

Lamivudine and Dolutegravir Simultaneous Dosage forms Determined and Validated Using the RP-UPLC Method

Sadaf Sultana^{1*}, Shaik Ejaz²

Abstract

RP-UPLC has been used to produce a straightforward, precise, and accurate approach for the simultaneous estimation of dolutegravir and lamivudine in a pharmaceutical dose form. A HSS C18 column of 2.8 x 50 mm and featuring particles with a size of 1.6 μ m was used to process the chromatogram. A mixture of 70:30 Buffer Na₂HPO₄ and methanol made up the mobile phase, which was pushed across the column at a flow rate of 0.3 mL/min. This approach maintained a temperature of 30°C and employed potassium dihydrogen phosphate as the buffer. 260 nm was chosen as the ideal wavelength for detection. Dolutegravir and Lamivudine were shown to have retention durations of 1.739 and 1.408 minutes, respectively. The precision and accuracy of the approach were confirmed. Good precision was shown by the percentage relative standard deviation (%RSD) of 0.8% for both dolutegravir and lamivudine. As evidence of the method's accuracy, the percentage recovery for dolutegravir (100.37%) and lamivudine (100.39%) was found. The limit of detection (LOD) and limit of quantification (LOQ) were also determined to assess the sensitivity of the approach. In the regression models, the LOD and LOQ values for lamivudine and dolutegravir were 1.25 and 0.41 and 0.09 and 0.26, respectively. These results show that the analytes may be detected and quantified at low quantities using this approach. The effectiveness of this approach in terms of resources and time is one of its main advantages. The compounds' retention durations were shortened, which resulted in a shorter run time overall. Because of this, the method can be applied to businesses where consistent, fast analysis is necessary for quality control testing. Additionally, the method's simplicity makes it easy to apply in laboratory settings and cost-effective. To summarize, the RP-UPLC method that has been developed provides a simple and efficient way for estimating both dolutegravir and lamivudine simultaneously in pharmaceutical formulations. It is a useful instrument for quality control in the pharmaceutical business due to its high levels of precision, accuracy, sensitivity, and efficiency.

Keywords: RP-UPLC, Lamivudine, Dolutegravir, Pharmaceutical dosage form, Method validation

INTRODUCTION

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Received Date: March 02, 2024

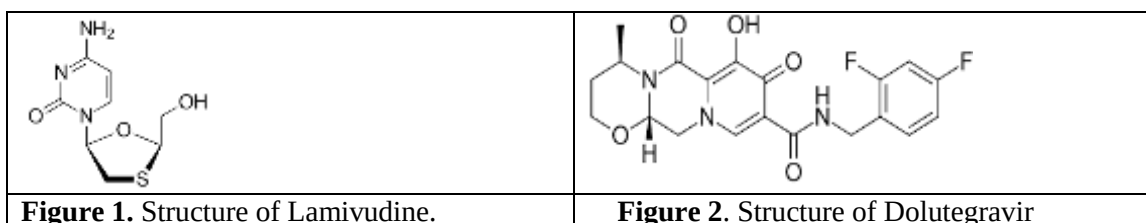
Accepted Date: March 12, 2024

Published Date: March 20, 2024

Citation: Sadaf Sultana, Shaik Ejaz. Lamivudine and Dolutegravir Simultaneous Dosage Forms Determined and Validated Using the RP-UPLC Method. Emerging Trends in Personalized Medicines. 2024; 1(1): 5–13p

Hepatitis B and HIV infections are managed using reverse transcriptase inhibitors like lamivudine. The analogue of lamivudine is lamivudine triphosphate (L-TP), the active 5'-triphosphate metabolite that is produced when lamivudine is phosphorylated within cells. When HBV polymerase and HIV reverse transcriptase incorporate this nucleoside analog into viral DNA, DNA chain termination takes place. [1–2]. The 4-amino IUPAC name [(2R,5S)-2-(hydroxymethyl)] 1 Five-yl -1,3-oxathiolanPyrimidine-2-one, 1,2-dihydro shown in Figure 1. The formula for molecules is C₈H₁₁N₃O₃S. There is 229.2 molecular weight.

When used with other antiretroviral medications, dolutegravir is an antiviral treatment for HIV-1 infections. One HIV-1 antiviral drug is dolutegravir [3–4]. Thus HIV integrase is inhibited because it binds to the active site of the enzyme and blocks the strand transfer portion of retroviral DNA integrated into the infection. In the HIV replication cycle, the strand transfer phase is crucial because it stops viral activity. The EC₅₀ values of Dolutegravir in peripheral blood mononuclear cells (PBMCs) and MT-4 cells vary from 0.5 nM (0.21 ng/mL) to 2.1 nM (0.85 ng/mL). The term N-[(2,4-difluorophenyl)methyl] is in IUPAC. 7-hydroxy-11-methyl-9,12-dioxo-4-oxa-1,8-diazatricyclo[8.4.0.0^{3,8}]Tetradeca-13,Diene-13-Carboxamide shown in Figure 2. C₂₀H₁₉F₂N₃O₅ is the molecular formula. There is 419.3 molecular weight.



Few techniques are available for measuring dolutegravir and lamivudine at the same time, according to a review of the literature Lamivudine, abacavir, and several other antiretroviral drugs have been analyzed individually and in combination with other medications using various assay techniques such as HPLC [5–14], LC/MS/MS [15–19], HPTLC [20, 21], UV [22–24], and UPLC [25], as documented in the literature. A suitable, affordable RP-UPLC method for the regular analysis of lamivudine and dolutegravir synchronised evaluation of pharmaceutical dose type is required. The estimation of lamivudine and dolutegravir had to be done as simply, precisely, accurately, and economically as possible. In compliance with ICH norms, the suggested method will be verified. Establishing a novel, easy, careful, precise, and cost-effective logical approach, and recognition for the synchronised evaluation of Lamivudine and Dolutegravir in pharmaceutical dose kind by using RP-UPLC is the goal of the suggested study. to confirm, for the intended analytical application, the established procedure based on ICH standards.

MATERIALS AND METHODS

Chemicals and Reagents Pure active pharmaceutical ingredients (APIs) of lamivudine and dolutegravir, Dovato combination oral tablets containing both lamivudine and dolutegravir, as well as distilled water, acetonitrile, phosphate buffer, methanol, potassium dihydrogen phosphate buffer, and ortho-phosphoric acid. All the mentioned chemicals and formulations were sourced from Rankem.

Diluent: A 50:50 mixture of methanol and water was used as the diluent, chosen based on the medicines' solubility.

Standard stock solution preparation: 75 mg of lamivudine and 12.5 mg of dolutegravir were precisely weighed before being divided into two separate 50 mL volumetric flasks. After adding one-third of the diluents to each of these flasks, they were Sonicated for ten minutes. Standard stock solutions 1 and 2 were written on flasks that were prepared using diluents. Dolutegravir at 250 µg/mL and Lamivudine at 1500 µg/mL.

Standard working solutions preparation (100% solution): Each stock solution was pipetted to yield one milliliter, which was then transferred to a ten-milliliter flask. Dolutegravir and lamivudine at 150 µg and 25 µg/mL, respectively

Sample Stock Solution Preparation: The procedure involved weighing ten compounds and placing them in a 100 mL volumetric flask, adding 50 mL of dilution, and then sonication of the mixture for

25 minutes. The volume was modified using the diluent, and the mixtures were filtered through HPLC filters containing 3000 µg/mL of lamivudine and 500 µg/mL of dolutegravir.

Sample working solution preparation (100 percent solution): A 0.5 mL portion of the filtered sample stock solution was transferred to a 10 mL volumetric flask, yielding concentrations of 150 µg/mL for lamivudine and 25 µg/mL for dolutegravir.

Preparation of buffer: 0.01N NaHPO₄ Buffer: Weigh 1.41 g of sodium dihydrogen orthophosphate precisely into a 1000 mL volumetric flask. Add around 900 mL of milli-Q water, degas to sonicate, and then add water to make up the volume.

Procedure: As you execute the validation parameters in accordance with ICH guidelines, note the conditions of appropriate peak elution and record the chromatograms after injecting the samples under modified chromatographic conditions.

RESULTS AND DISCUSSION

Method

In accordance with ICH criteria, the devised chromatographic technique was verified for system appropriateness, linearity accuracy, precision, ruggedness, and robustness.

Parameters of system suitability: To assess system appropriateness parameters such tailing factor, retention duration, and USP theoretical plate count, the column was equilibrated at room temperature using the mobile phase flowing over it at a rate of 0.3 mL/min. A volume of 1 µL of standard was injected into HSS C18 (2.6 x 50 mm, 1.6 µm) to achieve chromatographic separation. The mobile phase containing 70% 0.01N Na₂HPO₄: 30% methanol was flowed through the column at 0.3 mL/min. Table 1 displays the retention time, tailing factor, and theoretical plate count for the developed method.

Table 1. System suitability parameters.

S.N.	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

Analysis of pharmaceutical formulations: The pharmaceutical formulation of dolutegravir and lamivudine was accurately analyzed using the proposed validated method. The results are shown in Tables 2 and 3, where the numbers labeled in each case were comparable. Standard chromatogram, Sample chromatogram, Blank chromatogram analysis data graphically in Figure 3–5.

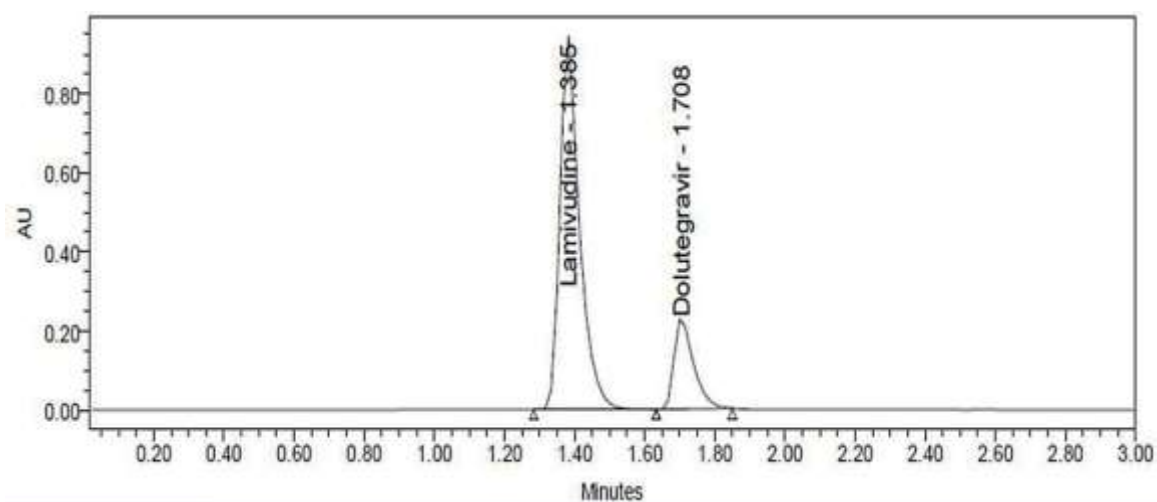
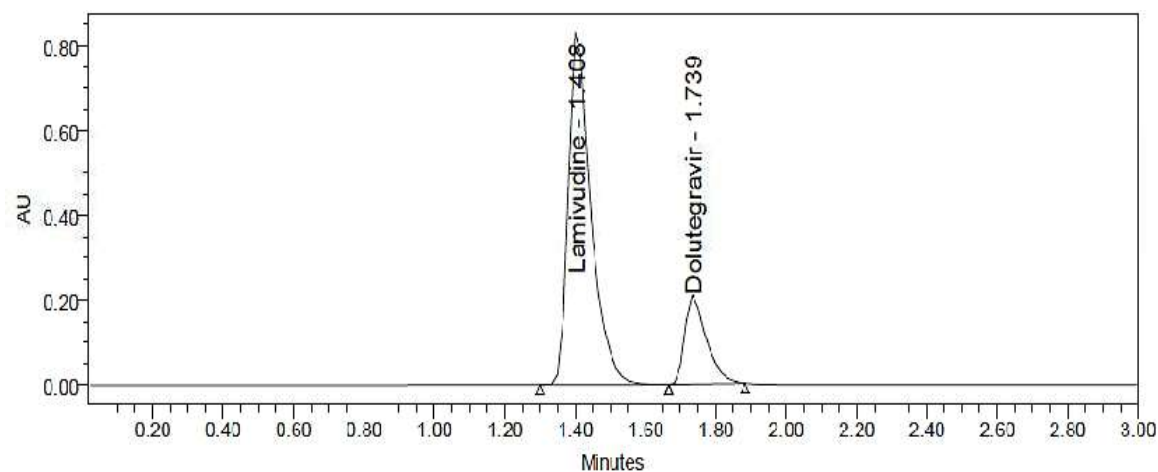
Table 2. Assay results for Lamivudine.

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
Stdev	29577.7	20068.2	0.55
%RSD	0.8	0.6	0.6

Table 3. Assay results for Dolutegravir.

S.N.	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.

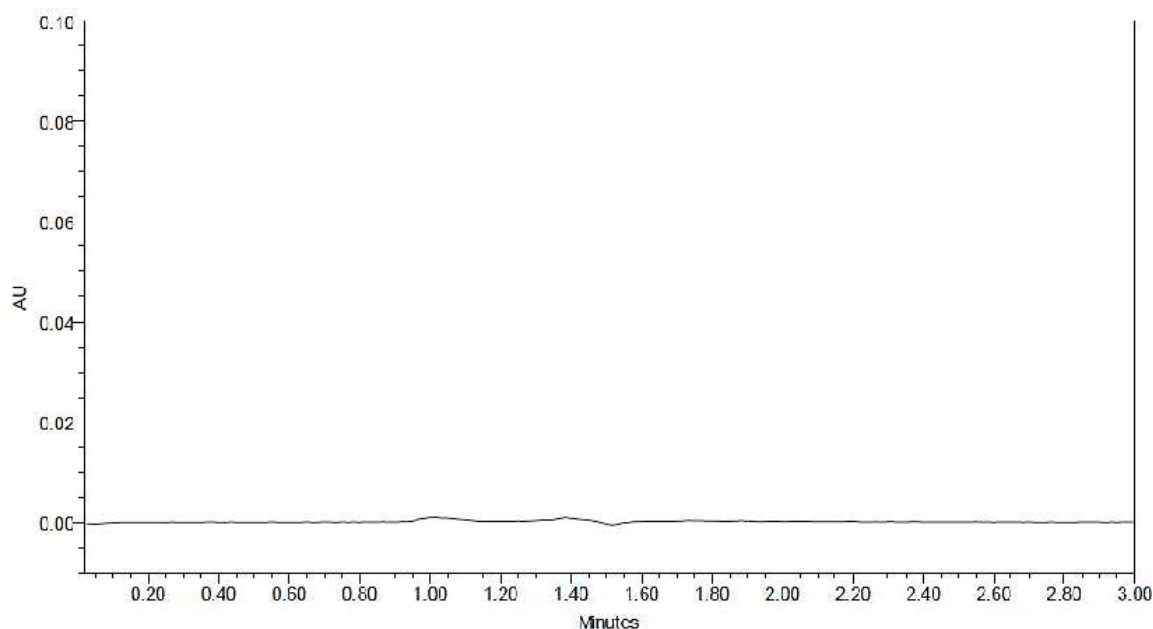


Figure 5. Blank chromatogram.

Validation of Analytical Method

Linearity: For the concentration range of 0 µg/ml to 225 µg/ml and 0 µg/ml to 37.5 µg/ml level, the linearity investigation was conducted. A chromatographic system received an injection from each level. The correlation coefficient was determined by using the area at each level. Enter each quantity in the chromatographic device and measure the peak area. Plot the concentration on the X-axis and the peak area on the Y-axis. Additionally, calculate the correlation coefficient between these two variables. Table 4 and Figures 6,7 displays the results.

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

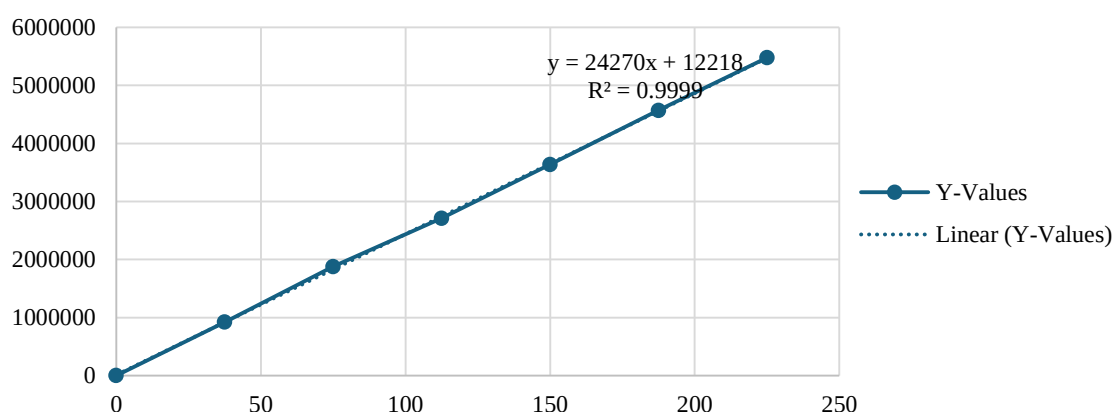


Figure 6. Linearity graph for Lamivudine.

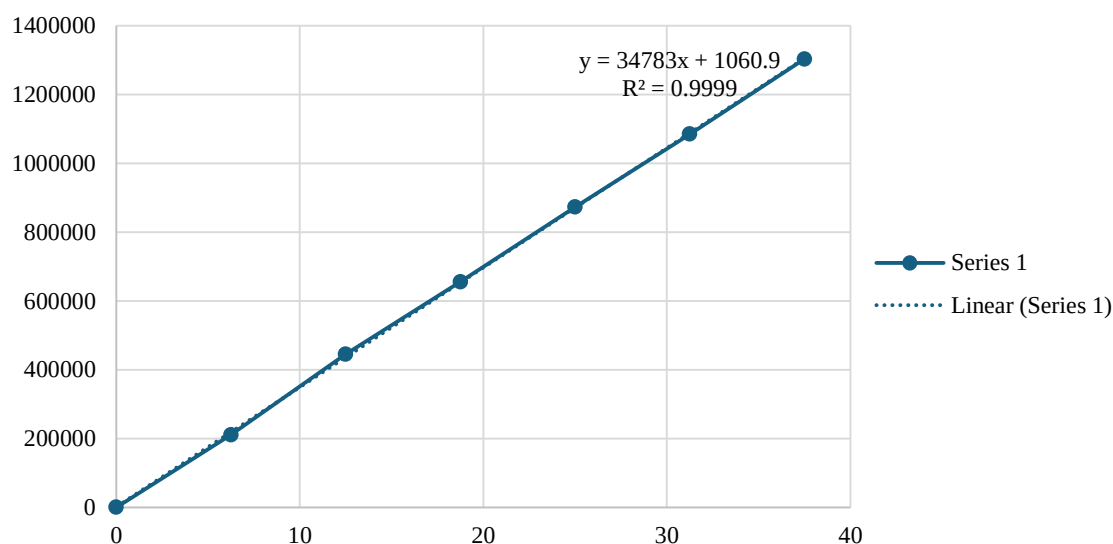


Figure 7. Linearity graph for Dolutegravir.

Accuracy studies: The recovery study proved to be helpful in determining the accuracy. The recuperation process was implemented at three different levels: 50%, 100%, 150%, and 50%, 100%, 150%. Fill the chromatographic apparatus with the standard solutions. Determine the amounts of lamivudine and dolutegravir that were found and added, together with the average and specific recovery values. Tables 5 and 6 display the results.

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.

S.N.	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: Precision was conducted on a different day in order to assess the method's intermediate precision. The area was measured by UPLC after six dilutions of standard solution. It was possible to find the %RSD for the six replicate injections. Table 8 displays the results.

Table 7. Ruggedness results of Lamivudine and Dolutegravir.

S. N.	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: To assess the method's robustness, intentional modifications were made to the temperature variation, flow rate, and composition of the mobile phase. From 0.2 to 0.4 ml per minute, the flow rate was adjusted. Table 9 shows results.

Table 8. Robustness results of Dolutegravir by RP-UPLC

S. N.	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: RP-UPLC's sensitivity was ascertained using LOD and LOQ. which, in accordance with ICH rules, were computed from the calibration curve using the following formulae. Table 10 shows the results.

$$\text{LOD} = 3.3\sigma/S \text{ and}$$

$$\text{LOQ} = 10 \sigma/S, \text{ where}$$

σ = Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

Table 9. LOD, LOQ of Lamivudine and Dolutegravir

Molecule	LOD	LOQ
Lamivudine	0.41	1.25
Dolutegravir	0.09	0.26

CONCLUSION

Following validation, it was discovered that the developed UPLC method for the simultaneous measurement of lamivudine and dolutegravir in their pure and pharmaceutical dose form was straightforward, exact, accurate, and sensitive. Thus, dolutegravir and lamivudine in their pure and medicinal dosage form can be routinely analysed for quality control with ease and convenience using this technology.

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