

Stability Indicating Method Development For Estimation Of Dolutegravir (DLT) And Lamivudine (LAM) By HPLC

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Abstract

A stability-indicating HPLC method was developed and validated for the simultaneous estimation of Dolutegravir (DLT) and Lamivudine (LAM) in pharmaceutical formulations. The method demonstrated excellent linearity for DLT (1–5 µg/mL, $r^2 = 0.9991$) and LAM (10–50 µg/mL, $r^2 = 0.9996$). System suitability parameters, including retention time, theoretical plates, and tailing factor, were within acceptable limits, indicating precise and reliable chromatographic performance. Accuracy studies showed mean recoveries of 97.97–98.54% for DLT and 98.32–99.06% for LAM with %RSD <1%. Precision, assessed through repeatability, intermediate precision, analyst-to-analyst variation, and reproducibility, exhibited %RSD values <2%, confirming robustness. The method was successfully applied to commercial tablet formulations, and forced degradation studies revealed susceptibility to acidic, alkaline, oxidative, and photolytic conditions, demonstrating the stability-indicating nature of the method. The developed method is accurate, precise, sensitive, and suitable for routine quality control and stability studies of DLT and LAM.

Keywords: Dolutegravir, Lamivudine, HPLC, Stability-indicating method, Forced degradation, Pharmaceutical analysis.

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Introduction

Human Immunodeficiency Virus (HIV) infection remains a major global health concern, and combination antiretroviral therapy (ART) is the standard approach for its effective management [1]. Dolutegravir (DLT), an integrase strand transfer inhibitor, and Lamivudine (LAM), a nucleoside reverse transcriptase inhibitor, are widely used in fixed-dose combination therapy due to their potent antiviral activity, favorable pharmacokinetic profile, and reduced resistance potential. The combination improves therapeutic efficacy and enhances patient compliance [2].

For pharmaceutical quality control, it is essential to develop a reliable analytical method capable of accurately quantifying both drugs in bulk and combined dosage forms. High-Performance Liquid Chromatography (HPLC) is the most widely employed technique for such analyses due to its high sensitivity, precision, specificity, and suitability for multicomponent estimation [3].

A stability-indicating method is particularly important for combination formulations. According to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

guidelines (ICH Q1A(R2) and Q2(R1)), a stability-indicating analytical method should accurately and specifically measure active pharmaceutical ingredients (APIs) in the presence of degradation products, impurities, and excipients [4-7].

Forced degradation studies under stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic environments are performed to demonstrate the method's specificity and stability-indicating capability [8-9].

Dolutegravir contains functional groups susceptible to hydrolytic and oxidative degradation, while Lamivudine may undergo degradation under acidic and oxidative conditions. Therefore, simultaneous estimation requires careful optimization of chromatographic conditions such as mobile phase composition, pH, column selection (commonly C18), flow rate, and detection wavelength to achieve adequate resolution between the drugs and their degradation products.

The development of a validated stability-indicating RP-HPLC method ensures accurate quantification, detection of degradation behavior, and compliance with regulatory standards. Such a method is essential for routine quality control, stability testing, and shelf-life

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determination of combined Dolutegravir and Lamivudine formulations.

Material and Methods

Material

Dolutegravir (DLT) and Lamivudine (LAM) were obtained as gift samples from Pharmaceutical Company. HPLC-grade methanol, acetonitrile, and water were procured from Merck, India. Analytical-grade reagents such as orthophosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were used for preparation of mobile phase and forced degradation studies. Tablet formulations containing DLT (50 mg) and LAM (300 mg) were purchased from local pharmacies for assay and method validation. All chemicals and solvents were of analytical or HPLC grade and used without further purification.

Methods

Selection of mobile phase

Initially, a variety of mobile phase ratios were tested in order to estimate DLT and LAM in a fixed dosage form. The mobile phase that was determined to be most appropriate for analysis was 25mM KH_2PO_4 : Acetonitrile (pH 4.0 with OPA) in a ratio of 20:80 v/v, taking into account system appropriateness parameters such as RT, Tailing factor, number of theoretical plates, and HETP. After removing particulate matter using 0.45m filter paper, the mobile phase was sonicated to degas it. 1.0 ml/min was the flow rate used for analysis.

Procedure for preparation of mobile phase

25 mM KH_2PO_4 : Acetonitrile (20:80v/v) at pH 4.0 with orthophosphoric acid (pH 4.0 with OPA) passed through filter paper with a pore size of 0.45.

Selection of Diluent

The diluent used to prepare the sample was compatible with the mobile phase and had no discernible effect on the analytes retention or resolution. Acetonitrile was employed as a diluent following a number of experiments.

Preparation of standard Stock solution

10 mg of DLT and LAM, each precisely weighed, were put into separate 10 ml volumetric flasks, dissolved in 5 ml of acetonitrile, and sonicated for 10 minutes before the volume was adjusted to 10 ml using acetonitrile. DLT and LAM concentrations in methanol were 1000 $\mu\text{g/ml}$ (stock-A).

Preparation of Sub Stock Solution

To get a concentration of 100 $\mu\text{g/ml}$ (Stock-B), 1 ml of the solution was extracted from DLT and LAM (stock-

A), put into a separate 10 ml volumetric flask, and diluted with 10 ml of diluent (acetonitrile).

Preparation of Different Solution

A 10 ml volumetric flask was filled with 0.1 ml, 0.2 ml, 3 ml, 4 ml, and 5 ml of stock-B, and the volume was increased to 10 ml using acetonitrile. This results in drug solutions containing 1 $\mu\text{g/ml}$, 2 $\mu\text{g/ml}$, 3 $\mu\text{g/ml}$, 4 $\mu\text{g/ml}$, and 5 $\mu\text{g/ml}$ for DLT. Similarly, LAM was generated at 10 $\mu\text{g/ml}$, 20 $\mu\text{g/ml}$, 30 $\mu\text{g/ml}$, 40 $\mu\text{g/ml}$, and 50 $\mu\text{g/ml}$.

Linearity and Calibration Graph

A series of dilutions ranging from 10–50 $\mu\text{g/ml}$ for DLT and 1–5 $\mu\text{g/ml}$ for LAM were created in order to demonstrate the linearity of the analytical procedure. Chromatograms were acquired at 254 nm after all of the solution had been filtered through a 0.2 nm membrane filter and injected three times. The regression equation was obtained by plotting a calibration graph between the mean peak area and the corresponding concentration.

System suitability parameters

After setting the separation parameters, the mobile phase was allowed to fill the column at a rate of 1 ml/minute. Three replicates of the working standard of DLT 5 $\mu\text{g/ml}$ and LAM 10 $\mu\text{g/ml}$ were injected independently after the column had fully saturated. Every chromatogram had a peak report and a column performance report.

Validation of HPLC method development [10]

Linearity

Drug response ratios were used to determine the linearity of both drugs. The drugs response ratio is determined by dividing the absorbance by the corresponding concentration. The relationship between concentration and response ratio was then shown on a graph.

Specificity

To determine the analyte presence of the components that would be predicted to be present, such as contaminants, degradation products, and matrix components, the specificity of the approach was tested.

Accuracy

Recovery studies evaluated the suggested approaches accuracy at three different levels: 80%, 100%, and 120%. In order to conduct the recovery investigations, preanalyzed tablet powder was mixed with a specified quantity of standard DLT and LAM solution. The suggested techniques were then used to re-analyze the final results. To determine the recovery of the additional drug sample, the entire analysis process

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was performed. Three replicates of five concentration levels were used for this recovery analysis.

Precision

Three levels of method precision were examined: reproducibility, intermediate precision (day to day and analyst to analyst), and repeatability. By analyzing the same drug concentration 5 times, repeatability was achieved. 5 different drug concentrations were analyzed every day for 3 days of the week.

Detection Limit and Quantitation Limit

The slope of the linearity curve and the response standard deviation were used to compute the LOD and LOQ of the developed approach.

Analysis of tablets formulation

After the tablets were weighed and broken into a fine powder, take equivalent to 5mg DLT and 30 mg LAM were taken in a 10 ml volumetric flask. After adding 5 ml of acetonitrile, the flask was sonicated for approximately 10 minutes to dissolve the drug in the tablet powder. Acetonitrile was then added to bring the volume up to the desired level. Following sonication, a 0.45 μ membrane filter was used for filtration. To obtain the final drug concentrations within the working range, the filtrate was gathered and further diluted with methanol. The calibration curve method was used to determine the concentrations, and the mean area of the final dilutions was measured. Five iterations of the process were conducted.

Forced degradation studies

Drug powder was subjected to forced degradation experiments in order to ascertain whether the procedure is stability suggesting. HPLC with a UV detector was used for the analysis. We injected 20 μ l of each forced degradation sample.

Acid degradation:

A 50 ml round bottom flask containing 10 mg of each drug was filled with 50 ml of 0.1 M HCl solution. The contents were thoroughly mixed and allowed to stir continuously for 8 hours at 80°C. After the samples were extracted and diluted to 10 μ g/ml, they were submitted to HPLC, and the percentage degradation was calculated using the drug calibration curve.

Alkaline hydrolysis:

A 50 ml round bottom flask containing 10 mg of each drug was filled with 50 ml of 0.1 M NaOH solution. The contents were thoroughly mixed and allowed to stir continuously for 8 hours at 80°C. After removing and diluting the samples to 10 μ g/ml, they were exposed to

HPLC, and the percentage degradation was calculated using the drug calibration curve.

Oxidative degradation:

A 50 ml round-bottom flask containing 10 mg of each was filled with 50 ml of a 3% hydrogen peroxide solution. The contents were thoroughly mixed and allowed to stand at room temperature for 24 hours while being constantly stirred. After the samples were extracted and diluted to 10 μ g/ml, they were submitted to HPLC, and the % degradation was calculated using the drug calibration curve.

Thermal degradation:

For 4 weeks, 10 mg of each drug was placed in a petri dish and baked at 50°C. After removing and diluting the samples to 10 μ g/ml, they were exposed to HPLC, and the percentage degradation was calculated using the drug calibration curve.

Results and Discussion

The present study focused on the development and validation of a stability-indicating HPLC method for the simultaneous estimation of Dolutegravir (DLT) and Lamivudine (LAM).

Linearity: Both DLT and LAM exhibited excellent linearity over the tested concentration ranges (1–5 μ g/mL for DLT and 10–50 μ g/mL for LAM), with correlation coefficients (r^2) of 0.9991 and 0.9996, respectively (Table 1). The slopes and intercepts further confirmed the proportionality between concentration and response. Chromatograms of individual drugs and their combination are shown in Figures 1–3.

System Suitability: System suitability parameters, including retention time, area under curve (AUC), theoretical plates, and tailing factor, were within acceptable limits (Table 2). The %RSD for DLT and LAM was below 2%, confirming the precision and reliability of the chromatographic system.

Recovery Studies: Accuracy was evaluated through recovery studies at 80%, 100%, and 120% levels. DLT showed recoveries between 97.97–98.54%, while LAM ranged from 98.32–99.06%, with %RSD values consistently below 1% (Table 3), demonstrating that the method is accurate and free from interference.

Precision Studies: Intermediate precision (day-to-day), analyst-to-analyst variation, and reproducibility studies further confirmed the robustness of the method (Table 4). All %RSD values were <2%, highlighting excellent method precision for both drugs.

LOD and LOQ: The low LOD and LOQ values (DLT: 0.10 μ g/mL and 0.30 μ g/mL; LAM: 0.45 μ g/mL and

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1.25 µg/mL) indicate the high sensitivity of the developed method (Table 5), making it suitable for detecting even trace amounts of the drugs.

Tablet Analysis: The method was successfully applied to commercial formulations. The % assay results for DLT (99.70%) and LAM (99.62%) were within acceptable limits, with low SD and %RSD (Table 6), confirming the method's applicability for routine quality control.

Forced Degradation Studies: Both drugs showed significant degradation under stress conditions, indicating susceptibility to acidic, alkaline, oxidative, and photolytic stress (Tables 7 and 8). DLT decomposed most under acidic hydrolysis (12.07%), while LAM was most sensitive to photolytic degradation (13.30%). These results confirm the stability-indicating nature of the method, capable of distinguishing the intact drug from its degradation products.

Chromatograms: The HPLC chromatograms (Figures 1–3) demonstrated clear, well-resolved peaks for DLT and LAM, with no interference from degradation products, excipients, or other matrix components, confirming the specificity of the method.

The developed HPLC method is accurate, precise, robust, and stability-indicating, making it suitable for the routine analysis of DLT and LAM in pharmaceutical formulations as well as for forced degradation studies.

Table 1: Linearity of DLT and LAM

Parameter	DLT	LAM
Concentration Range (µg/mL)	1–5	10–50
Correlation Coefficient (r ²)	0.9991	0.9996
Slope (m)	786.56	607.65
Intercept (c)	53.416	20.662

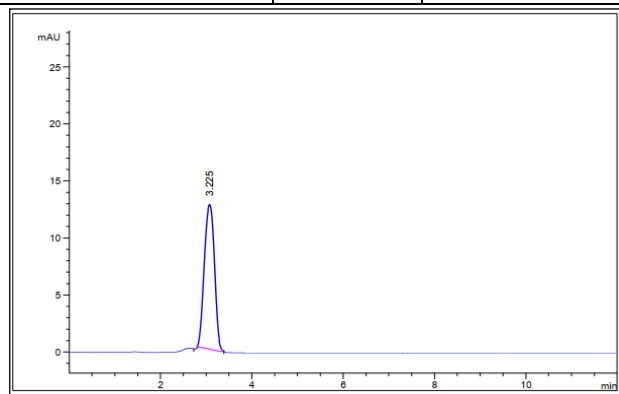


Figure 1: Chromatogram of DLT

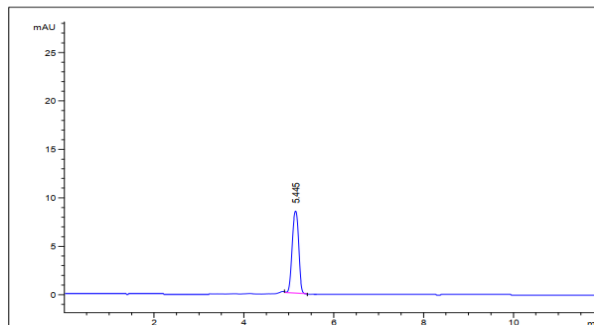


Figure 2: Chromatogram of LAM

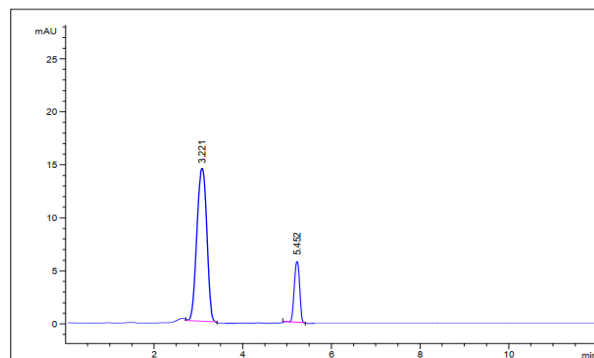


Figure 3: Chromatogram of DLT and LAM

Table 2: System Suitability Parameters of DLT and LAM

Drug	Statistic	RT	AUC	Theoretical Plates	Tailing Factor
DLT	Mean	3.22	3969.76	2872.833	1.142
	SD	0.00	6.624	11.974	0.015
	%RSD	0.06	0.167	0.417	1.313
LAM	Mean	5.44	4583.39	2673.167	1.182
	SD	0.00	11.895	17.452	0.020
	%RSD	0.05	0.260	0.653	1.692

Table 3: Results of Recovery Study of DLT and LAM

Drug	Level	Mean % Recovery	SD	%RSD
DLT	80%	98.20	0.892	0.909
DLT	100%	97.97	0.447	0.456
DLT	120%	98.54	0.552	0.561

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LAM	80%	98.52	0.186	0.188
LAM	100%	98.32	0.707	0.719
LAM	120%	99.06	0.667	0.673

Table 4: Results of Precision Study of DLT and LAM

Drug	Parameter	Mean	SD	%RSD
DLT	Intermediate Precision (Day-to-Day)	0.96–4.75	0.025	0.026
DLT	Analyst-to-Analyst	0.97–4.95	0.025	0.025
DLT	Reproducibility	0.96–4.918	0.019–0.054	0.012
LAM	Repeatability	9.866–48.764	0.025	0.025–0.026
LAM	Intermediate Precision (Day-to-Day)	9.88–48.85	0.025	0.025
LAM	Analyst-to-Analyst	9.96–48.88	0.025	0.025
LAM	Reproducibility	9.86–49.424	0.025	0.025

Table 5: LOD and LOQ of DLT and LAM

Name	LOD (µg/ml)	LOQ (µg/ml)
DLT	0.10	0.30
LAM	0.45	1.25

Table 6: Mean analysis of tablets formulation of DLT and LAM

Name of Drugs	Label Claim (mg)	Amount Found (mg)	% Found	S.D.	%RSD
DLT	50	49.85	99.700	0.255	0.269
LAM	300	298.85	99.617	0.365	0.374

Table 7: Results of Forced degradation studies of DLT

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.85	0
Acidic hydrolysis	87.78	12.07
Alkaline	93.32	6.53

hydrolysis		
Oxidative degradation	94.45	5.4
Photolytic degradation	91.32	8.53

Table 8: Results of Forced degradation studies of LAM

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.75	0
Acidic hydrolysis	92.25	7.50
Alkaline hydrolysis	88.12	11.63
Oxidative degradation	93.25	6.50
Photolytic degradation	86.45	13.30

Conclusion

A simple, accurate, precise, and stability-indicating HPLC method was successfully developed and validated for the simultaneous estimation of Dolutegravir (DLT) and Lamivudine (LAM) in pharmaceutical formulations. The method demonstrated excellent linearity, accuracy, precision, and robustness, and was able to effectively separate the drugs from their degradation products under various stress conditions. This validated method is suitable for routine quality control, stability studies, and assay of DLT and LAM in commercial formulations.

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