded and evaluated for the peak purity. The % degradation of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate were calculated. The chromatograms of the stressed samples were evaluated for peak purity as shown in the figures 10 to 11.

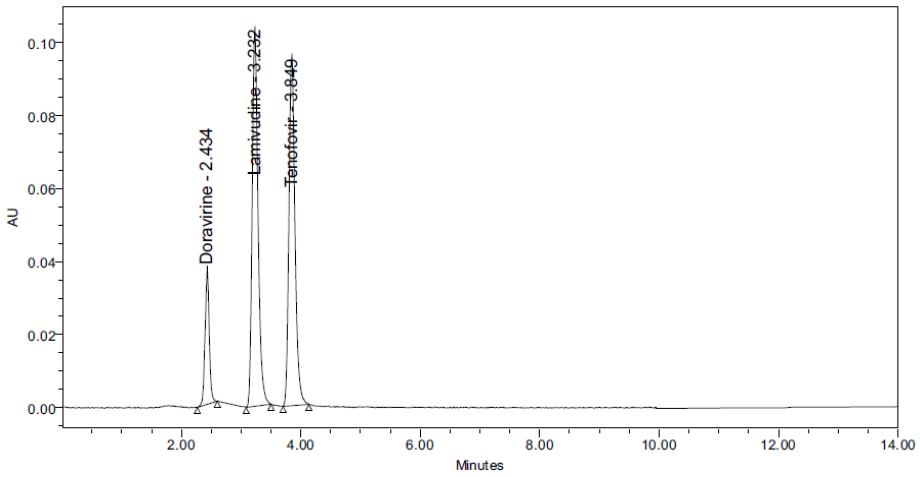

Figure 10: Chromatogram for acid degradation

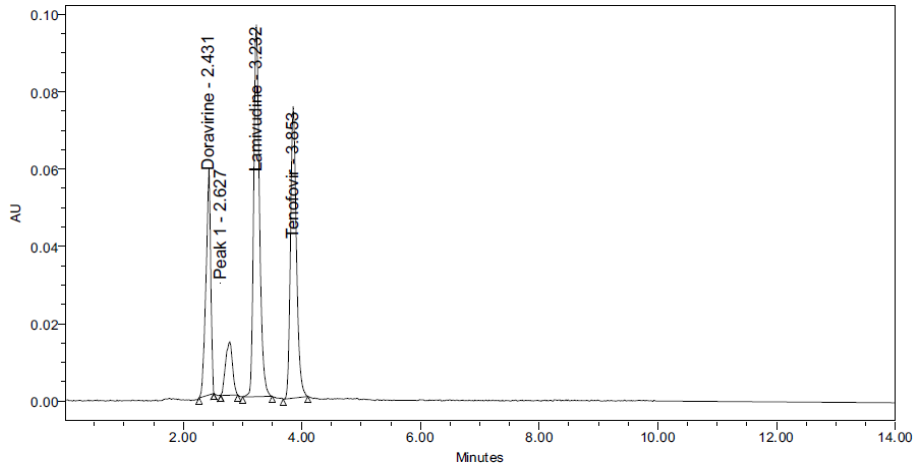

Figure 11: Chromatogram for base degradation

Proposed Procedure for Marketed Pharmaceutical Formulation:

The marketed formulation Delstrigo was analyzed separately by injecting 10µL of standard and sample solution into the HPLC system and chromatograms were then recorded The amount of the drug present in marketed tablets was calculated by comparing the peak area of standard and sample. The typical chromatograms values of standard and sample solutions using the proposed method % Assay values 99.46, 100.17 and 99.87 of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate.

CONCLUSION

A new precise, accurate and simple RP-HPLC method was developed and validated for simultaneous estimation of Doravirine, Lamivudine and Tenofovir Disoproxil Fumarate in tablet dosage form. This method is fast, accurate, precise and sensitive hence, it can be

employed for routine quality control of tablets containing both drugs in quality control laboratories and in Pharmaceutical industries.

CONFLICTS OF INTEREST

No conflicts of interest

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