RESEARCH PAPER

Simultaneous Estimation of Lamivudine and Dolutegravir in Tablet Dosage Form by RP-HPLC: Development and Validation

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Received: 16th Dec, 2025; Revised: 8th Feb 2026; Accepted: 24th Feb, 2026; Available Online: 30th March, 2026

ABSTRACT
Reliable and validated analytical methods are indispensable for ensuring the quality and safety of pharmaceutical products. The present work aimed to develop and validate a sensitive, accurate, precise, and economical reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous quantification of Lamivudine and Dolutegravir in tablet dosage form.
Chromatographic separation was achieved on an Agilent C18 column (150 mm × 4.6 mm, 5 µm) using a mobile phase of orthophosphoric acid and acetonitrile (60:40 v/v) at a flow rate of 1 mL/min with UV detection at 260 nm. Column temperature was maintained at 30°C with an injection volume of 10 µL. Retention times were 2.250 min for Lamivudine and 2.875 min for Dolutegravir. The method was validated for linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ) as per ICH Q2(R1) guidelines.
Excellent linearity was observed over the concentration ranges studied, with regression equations y = 3455x + 10272 (Lamivudine) and y = 30992x + 5402 (Dolutegravir). Precision studies yielded %RSD values of 0.6% and 0.3% for Lamivudine and Dolutegravir, respectively. Mean percentage recovery values were 99.95% and 99.62%, confirming method accuracy. LOD and LOQ were 0.27 and 0.81 µg/mL for Lamivudine and 0.11 and 0.34 µg/mL for Dolutegravir.
The developed RP-HPLC method was found to be simple, rapid, accurate, and suitable for routine quality control analysis of Lamivudine and Dolutegravir in tablet dosage forms.
Keywords: Lamivudine; Dolutegravir; RP-HPLC; Tablet dosage form; Validation; ICH guidelines; LOD; LOQ; Precision; Accuracy
How to cite this article: Palur K, Archakam SC, Sujitha YS, Chaitanya P, Jyothika M. Simultaneous Estimation of Lamivudine and Dolutegravir in Tablet Dosage Form by RP-HPLC: Development and Validation. Int J Drug Deliv Technol. 2026;16(24s): 650-655. DOI: 10.25258/ijddt.16.24s.82
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Pharmaceutical quality assurance relies heavily on the development of robust, validated analytical methods capable of detecting and quantifying active pharmaceutical ingredients (APIs) with high precision and accuracy. The simultaneous quantification of multiple APIs in a single dosage form presents additional challenges that demand well-optimized chromatographic conditions. Reverse-phase high-performance liquid chromatography (RP-HPLC) has emerged as the method of choice for pharmaceutical analysis due to its exceptional resolution, sensitivity, and reproducibility. [1-4]

Lamivudine (3TC) is a nucleoside reverse transcriptase inhibitor (NRTI) widely used in the management of human immunodeficiency virus (HIV) infection and chronic hepatitis B. Dolutegravir (DTG) is an integrase strand transfer inhibitor (INSTI) that prevents the integration of viral DNA into the host genome, offering a high barrier to resistance.[5] The fixed-dose combination of Lamivudine and Dolutegravir (marketed as Dovato) has been recognized as a simplified two-drug regimen for HIV treatment, offering comparable efficacy to three-drug regimens with a more favorable safety profile.[6-10]

Several analytical methods have been reported for the individual or simultaneous determination of these drugs; however, many are characterized by prolonged run times, complex mobile phase compositions, or high operational costs. In the present study, a simple, rapid, and validated RP-HPLC method was developed for the simultaneous estimation of Lamivudine and Dolutegravir in tablet dosage form, with a run time of only 5 minutes, making it suitable for routine quality control applications. [11-20]

MATERIALS

Instruments

An electronic analytical balance (Denver Instruments) was used for accurate weighing. A pH meter (BVK Enterprises, India) was employed for buffer pH adjustment. Sonication was performed using an ultrasonicator (BVK, India). Chromatographic analysis was carried out on a WATERS

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HPLC 2695 system equipped with quaternary pumps, a photodiode array (PDA) detector, and an autosampler, controlled through Empower-2 software. UV scanning was performed using a PG Instruments T60 UV-VIS spectrophotometer with a 2 nm spectral bandwidth and 10 mm matched quartz cells, operated with UV Win 6 software.

Chemicals and Reagents

Lamivudine (300 mg) and Dolutegravir (50 mg) were obtained from the commercial tablet formulation Dovato. HPLC-grade acetonitrile, orthophosphoric acid, potassium dihydrogen orthophosphate, methanol, and Milli-Q water were procured from standard suppliers. All chemicals and reagents used were of analytical or HPLC grade.

RP-HPLC Method Development

Selection of Chromatographic Mode

Based on the physicochemical properties of Lamivudine and Dolutegravir, reverse-phase HPLC was selected as the appropriate chromatographic mode. Both compounds exhibit polar characteristics suited to RP-HPLC, which provides adequate retention and resolution. An Agilent C18 bonded stationary phase column (150 mm × 4.6 mm, 5 µm) was selected as the preferred column based on its widespread use for similar analytes.

UV Spectral Analysis

Lamivudine and Dolutegravir were scanned in the UV range of 200–400 nm using a UV-VIS spectrophotometer. Both drugs exhibited absorption maxima at 260 nm; therefore, 260 nm was selected as the detection wavelength for HPLC analysis.

Optimization of Chromatographic Conditions

The mobile phase composition was optimized by evaluating various combinations of organic solvents and aqueous buffers. A mobile phase consisting of orthophosphoric acid buffer and acetonitrile (60:40 v/v) provided well-resolved peaks with acceptable tailing factors and theoretical plate counts. The flow rate was set at 1 mL/min, column temperature at 30°C, and injection volume at 10 µL, with a total run time of 5 minutes. The optimized chromatogram was depicted in figure no.1

Table 1. Optimized chromatographic conditions

Parameter	Condition
Column	Agilent C18 (150 mm × 4.6 mm, 5 µm)
Mobile phase	Orthophosphoric acid : Acetonitrile (60:40 v/v)
Flow rate	1 mL/min
Detection wavelength	260 nm
Column temperature	30°C
Injection volume	10 µL
Run time	5 min
Diluent	Milli-Q water

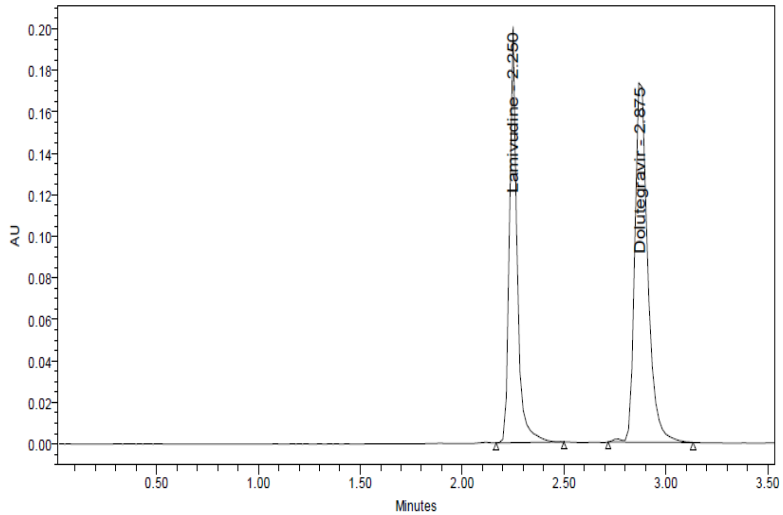


Fig.1: Optimized chromatogram

Preparation of Buffer Solutions:

0.1N Potassium Dihydrogen Orthophosphate Buffer: Exactly 1.36 g of potassium dihydrogen orthophosphate was accurately weighed and dissolved in approximately 900 mL of Milli-Q water in a 1000 mL volumetric flask. The solution was degassed by sonication, followed by addition of 1 mL of triethylamine, and the pH was adjusted to 3.3 using dilute orthophosphoric acid, then made up to volume.

0.1% Orthophosphoric Acid Solution: Exactly 1 mL of orthophosphoric acid was transferred to a 1000 mL volumetric flask containing approximately 100 mL of Milli-Q water, and the volume was made up to 1000 mL with Milli-Q water.

Preparation of Standard Stock Solutions

Accurately weighed 37.5 mg of Lamivudine and 6.25 mg of Dolutegravir working standards were transferred into a clean, dry 25 mL volumetric flask. Three-quarters of the volume was added with diluent, and the mixture was sonicated for 5 minutes, followed by making up to the final volume with diluent to obtain stock concentrations of 1500 µg/mL for Lamivudine and 250 µg/mL for Dolutegravir.

Preparation of Standard Working Solutions

One milliliter of each of the above stock solutions was transferred into a 10 mL volumetric flask and diluted to volume with diluent to yield working standard concentrations of 150 µg/mL for Lamivudine and 25 µg/mL for Dolutegravir (100% concentration level).

Preparation of Sample Stock Solutions

Five tablets were accurately weighed and the mean tablet weight was calculated. A quantity of powder equivalent to one tablet was transferred into a 100 mL volumetric flask, 5 mL of diluent was added, and the mixture was sonicated for 25 minutes. The volume was made up to the mark with diluent and filtered through HPLC-grade syringe filters to obtain concentrations of 3000 µg/mL for Lamivudine and 500 µg/mL for Dolutegravir.

Preparation of Sample Working Solutions

A volume of 0.5 mL of the filtered sample stock solution was transferred into a 10 mL volumetric flask and diluted to volume with diluent to obtain concentrations of 150 µg/mL for Lamivudine and 25 µg/mL for Dolutegravir (100% level).

Analytical Method Validation

The developed RP-HPLC method was validated in accordance with ICH Q2(R1) guidelines. Validation parameters assessed included system suitability, linearity, precision (repeatability and intermediate precision), accuracy, robustness, LOD, and LOQ.

System Suitability

System suitability was evaluated by injecting six replicate injections of the standard working solution. Parameters including retention time (RT), USP tailing factor, USP plate count (N), and resolution (Rs) were determined and compared against acceptance criteria.

Linearity

Linearity was assessed by preparing calibration standards at six concentration levels covering 25–150% of the working concentration. The calibration curve was constructed by plotting peak area against concentration, and the data were subjected to linear regression analysis to determine the slope, intercept, and correlation coefficient (R^2).

Precision

System precision (repeatability) was evaluated by injecting six replicate injections from a single working standard solution and calculating the mean peak area, standard deviation (SD), and percentage relative standard deviation (%RSD). Method precision was assessed by preparing six independent sample working solutions of the same concentration and injecting each once. %RSD was calculated for both Lamivudine and Dolutegravir.

Accuracy

Accuracy was determined using the standard addition method at three concentration levels (80%, 100%, and 120% of the working concentration), with triplicate injections at each level. Mean percentage recovery was calculated for both drugs.

Robustness

Robustness was studied by introducing deliberate small changes to the optimized chromatographic parameters, including flow rate (0.9 and 1.1 mL/min), mobile phase ratio (55B:45A and 65B:35A), and column temperature (25°C and 35°C). Duplicate injections were performed under each condition, and the effect on system suitability parameters was evaluated.

Limit of Detection and Limit of Quantification

The LOD and LOQ were determined from the linear regression data using the following equations:

$$LOD = 3.3 \times \sigma / S \qquad (Eq.\ 1)$$

$$LOQ = 10 \times \sigma / S \qquad (Eq.\ 2)$$

where σ is the standard deviation of the y-intercept and S is the mean slope of the calibration curve.

RESULTS AND DISCUSSION

UV Spectral Analysis

UV scanning of Lamivudine and Dolutegravir over the range of 200–400 nm in aqueous solution revealed absorption maxima at 260 nm for both drugs. This wavelength was selected for simultaneous detection during RP-HPLC analysis.

System Suitability

System suitability results are summarized in Table 2. The retention times for Lamivudine and Dolutegravir were 2.252 and 2.871 min, respectively. All system suitability parameters including USP plate count, tailing factor, and resolution met the prescribed acceptance criteria, confirming the reliability of the chromatographic system.

Table 2. System suitability parameters

Inj.	Lamivudine	Dolutegravir

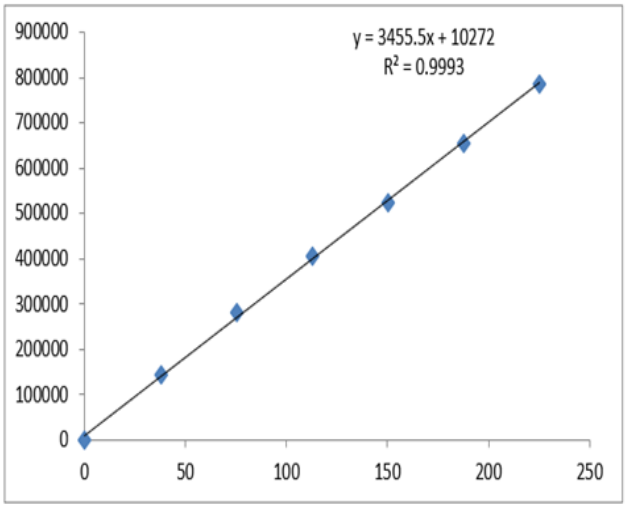
No.	RT (min)	Plate Count	Tailing	RT (min)	Plate Count	Tailing	Resolution
1	2.252	14261	1.39	2.871	10527	1.39	6.3
2	2.253	15639	1.45	2.872	10156	1.42	6.6
3	2.254	16316	1.50	2.873	9974	1.43	6.6
4	2.266	16795	1.33	2.884	10925	1.34	6.6
5	2.267	15612	1.34	2.885	11018	1.35	6.4
6	2.268	15669	1.35	2.886	11094	1.36	6.3

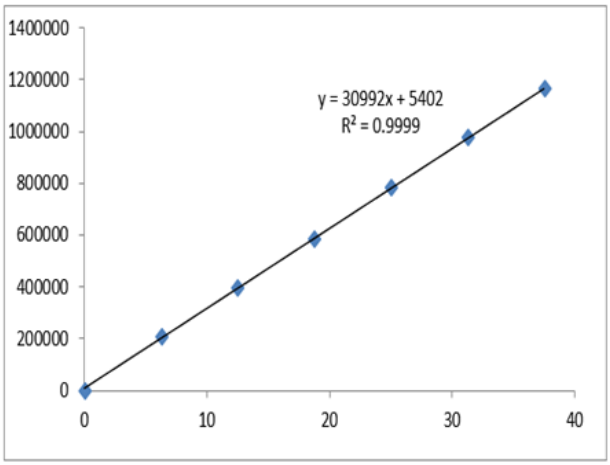
Linearity

The calibration curves for both Lamivudine and Dolutegravir were linear over the studied concentration ranges. The regression equations were y = 3455x + 10272 (R^2 = 0.999) for Lamivudine and y = 30992x + 5402 (R^2 = 0.999) for Dolutegravir, confirming excellent linearity. Linearity data are presented in Table 3 and fig no.2.

Table 3. Linearity data for Lamivudine and Dolutegravir

Lamivudine Conc. (µg/mL)	Peak Area	Dolutegravir Conc. (µg/mL)	Peak Area
0	0	0	0
37.5	142026	6.25	204515
75	281172	12.5	392948
112.5	404673	18.75	586007
150	523158	25	781670
187.5	655878	31.25	977173
225	786185	37.5	1163176





Precision

System precision was evaluated from six replicate injections of the standard working solution. The %RSD values were 0.4% and 0.2% for Lamivudine and Dolutegravir, respectively, confirming acceptable system repeatability (acceptance criteria: %RSD ≤ 2%).

Method precision (repeatability) was assessed from six independently prepared sample solutions. The %RSD values were 0.6% for Lamivudine and 0.3% for Dolutegravir, well within the ICH acceptance criterion of ≤ 2%, indicating that the method yields consistent and reproducible results.

Accuracy

Accuracy was evaluated through percentage recovery studies at 80%, 100%, and 120% concentration levels. Mean recovery values of 99.95% for Lamivudine and 99.62% for Dolutegravir were obtained, demonstrating that the method is accurate and free from significant matrix interference. All recovery values fell within the acceptable range of 98–102%.

Robustness

Robustness evaluation was performed by deliberately varying the flow rate (±0.1 mL/min), mobile phase ratio (±5%), and column temperature (±5°C). Under all modified conditions, the %RSD remained within the acceptable limit, and system suitability parameters were not significantly affected, indicating that the method is robust and reliable under minor variations in chromatographic conditions.

Limit of Detection and Limit of Quantification

The LOD and LOQ values were calculated from the calibration curve data. For Lamivudine, the LOD was 0.27 µg/mL and LOQ was 0.81 µg/mL. For Dolutegravir, the LOD was 0.11 µg/mL and LOQ was 0.34 µg/mL. These low values indicate that the method possesses adequate sensitivity for the intended purpose.

Drug Assay

The assay of Lamivudine and Dolutegravir in the commercial tablet formulation (Dovato) was performed using the validated method. The percentage label claim was found to be within the acceptable limits, confirming that the method is suitable for routine drug content estimation in pharmaceutical formulations.

CONCLUSION

A simple, rapid, accurate, and validated RP-HPLC method was successfully developed for the simultaneous estimation of Lamivudine and Dolutegravir in tablet dosage form. Chromatographic separation was achieved within 5 minutes using an Agilent C18 column and a mobile phase of orthophosphoric acid and acetonitrile (60:40 v/v) at 260 nm. The retention times for Lamivudine and Dolutegravir were 2.250 and 2.875 min, respectively. All validation parameters including linearity ($R^2 > 0.999$), precision (%RSD < 2%), accuracy (mean recovery 99.95% and 99.62%), robustness, LOD, and LOQ were within the ICH Q2(R1) acceptance criteria. The short run time and straightforward sample preparation make this method economical and practical for routine quality control testing in the pharmaceutical industry.

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