STM JOURNALS

Emerging Trends in
Personalized Medicines

Volume 1, Issue 1, 2024
January-June
(Journal):10.37591/ETPM
DOI: 10.37591/ETPM

https://journals.stmjournals.com/etpm

Research ETPM

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

Sadaf Sultana[1,*], Shaik Ejas[2]

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 µm 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. The LOD and LOQ values derived from the regression equations were 1.25 and 0.41 for lamivudine and 0.09 and 0.26 for dolutegravir, 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

*Author for Correspondence
Sadaf Sultana
E-mail: sadafsultana127@gmail.com

[1]Student, Department of Pharmaceutical Analysis, Mak college of Pharmacy, Moinabad, Hyderabad, Telangana, India.
[2]Associate professor, Department of Pharmaceutical Analysis, Mak college of Pharmacy, Moinabad, Hyderabad, Telangana, India.

Received Date: March 02, 2024
Accepted Date: March 12, 2024
Published Date: March 20, 2024

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

Reverse transcriptase inhibitors like lamivudine are prescribed to treat hepatitis B and HIV infections. Lamivudine is an analogue of a synthetic nucleoside that undergoes intracellular phosphorylation to provide its active 5'-triphosphate metabolite, lamivudine triphosphate (L-TP). DNA chain termination occurs when HIV reverse transcriptase and HBV polymerase integrate this nucleoside analogue into viral DNA [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]. It binds to the active site of HIV integrase and prevents the strand transfer stage of retroviral DNA integration in the host cell, hence inhibiting HIV integrase. In the HIV replication cycle, the strand transfer phase is crucial because it stops viral activity. In peripheral blood mononuclear cells (PBMCs) and MT-4 cells, dolutegravir has a mean EC50 value ranging 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.

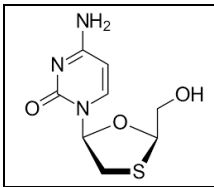

Figure 1. Structure of Lamivudine.

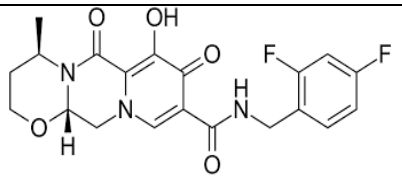

Figure 2. Structure of Dolutegravir

Few techniques are available for measuring dolutegravir and lamivudine at the same time, according to a review of the literature. Lamivudine, abacavir, and a few other anti-retroviral drugs were estimated separately and in combination with other drugs using a variety of HPLC [5–14], LC/MS/MS [15–19], HPTLC [20, 21], UV [22–24], and UPLC [25] assay methods that were described 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 medications (API) containing lamivudine and dolutegravir, combination oral tablets (Dovato) containing lamivudine and dolutegravir, distilled water, acetonitrile, phosphate buffer, methanol, potassium dehydrogenate ortho phosphate buffer, and ortho-phosphoric acid. Rankem is the source of all of the compounds and solvents mentioned above.

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

Preparation of Standard stock solutions: 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.

Preparation of Standard working solutions (100% solution): Each stock solution was pipetted out to yield 1ml, which was then put into a 10 ml volumetric flask and diluted. (Lamivudine at 150 µg/mL and Dolutegravir at 25 µg/mL)

Preparation of Sample stock solutions: After 10 tablets were weighed and placed in a 100 mL volumetric flask, 50 mL of diluents were added, and the mixture was Sonicated for 25 minutes. The

volume was then adjusted with diluent, and HPLC filters (3000 µg/mL of Lamivudine and 500 µg/mL of Dolutegravir) were used to filter the mixtures.

Preparation of Sample working solutions (100% solution): After filtering, 0.5 mL of the sample stock solution was added to a 10 mL volumetric flask and diluted. (Lamivudine at 150 µg/mL and Dolutegravir at 25 µg/mL)

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, which consisted of 70% 0.01N Na2HPO4: 30% Methanol, was allowed to pass through the column at a flow rate of 0.3 ml per minute. The developed method's retention time, tailing factor, and theoretical plate count are shown in Table 1.

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

Assay of pharmaceutical formulation: Dolutegravir and Lamivudine in their pharmaceutical dosage form were effectively determined using the suggested validated approach. Tables 2 and 3 display the results, which were comparable with the corresponding labelled amounts**.** 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

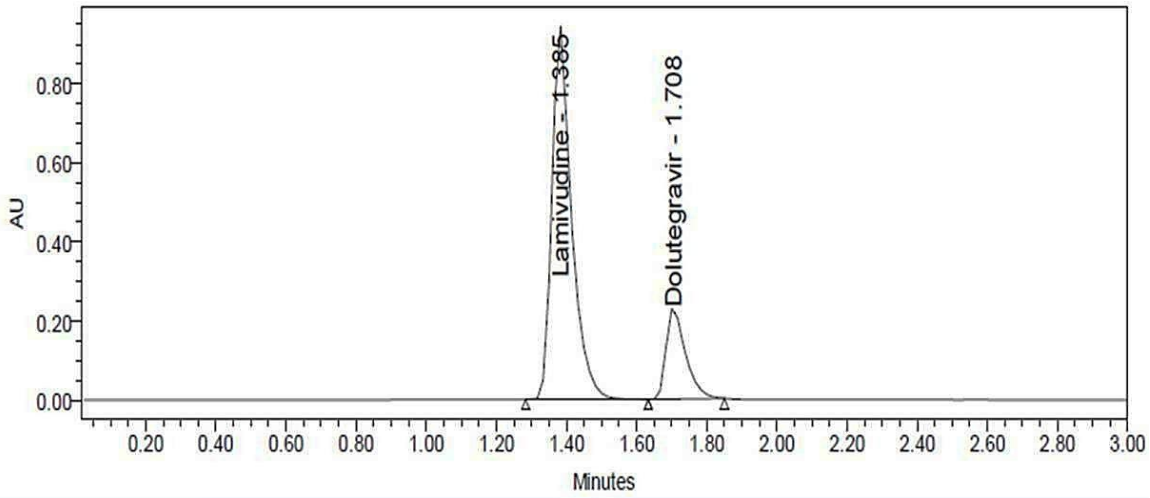

Figure 3. Standard chromatogram.

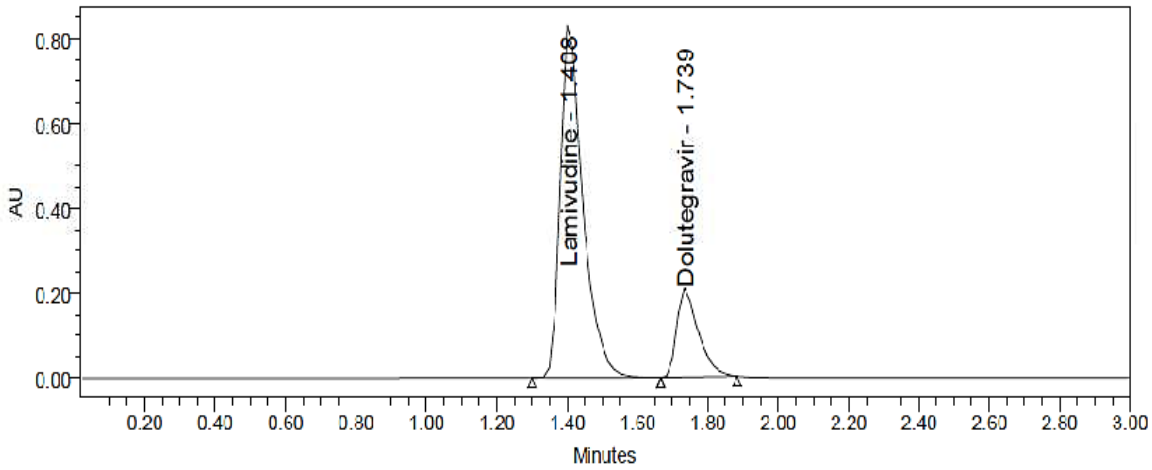

Figure 4. Sample chromatogram.

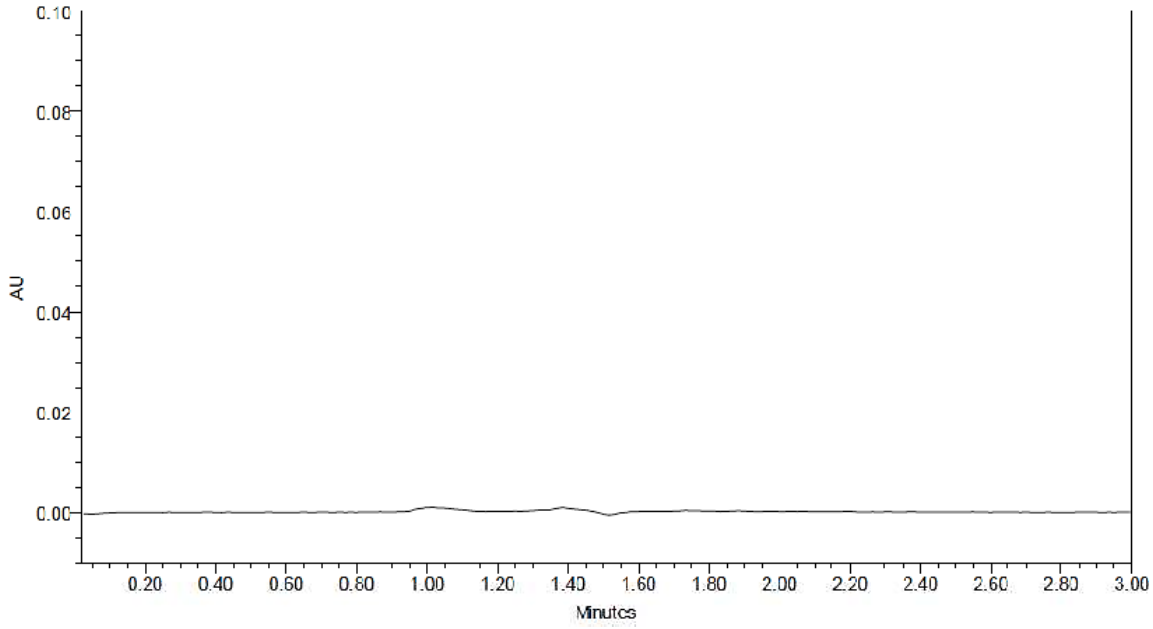

Figure 5. Blank chromatogram.

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 calculated using each level's area. Measure the peak area after each level has been injected into the chromatographic apparatus. Plot the concentration vs peak area (peak area on the Y-axis and concentration on the X-axis) and find the correlation coefficient. 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

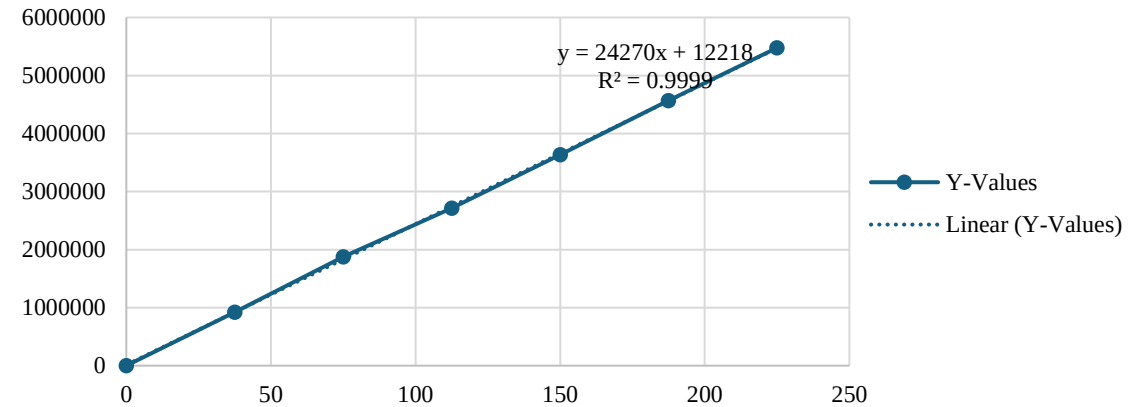

Figure 6. Linearity graph for Lamivudine.

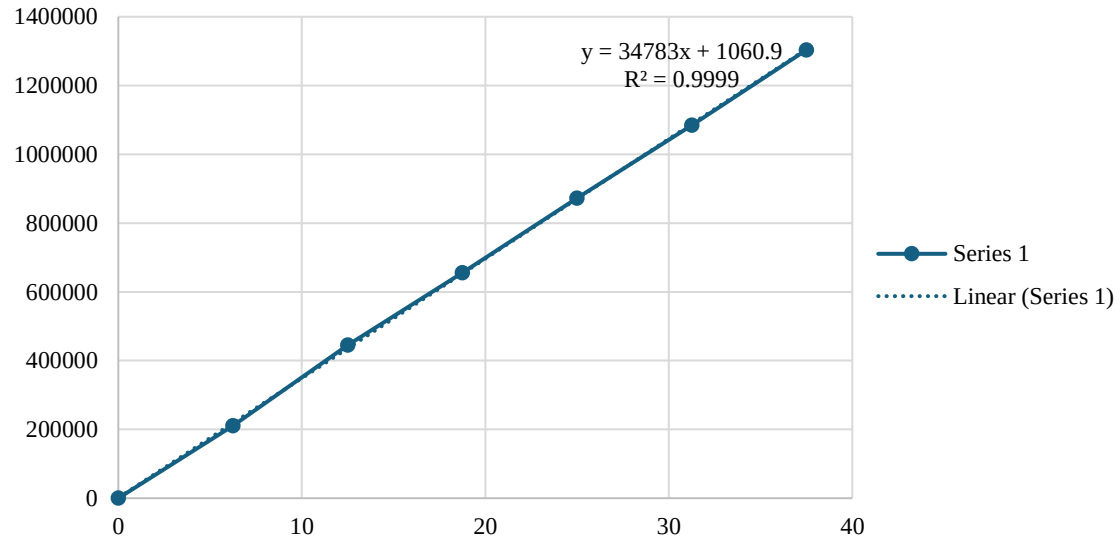

Figure 7. Linearity graph for Dolutegravir.

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. Compute the amounts for lamivudine and dolutegravir that were identified and added, as well as the mean and individual recovery values. Tables 5 and 6 display the results.

Table 5. Showing accuracy results for Lamivudine.

% Level	AmountSpiked (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 in UPLC after the standard solution was injected six times. It was possible to find the %RSD for the six replicate injections. Table 7 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 8 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 as certained using LOD and LOQ. which, in accordance with ICH rules, were computed from the calibration curve using the following formulae. Table 9 shows the results.

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 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.

REFERENCES

1. Fox Z, Dragsted UB, Gerstoft J, Phillips AN, Kjaer J, Mathiesen L, Youle M, Katlama C, Hill A, Bruun JN, Clumeck N. A randomized trial to evaluate continuation versus discontinuation of lamivudine in individuals failing a lamivudine-containing regimen: the COLATE trial. Antiviral therapy. 2006 Aug;11(6):761–70.
2. FDA Approved Drug Products: Triumeq/Triumeq PD (abacavir, dolutegravir, and lamivudine) for oral administration, https://www.fda.gov/drugs/human-immunodeficiency-virus-hiv/food-and-drug-administration-approved-revisions-triumeq-abacavirdolutegravirlamivudine-and-dovato.
3. Min S, Song I, Borland J, Chen S, Lou Y, Fujiwara T, Piscitelli SC. Pharmacokinetics and safety of S/GSK1349572, a next-generation HIV integrase inhibitor, in healthy volunteers. Antimicrobial agents and chemotherapy. 2010 Jan;54(1):254–8.
4. Lenz JC, Rockstroh JK. S/GSK1349572, a new integrase inhibitor for the treatment of HIV: promises and challenges. Expert Opinion on Investigational Drugs. 2011 Apr 1;20(4):537–48.
5. Kumar DA, Rao GS, Rao JV. Simultaneous determination of lamivudine, zidovudine and abacavir in tablet dosage forms by RP HPLC method. Journal of Chemistry. 2010 Jan 1;7:180–4.
6. Ashok G, Mondal DS. Development and validation of stability indicating method for the simultaneous estimation of batcaver sulfate, Lamivudine and Dolutegravir sodium in pharmaceutical dosage forms by RP-HPLC. Saudi J Med Pharm Sci. 2018;4(2):289–96.
7. Khaleel N, Abdul Rahaman SK. A validated stability indicating RP-HPLC method for simultaneous determination of abacavir, lamivudine and dolutegravir in bulk and pharmaceutical dosage form. World J Pharm Res. 2015 May 4;4(7):1453–76.
8. Rao NM, Sankar DG. Development and validation of stability-indicating HPLC method for simeltaneous determination of Lamivudine, Tenofovir, and Dolutegravir in bulk and their tablet dosage form. Future Journal of Pharmaceutical Sciences. 2015 Dec 1;1(2):737
9. Vijayalakshmi R, Kalyani P, Sandya P, Dhanaraju MD. Method development and validation of a reverse phase liquid chromatographic method for simultaneous determination of lamivudine and abacavir sulphate in tablets. AJ of Phytomed and Clin. Therapeutics. 2013;1(2):208–14.
10. Raja T, Lakshmana Rao A. Development and validation of RP-HPLC method for the estimation of abacavir, lamivudine and zidovudine in pharmaceutical dosage form. Int J Pharm Tech Res. 2011 Apr;3:852–7.
11. Nodagala AY, Mangamma K, Kumar GM, Rao DV. Analytical Method Development And Validation By Rp-Hplc For The Simultaneous Estimation Of Abacavir Sulphate And Lamivudine In Tablet Dosage Forms. International Journal of Pharmaceutical, Chemical & Biological Sciences. 2013 Jul 1;3(3).
12. Mastanamma SK, Jyothi JA, Saidulu P, Varalakshmi M. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Lamivudine, Tenofovir Alafenamide and Dolutegravir Bulk and their Combined Dosage Form. Pharmaceutical Methods. 2018 Jul 1;9(2).
13. Sudha T, Ravi Kumar VR, Hemalatha PV (2008) RP-HPLC method for simultaneous estimation of Lamivudine and Abacavir sulfate in tablet form. Int. J. on Pharm. Biomed. Res. 1(4):108–113
14. Pal N, Rao AS, Ravikumar P. Simultaneous HPLC method development and validation for estimation of Lamivudine, Abacavir and Dolutegravir in combined dosage form with their stability studies. Asian journal of chemistry. 2016 Jan 1;28(2):273.
15. Kenney KB, Wring SA, Carr RM, Wells GN, Dunn JA. Simultaneous determination of zidovudine and lamivudine in human serum using HPLC with tandem mass spectrometry. Journal of pharmaceutical and biomedical analysis. 2000 Jul 1;22(6):967–83.
16. Pereira AS, Kenney KB, Cohen MS, Hall JE, Eron JJ, Tidwell RR, Dunn JA. Simultaneous determination of lamivudine and zidovudine concentrations in human seminal plasma using high-performance liquid chromatography and tandem mass spectrometry. Journal of Chromatography B: Biomedical Sciences and Applications. 2000 May 26;742(1):173–83.
17. Bennetto-Hood C, Tabolt G, Savina P, Acosta EP. A sensitive HPLC–MS/MS method for the determination of dolutegravir in human plasma. Journal of Chromatography B. 2014 Jan 15;945:225–32.

18. Sparidans RW, Hoetelmans RM, Beijnen JH. Liquid chromatographic assay for simultaneous determination of abacavir and mycophenolic acid in human plasma using dual spectrophotometric detection. Journal of Chromatography B: Biomedical Sciences and Applications. 2001 Jan 5;750(1):155–61.
19. Singh AV, Nath LK, Pani NR. Development and validation of analytical method for the estimation of lamivudine in rabbit plasma. Journal of Pharmaceutical Analysis. 2011 Nov 1;1(4):251–7.
20. Sudha T, Ravikumar VR, Hemalatha PV. Validated HPTLC method for simultaneous determination of lamivudine and abacavir sulphate in tablet dosage form. International Journal of Pharmaceutical Sciences and Research. 2010 Nov 1;1(11):107.
21. Bhavar GB, Pekamwar SS, Aher KB, Thorat RS, Chaudhari SR. High-Performance Liquid Chromatographic and High-Performance Thin-Layer Chromatographic Method for Quantitative Estimation of Dolutegravir Sodium in Bulk Drug and Pharmaceutical Dosage Form. Scientia pharmaceutica. 2016 Jun;84(2):305–20.
22. Deepali G, Elvis M. UV Spectrophotometric Method for assay of the anti-retroviral agent lamivudine in active pharmaceutical ingredient in its tablet formulation. Journal of Young Pharmacists. 2010 Oct 1;2(4):417–9.
23. Balasaheb BG, Balasaheb AK, Subhash TR, Jijabapu K, Sudhakar PS. Development and validation of UV spectrophotometric method for estimation of dolutegravir sodium in tablet dosage form. Malaysian Journal of Analytical Sciences. 2015;19(6):1156–63.
24. Madu KC, Ukoha PO, Attama AA. Spectrophotometric Determination of Lamivudine Using Chloranilic Acid and 2, 3-Dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ).
25. Reddy GS, Kumar SA, Kumar VR. A new, simple, sensitive, accurate & rapid analytical method development & validation for simultaneous estimation of lamivudine, abacavir & zidovudine in tablet dosage form by using UPLC. International Journal of Pharmaceutical Sciences and Research. 2014 Sep 1;5(9):3852.

