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DEVELOPMENT AND VALIDATION OF RP- UPLC METHOD FOR SIMULTANEOUS DETERMINATION OF LAMIVUDINE AND DOLUTEGRAVIR IN COMBINED DOSAGE FORM

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Abstract:
A simple, accurate, precise method was developed for the simultaneous estimation of the Lamivudine and Dolutegravir in pharmaceutical dosage form by RP-UPLC. Chromatogram was run through HSS C18 (2.8 x 50 mm x 1.6 □m). Mobile phase containing Buffer Na2HPO4: Methanol taken in the ratio 70:30 was pumped through column at a flow rate of 0.3 mL/min. Buffer used in this method was Potassium dihydrogen phosphate. Temperature was maintained at 30°C. Optimized wavelength selected was 260 nm. Retention time of Lamivudine and Dolutegravir were found to be 1.408 min and 1.739 min. The percentage RSD of the Lamivudine and Dolutegravir were and found to be 0.8 and 0.8 respectively. The percentage recovery was obtained as 100.39% and 100.37% for Lamivudine and Dolutegravir respectively. LOD, LOQ values obtained from regression equations of Lamivudine and Dolutegravir were 0.41, 1.25 and 0.09, 0.26 respectively. Retention times were decreased and the run time was decreased, so the developed method was simple, economical and effective for the routine quality control test in industries.

Keywords: *Lamivudine, Dolutegravir, RP-UPLC*

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INTRODUCTION:

Lamivudine is a reverse transcriptase inhibitor used to treat HIV and hepatitis B infections. Lamivudine is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination.[1-2] IUPAC name is 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. Molecular Formula is $C_8H_{11}N_3O_3S$. Molecular weight is 229.2.

Dolutegravir is an antiviral agent used for the treatment of HIV-1 infections in combination with other antiretroviral agents. Dolutegravir is an HIV-1 antiviral agent.[3-4]It inhibits HIV integrase by binding to the active site and blocking the strand transfer step of retroviral DNA integration in the host cell. The strand transfer step is essential in the HIV replication cycle and results in the inhibition of viral activity. Dolutegravir has a mean EC50 value of 0.5 nM (0.21 ng/mL) to 2.1 nM (0.85 ng/mL) in peripheral blood mononuclear cells (PBMCs) and MT-4 cells. IUPAC name is N-[(2,4-difluorophenyl)methyl]-11-hydroxy-7-methyl-9,12-dioxo-4-oxa-1,8-diazatricyclo[8.4.0.0^{3,8}]tetradeca-10,13-diene-13-carboxamide. Molecular Formula is $C_{20}H_{19}F_2N_3O_5$. Molecular weight is 419.3.

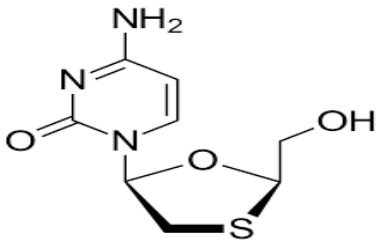

Figure 1: Structure of Lamivudine

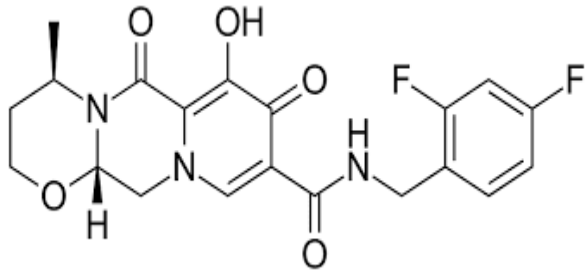

Figure 2: Structure of Dolutegravir

A literature survey conveyed that, limited methods are available for simultaneous estimation of Lamivudine and Dolutegravir. Various HPLC [5,6,7,8,9,10,11,12,13,14], LC/MS/MS [15,16,17,18,19], HPTLC [20, 21], UV [22,23,24] and UPLC [25] assay methods were described within the literature regarding the estimation of lamivudine, abacavir, and a few other anti-retroviral drugs individually as well as in combination with other drugs. In view of the demand for an appropriate, cost-effective RP-UPLCmethod for routine analysis of Lamivudine and Dolutegravir synchronized evaluation of in pharmaceutical dose type. Attempts were made to establish easy, precise, accurate as well as cost-efficient logical method for the estimate of Lamivudine and Dolutegravir. The recommended approach will be validated according to ICH guidelines. The objective of t he recommended work is to establish a brand-new, simple, delicate, exact and economical logical method as well as recognition for the Synchronized evaluation of Lamivudine and Dolutegravir in pharmaceutical dose kind by utilizing RP-UPLC. To verify the established method based on ICH standards for the desired analytical application.

MATERIALS AND METHODS:

Chemicals and Reagents: Lamivudine and Dolutegravir pure drugs (API), Combination Lamivudine and Dolutegravir oral tablets (Dovato), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Diluent: Based up on the solubility of the drugs, diluent was selected, Methanol and Water taken in the ratio of 50:50 as diluent.

Preparation of Standard stock solutions: Accurately weighed 75 mg of Lamivudine, 12.5 mg of Dolutegravir and transferred to individual 50 mL volumetric flasks separately. 3/4th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (1500 µg/mL of Lamivudine and 250 µg/mL of Dolutegravir)

Preparation of Standard working solutions (100% solution): 1mL from each stock solution was pipetted out and taken into a 10mL volumetric flask and made up with diluent. (150 µg/mL Lamivudine of and 25 µg/mL of Dolutegravir)

Preparation of Sample stock solutions: 10 tablets were weighed and was transferred into a 100 mL volumetric flask, 50 mL of diluents was added and

sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (3000 µg/mL of Lamivudine and 500 µg/mL of Dolutegravir)

Preparation of Sample working solutions (100% solution): 0.5 mL of filtered sample stock solution was transferred to 10 mL volumetric flask and made up with diluent. (150 µg/mL of Lamivudine and 25 µg/mL of Dolutegravir)

Preparation of buffer:

0.01N Na2HPO4 Buffer: Accurately weighed 1.41 gm of sodium dihyrogen Ortho phosphate in a 1000 mL of Volumetric flask add about 900 mL of milli-Q water added and degas to sonicate and finally make up the volume with water.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

RESULTS AND DISCUSSION:

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 0.3 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 1 µL of standard into HSS C18 (2.6 x 50 mm, 1.6 µm), the mobile phase of composition 70% 0.01N Na_2HPO_4: 30% Methanol was allowed to flow through the column at a flow rate of 0.3 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Table 1: System suitability parameters

S.No.	Lamivudine			Dolutegravir			Resolution
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	
1	1.376	2685	1.39	1.702	4512	1.37	3.1
2	1.385	2903	1.39	1.708	4172	1.42	3.0
3	1.390	3047	1.39	1.717	4553	1.38	3.1
4	1.391	2685	1.42	1.72	4483	1.39	3.0
5	1.400	2933	1.34	1.722	4448	1.39	3.0
6	1.408	2147	1.46	1.739	3764	1.46	2.8

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Lamivudine and Dolutegravir in their pharmaceutical dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2,3.

Table 2: Assay results for Lamivudine

S. No.	Standard Area	Sample area	% Assay
1	3640080	3639645	99.46
2	3662158	3653746	99.85
3	3623764	3640033	99.48
4	3646966	3601832	98.43
5	3650353	3616126	98.82
6	3710141	3612258	98.72
Avg	3655577	3627273	99.13
Std ev	29577.7	20068.2	0.55
%RSD	0.8	0.6	0.6

Table 3: Assay results for Dolutegravir

S. No.	Standard Area	Sample area	% Assay
1	882110	886576	100.74
2	882532	888945	101.00
3	870302	882568	100.28
4	872703	880422	100.04
5	878651	889162	101.03
6	889046	887665	100.86
Avg	882557	885890	100.66
Stdev	6902.1	3594.3	0.4
%RSD	0.8	0.4	0.4

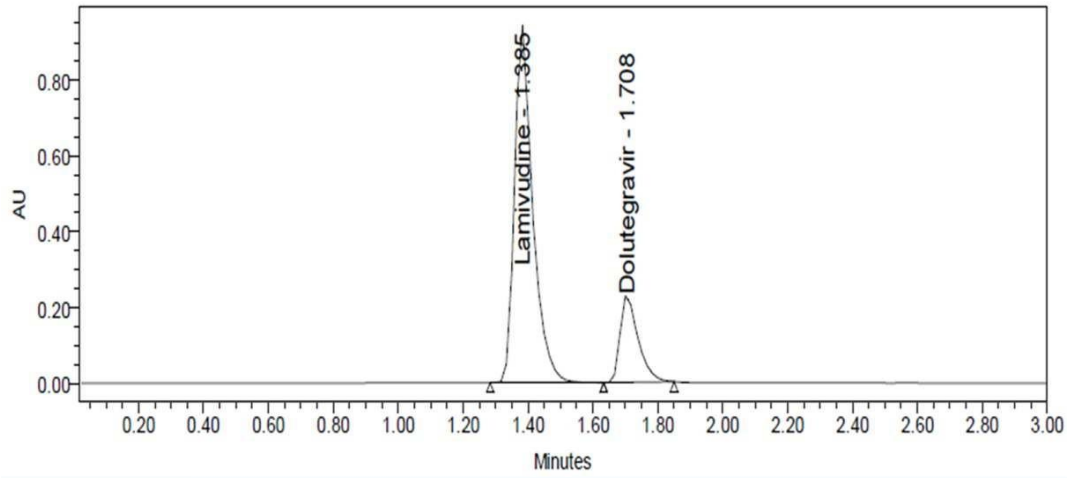

Figure 3: Standard chromatogram

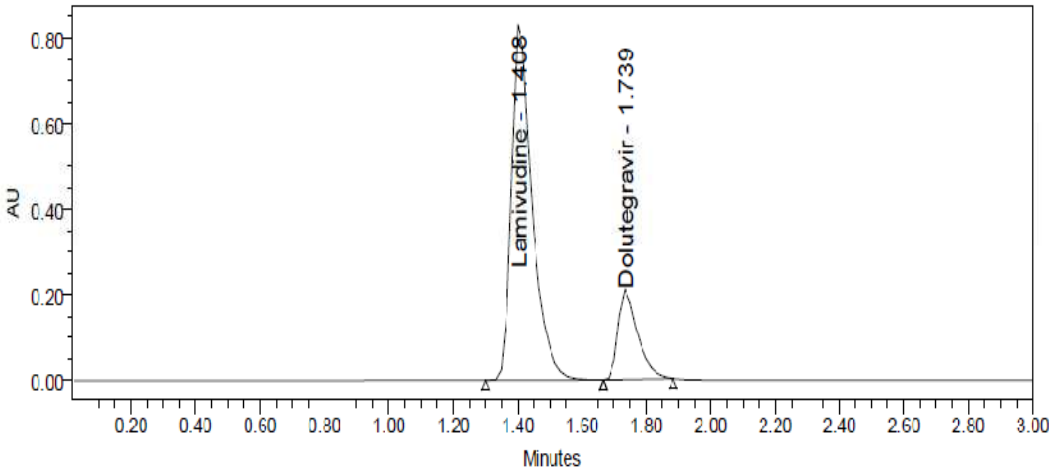

Figure 4: Sample chromatogram

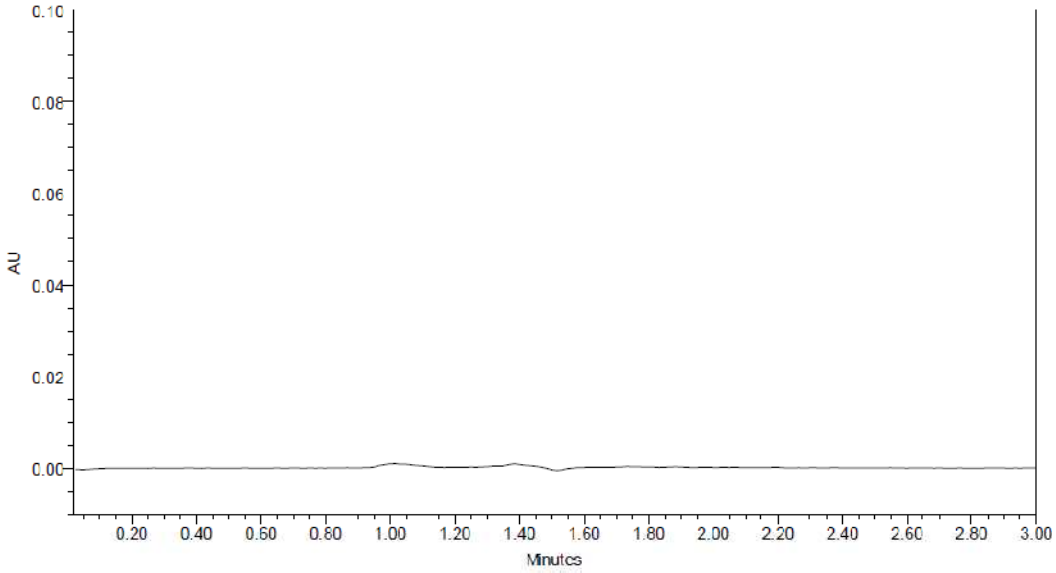

Figure 5: Blank chromatogram

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 0 **µg/ml** to 225 **µg/ml** and 0 **µg/ml** to 37.5 **µg/ml** level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 4.

Table 4: Results of linearity for Lamivudine& Dolutegravir

Lamivudine		**Dolutegravir**	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
37.5	922806	6.25	210556
75	1878662	12.5	445258
112.5	2712498	18.75	655630
150	3638097	25	872691
187.5	4569129	31.25	1084994
225	5477284	37.5	1303577

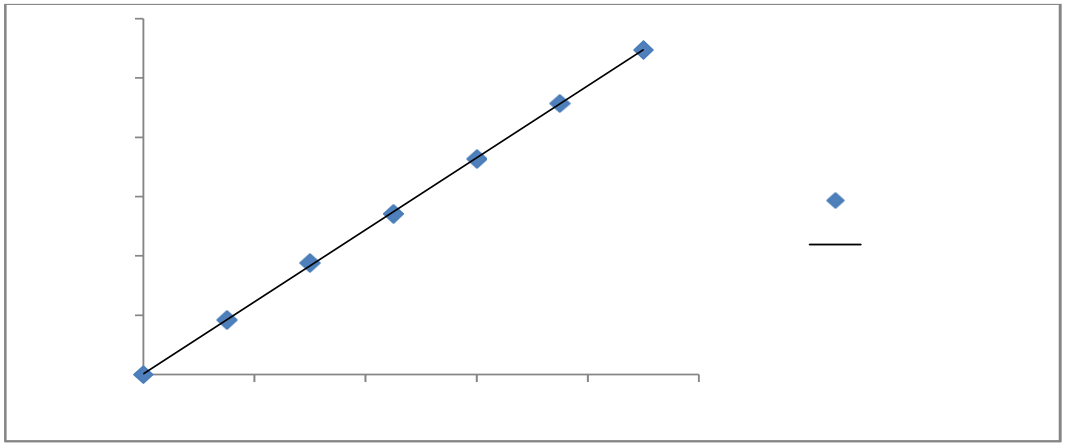

Figure 6: Linearity graph for Lamivudine

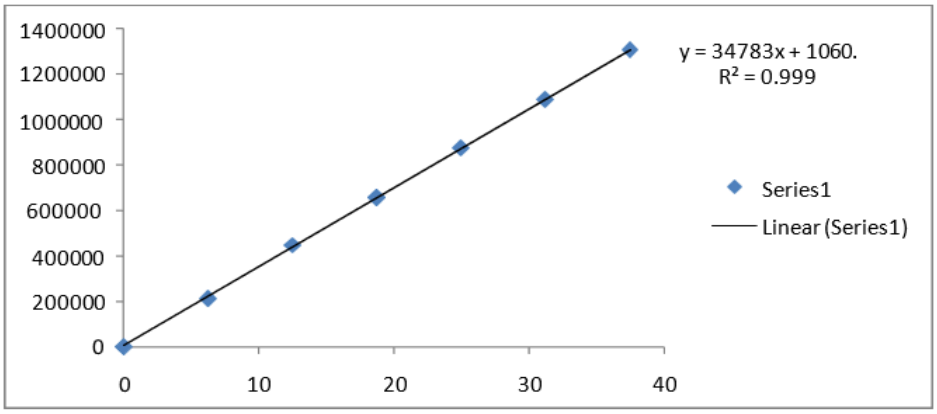

Figure 7: Linearity graph for Dolutegravir

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150% and 50%, 100%, 150% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Lamivudine and Dolutegravir and calculate the individual recovery and mean recovery values. The results are shown in table 5,6.

Table 5: Showing accuracy results for Lamivudine

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	75	74.63	99.50	100.39%
	75	74.93	99.91	
	75	75.55	100.74	
100%	150	147.86	98.58	
	150	151.98	101.32	
	150	150.99	100.66	
150%	225	228.58	101.59	
	225	226.97	100.88	
	225	225.78	100.34	

Table 6: Showing accuracy results for Dolutegravir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	12.5	12.49	99.94	100.37%
	12.5	12.48	99.83	
	12.5	12.54	100.34	
100%	25	25.34	101.36	
	25	25.28	101.12	
	25	25.25	101.00	
150%	37.5	37.32	99.51	
	37.5	37.69	100.50	
	37.5	37.38	99.68	

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 7.

Table 7: Precision results for Lamivudine and Dolutegravir

S. No	Area of Lamivudine	Area of Dolutegravir
1.	3639645	886576
2.	3653746	888945
3.	3640033	882568
4.	3601832	880422
5.	3616126	889162
6.	3612258	887665
Mean	3627273	885890
S.D	20068.2	3594.3
%RSD	0.6	0.4

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in UPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 8.

Table 8: Ruggedness results of Lamivudine and Dolutegravir

S. No	Area of Lamivudine	Area of Dolutegravir
1.	3582461	868029
2.	3575456	865657
3.	3603542	873452
4.	3573254	859756
5.	3580782	864018
6.	3587303	856778
Mean	3583800	864615
S.D	10897.3	5936.6
%RSD	0.3	0.7

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.2 ml/min to 0.4 ml/min. The results are shown in table 9.

Table 9: Robustness results of Dolutegravir by RP-UPLC

S. No.	Condition	% RSD of Lamivudine	% RSD of Dolutegravir
1	Flow rate (-) 0.2 mL/min	0.7	1.0
2	Flow rate (+) 0.4 mL/min	0.3	0.5
3	Mobile phase (-) 75B:25M	0.2	0.4
4	Mobile phase (+) 65B:35M	0.7	1.1
5	Temperature (-) 25°C	0.1	0.1
6	Temperature (+) 35°C	0.2	0.7

LOD and LOQ: The sensitivity of RP-UPLCwas determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 10.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Table 10: LOD, LOQ of Lamivudineand Dolutegravir

Molecule	LOD	LOQ
Lamivudine	0.41	1.25
Dolutegravir	0.09	0.26

CONCLUSION:

The Developed UPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Lamivudine and Dolutegravir in its pure and pharmaceutical dosage form. Hence, this method can easily and conveniently adopt for routine quality control analysis of Dolutegravir and Lamivudine in its pure and pharmaceutical dosage form.

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