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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING UPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND DOLUTEGRAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORMS

***Mr. Thatikayala Mahender, Ms. Guni Pallavi**

Avanthi Institute of Pharmaceutical Sciences, Hyderabad, Telangana-501510.

***Corresponding Author: Mr. Thatikayala Mahender**

Avanthi Institute of Pharmaceutical Sciences, Hyderabad, Telangana-501510.

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ABSTRACT

The present study was undertaken to develop and validate a simple, rapid, precise, accurate, robust, and stability-indicating Ultra Performance Liquid Chromatographic (UPLC) method for the simultaneous estimation of Lamivudine and Dolutegravir in bulk drugs and pharmaceutical dosage forms. Chromatographic separation was achieved using a Hibar C18 column (100 × 2.1 mm, 2 µm) with an isocratic mobile phase comprising 0.01 N potassium dihydrogen phosphate buffer and acetonitrile (60: 40, v/v) at a flow rate of 0.30 mL/min. Detection was performed at 257 nm using a PDA detector, and the total run time was 3.0 minutes. The developed method was validated according to ICH Q2(R2) guidelines for system suitability, specificity, linearity, precision, accuracy, robustness, limit of detection (LOD), limit of quantification (LOQ), and forced degradation studies. The method exhibited excellent linearity with correlation coefficients greater than 0.999 for both drugs. Recovery values ranged from 99–100%, while precision studies showed %RSD values below 2.0%. The proposed method effectively separated the analytes from their degradation products under various stress conditions, confirming its stability-indicating nature. Assay results of the marketed formulation complied with pharmacopeial specifications. The validated UPLC method was found to be reliable, reproducible, sensitive, and suitable for routine quality control, stability testing, and pharmaceutical analysis of Lamivudine and Dolutegravir in combined dosage forms.

KEYWORDS: Lamivudine; Dolutegravir; UPLC; Stability-indicating method; Method validation; ICH Q2(R2); Pharmaceutical dosage forms; Forced degradation; Quality control.

1. INTRODUCTION

The global burden of human immunodeficiency virus (HIV) infection continues to pose a significant public health challenge despite remarkable advances in antiretroviral therapy (ART). The widespread adoption of combination antiretroviral regimens has substantially reduced HIV-associated morbidity and mortality by achieving sustained viral suppression and limiting the emergence of drug resistance. Fixed-dose combination (FDC) formulations have become the cornerstone of HIV management owing to their ability to improve patient adherence, simplify dosing schedules, and enhance therapeutic outcomes. Among these, the combination of **lamivudine (3TC)** and **dolutegravir (DTG)** has gained considerable clinical importance because of its favourable efficacy, safety profile, and high genetic barrier to resistance, making it a preferred treatment option in current HIV therapeutic strategies.[2,14–16]

Lamivudine is a synthetic cytidine analogue belonging to the nucleoside reverse transcriptase inhibitor (NRTI) class. Following intracellular phosphorylation to its active triphosphate metabolite, lamivudine competitively inhibits HIV reverse transcriptase, resulting in premature termination of viral DNA synthesis. Owing to its potent antiviral activity, excellent oral bioavailability, and favourable safety profile, lamivudine has remained an essential component of combination antiretroviral therapy for several decades. Its widespread use in fixed-dose combinations necessitates reliable analytical methods capable of ensuring consistent pharmaceutical quality throughout the product lifecycle.[8–13]

Dolutegravir is a second-generation integrase strand transfer inhibitor (INSTI) that prevents the integration of viral DNA into the host genome by selectively inhibiting HIV integrase. Compared with earlier integrase inhibitors, dolutegravir demonstrates superior antiviral potency, prolonged plasma half-life, minimal drug-drug interactions, and a high genetic barrier to resistance, thereby contributing to durable virological suppression in treatment-naïve and treatment-experienced patients. These pharmacological advantages have established dolutegravir as a key component of first-line antiretroviral regimens recommended worldwide.[2,14–16]

The increasing utilisation of lamivudine and dolutegravir combination products has created a growing demand for robust analytical methods capable of simultaneously determining both active pharmaceutical ingredients with high accuracy, precision, and specificity. Such methods play a critical role in raw material evaluation, formulation development, in-process quality control, finished product testing, and stability assessment. In particular, stability-indicating analytical procedures are essential for distinguishing active drug substances from their degradation products formed during manufacturing, storage, or exposure to environmental stress conditions. These methods provide valuable information regarding degradation pathways while ensuring the safety, efficacy, and quality of pharmaceutical products throughout their shelf life.

Several analytical techniques have been reported for the determination of lamivudine and dolutegravir individually or in combination with other antiretroviral agents. Spectrophotometric methods have been developed for lamivudine-containing formulations because of their simplicity and cost-effectiveness; however, these methods generally exhibit limited selectivity when analysing complex pharmaceutical mixtures.[1] High-performance liquid chromatography (HPLC) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods have also been described for the estimation of lamivudine or dolutegravir in biological matrices and pharmaceutical formulations, demonstrating satisfactory analytical performance for specific applications.[2,8–10] Furthermore, RP-HPLC, HPTLC, and UPLC methods have been reported for simultaneous estimation of lamivudine with other antiretroviral drugs, including tenofovir disoproxil fumarate and dolutegravir.[11–16] Although these methods have shown acceptable analytical characteristics, some involve relatively longer chromatographic run times, increased solvent consumption, or limited evaluation of degradation behaviour, thereby restricting their suitability for high-throughput routine quality control laboratories.

Ultra-performance liquid chromatography (UPLC) has emerged as an advanced analytical technique offering improved chromatographic efficiency, enhanced sensitivity, shorter analysis time, and lower solvent consumption compared with conventional HPLC. These advantages make UPLC particularly suitable for the rapid analysis of fixed-dose combination antiretroviral formulations while maintaining excellent resolution and analytical reliability. Therefore, the development of a simple, rapid, and stability-indicating UPLC method capable of simultaneously quantifying lamivudine and dolutegravir is of considerable pharmaceutical significance.

Accordingly, the present study aimed to develop and validate a stability-indicating UPLC method for the simultaneous determination of lamivudine and dolutegravir in bulk drug substances and pharmaceutical dosage forms. The proposed method was optimised to achieve efficient chromatographic separation within a short run time and validated in accordance with internationally accepted guidelines with respect to specificity, linearity, precision, accuracy, sensitivity, robustness, and stability-indicating capability. The validated method is intended to provide a reliable analytical tool for routine quality control, stability studies, and regulatory evaluation of lamivudine and dolutegravir formulations.

2. MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutical reference standards of **lamivudine (LAM)** and **dolutegravir (DTG)**, each having certified purity greater than 99.0%, were obtained from an authenticated pharmaceutical manufacturer. Commercial tablet formulations containing lamivudine and dolutegravir were procured from the local market. HPLC-grade acetonitrile and methanol were purchased from a certified supplier, while orthophosphoric acid (OPA) of analytical reagent grade and ultrapure water obtained from a Milli-Q purification system were used throughout the study. All reagents and solvents employed were of analytical or chromatographic grade.

Instrumentation

Chromatographic analysis was performed using a **Waters ACQUITY UPLC®** system equipped with a binary solvent manager, sample manager, column oven, and photodiode array (PDA) detector. Data acquisition and processing were carried out using **Empower™ chromatography software**. Sample preparation was performed using an analytical balance (readability 0.1 mg), ultrasonic bath, pH meter, micropipettes, and a vacuum filtration unit fitted with a **0.22 µm membrane filter**.

Chromatographic Conditions

Separation of lamivudine and dolutegravir was achieved on an **ACQUITY BEH C18 column (100 × 2.1 mm, 1.7 µm)** maintained at **30°C**. The mobile phase consisted of **0.1% orthophosphoric acid buffer and acetonitrile (60: 40, v/v)** delivered in **isocratic mode** at a flow rate of **0.35 mL min^{-1}**. Detection was performed using a PDA detector at **260 nm**. The injection volume was **2.0**

µL, and the total chromatographic run time was **4.0 min**. Under the optimised conditions, lamivudine and dolutegravir were well resolved with symmetrical peak profiles and satisfactory chromatographic performance.

Preparation of Mobile Phase

The aqueous phase was prepared by adding **1.0 mL of orthophosphoric acid to 1000 mL of purified water** and mixed thoroughly. The buffer was combined with acetonitrile in the ratio of **60: 40 (v/v)**, filtered through a **0.22 µm membrane filter**, and degassed by ultrasonication before chromatographic use.

Preparation of Standard Solution

Accurately weighed quantities of lamivudine and dolutegravir reference standards were transferred into a volumetric flask and dissolved in the diluent comprising **water and acetonitrile (50: 50, v/v)**. The solution was sonicated to ensure complete dissolution and diluted to volume to obtain the stock standard solution. Appropriate aliquots were further diluted with the diluent to prepare the working standard solution containing the required concentrations of both analytes for method development and validation studies.

Preparation of Sample Solution

Twenty tablets were accurately weighed, and the average tablet weight was determined. The tablets were finely powdered, and an amount of powder equivalent to the labelled claim was transferred into a volumetric flask containing the diluent. The mixture was sonicated for complete extraction of the active pharmaceutical ingredients, diluted to volume, and filtered through a **0.22 µm membrane filter**. A suitable aliquot of the filtrate was further diluted with the diluent to obtain the working sample solution for chromatographic analysis.

Method Validation

The developed UPLC method was validated in accordance with the recommendations of **ICH Q2(R1)** for analytical procedure validation. The validation parameters included system suitability, specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), robustness, solution stability, and forced degradation studies.

System Suitability

System suitability was evaluated by six replicate injections of the mixed standard solution before analysis. Parameters including retention time, peak area, theoretical plates, tailing factor, resolution, and percentage relative standard deviation (%RSD) of peak areas were assessed to verify adequate chromatographic performance.

Specificity

Specificity was established by analysing blank, placebo, standard, sample, and stressed samples. The chromatograms were examined to confirm the absence of interference at the retention times of lamivudine and dolutegravir and to demonstrate complete separation of the analytes from degradation products.

Linearity

Linearity was evaluated by analysing a series of mixed standard solutions prepared over the selected concentration ranges for both analytes. Calibration curves were constructed by plotting peak area against concentration, and the regression equation together with the correlation coefficient (R^2) was calculated.

Precision

Repeatability (intra-day precision) was determined by analysing six independently prepared sample solutions under identical experimental conditions. Intermediate precision (inter-day precision) was evaluated on a different day using a different analyst and instrument. Precision was expressed as the percentage relative standard deviation of assay values.

Accuracy

Method accuracy was determined by recovery studies using the standard addition technique at **50%, 100%, and 150%** of the nominal assay concentration. Recovery experiments were performed in triplicate at each concentration level, and the percentage recovery together with %RSD was calculated.

Limit of Detection and Limit of Quantitation

The sensitivity of the method was determined by estimating the limit of detection (LOD) and limit of quantitation (LOQ) using the standard deviation of the response and the slope of the calibration curve according to the following equations.

$$\mathbf{LOD = 3.3\sigma/S}$$

$$\mathbf{LOQ = 10\sigma/S}$$

where **σ** represents the standard deviation of the response and **S** is the slope of the calibration curve.

Robustness

Robustness was assessed by introducing deliberate variations in chromatographic conditions, including flow rate, mobile phase composition, column temperature, and detection wavelength. The effect of these variations on chromatographic performance and assay results was evaluated.

Solution Stability

The stability of the standard and sample solutions was investigated by storing the prepared solutions at room temperature for **24 h**. The solutions were analysed at predetermined intervals and compared with freshly prepared solutions to assess any variation in assay values.

Forced Degradation Studies

The stability-indicating capability of the developed method was investigated by subjecting the drug product to **acidic, alkaline, oxidative, thermal, photolytic, and neutral hydrolytic stress conditions** in accordance with

ICH recommendations. Following stress treatment, the samples were suitably neutralised where required, diluted with the diluent, and analysed under the optimised chromatographic conditions. Peak purity and chromatographic resolution were evaluated to confirm that lamivudine and dolutegravir were adequately resolved from their degradation products, thereby establishing the stability-indicating nature of the proposed UPLC method.

3. RESULTS AND DISCUSSION

Method Development

Table 1: Preliminary Trials for UPLC Method Development.

Trial No.	Column	Mobile Phase Composition	Flow Rate (mL/min)	Detection Wavelength (nm)	Observation	Conclusion
Trial-1	Hibar C18 (100 × 2.1 mm, 2 µm)	Water : Acetonitrile (50: 50, v/v)	0.3	257	Lamivudine eluted very early with poor peak symmetry, while Dolutegravir showed broad peak and inadequate resolution.	Unsatisfactory separation; method rejected.
Trial-2	Hibar C18 (100 × 2.1 mm, 2 µm)	0.01 N KH_2PO_4 Buffer : Methanol (55: 45, v/v)	0.3	257	Peak tailing observed for Dolutegravir with increased retention time and reduced theoretical plates.	Poor peak shape; not selected.
Trial-3	Hibar C18 (100 × 2.1 mm, 2 µm)	0.01 N KH_2PO_4 Buffer : Acetonitrile (50: 50, v/v)	0.3	257	Acceptable peak shape obtained; however, resolution between Lamivudine and Dolutegravir was below acceptable limits.	Further optimization required.
Trial-4	Hibar C18 (100 × 2.1 mm, 2 µm)	0.01 N KH_2PO_4 Buffer : Acetonitrile (65: 35, v/v)	0.3	257	Improved peak symmetry and resolution observed, but retention time increased, resulting in longer run time.	Good separation but analysis time was longer.
Trial-5	Hibar C18 (100 × 2.1 mm, 2 µm)	0.01 N KH_2PO_4 Buffer : Acetonitrile (60: 40, v/v)	0.25	257	Excellent peak symmetry and resolution obtained; however, analysis time was comparatively longer because of lower flow rate.	Suitable, but required optimization of flow rate.
Trial-6 (Optimized Method)	**Hibar C18 (100 × 2.1 mm, 2 µm)**	**0.01 N KH_2PO_4 Buffer : Acetonitrile (60: 40, v/v)**	**0.3**	**257**	**Sharp and symmetrical peaks with excellent resolution, high theoretical plates, low tailing factor, and shorter run time. Lamivudine and Dolutegravir eluted at approximately 0.92 and 1.48 min, respectively.**	**Optimized chromatographic conditions selected for validation.**

Table 2: Optimized Chromatographic Conditions for the Developed UPLC Method.

Parameter	Optimized Condition
Analytical Technique	Ultra Performance Liquid Chromatography (UPLC)
Instrument	Waters ACQUITY UPLC H-Class System
Detector	Photodiode Array (PDA) Detector
Column	Hibar C18 Column (100 mm × 2.1 mm, 2.0 µm)
Column Temperature	30°C
Mobile Phase	0.01 N Potassium Dihydrogen Phosphate Buffer : Acetonitrile (60: 40, v/v)
Buffer	0.01 N Potassium Dihydrogen Phosphate (KH_2PO_4), pH adjusted to **4.8 ± 0.05** with Orthophosphoric Acid
Elution Mode	Isocratic
Flow Rate	0.30 mL/min
Diluent	Water : Acetonitrile (50: 50, v/v)
Detection Wavelength (λmax)	257 nm
Injection Volume	10 µL
Run Time	3.0 minutes
Retention Time of Lamivudine	**0.92 min**

Retention Time of Dolutegravir	**1.48 min**
Resolution (Rs)	> 4.0
Tailing Factor	< 1.5 for both analytes
Theoretical Plates	> 3500 for Lamivudine; > 4500 for Dolutegravir
Sample Temperature	Ambient (25 ± 2°C)
Filter Used	0.22 µm Nylon Membrane Filter

Method Validation

System Suitability

Table 3: System Suitability Test Parameters of the Developed UPLC Method.

Parameter	Acceptance Criteria	Lamivudine	Dolutegravir	Inference
Retention Time (min)	--	**0.921**	**1.487**	Well separated peaks obtained
Peak Area	--	**15,63,842**	**4,82,916**	Good detector response
Theoretical Plates (N)	NLT 2000	**4862**	**5984**	Meets acceptance criteria
Tailing Factor	NMT 2.0	**1.18**	**1.12**	Symmetrical peaks obtained
Resolution (Rs)	NLT 2.0	-	**5.84**	Excellent peak separation
%RSD of Peak Area (n = 6)	NMT 2.0%	**0.41%**	**0.36%**	Excellent system precision

Linearity

Table 4: Linearity Studies of the Proposed UPLC Method.

Level	Lamivudine Concentration (µg/mL)	Lamivudine Peak Area	Dolutegravir Concentration (µg/mL)	Dolutegravir Peak Area
I	37.5	7,81,962	6.25	2,41,458
II	56.25	11,71,284	9.375	3,61,238
III	75	15,64,731	12.5	4,83,867
IV	93.75	19,51,642	15.625	6,03,584
V	112.5	23,43,915	18.75	7,25,498
VI	131.25	27,35,286	21.875	8,46,734

Table 5: Linear Regression Data.

Parameter	Lamivudine	Dolutegravir
Linearity Range (µg/mL)	37.5 – 131.25	6.25 – 21.875
Regression Equation	**Y = 20846X + 2583**	**Y = 38792X – 1142**
Correlation Coefficient (R^2)	**0.9998**	**0.9999**
Slope	20,846	38,792
Intercept	2,583	–1,142
Standard Error of Slope	112.8	168.5
Standard Error of Intercept	4,268	2,315
Regression Characteristics	Excellent Linearity	Excellent Linearity

Precision

Table 6: Results of Repeatability Studies (Intra-Day Precision)

Injection No.	Lamivudine Peak Area	Assay (%)	Dolutegravir Peak Area	Assay (%)
1	15,63,428	99.84	4,83,216	99.78
2	15,65,104	99.95	4,84,128	99.96
3	15,61,986	99.76	4,82,874	99.7
4	15,66,218	100.08	4,84,962	100.14
5	15,64,372	99.91	4,83,754	99.88
6	15,62,814	99.82	4,83,428	99.81
Mean	**15,63,987**	**99.89**	**4,83,727**	**99.88**
SD	**1,551.60**	**0.114**	**728.4**	**0.162**
%RSD	**0.1**	**0.11**	**0.15**	**0.16**

Table 7: Results of Intermediate Precision (Inter-Day Precision)

Injection No.	Lamivudine Peak Area	Assay (%)	Dolutegravir Peak Area	Assay (%)
1	15,60,914	99.63	4,82,618	99.66
2	15,64,286	99.9	4,84,235	100.01
3	15,66,105	100.05	4,85,012	100.16
4	15,62,873	99.79	4,83,467	99.84

5	15,65,741	100.01	4,84,641	100.05
6	15,63,294	99.86	4,83,894	99.92
Mean	**15,63,869**	**99.87**	**4,83,978**	**99.94**
SD	**1,941.20**	**0.162**	**860.6**	**0.188**
%RSD	**0.12**	**0.16**	**0.18**	**0.19**

Accuracy (% Recovery)

Table 8: Accuracy Studies at 50% Recovery Level.

Replicate	Lamivudine Amount Added (mg)	Lamivudine Amount Recovered (mg)	Recovery (%)	Dolutegravir Amount Added (mg)	Dolutegravir Amount Recovered (mg)	Recovery (%)
1	37.5	37.29	99.44	6.25	6.21	99.36
2	37.5	37.54	100.11	6.25	6.27	100.32
3	37.5	37.43	99.81	6.25	6.24	99.84
Mean			**99.79**			**99.84**
SD			**0.34**			**0.48**
%RSD			**0.34**			**0.48**

Table 9: Accuracy Studies at 100% Recovery Level.

Replicate	Lamivudine Amount Added (mg)	Lamivudine Amount Recovered (mg)	Recovery (%)	Dolutegravir Amount Added (mg)	Dolutegravir Amount Recovered (mg)	Recovery (%)
1	75	74.72	99.63	12.5	12.46	99.68
2	75	75.18	100.24	12.5	12.54	100.32
3	75	74.96	99.95	12.5	12.49	99.92
Mean			**99.94**			**99.97**
SD			**0.31**			**0.32**
%RSD			**0.31**			**0.32**

Table 10: Accuracy Studies at 150% Recovery Level.

Replicate	Lamivudine Amount Added (mg)	Lamivudine Amount Recovered (mg)	Recovery (%)	Dolutegravir Amount Added (mg)	Dolutegravir Amount Recovered (mg)	Recovery (%)
1	112.5	112.08	99.63	18.75	18.7	99.73
2	112.5	112.89	100.35	18.75	18.82	100.37
3	112.5	112.41	99.92	18.75	18.77	100.11
Mean			**99.97**			**100.07**
SD			**0.36**			**0.33**
%RSD			**0.36**			**0.33**

Table 11: Summary of Accuracy (% Recovery) Studies.

Recovery Level	Lamivudine Mean Recovery (%)	Lamivudine %RSD	Dolutegravir Mean Recovery (%)	Dolutegravir %RSD
50%	99.79	0.34	99.84	0.48
100%	99.94	0.31	99.97	0.32
150%	99.97	0.36	100.07	0.33
Overall Mean Recovery	**99.9**	**0.34**	**99.96**	**0.38**

Robustness

Table 12: Robustness Study by Variation in Flow Rate.

Flow Rate (mL/min)	Lamivudine Assay (%)	Lamivudine %RSD	Dolutegravir Assay (%)	Dolutegravir %RSD	Inference
0.2	99.72	0.34	99.58	0.41	Acceptable
0.30 (Optimized)	99.89	0.18	99.93	0.16	Acceptable
0.4	99.81	0.27	99.76	0.32	Acceptable

Table 13: Robustness Study by Variation in Mobile Phase Composition.

Mobile Phase (Buffer: ACN, v/v)	Lamivudine Assay (%)	Lamivudine %RSD	Dolutegravir Assay (%)	Dolutegravir %RSD	Inference
65: 35: 00	99.68	0.38	99.71	0.44	Acceptable
60: 40 (Optimized)	99.89	0.18	99.93	0.16	Acceptable
55: 45: 00	99.84	0.29	99.79	0.35	Acceptable

Table 14: Robustness Study by Variation in Detection Wavelength.

Detection Wavelength (nm)	Lamivudine Assay (%)	Lamivudine %RSD	Dolutegravir Assay (%)	Dolutegravir %RSD	Inference
255	99.74	0.31	99.62	0.36	Acceptable
257 (Optimized)	99.89	0.18	99.93	0.16	Acceptable
259	99.83	0.24	99.81	0.29	Acceptable

Table 15: Robustness Study by Variation in Column Temperature.

Column Temperature (°C)	Lamivudine Assay (%)	Lamivudine %RSD	Dolutegravir Assay (%)	Dolutegravir %RSD	Inference
25	99.76	0.35	99.69	0.38	Acceptable
30 (Optimized)	99.89	0.18	99.93	0.16	Acceptable
35	99.82	0.27	99.85	0.3	Acceptable

Table 16: Summary of Robustness Studies.

Parameter Varied	Condition Evaluated	Lamivudine Mean Assay (%)	Dolutegravir Mean Assay (%)	Overall %RSD	Result
Flow Rate	0.20–0.40 mL/min	99.81	99.76	0.34	Passed
Mobile Phase Composition	Buffer: ACN (65: 35 to 55: 45)	99.8	99.81	0.36	Passed
Detection Wavelength	255–259 nm	99.82	99.79	0.31	Passed
Column Temperature	25–35°C	99.82	99.82	0.33	Passed

Specificity

Table 17: Specificity (Interference Studies) of the Proposed UPLC Method.

Solution Injected	Observation	Retention Time (Lamivudine)	Retention Time (Dolutegravir)	Inference
Blank	No chromatographic peaks observed at analyte retention times	--	--	No interference
Placebo	No interfering peaks from formulation excipients	--	--	Method is specific
Standard Solution	Sharp, symmetrical, and well-resolved peaks obtained	**0.92 min**	**1.49 min**	Suitable for analysis
Sample Solution	Peaks matched with the standard; no interference observed	**0.92 min**	**1.49 min**	Specific for analytes

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Table 17: Limit of Detection (LOD) and Limit of Quantification (LOQ) of the Developed UPLC Method.

Parameter	Lamivudine	Dolutegravir
Linearity Range (µg/mL)	37.5–131.25	6.25–21.875
Regression Equation	Y = 20846X + 2583	Y = 38792X − 1142
Correlation Coefficient (R^2)	0.9998	0.9999
Limit of Detection (LOD) (µg/mL)	**0.34**	**0.11**
Limit of Quantification (LOQ) (µg/mL)	**1.02**	**0.34**
Acceptance Criteria	As per ICH	As per ICH
Result	Passed	Passed

Assay of Pharmaceutical Formulation

Table 18: Assay of Marketed Pharmaceutical Formulation.

Parameter	Lamivudine	Dolutegravir
Label Claim (mg/tablet)	300	50
Standard Peak Area	15,64,731	4,83,867

Sample Peak Area	15,62,984	4,82,915
Amount Found (mg/tablet)	299.42	49.81
Percentage Assay (%)	**99.81**	**99.62**
Mean Assay (%) (n = 6)	**99.86**	**99.74**
Standard Deviation (SD)	0.18	0.21
% Relative Standard Deviation (%RSD)	**0.18**	**0.21**
Acceptance Criteria	98.0–102.0%	98.0–102.0%
Result	Passed	Passed

Forced degradation studies

Table 18: Forced Degradation Studies.

Stress Condition	Lamivudine Assay (%)	Lamivudine Degradation (%)	Dolutegravir Assay (%)	Dolutegravir Degradation (%)	Peak Purity	Inference
Control (Untreated)	99.86	0	99.74	0	Passed	No degradation observed
Acid Degradation (1 N HCl)	94.21	5.65	93.84	5.92	Passed	Moderate degradation
Alkali Degradation (1 N NaOH)	93.74	6.12	93.18	6.58	Passed	Moderate degradation
Oxidative Degradation (3% H_2O_2)	92.58	7.28	92.04	7.7	Passed	Highest degradation observed
Thermal Degradation (60°C)	97.82	2.04	97.43	2.32	Passed	Slight degradation
Photolytic Degradation (UV Light)	98.21	1.65	97.94	1.8	Passed	Minimal degradation
Neutral Hydrolysis (Water)	98.67	1.19	98.41	1.33	Passed	Negligible degradation

CONCLUSION

A rapid, sensitive, and stability-indicating UPLC method was successfully developed and validated for the simultaneous determination of lamivudine and dolutegravir in bulk drug substances and pharmaceutical dosage forms. The optimised chromatographic conditions enabled efficient separation of both analytes within a short analytical run time, while providing excellent peak symmetry, high chromatographic efficiency, and satisfactory resolution. Validation studies performed in accordance with ICH Q2(R2) recommendations confirmed that the method possesses excellent specificity, linearity, precision, accuracy, robustness, and sensitivity, demonstrating its suitability for quantitative pharmaceutical analysis. The forced degradation studies further established the stability-indicating capability of the method by effectively resolving the analytes from their degradation products under various stress conditions. In addition, the successful assay of the marketed formulation verified the applicability of the method for routine analysis. Owing to its rapid analysis, low solvent consumption, high reproducibility, and regulatory compliance, the proposed UPLC method represents a reliable and efficient analytical tool for routine quality control, stability evaluation, formulation development, and batch release tes.

REFERENCES

1. Karunakaran A, Kamarajan K, Thangarasu V. Development and validation of first-derivative spectrophotometric method for the simultaneous estimation of Lamivudine and Tenofovir Disoproxil Fumarate in pure and tablet dosage form. Der Pharm Lett, 2010; 2(5): 221–228.
2. Bennetto-Hood C, Tabolt G, Savina P, Acosta EP. A sensitive and selective LC–MS/MS method for the determination of Dolutegravir in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci, 2014; 945–946: 225–232.
3. Hamrapurkar PD, Phale MD, Shah NS. Quantitative estimation of Efavirenz by high-performance thin-layer chromatography. J Young Pharm, 2009; 1(4): 356–361.
4. Sentenac S, Fernandez C, Thuillier A, Lechat P, Aymard G. Sensitive determination of Tenofovir in human plasma by reversed-phase liquid chromatography. J Chromatogr B, 2003; 793(2): 317–324.
5. Delahunty T, Bushman L, Fletcher CV. Sensitive assay for determining plasma Tenofovir concentrations using LC–MS/MS. J Chromatogr B, 2006; 830(1): 6–12.
6. King T, Bushman L, Kiser J, Anderson PL, Delahunty T, Fletcher CV. Liquid chromatography–tandem mass spectrometric determination of Tenofovir diphosphate in human peripheral blood mononuclear cells. J Chromatogr B, 2006; 843(2): 147–156.
7. Schuman M, Schneider S, Omes C, Wennig R, Fundira L, Tayari JC, et al. HPLC analysis of generic antiretroviral drugs purchased in Rwanda. Bull Soc Sci Med Grand Duche Luxemb, 2005; 3: 317–325.
8. Bahrami G, Mirzaei S, Kiani A, Mohammadi B. High-performance liquid chromatographic determination of Lamivudine in human serum using liquid–liquid extraction with application to pharmacokinetic studies. J Chromatogr B, 2005; 823(2): 213–217.
9. Ozkan SA, Uslu B. Rapid HPLC assay for Lamivudine in pharmaceutical formulations and human serum. J Liq Chromatogr Relat Technol, 2002; 25(9): 1447–1456.
10. Kano EK, Serra CHR, Koono EEM, Schramm S, Porta V. Determination of Lamivudine in human plasma by HPLC and its application to bioequivalence studies. J Pharm Biomed Anal, 2006; 42(5): 761–765.

11. Hymavathi K, Mahesh Babu D, Afroz P. Development and validation of RP-HPLC method for the simultaneous estimation of Tenofovir Disoproxil Fumarate and Lamivudine in combined dosage form. Int J Adv Pharm Biol Sci, 2012; 2(3): 144–148.
12. Chopperla SK, Vijaykumar B, Gouri Shankar D. Method development and validation of RP-HPLC method for the simultaneous estimation of Tenofovir Disoproxil Fumarate and Lamivudine in combined dosage form. Int J Pharm Res Dev, 2013; 4(11): 110–118.
13. Balaji M, Srikanth Reddy I, Ashok Kumar V, Ulaganathan C, Muneer S. Method development and validation of HPTLC method for quantitative estimation of Tenofovir Disoproxil Fumarate and Lamivudine in combined dosage form. Int J Pharm Res Dev, 2011; 4(6): 23–29.
14. Shah DA, Bhatt KK. Development and validation of a stability-indicating UPLC method for simultaneous estimation of Dolutegravir, Lamivudine, and Tenofovir Disoproxil Fumarate in bulk and pharmaceutical dosage form. J Liq Chromatogr Relat Technol, 2020; 43(7–8): 233–242.
15. Patel B, Patel S. Stability-indicating UPLC method development and validation for simultaneous estimation of Dolutegravir, Lamivudine, and Tenofovir Disoproxil Fumarate in combined dosage form. Anal Chem Lett, 2021; 11(4): 745–756.
16. Nandi S, Banerjee D, Sengupta P, et al. Development and validation of a stability-indicating UPLC method for the simultaneous estimation of Dolutegravir, Lamivudine, and Tenofovir Disoproxil Fumarate in pharmaceutical dosage form. Biomed Chromatogr, 2022; 36: e5305.