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REVIEW ARTICLE: STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION DOLUTEGRAVIR, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE BY RP –HPLC

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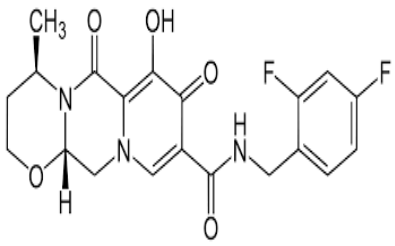
ABSTRACT

Objective: To develop a simple, accurate, precise specific and rugged reverse phase liquid chromatographic method was developed for the simultaneous estimation of lamivudine, dolutegravir, tenofovir in bulk and tablet dosage form. The analytical method was validated according to ICH guidelines [ICH Q_2(R1)]. **Method:** A reverse phase gradient program has been developed to separate all the four active compounds. The compounds present in different concentrations and chromatographic behaviour KH_2PO_4 and 1-octane sulfonic acid buffer pH 3.2 ±0.05 adjusted with trifluroacetic acid, acetonitrile and methanol was used as mobile phase (B). A gradient programming has been done, on a reverse phase kinetex biphenyl (250×4.6 mm, 5µ) with a flow rate 1mL/ min, monitored at 260nm. **Results:** The mean retention times of lamivudine tenofovir, dolutegravir was found to be 11.5, 26.5 and 30.0 min respectively. **Conclusion:** The proposed method was validated in terms of linearity, range, accuracy, precision, specificity, robustness, and stability studies and the method was successfully applied to estimation of lamivudine, tenofovir and dolutegravir in combined tablet dosage forms.

KEYWORDS: Gradient Lamivudine, tenofovir, dolutegravir, RP –HPLC.

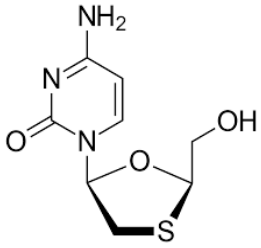
INTRODUCTION

Dolutegravir {figure 1} (DTG, is a newly developed human immunodeficiency virus (HIV) integrase inhibitor from Viiv Healthcare (Research Triangle Park, NC,USA)). Chemically known as (4R, 12As)-N-[(2,4-difluorophenyl)methyl]-7- hydroxyl -4-methyl-6,8-dioxo-3,4,12,12a-tetrehydro-2H –pyrido [5,6]pyrazino[2,6-b][1,3]oxazine -9 carboxamide.DTG is an integrase strand transfer inhibitor (INSTI) that does not require ritonavir for cytochrome P450 3A4 inhibition, and preferentially blocks the strand transfer step of integration of the viral genome into the host cell's DNA, which is a two –step process mediated by the viral integrase enzyme. Like the other approved INSTIs raltegravir (RAL) and elvitegravir (EVG), DTG inhibits the binding of the integrase viral DNA complex to host cell DNA by chelating Mg^{2+} ions in the active site. Once integration is blocked HIV-1 can no longer replicate, and the viral replication cycle is interrupted. Lamivudine {figure2} is a nucleoside transcriptase inhibitor (NRTI) reported to be active against HIV-1, and hepatitis B virus. Chemically known as 4-amino -1-[(2R,5S)-2- (hydroxyl methyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrmidin-2-one. Lamivudine has been used for treatment of chronic hepatitis B at lower dose than for treatment of HIV. It improves the sero-conversion of e- antigen positive hepatitis-B and also histology staging of liver. Tenofovir Disoproxil Fumarate{figure3} is a fumaric acid salt of the bis iso –propoxy carbonyl oxy methyl ester derivative of Tenofovir Chemically it is 9-[(R) -2-[[(isopropoxcarbonyl)-oxy]methoxy]phosphinyl]methoxy] propyl] adenine fumarate [4e7]. It exhibits activity against HIV reverse transcriptase.



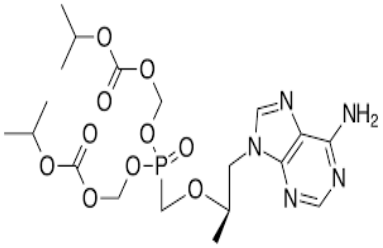
1. Dolutegravir

Figure 1



(b) Lamivudine

Figure 2



(c) Tenofovir

Figure 3

Our present study aim was to develop and validate RP-HPLC method, being simple, accurate selective and proposed method can be used for estimation of these drugs in combined dosage forms.

MATERIALS AND METHODS

Chemicals and reagents

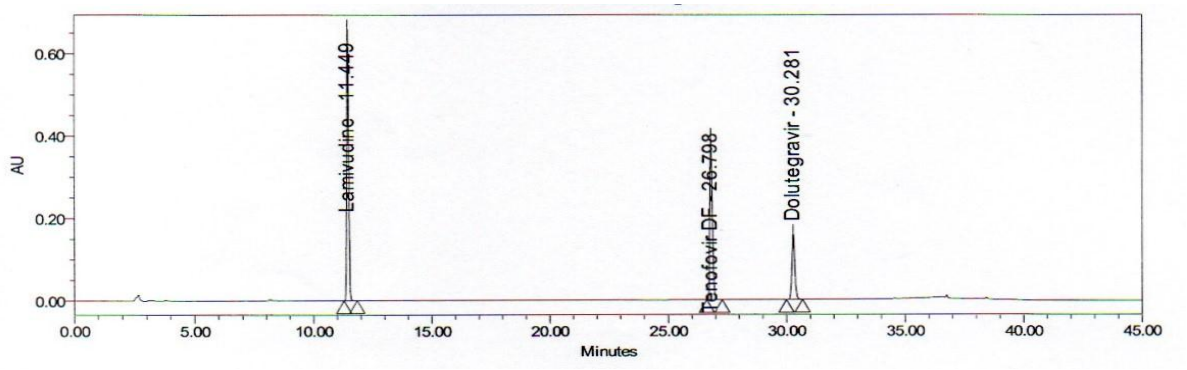
Methanol, acetonitrile, water were purchased from merck chemical company (HPLC grade). Potassium dihydrogen phosphate, orthophosphoric acid, 1- octane sulfonic acid, trifluroacetic acid were purchased from merck chemical company (GR grade). TIVICAY (Dolutegravir Sodium, Lamiviudine) was supplied by V ii V Healthcare company and VIREAD (TENOFOVIR) was supplied by Gliead sciences Inc.

Instrumentation

HPLC instrument waters was equipped with photodiode array detector. A pH meter from lab india was used to check p^H of the solution.

Chromatographic conditions

Column	Kinetex biphenyl 250× 4.6 mm, 5µm
Flow rate	1.0mL/min
Detection	UV 260nm
Injection volume	10 µL
Column temperature	30^0 C
Run time	45 minutes
Sample cooler	5^0C



Optimized chromatogram

Preparation of stock solution

Dolutegravir: weigh accurately 53mg of dolutegravir into 100 mL volumetric flask and add 60Ml of methanol and sonicate to dissolve .Dilute up-to mark with methanol.

Lamivudine & Tenofovir disoproxil fumarate: Weigh accurately 60mg of lamivudine and tenofovir disoproxil fumarate into 50 ml volumetric flask and add 30 ml of methanol and sonicate to dissolve. Dilute up-to mark with methanol.

Preparation of standard solution

Transfer each 5 ml of lamivudine &tenofovir disoproxil fumarate stock solution and 2ml of dolutegravir into 25ml of volumetric flask dilute it up-to the mark with diluent.

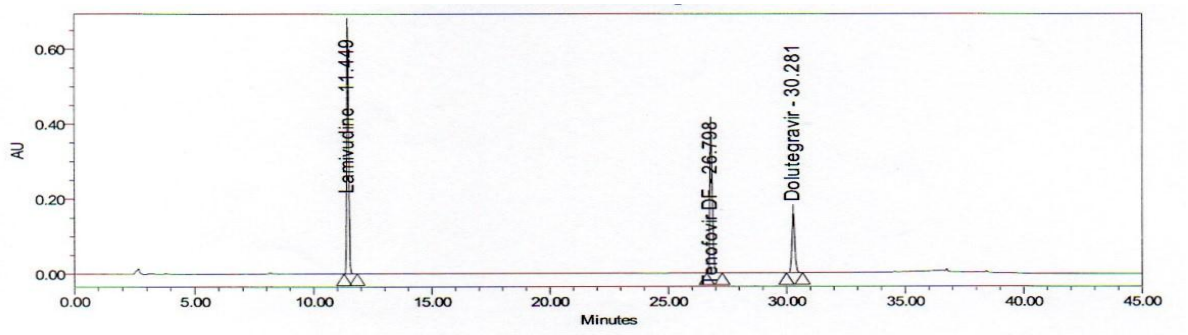
Preparation of sample solution

Transfer 5 tablets into a 500ml volumetric flask and add 50ml of 0.1% OPA buffer and sonicate not less than 45 minutes with intermediate shaking. add about 300ml of methanol sonicate for not less than 30 minutes with occasional shaking {maintain the sonicator bath temperature between 20-25^{0}c}. Dilute with methanol and mix. Centrifuge a portion of the solution at 5000 rpm for about 10 minutes .Filter the solution through 0.45 µm membrane filter and discard first few ml of the filterate. Transfer 4ml of above solution into 50 ml volumetric flask, dilute to volume with diluent and mix.

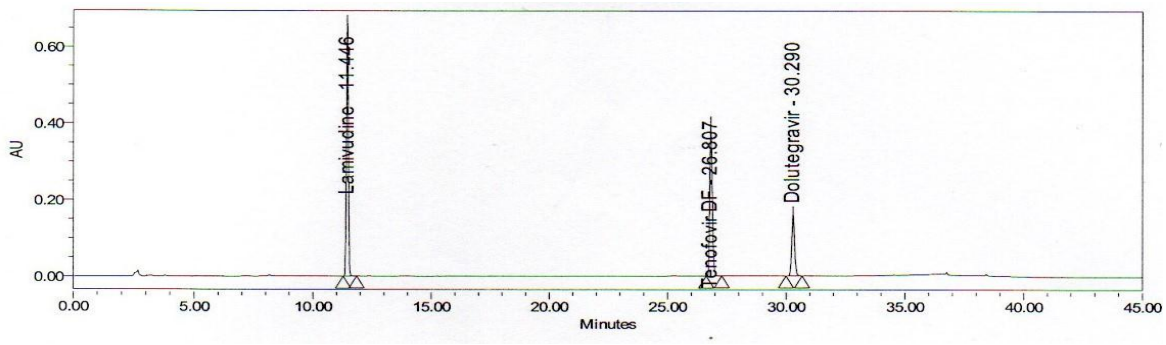
Method validation

1. System Suitability

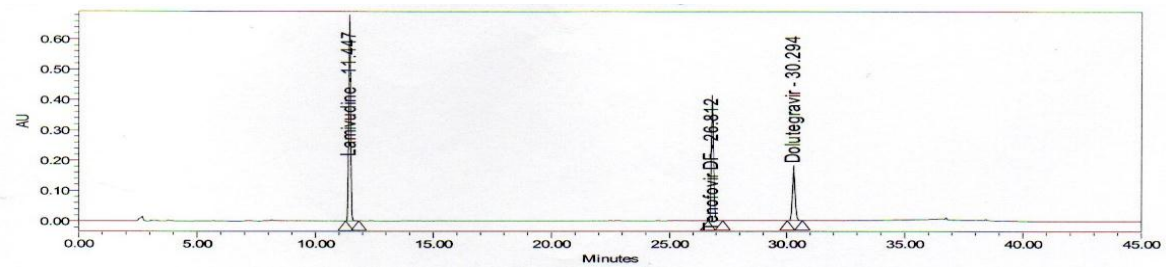
The %RSD of the peak areas of lamivudine, tenofovir, dolutegravir obtained from five replicate injections of standard solution is not more than 2.0. The retention time for lamivudine, tenofovir, disoproxil fumarate & dolutegravir peaks were about 11.5, 26.5, 30minutes respectively. five sample solutions were prepared and were injected into HPLC system.



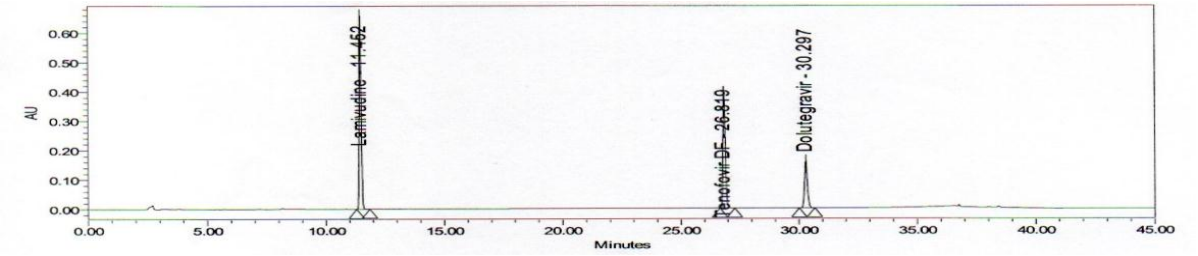
Chromatogram of system suitability 1.



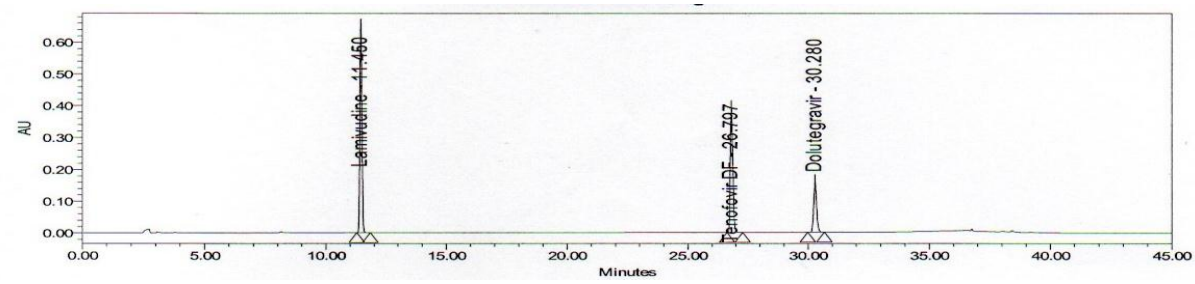
Chromatogram of system suitability 2.



Chromatogram of system suitability 3.



Chromatogram of system suitability 4.



Chromatogram of system suitability 5.

	SAMPLE NAME	NAME	RT	AREA	USP PLATE COUNT	USP TAILING
1	Standard	Lamivudine	11.450	3780080	89014	1.1
2	Standard	Lamivudine	11.452	3766628	89652	1.1
3	Standard	Lamivudine	11.447	3758460	90050	1.1
4	Standard	Lamivudine	11.446	3772060	90237	1.1
5	Standard	Lamivudine	11.449	3772248	89905	1.0

Mean			11.449	3769895	89766	1.1
%RSD			0.0	0.2		

System suitability data of lamivudine

	SAMPLE NAME	NAME	RT	AREA	USP PLATE COUNT	USP TAILING
1	Standard	Tenofovir DF	26.797	2926285	294333	1.3
2	Standard	Tenofovir DF	26.819	2916393	297158	1.3
3	Standard	Tenofovir DF	26.812	2916538	297375	1.3
4	Standard	Tenofovir DF	26.807	2924840	297012	1.3
5	Standard	Tenofovir DF	26.798	2926792	296641	1.3
Mean			26.807	2922170	296504	1.3
%RSD			0.0	0.2		

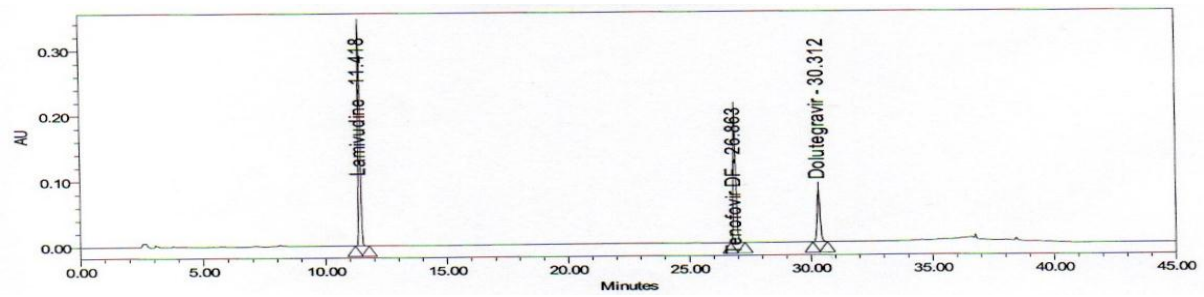
System suitability data of tenofovir disoproxil fumarate

	Sample name	Name	RT	Area	USP Plate Count	USP Tailing
1	Standard	Dolutegravir	30.280	1366233	291928	1.1
2	Standard	Dolutegravir	30.297	1363394	291720	1.1
3	Standard	Dolutegravir	30.294	1364640	290547	1.1
4	Standard	Dolutegravir	30.290	1368064	291322	1.1
5	Standard	Dolutegravir	30.281	1369696	291745	1.1
Mean			30.289	1366406	291452	1.1
%RSD				0.2		

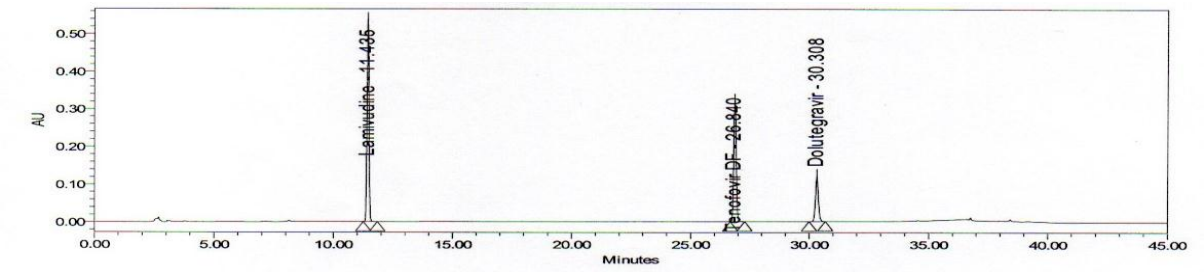
System suitability data of dolutegravir

2. Linearity

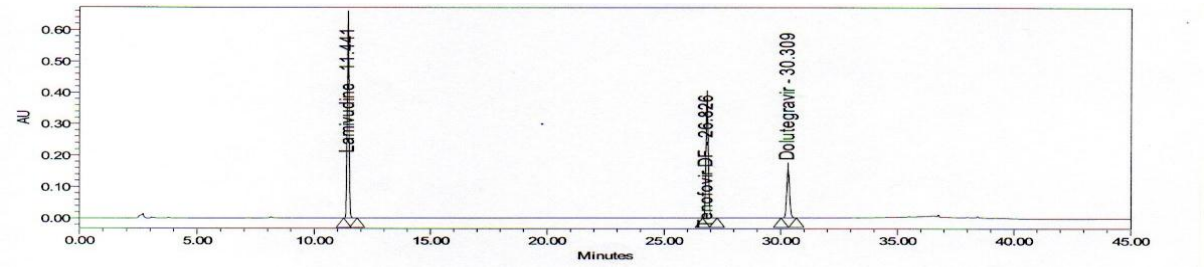
The method was demonstrated over the concentration range of 50%- 150% of the target concentration. Aliquots of 50%, 75%, 100%, 125%, 150% were prepared from stock solution.



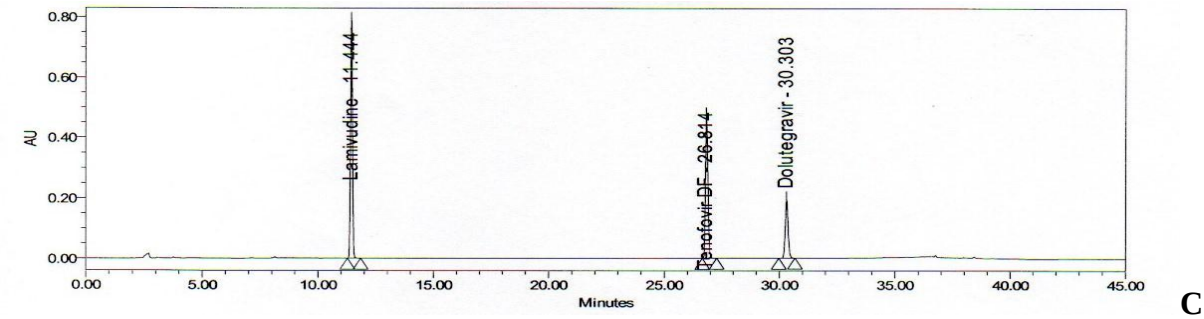
Chromatogram of linearity - 50%.



hromatogram of linearity –75%.

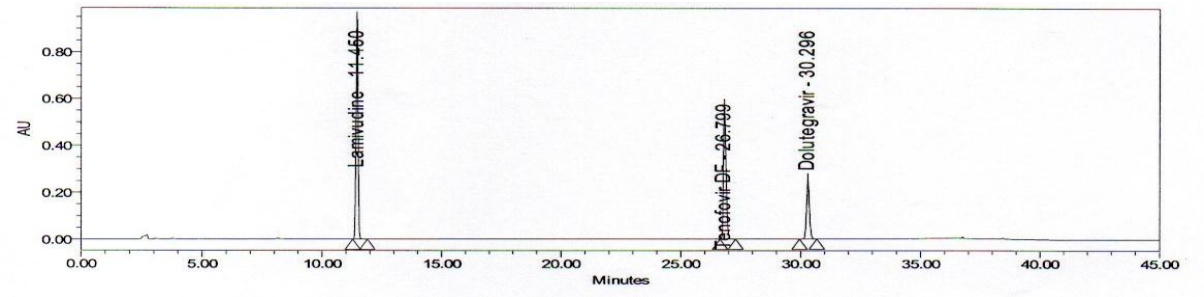


Chromatogram of linearity -100%.



C

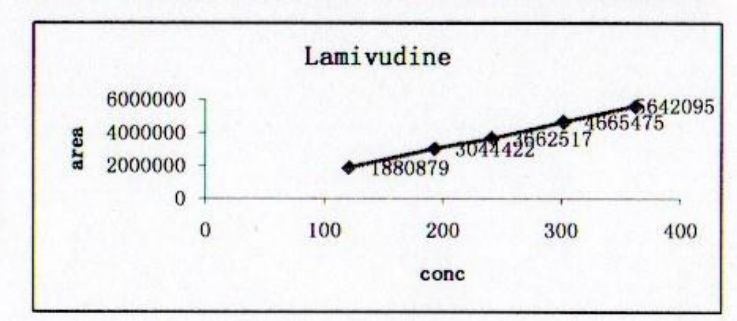
hromatogram of linearity – 125%.



Chromatogram of linearity -150%.

S.no	Linearity level	Concentration (ppm)	Response
1	Level -1	120.72	1880879
2	Level -2	193.15	3044422
3	Level -3	241.44	3662517
4	Level -4	301.8	4665475
5	Level-5	362.16	5642095
Correlation coefficient			0.99953
%y –intercept			-6952.543222

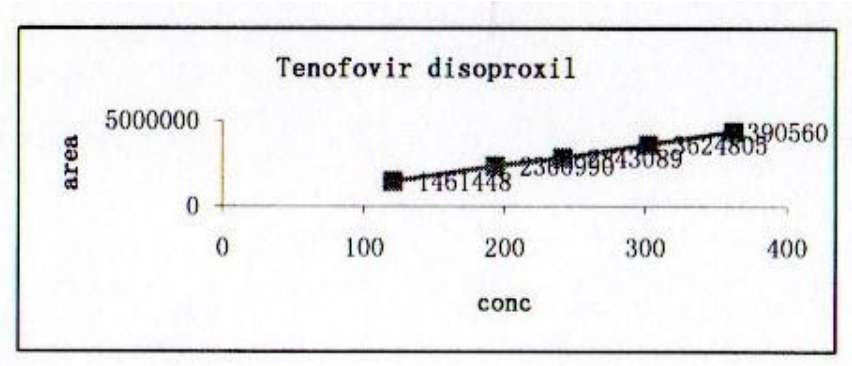
Linearity data of lamivudine



Linearity of lamivudine

S.NO	LINEARITY LEVEL	CONCENTRATION (ppm)	RESPONSE
1	Level-1	120.72	1461448
2	Level-2	193.15	2360990
3	Level-3	241.44	2843089
4	Level-4	301..8	3624805
5	Level-5	362.16	4390560
Co –relation coefficient			0.999485
% y-intercept			-7737.004314

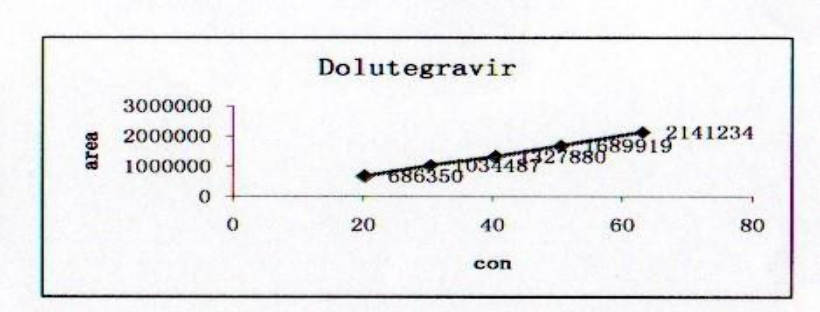
Linearity data of tenofovir DF



Linearity of tenofovir DF

S.NO	LINEARITY LEVEL	CONCENTRATION (ppm)	Response
1	Level-1	20.18	686350
2	Level-2	30.27	1034487
3	Level-3	40.36	1327880
4	Level-4	50.46	1689919
5	Level-5	63.07	2141234
Correlation coefficient			0.999484
% y –intercept			-7084.209455

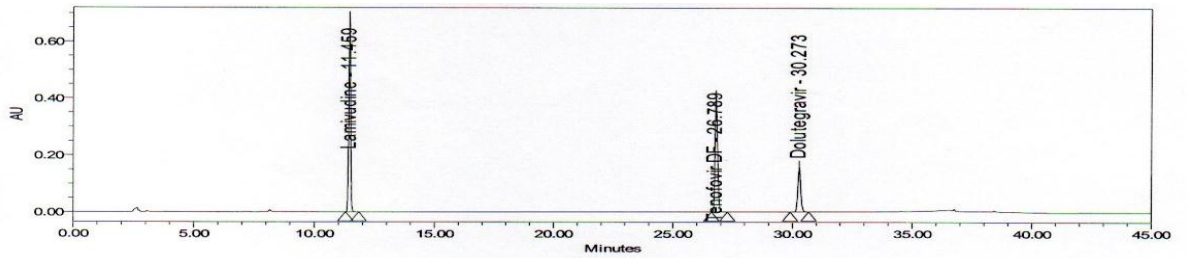
Linearity data of dolutegravir



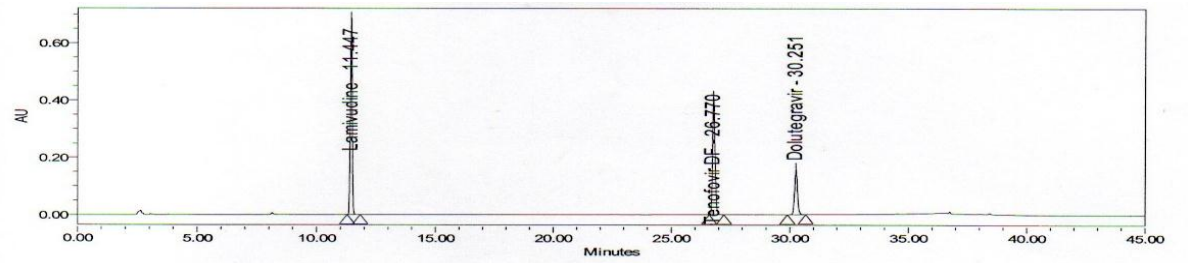
Linearity of dolutegravir

3. Precision

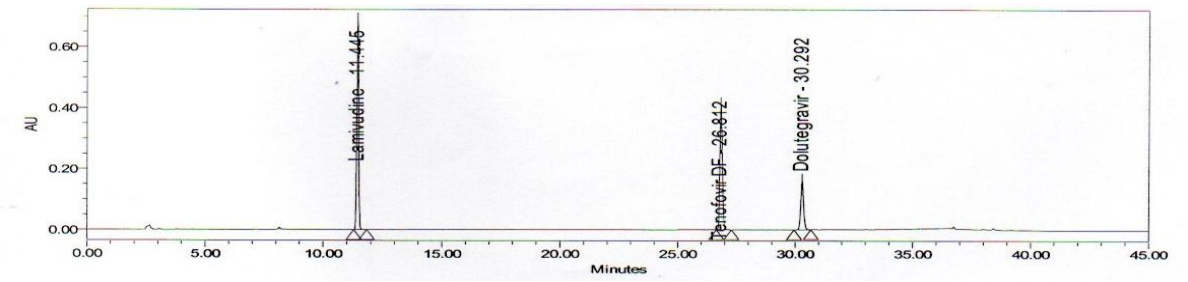
The method was determined by system precision and method precision using 100%standard and sample solutions Each level was investigated by 6 replicate injections.



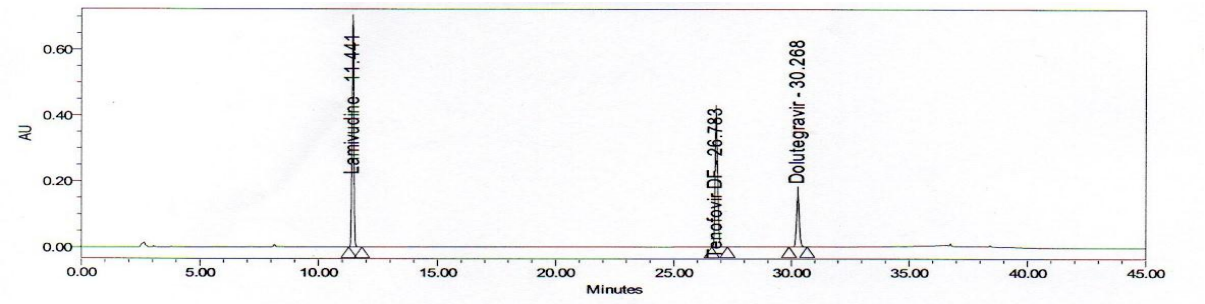
Chromatogram of precision sample -1.



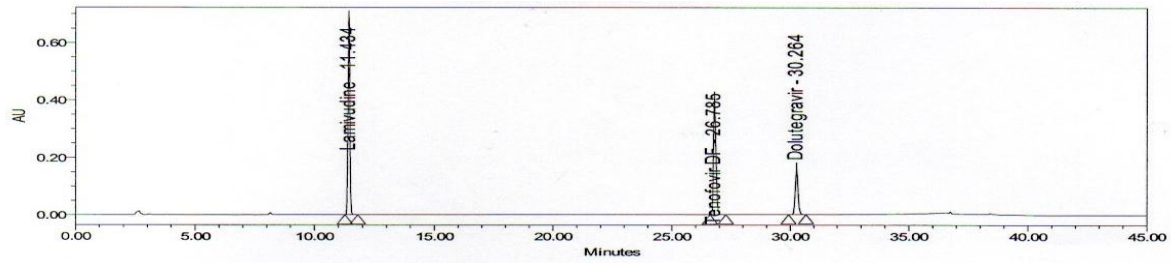
Chromatogram of precision sample-2.



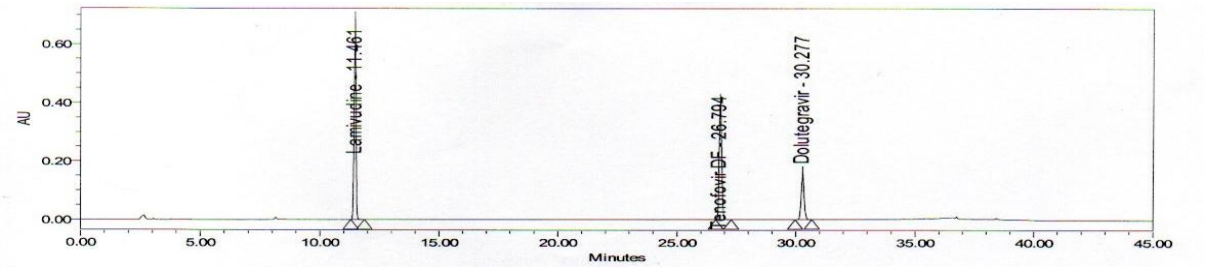
Chromatogram of precision of sample-3.



Chromatogram of precision sample -4.



Chromatogram of precision sample-5.



Chromatogram of precision sample -6.

System Precision Data of Lamivudine Tenofovir Df And Dolutegravir.

S.NO	Sample name	Channel	Lamivudine	Tenofovir DF	Dolutegravir
1	Precision sample -1	DAD.0.0.S	3698305	3040039	1351772
2	Precision sample -2	DAD.0.0.S	3716903	3046959	1355486
3	Precision sample-3	DAD.0.0.S	3728306	3059514	1362600
4	Precision sample -4	DAD.0.0.S	3732048	3059226	1361095
5	Precision sample-5	DAD.0.0.S	3706293	3037375	1351463
6	Precision sample-6	DAD.0.0.S	3720306	3048802	1358159
Mean			3717027	3048653	1356763
Std. dev.			12879	9317	4678
%RSD			0.3	0.3	0.3

Method precision data of lamivudine, tenofovir,dolutegravir.

S.NO	Sample name	Lamivudine	Tenofovoir DF	Dolutegravir
1	Precision sample -1	98.2	102.3	99.4
2	Precision sample-2	98.7	102.6	99.7
3	Precision sample-3	99	103	100.2
4	Precision sample-4	99.1	103	100.1

5	Precision sample-5	98.4	102.2	99.4
6	Precision sample-6	98.8	102.6	99.9
Mean		98.7	102.6	99.8
%RSD		0.4	0.3	0.3

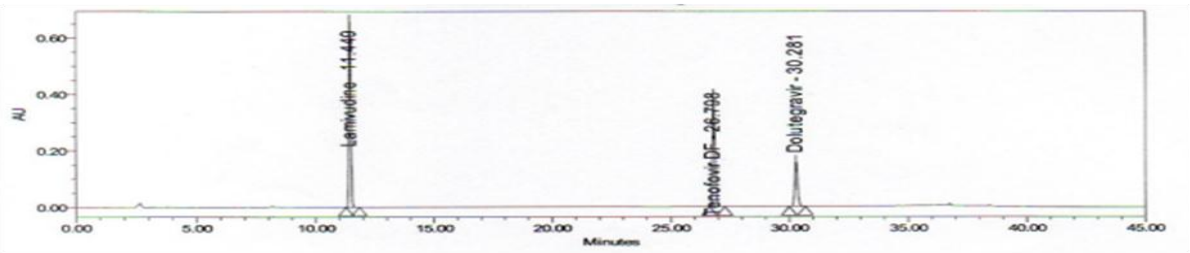
4. Accuracy

To determine the accuracy the recovery studies are conducted known amount of pure drug concentrations were spiked in placebo at three levels i.e. 50%,100%,150% respectively.

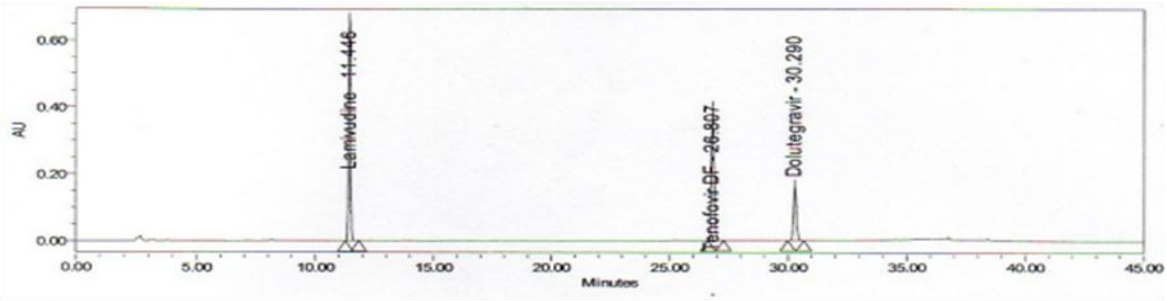
Parameter	%Recovery	Mean recovery	Overall mean recovery
Dolutegravir			
50%level	98.9,99.6,99.6	99.4	
100%level	100.04,99.4,99.9	99.9	99.6
150%level	101.1,99.2,98.4	99.6	
Lamivudine			
50%level	100.2,101.1,100.3	100.5	
100%level	99.7,98.4,98.9	99	99.6
150%level	101.1,98.4,98.6	99.4	
Tenofovir DF			
50%LEVEL	99.7,100.7,100.4	100.3	
100%LEVEL	99.2,99.1,98.7	99	99.6
150%LEVEL	100,99.8,98.4	99.4	

5. Assay

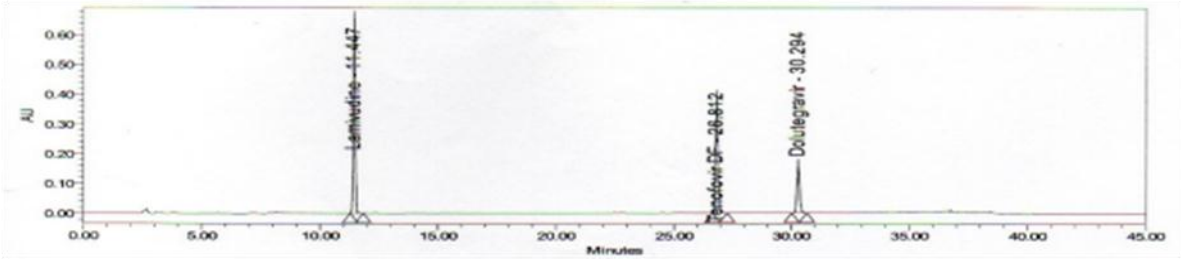
Weigh accurately 1.36g of potassium dihydrogen phosphate and 1g 1-octane sulfonic acid sodium salt monohydrate into a beaker containing 1000ml of Milli-Q water and sonicated to dissolve Adjust the p^H TO 3.0 ±0.05 with trifluroacetic acid solution. Filter the solution through 0.45 µm memnbrane filter .Preparation of mobile phase –A. use buffer preparation of mobile phase –B mixture of acetonitrile and methanol in ratio of 80:20 (%v/v).



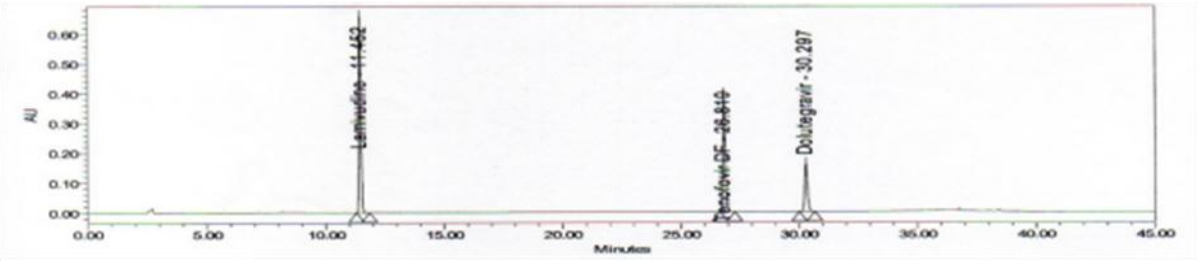
Chromatogram of standard 1



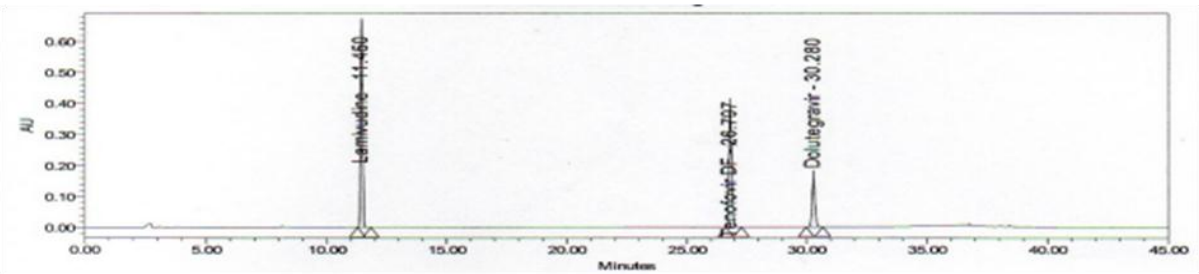
Chromatogram of standard 2



Chromatogram of standard 3



Chromatogram of standard 4



Chromatogram of standard 5

S. no.	Lamivudine %Assay	Tenofovir %Assay	Dolutegravir %assay
1	98.2	102.3	99.4
2	98.7	102.6	99.7
3	99	103	100.2
4	99.1	103	100.1
5	98.4	102.2	99.4
6	98.8	102.6	99.9
Mean	98.7	102.6	99.8
%RSD	0.4	0.3	0.3

Summary

Parameters	Dolutegravir	Lamivudine	Tenofovir DF	LIMIT
SYSTEM SUITABILITY (PLATE COUNT)	291452	89766	296504	NMT5000
USP TAILING	1.1	1.1	1.3	NMT2.0
%RSD	0.2	0.2	0.2	NMT2.0
LINEARITY RANGE(ppm**)**	20.2-63.1ppm	121-362.1ppm	120-362.2ppm	R<1
REGRESSION COEFFICIENT	0.999484	0.99953	0.99485	
SLOPE(m)	33894	15542	12092	
INTERCEPT(c)	7084	6952	7737	
REGRESSION EQUATION **(y= mx +c)**	Y=33894.x+7084	Y=15542.x+6952	Y=12092.x+7737	
ASSAY	99.6%	99.0%	99.2%	90-110%
ACCURACY %RECOVERY	99.6%	99.6%	99.6%	98-102%
PRECISION SYSTEM PRECISION (%RSD)	0.3	0.3	0.3	NMT2.0%
METHOD PRECISION (%RSD)	0.4	0.3	0.3	NMT2.0%

CONCLUSION

A simple accurate precise method was developed for the simultaneous of the dolutegravir, lamivudine, tenofovir in tablet dosage form. Retention time of dolutegravir lamivudine, tenofovir were found to be 30min, 11.5min, 26.5min. %RSD of dolutegravir lamivudine tenofovir were found to be o.3,0.4,0.7 respectively. %Recovery obtained was 99.6% 99%,99.2% fordolutegravir, lamivudine, tenofovir. LOD, LOQ Values are obtained from regression equation. Retention time and run time was high so that this method can be adopted in quality control test.

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