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A novel quantitative estimation of antiviral drugs in combined dosage form by using RP-HPLC method

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Abstract

Objective: A Simple, accurate, specific and rugged reverse phase liquid chromatographic method was developed and validated for the simultaneous estimation of Lamivudine, Tenofovir, and Dolutegravir in bulk and tablet dosage form.

Method: A reverse phase gradient program has been developed to separate the all four active ingredients using 0.1% triflouro acetic acid, acetonitrile was used as mobile phase. A gradient programing has been developed and validated, on a reverse phase C18 column (150 X4.6 mm, 3µ) with a flow rate of 0.9 mL/min by monitoring at 258 nm of wavelength.

Results: The mean retention times of Lamivudine, Tenofovir, and Dolutegravir were found to be 3.06, 9.37 and 10.08 min respectively. Linearity of Lamivudine, Tenofovir, and Dolutegravir was found to be 10-50 µg/mL, 10-50 µg/mL and 1-10 µg/mL respectively. The accuracy of the proposed method was determined by performing recovery studies and was found to be between 98-102%. The repeatability testing for both sample and standard solutions was found as %RSD<2.0% which is within the acceptable limits showing that the method is precise as well. The LOD and LOQ were found to be 0.18 and 0.53 µg/ml for Lamivudine, 0.18 and 0.53 µg/ml for Tenofovir, 0.08 and 0.25 µg/ml for Dolutegravir respectively.

Conclusion: The proposed method was validated in terms of linearity, range, accuracy, precision, specificity, robustness and stability studies and the method is successfully applied for the estimation of lamivudine, tenofovir, and dolutegravir in combined tablet dosage form.

Keywords: lamivudine, tenofovir, dolutegravir and RP-HPLC

Introduction

Lamivudine, commonly called 3TC, is an antiretroviral medication used to prevent and treat HIV/AIDS. It is also used to treat chronic hepatitis B when other options are not possible. It is effective against both HIV-1 and HIV-2.

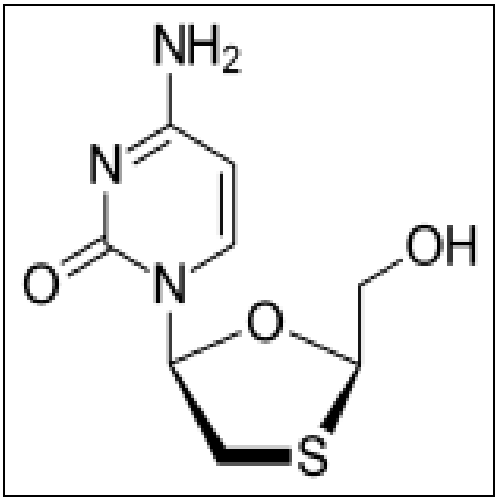

Fig 1: Structure of Lamivudine

Tenofovir disoproxil, sold under the trade name Viread among others, is a medication used to treat chronic hepatitis B to prevent and treat HIV/AIDS. It is generally recommended for use with other antiretrovirals.

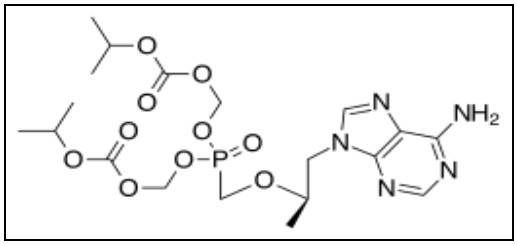

Fig 2: Structure of Tenofovir Disoproxil

Dolutegravir (DTG), sold under the brand name Tivicay, is an antiretroviral medication used, together with other medication, to treat HIV/AIDS. It may also be used, as part of post exposure prophylaxis, to prevent HIV infection following potential exposure. It is taken orally.

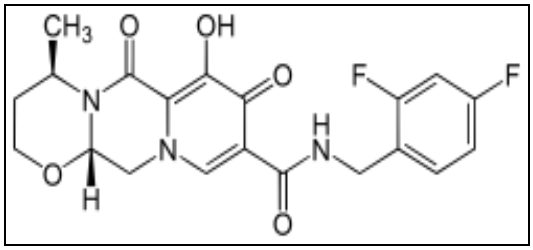

Fig 3: Structure of Dolutegravir

There are no quantitative methods available for the estimation Dolutegravir, Lamivudine, and Tenofovir

disoproxil. Hence a new and simple analytical method was developed and validated as per the ICH Q2 (R1) guidelines which can be applied for the regular analysis of commercial marketed formulations.

Materials and Methods

Chemicals and reagents

Laurus pharma Ltd, Hyderabad, India kindly supplied the pure working standards of known potency of Dolutegravir, Lamivudine and Tenofovir disoproxil are as gift samples. The reagents like water, acetonitrile, methanol are merk, potassium dihydrogen phosphate are Thermo Fisher Scientific India Pvt. Ltd.

Instrumentation

The HPLC system consists shimadzu prominence-I, LC-2030C series HPLC consisting quaternary pump, Auto sampler, Auto injector and photo diode array detector, thermostatic column compartment connected with lab solutions software connected with a YMC-pack pro C_{18} (150 × 4.6 mm, 3µ) column.

Preparation Standard Solution

Accurately weighed **10mg** of each drug transferred into separate 10ml volumetric flasks added 6ml of diluent and sonicated for 15min to dissolve compound and then volume was made up with diluent to 10ml. Further the concentration of 5 µg/ml, 30 µg/ml, 30 µg/ml of Dolutegravir, Lamivudine, and Tenofovir disoproxil were prepared.

Preparation of sample solution

Accurately weighed 1102 mg of tablet powder and transferred into 100 ml volumetric flask dissolved in diluent and sonicated for 30mins, the volume was made up with diluent, filtered with 0.45µ PVDF filter. Further 1ml diluted 100ml with diluent.

Optimazation of HPLC Method

The HPLC method was optimized with shimadzu prominence-I, LC-2030C series HPLC consisting of quaternary pump, Auto sampler, Auto injector & photo diode array detector, thermostatic column compartment connected with lab solutions software and YMC-pack pro C_{18} (150 × 4.6 mm, 3µ) column. Mobile phase-A 0.1% trifluoracetic acid buffer and mobile phase-B acetonitrile with gradient program 14 minutes runtime performed. The flow rate of the mobile phase was maintained at 0.9 mL/min and the detection was carried out at 258 nm with an injection volume of 20 µl.

Gradient program

Table 1: Gradient program

Time	Mobile phase-A	Mobile phase-B	Elution
0	95	5	Gradient
5	60	40	Gradient
7	70	30	Gradient
9	10	90	Gradient
11	10	90	Gradient
12	95	5	Gradient
14	95	5	Gradient

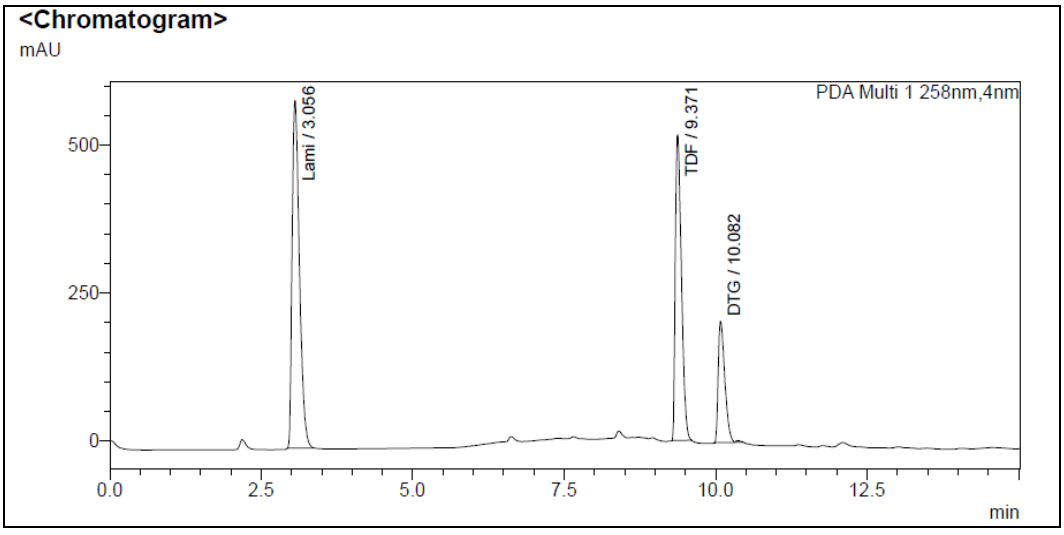

Fig 4: Chromatogram of Formulation

Results and Discussion

After RP-HPLC method development was completed and validation was performed for following parameters as per ICH Q2 (R1) guidelines.

System Suitability

System suitability test is performed to determine the suitability and effectiveness of chromatographic system. Chromatographic parameters such as the number of theoretical plates, resolution, asymmetry, detection limit and selectivity were taken into consideration. Standard solution of 10µg/ml, 10µg/ml, 4µg/ml Dolutegravir, Lamivudine, and Tenofovir disoproxil was prepared and injected into HPLC system and. Observed results were tabulated in table no. 1.

Table 2: System suitability data

S.No	Injection number	Peak area for Dolutegravir	Peak area for Lamivudine	Peak area for Tenofovir	Acceptance criteria
1	01	828516	6446765	4124921	The % RSD of peak areas should not be more than 2.0.
2	02	824587	6398562	4212581	
3	03	826498	6439561	4156894	
4	04	827856	6452689	4165854	
5	05	825189	6456254	4144654	
6	06	825893	6445689	4172541	
Mean		826423	6439920	4162908	
%RSD		0.18	0.33	0.71	

System suitability parameters	Observed value			
	Dolutegravir	Lamivudine	Tenofovir	Acceptance criteria
Tailing for Dolutegravir, Lamivudine, and Tenofovir disoproxil in standard solution	1.11	1.14	1.03	NMT 2.0
Theoretical plates for Dolutegravir, Lamivudine, and Tenofovir disoproxil in standard solution	10241	16547	19412	NLT 2000
Resolution Dolutegravir, Lamivudine, and Tenofovir disoproxil peaks in standard solution	3.3	--	--	NLT 2.0

Precision

Precision can be defined as the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. The relative standard deviation of individual areas of Dolutegravir, Lamivudine, and Tenofovir disoproxil were found to be within limits. (≥2.0)

Intra-day and Inter-day Precision

Table 3: Intra-day precision and Inter-day for Dolutegravir, Lamivudine, and Tenofovir Disoproxil

		Intra-day precision			Inter-day precision		
S.No	No of INJ	Peak area for DLT	Peak area for Lam	Peak area for TNF	Peak area for DLT	Peak area for LAM	Peak area for TNF
1	1	828369	6448562	4214569	825968	6464592	4128961
2	2	826598	6454897	4205894	826598	6398561	4202569
3	3	827854	6452681	4109562	827258	6459681	4168952
4	4	826421	6466895	4165854	826985	6459685	4154698
5	5	824968	6444851	4124582	828156	6462564	4164587
6	6	825468	6443961	4132561	825689	6461689	4135698
Mean		826613	6451975	4158837	826776	6451129	4159244
%RSD		1.06	0.13	0.16	0.23	0.19	0.15

Inearity

Linearity is the method's ability to obtain test results which are directly proportional to the concentration of analyte in the sample. A series of standard solutions were prepared in the range of 10µg/ml-50µg/ml for Lamivudine, Tenofovir disoproxil and Dolutegravir in the range for 1µg/ml-10µg/ml. The mixture of standard solutions was injected into HPLC system and calculated the correlation coefficient value, Y-intercept for area and concentrations of the standard injected.

Table 4: Report of Linearity

Standard Conc(µg/ml)	Area of Dolutegravir	Standard Conc(µg/ml)	Area of Lamivudine	Standard Conc(µg/ml)	Area of Tenofovir
1	165194	10	2232581	10	1382569
2.5	406056	20	4298254	20	2814569
5	827968	30	6442854	30	4162918
7.5	1265952	40	8592540	40	5648557
10	1646936	50	10747155	50	6938197

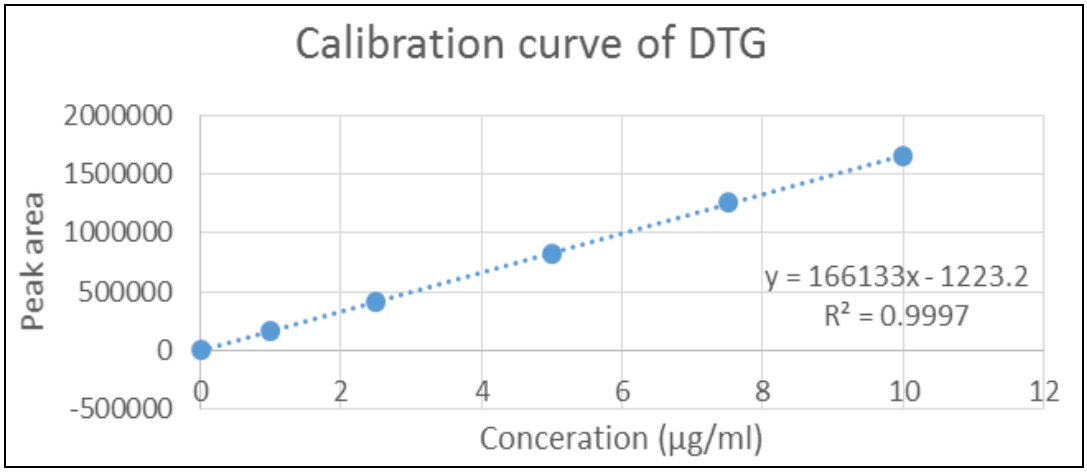

Fig 5: Calibration curve of Dolutegravir

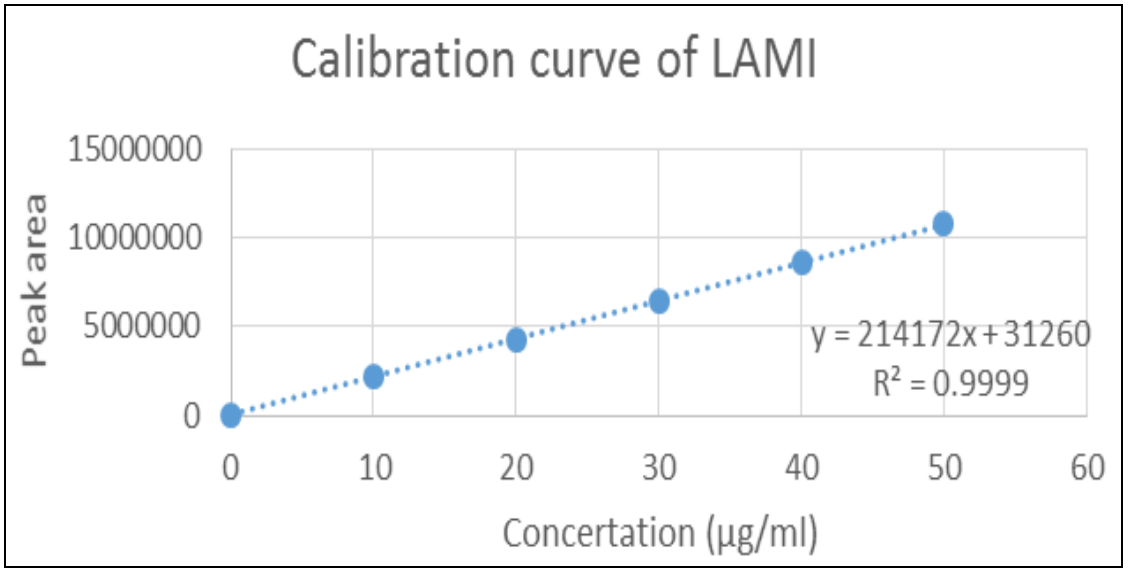

Fig 6: Calibration curve of Lamivudine

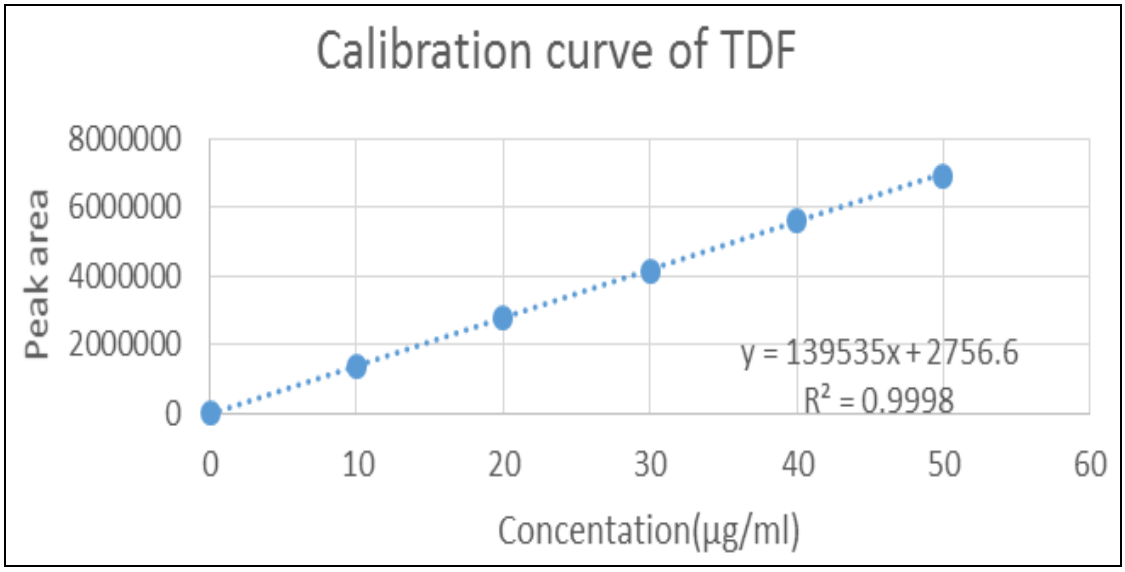

Fig 7: Calibration curve of Tenofovir

Accuracy

To determine the Accuracy of the proposed method, recovery studies were conducted. The known amount of pure drug concentrations were spiked in three different levels which were 50%, 100% and 150% accuracy was calculated and tabulated below in Table 5.

Table 5: Accuracy data

Level	Peak area			% recovery			Mean % recovery			Over all recovery		
	DTG	LAMI	TDF	DTG	LAMI	TDF	DTG	LAMI	TDF	DTG	LAMI	TDF
50	424818	3254521	2108691	99.94	100.22	101.06	100.08	100.35	100.89	99.73	99.79	100.29
	425124	3264214	2104546	100.04	100.55	100.90						
	426258	3256521	2101568	100.27	100.28	100.71						
100	846985	6440124	4154128	99.66	99.20	99.58	99.62	99.19	99.67			
	845868	6438956	4155265	99.51	99.17	99.60						
	847256	6439854	4164587	99.70	99.21	99.85						
150	1268994	9739654	6285471	99.55	100.02	100.46	99.47	99.83	100.30			
	1270258	9715240	6274561	99.64	99.76	100.27						
	1264581	9709651	6266895	99.22	99.72	100.17						

Robustness

Robustness of the proposed method demonstrated a non-significant alteration through analysis of the sample and standard Dolutegravir, Lamivudine, and Tenofovir disoproxil solution (Table 6). The results obtained were compared with that of optimized method. It was confirmed that by making the deliberate changes in the parameters there were no significant changes in standard deviation, relative standard deviation, theoretical plates, retention time and USP tailing factor were found within acceptance criteria and tabulated below.

Table 6: Report of Robustness – Dolutegravir, Lmividine, Tenofovir Disoproxil

S.No.	Parameter	Condition	System suitability results								
			% RSD			USP tailing			USP plate count		
			DTG	LAMI	TDF	DTG	LAMI	TDF	DTG	LAMI	TDF
1	Flow rate by ± 2%	0.8 ml	0.94	0.45	0.18	0.99	1.21	1.24	16878	17638	19531
		0.9 ml	0.74	0.24	0.25	1.05	1.23	1.15	16695	17410	19456
		1.0 ml	0.65	0.19	0.15	1.01	1.10	1.04	16308	17308	19210
2	Column oven temperature by ± 2°C	23°C	0.28	0.78	0.22	1.02	1.24	1.32	16603	17603	19900
		25°C	0.19	0.65	0.14	1.11	1.22	1.21	16256	17850	19533
		27°C	0.45	0.44	0.17	1.23	1.14	1.17	16968	17652	19411
3	Wavelength of analysis ± 2nm	256 nm	0.59	0.21	0.56	1.10	0.91	1.16	16965	17921	19865
		258 nm	0.66	0.32	0.72	1.14	0.96	1.21	16664	17652	19456
		256 nm	0.80	0.71	0.65	1.01	0.86	1.09	16723	17121	19741
4	Concentration of TFA	0.05%	0.29	0.69	0.79	1.23	1.23	1.26	16527	17542	19648
		0.1%	0.45	0.58	0.73	1.14	1.16	1.15	16692	17721	19315
		0.15%	0.65	0.72	0.75	1.12	1.19	1.11	16589	17533	19145

Assay Results

Accurately weighed 20 tablets and crushed to fine powder. From the pooled powder weighed equivalent to 1102 mg and transferred into 100 ml volumetric flask dissolved in diluent and sonicated for 30mins, the volume was made up with diluent, filtered with 0.45µ PVDF filter. Further 1ml diluted 100 ml with diluent.

Table 10: Results of Assay

Drug	Label Claim	% Assay
Dolutegravir	300	100.14
Lamivudine	300	100.47
Tenofovir	50	99.8

Conclusion

A simple, specific and reliable isocratic HPLC-PDA method was developed for the estimation of Dolutegravir, Lamivudine, and Tenofovir disoproxil in their bulk and pharmaceutical formulation was given. The current method was validated according to ICH guidelines Q2 (R1) in terms of Linearity, Accuracy, Precision, Limit of detection, Limit of quantification, and Robustness. Linearity plot was constructed for concentration range of 10-50µg/ml for Lamivudine, 10-50µg/ml for Tenofovir disoproxil and 1-10µg/ml for Dolutegravir standard solutions. It shows best regression coefficient and y/s values. The accuracy of the proposed method was determined by performing recovery studies and was found between 98-102%. The repeatability testing for both sample and standard solutions was found as %RSD<2.0% which is within the acceptable limits showing that the method is precise as well. The LOD and LOQ were found to be 0.18 and 0.53 µg/ml for Lamivudine, 0.18 and 0.53 µg/ml for Tenofovir disoproxil, 0.08 and 0.25 µg/ml for Dolutegravir respectively. Hence the current developed Method can be fruitfully applied for the estimation of Dolutegravir, Lamivudine, and Tenofovir disoproxil in drug testing laboratories and pharmaceutical industries.

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