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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DOLUTEGRAVIR AND LAMIVUDINE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A Novel reversed phase high performance liquid chromatographic method was developed and validated for the simultaneous determination of human immune deficiency virus drug Dolutegravir (DGV) and Lamivudine(LMD) present in formulation known as Dovato which consists 50 mg of DGV and 300 mg LMD. Chromatographic separation achieved isocratically on thermo C18 column (5µm, 150mm x 4.60mm) and Acetonitrile Water(pH 5) in the ratio of 50:50 (v/v) as the mobile phase, at a flow rate of 0.8 ml/min. Detection was carried out at 267 nm. The retention times for DGV and LMD was found to be 2.56 min and 6.7 min respectively. Parameters like linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported within the ICH guidelines. The method was linear in the concentration range of 25-75 µg/ml for (DGV) and 150-450 µg/ml for (LMD). Correlation coefficient of Dolutegravir and Lamivudine was found to be 0.999 and 0.998 respectively. Developed method was found to be novel, accurate, precise, selective and rapid for simultaneous estimation of DGV and LMD.

KEYWORDS: Reverse Phase, Isocratic elution, Linearity, Chromatographic separation.

INTRODUCTION

Dolutegravir (DTG) may be a medication used for the treatment of HIV infection. Dolutegravir is chemically designated as 4-{[(2S, 4R)-1-(4-Biphenylyl)-5-ethoxy-4-methyl-5-oxo-2-pentanyl] amino}-4-oxobutanoic acid.[1,2] Dolutegravir is an HIV integrase inhibitor, a replacement class of drug with a high barrier to drug resistance and few side effects. Dolutegravir is a HIV-1 antiviral agent. It binds to the active site and blocks the strand transfer step of retroviral DNA integration in the host cell and also inhibits the HIV integrase. The most essential step in the HIV replication cycle is stand transfer step which results in the inhibition of viral activity.

Lamivudine,(2R-cis)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone,[1] is a synthetic nucleoside analogue with activity against the human immunodeficiency virus (HIV) and hepatitis B virus (HBV). Lamivudine is a nucleotide reverse transcriptase inhibitor and works by blocking the HIV reverse transcriptase and hepatitis B virus polymerase. It is effective against both HIV-1 and HIV-2.[4] Lamivudine is either formulated alone as a tablet/oral formulation or in combination with dolutegravir. HPLC is the most widely used technique for the estimation of lamivudine in human plasma, saliva, cerebrospinal fluid, and human blood cells, as well as for studying the drug metabolites in the urine. The present research work describes RP-HPLC method for estimation of Lamivudine and Dolutegravir in bulk and pharmaceutical dosage form.[7]

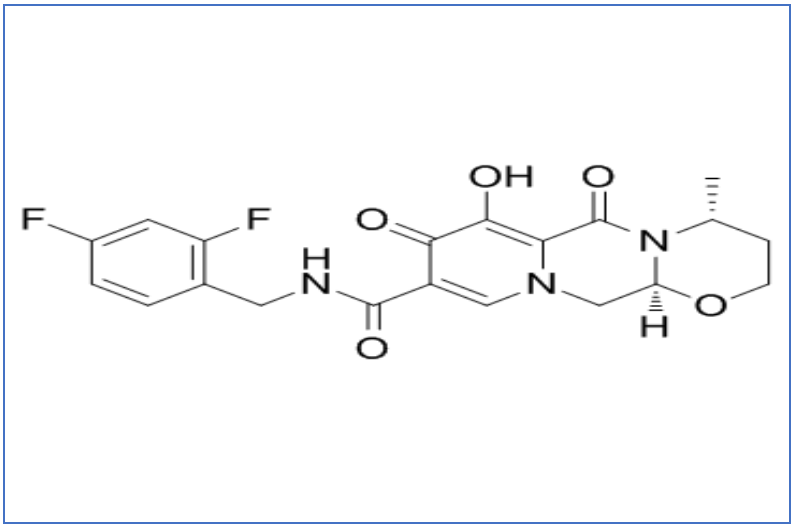


Fig. 1: Dolutegravir.

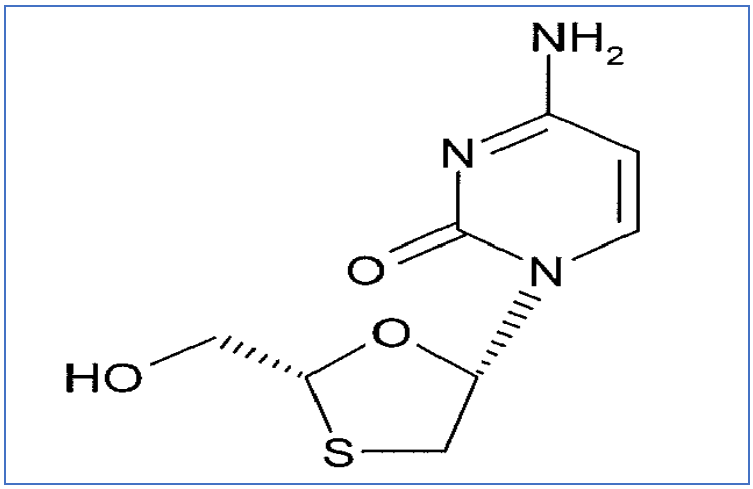


Fig. 2: Lamivudine.

Experimental[8-10]

Instrumentation

The analysis was performed on Agilent 1120 compact LC chromatographic system equipped with a quaternary pump and UV-visible detector. Chromatographic software EZ-Chrome was used for data collection. Reversed phase C18 column (5µm, 150mm x 4.60mm) as stationary phase, Ultrasonic cleaner, analytical balance, Vacuums micro filtration unit with 0.45µ membrane filter was used in the study.

Chemicals and reagents

Acetonitrile (HPLC grade), orthophosphoric acid (HPLC grade), and water (HPLC grade) were purchased from Merck (India) Ltd., Worli, Mumbai, India. Pharmaceutically pure sample of Lamivudine and Dolutegravir were obtained as gift samples from Emcure pharmaceuticals, Hinjewadi, pune, India.

Chromatographic conditions

Chromatographic analysis was done using gradient elution, mobile phase in the ratio of acetonitrile: water (pH 5.0) (50:50 v/v) was filtered through 0.45 µ membrane filter paper. The flow rate of the mobile phase was monitored at 0.8 ml/min and eluents were detected at 267 nm. By injecting the volume 10µl with a run time 20 min.

Preparation of mobile phase

pH adjustment (Water pH 5.0)

Taken accurately 1ml of OPA in1000 ml of water.

Mobile phase

Then taken 50 volumes of water and 50 volumes of acetonitrile and sonicated for 5 min.

Diluents

Water: Acetonitrile (50:50 v\v).

Preparation of standard stock solution

A 300 mg of pure Lamivudine and 50 mg of Dolutegravir were weighed and transferred to 50 ml of volumetric flask and dissolved in diluent. Accurate quantity of 30 ml of mobile phase was added and sonicated for 20 min to dissolve the components makeup to the mark with diluent and mixed the flask was shaken and volume was made up to mark with diluent to give primary stock solution. From the above solution 1ml of

solution is pipetted out into a 10 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 600µg/ml of Lamivudine and 100 µg/ml Dolutegravir.

Preparation of sample solution

Accurately weighed twenty tablets were ground to obtain fine powder equivalent to 300mg of Lamivudine and 50mg of Dolutegravir sample were weighed and transferred to 50 ml of volumetric flask and dissolved in diluent. The flask was shaken and volume was made up to mark with diluents to give a primary stock solution. From the above solution 1 ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with diluents to give a solution containing 600µg/ml of Lamivudine and 100 µg/ml Dolutegravir .

RESULTS AND DISCUSSION

Determination of Working Wavelength (λ max)

10 mg of the Lamivudine and Dolutegravir standard drug was taken in a 10 ml volumetric flask and dissolved in Diluent and volume was made up to the mark, from this solution 0.1ml was pipetted into 10 ml volumetric flask and made upto the mark with the Water to give a concentration of 10 µg/ml. The above prepared solution was scanned from 400 nm to 200 nm using Water as blank. The λ max was found to be 267.0 nm. After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of ACN: Water (pH 5) (50:50).The flow rate was 0.8 ml/ min. brought sharp peaks. The chromatogram was shown in Figure No.5

Method Validation

a) System Suitability Parameters

Six replicate injections of system suitability solutions (working standard solution) were injected. The retention time, areas, theoretical plates, peak asymmetry, and resolution were calculated for standard solutions.

b) Linearity

Linearity was studied by analysing ten standard solutions covering the range of 10-100 µg/ml for dolutegravir and 50-500 µg/ml lamivudine. For this concentration 100 mg of dolutegravir and lamivudine was taken separately in volumetric flask and further dilution was made up by using diluent. Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and therefore the obtained data were subjected to multivariate analysis using the smallest amount squares method.

c) Method Precision (Repeatability)

The precision of the method was checked by repeated preparation (n=6) of 60-360µg/ml of Lamivudine and 20-120µg/ml Dolutegravir for inter-day and intraday interval without changing the parameter of the proposed chromatographic method and measure the peak areas and retention times.

d) Accuracy (Recovery Study)

The accuracy of the method was determined by calculating the recoveries of Lamivudine and Dolutegravir by analysing solutions containing approximately 80%, 100% and 120% of the working strength of Lamivudine and Dolutegravir. The study was measured three times at each level. The percentage recovery results obtained are listed in Table No.5 and 6.

e) Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ± 2nm and flow rate was varied ± 0.2 ml/min.

f) Ruggedness

The ruggedness of the method was studied by analysing the sample and standard preparations by two analysts.

g) Limit Of Detection And Limit Of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

$LOD = 3.3\ \sigma/S$ ……. (1)

$LOQ = 10\ \sigma/S$ ……. (2)

Where, σ = the standard deviation of the response (STEYX)

S = the slope of the calibration curve

The slope S could also be estimated from the calibration curve of the analyte.

RESULT AND DISCUSSION

1) System Suitability Parameters

In system suitability test all the parameter i.e. peak area, USP plate count, USP tailing, and RT were shown within acceptance criteria. result shown in table 1 & 2.

2) Linearity

A linear relationship between peak areas versus concentrations was observed for Lamivudine and Dolutegravir in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.9992 and 0.9990 for both Lamivudine and Dolutegravir which prove that the method is linear in the range of 50% to 150%. Table 3 & 4.

3) Method Precision (Repeatability)

Results of variability were summarized in the above table. Percentage relative standard deviation (%RSD) was found to be less than 2.0% which proves that method is precise. Table 5 & 6.

4) Accuracy (Recovery Study)

Results of accuracy study are presented in the below table 7 & 8. The method is highly accurate.

5) Robustness

The results of Robustness of the present method had shown that changes are not significant we can say that the method is Robust. The results were shown in (Table No.9,10,11,12)

6) Ruggedness

The %RSD assay values between two analysts was calculated, this indicates the method was rugged. The results were shown in Table No.13 and 14.

7) Limit of Detection and Limit of Quantification

Table no 15 shows the result of Dolutegravir and Lamivudine.

Table 1: System Suitability.

Dolutegravir				
Injection	**RT(min)**	**Peak Area**	**USP Plate count(N)**	**USP Tailing (T)**
1	**6.53**	**8884868**	**10155**	**1.13**
2	**6.55**	**8876543**	**9970**	**1.10**
3	**6.35**	**8855324**	**9912**	**1.09**
Mean	**6.47**	**8872245**	**10012.33**	**1.10**
SD	**0.08**	**12438.29**	**103.62**	**0.016**
%RSD	**1.38**	**0.1401**	**1.03**	**1.53**

Table 2: Linearity.

Lamivudine				
Injection	**RT(min)**	**Peak Area**	**USP Plate count(N)**	**USP Tailing (T)**
1	**2.55**	**38545477**	**7856**	**1.04**
2	**2.46**	**38411765**	**7650**	**1.01**
3	**2.51**	**38516654**	**7621**	**1.06**
Mean	**2.50**	**38491299**	**7709**	**1.03**
SD	**0.036**	**57456.6**	**104.61**	**0.021**
%RSD	**1.46**	**0.149**	**1.35**	**1.98**

Table 3: Linearity of Dolutegravir.

Conc(ppm)	Area
10	1213843
20	2318214
30	3412931
40	4418163
50	5531926
60	6649486
70	7598890
80	8685676
90	9816443
100	11152309
Slope	1100953.1
Correlation coefficient	0.999

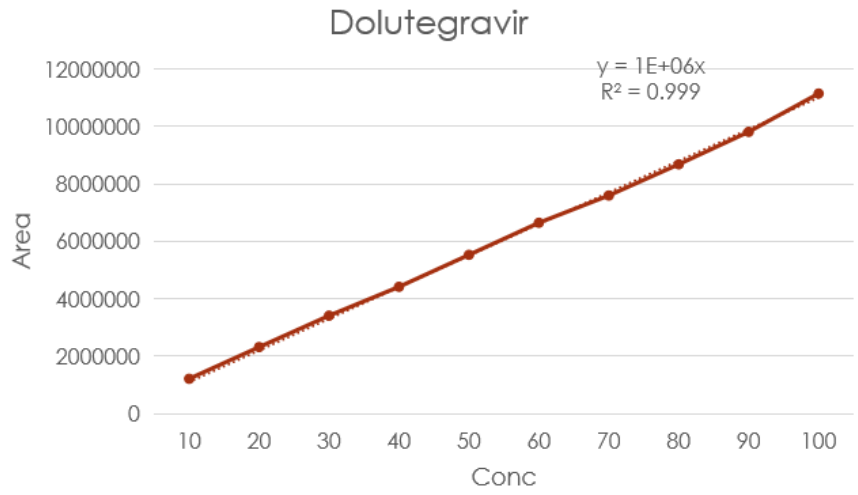
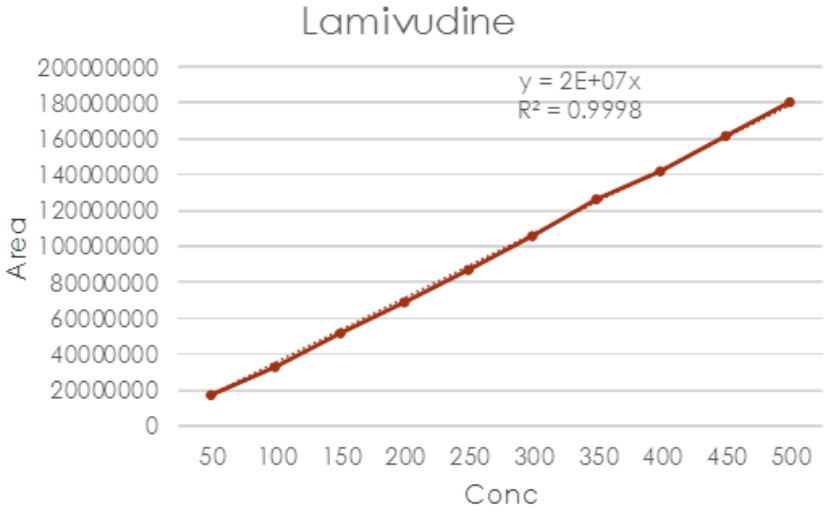


Table 4: Linearity of Lamivudine.

Conc(ppm)	Area
50	16851574
100	33113381
150	51266174
200	69185789
250	86979172
300	105895981
350	125892831
400	142110391
450	161411345
500	180149011
Slope	364972.3
Correlation Coefficient	0.999



Precision (Intra-day)

Table 5: Summary of peak areas for precision of Lamivudine.

Sr No	Sample No	Retention time	Peak area	% Assay
1	1	2.55	41322760	100.1
2	2	2.53	41332962	99.6
3	3	2.57	42123456	99.8
4	4	2.53	43123432	98.4
5	5	2.58	42122650	98.5
6	6	2.59	42231802	99.6
7	Mean	2.558	42042843	99.33
8	%RSD	0.914	1.45	0.65

Table 6: Summary of peak areas for precision of Dolutegravir.

Sr No	Sample No	Retention time	Peak area	% Assay
1	1	6.57	4123214	100.4
2	2	6.53	4212321	99.7
3	3	6.23	4312312	99.5
4	4	6.54	4122323	98.2
5	5	6.57	4312232	98.4
6	6	6.52	4167566	99.5
7	Mean	6.49	4208328	99.28
8	%RSD	1.83	1.74	0.76

Precision (Inter-day) 1) Dolutegravir

	Sample no	Retention time	Peak area	%%Assay
Day-1	1	6.52	4323256	99.40
	2	6.56	4312633	99.80
	3	6.45	4380344	99.20
Mean		6.50	4338744	99.46
%RSD		0.54	0.68	0.25

	Sample no	Retention time	Peak area	%%Assay
Day-2	1	6.33	4263298	99.50
	2	6.36	4355610	99.10
	3	6.48	4376348	99.40
Mean		6.39	4331752	99.33
%RSD		1.01	1.13	0.17

2) Lamivudine

	Sample no	Retention time	Peak area	%%Assay
Day-1	1	2.58	32448701	99.70
	2	2.54	32115405	99.90
	3	2.51	32995441	99.50
Mean		2.54	32519849	99.70
%RSD		1.12	1.11	0.16

	Sample no	Retention time	Peak area	%%Assay
Day-2	1	2.43	32746111	99.88
	2	2.47	32665401	99.45
	3	2.51	32455456	99.78
Mean		2.47	33658984	99.70
%RSD		1.32	1.26	0.18

Table 7: Recovery data of Dolutegravir.

Drug name	%concentration	Amount added(mg/ml)	Amount found(mg/ml)	%recovery	mean
Dolutegravir	80	4	3.92	98.4%	98.93%
	80	4	3.87	98.8%	
	80	4	3.89	99.6%	

	100	5	4.91	98.2%	98.33%
	100	5	4.93	98.6%	
	100	5	4.96	99.2%	
	120	6	5.87	99.6%	99.63%
	120	6	5.98	99.7%	
	120	6	5.88	99.6%	

Table 8: Recovery data of Lamivudine.

Drug name	%concentration	Amount added(mg/ml)	Amount found(mg/ml)	%recovery	mean
Lamivudine	80	24	23.85	99.0%	98.83%
	80	24	23.87	99.1%	
	80	24	23.76	98.4%	
	100	30	29.14	97.0%	98.1%
	100	30	29.53	98.3%	
	100	30	29.72	99.0%	
	120	36	35.92	99.11%	99.00%
	120	36	35.82	98.4%	
	120	36	35.79	99.5%	

Robustness

Table 9: Change in Flowrate 1ml/min.

Inj. No.	Dolutegravir			Lamivudine		
	Area	Tailing factor	Theoretical plates	Area	Tailing factor	Theoretical plates
1	4341214	1.4	10344	56322760	1.6	7135
2	4214321	1.4	10284	56782962	1.5	7335
3	4316312	1.4	10828	54563456	1.5	8768
4	4322323	1.4	10335	54224343	1.5	7862
5	4412232	1.4	10346	55432650	1.6	7762
Average	**4321280**			**55465234**		
STDEV	63459.64			982429		
% RSD	**1.46**			**1.77**		

Table 10: Change in Flowrate 0.6ml/min.

Inj. No.	Dolutegravir			Lamivudine		
	Area	Tailing factor	Theoretical plates	Area	Tailing factor	Theoretical plates
1	5341214	1.2	11234	98322760	1.5	8712
2	5494321	1.2	11234	98782962	1.5	8355
3	5377312	1.2	11218	94563456	1.5	8944
4	5382323	1.2	11235	98767743	1.5	8554
5	5482232	1.2	11236	98712350	1.5	8343
Average	**5415480**			**97829854**		
STDEV	61226.3			1641899		
% RSD	**1.13**			**1.67**		

Table 11: Change in Wavelength 271.

Inj. No.	Dolutegravir			Lamivudine		
	Area	Tailing factor	Theoretical plates	Area	Tailing factor	Theoretical plates
1	5141214	1.2	13234	97355765	1.5	8312
2	5277321	1.2	13224	97782962	1.5	8223
3	5261312	1.2	12218	96563456	1.5	8511
Average	**5226616**			**97234061**		
STDEV	60740.74			505244.2		
% RSD	**1.16**			**0.51**		

Table 12: Change in Wavelength 265.

Inj. No.	Dolutegravir			Lamivudine		
	Area	Tailing factor	Theoretical plates	Area	Tailing factor	Theoretical plates
1	5612565	1.3	13234	96542760	1.4	9382
2	5677399	1.3	13224	94322962	1.4	9663
3	5561377	1.3	12218	94653456	1.4	9566
Average	**5617114**			**95173059**		
STDEV	47474.86			977877.5		
% RSD	**0.84**			**1.02**		

Ruggedness

Table 13: Results of Dolutegravir.

Sr No			%Assay	%RSD
1	Analyst 1	Dolutegravir	100.8	0.54 %
2	Analyst 2		99.7	

Table 14: Results of Lamivudine.

Sr No			%Assay	%RSD
1	Analyst 1	Lamivudine	100.3	0.24 %
2	Analyst 2		99.8	

LOD & LOQ

Sr no		Dolutegravir	Lamivudine
1	LOD	4.26	2.52
2	LOQ	1.29	2.08

Table 15: LOD & LOQ.

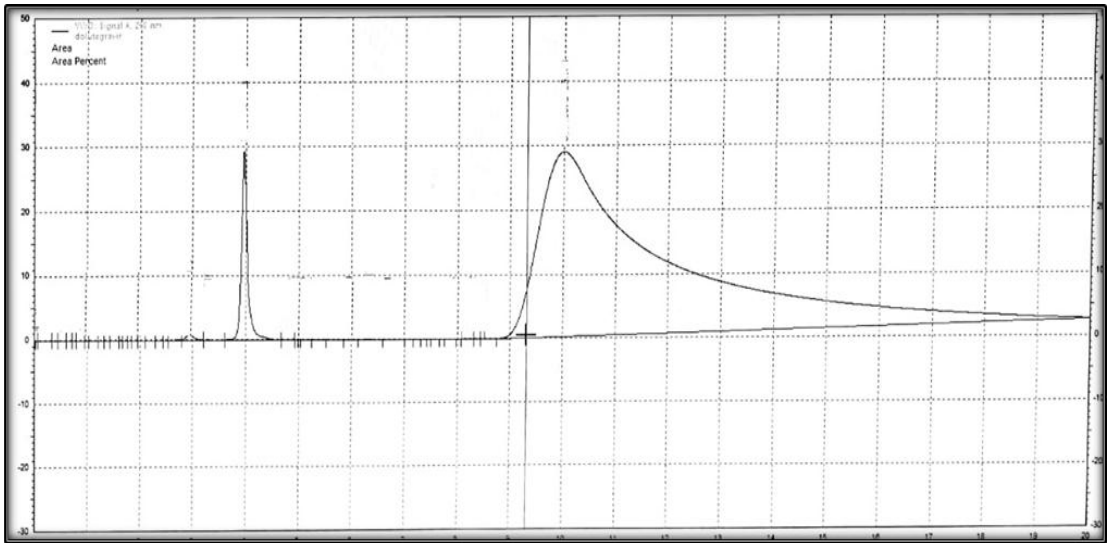
Summery Result of Lamivudine.

Sr no	Parameter	Result	Acceptance criteria
1)	System suitability a) Theoretical plates b) Tailing factor c) Retention time d) %RSD	 8312 1.4 2.56 1.02	 a) Not less than 3000 b) Not more than 2.0 d) Not more than 2.0
2)	Specificity	Specific	Specific
3)	Method precision(%RSD)	1.45	Not more than 2.0%
4)	Linearity Correlation coefficient (r2)	150-450 ug/ml 0.9992	Not less than 0.990
5)	Accuracy (Mean % recovery) 80% 100% 120%	 98.83% 98.1% 99.00%	97 - 103%
6)	Robustness	All the system suitability parameters are within the limits.	

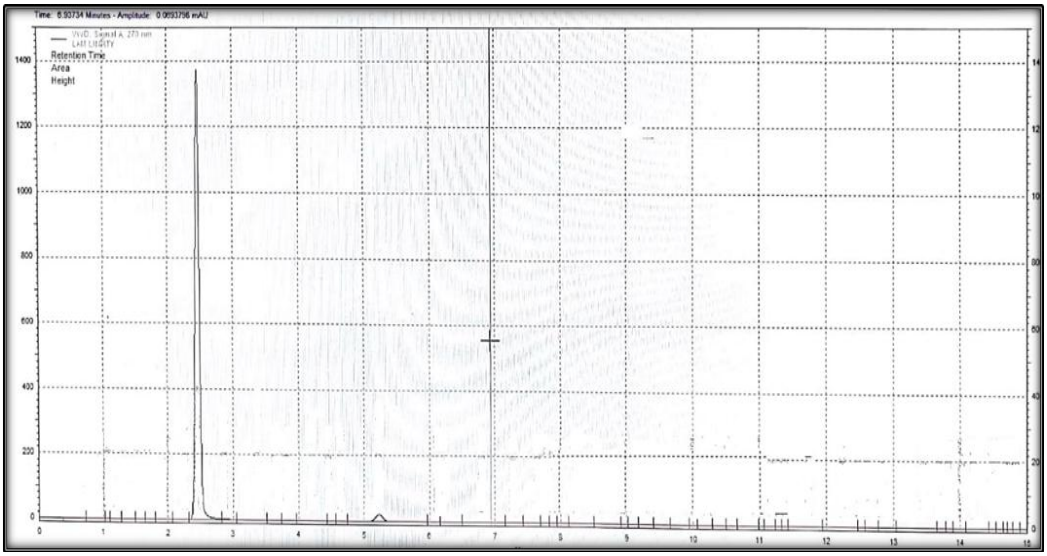
Summery Result of Dolutegravir

Sr no	Parameter	Result	Acceptance criteria
1)	System suitability a) Theoretical plates b) Tailing factor c) Retention time d) %RSD	 10344 1.3 6.80 0.84	 a) Not less than 3000 b) Not more than 2.0 d) Not more than 2.0
2)	Specificity	Specific	Specific
3)	Method precision(%RSD)	1.74	Not more than 2.0%
4)	Linearity Correlation coefficient (r2)	25-75 ug/ml 0.999	Not less than 0.990

5)	Accuracy (Mean % recovery) 80% 100% 120%	 98.93% 98.33% 99.63%	 97 - 103%
6)	Robustness	All the system suitability parameters are within the limits.	



Trial 1:



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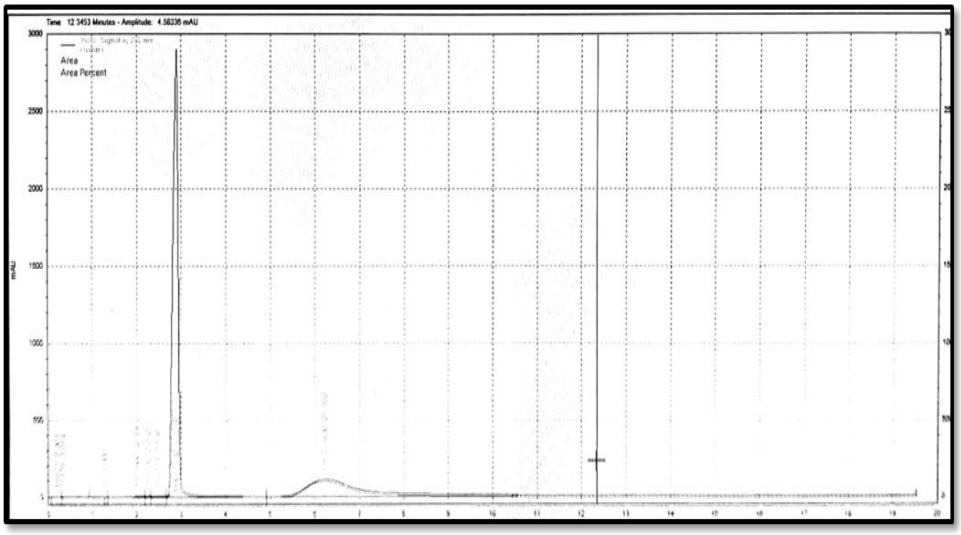

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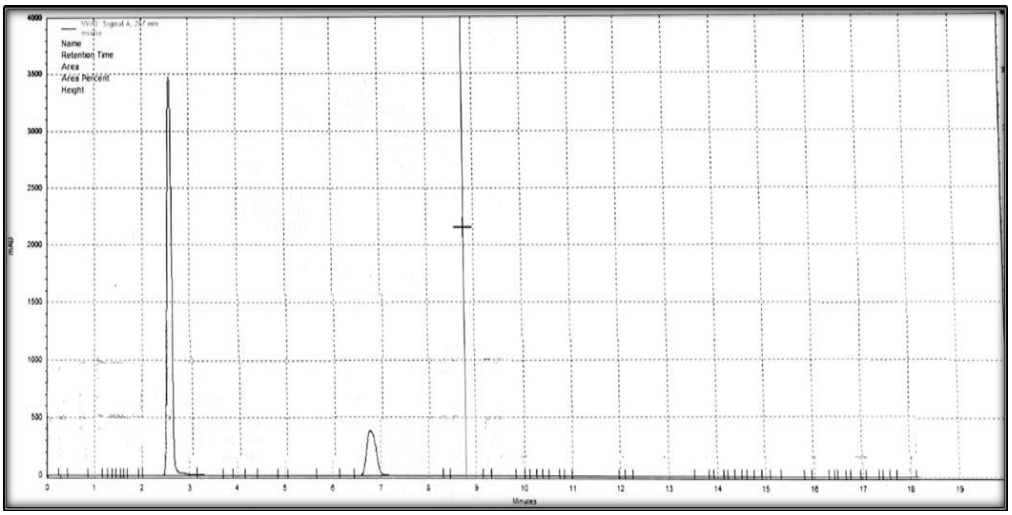

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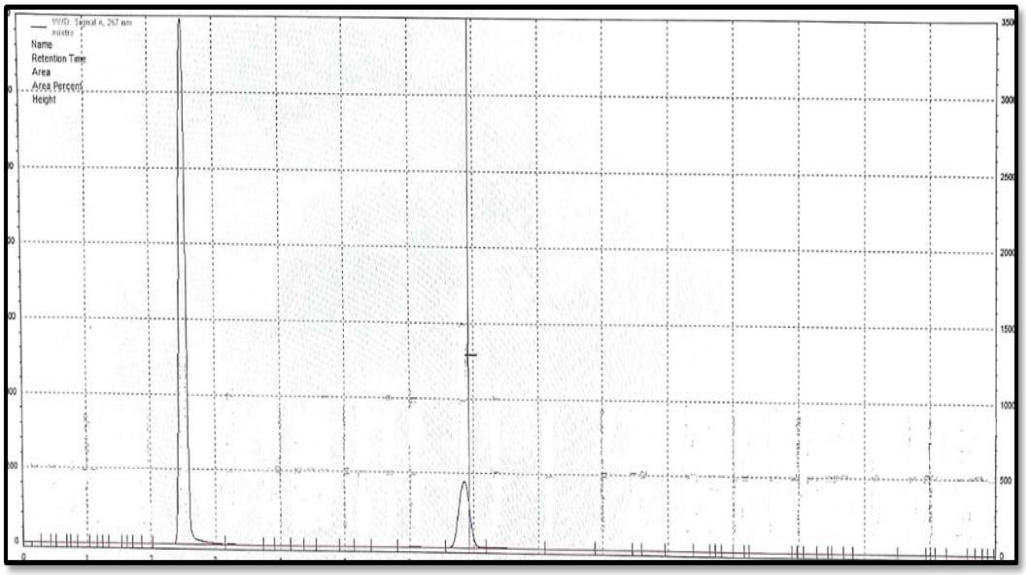

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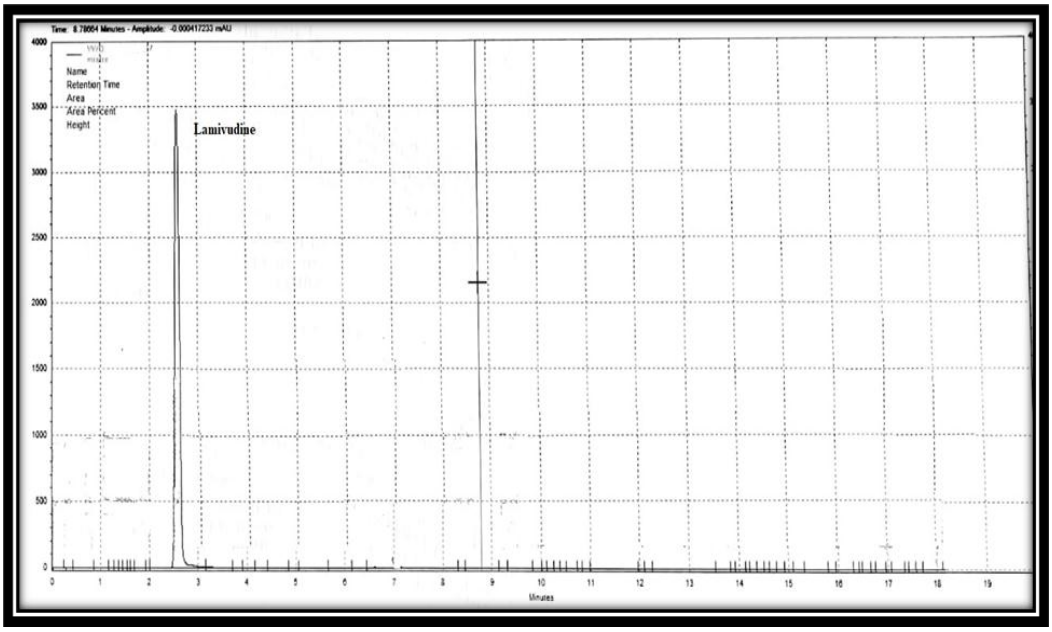
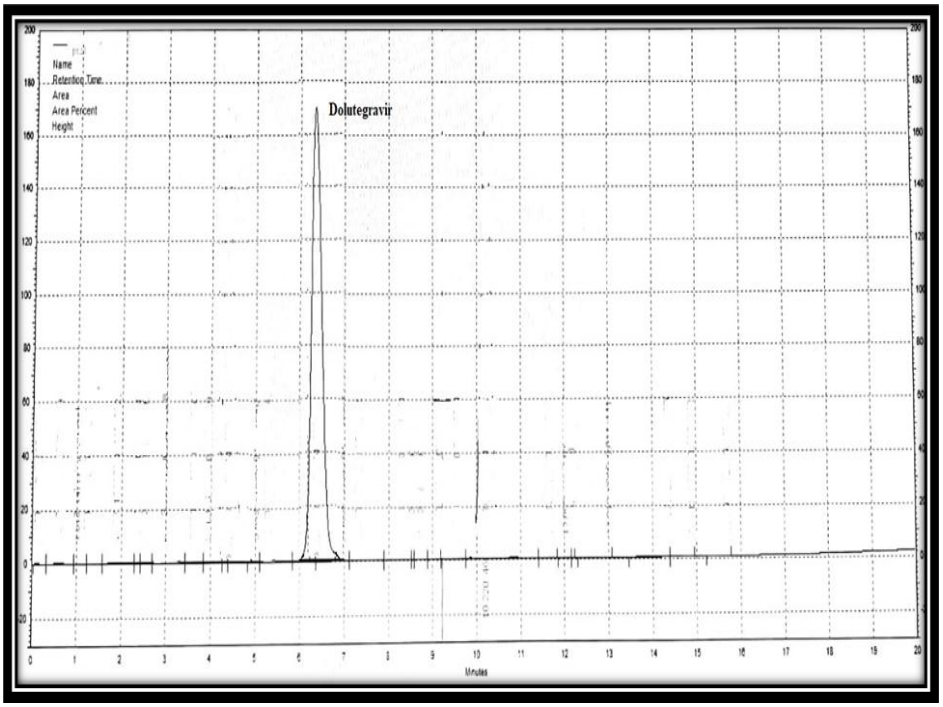
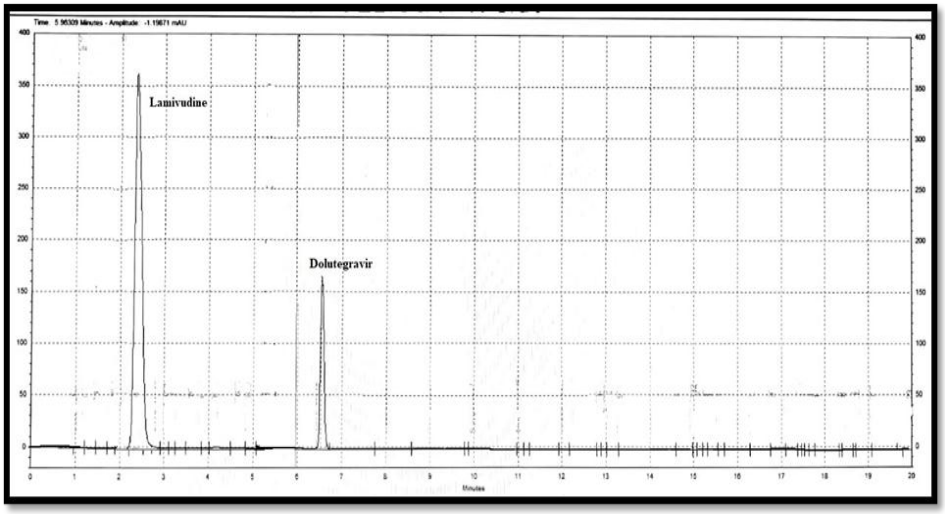


Lamivudine Peak.



Dolutegravir Peak:



Final Chromatogram of Dolutegravir & Lamivudine.

CONCLUSION

A simple and reliable RP- HPLC method has been developed and successfully validated for the Simultaneous estimation of dolutegravir and lamivudine. This method is precise, accurate and high resolution and shorter retention time which makes this method more acceptable and cost effective. Hence, the proposed HPLC method is suitable for routine determination of dolutegravir and lamivudine in pharmaceutical formulation in quality control laboratories, where economy and time are essential.

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