CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.24403805

Available online at: http://www.iajps.com Review Article

A QUALITY BY DESIGN-DRIVEN SHIFTING PARADIGM IN QUANTIFICATION AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR LAMIVUDINE IN BULK DRUG AND TABLET DOSAGE FORM

Jagdish V. Manwar[1]*, Ganesh R. Pawar[1], Bhushan R. Gudalwar[1], Abhijit R. Raut[1], Priyanka P. Patil[2], Priyanka S. Patil[3]

[1] Takshashila Institute of Pharmaceutical Education and Research, Kherda 444107, MS, India
[2] KLE College of Pharmacy, Belagavi 591124, Karnataka, India
[3] Genezen Institute of Pharmacy, Delol 389310, Gujarat, India
*Email: jvmanwar@gmail.com

Abstract:
A stability-indicating RP-HPLC method was developed and validated for the quantification of lamivudine in the bulk drug as well as tablet dosage forms using an Analytical Quality by Design (AQbD) approach. The Analytical Target Profile was mainly being defined, and critical analytical attributes were identified through the risk assessment and Failure Mode as well as Effects Analysis. It was found that the parameters of the critical method that were optimized after a 3^2 full factors design were the percentage of methanol in ammonium acetate buffer (pH 3.5) and the flow rate. Chromatography was used to extract the C18 column using methanol: ammonium acetate buffer (10:90, v/v; pH 3.5) at a rate of 0.9 mL/min and UV detection at 271 nm. Drug eluted at retention time of 3.9 min with the acceptable system suitability (theoretical plates 10533 ± 1600; tailing factor 0.98 ± 0.06). The method exhibited a high range of linearity (0-5 µg/mL, r^2 = 0.999), a high accuracy (99.21-100.84%), strong accuracy (% RSD = 2), and robustness. LOD and were 0.07 µg/mL and 0.18 µg/mL, respectively. The proposed AQbD-based on the RP-HPLC method is simple, robust, as well as suitable for the routine quality control as well as the stability assessment of lamivudine pharmaceutical formulations.

Keywords: *Lamivudine, RP-HPLC, Quality by Design, 3^2 full factorial design, Stability-indicating method, Method validation.*

Corresponding author:
Jagdish V. Manwar,
Takshashila Institute of Pharmaceutical Education and Research,
Kherda 444107, MS, India
**Email: jvmanwar@gmail.com*

QR CODE

Please cite this article in press ***Jagdish V. Manwar*** *et al* ***A Quality By Design-Driven Shifting Paradigm In Quantification And Validation Of A Stability-Indicating Rp-Hplc Method For Lamivudine In Bulk Drug And Tablet Dosage Form.,***. *Indo Am. J. P. Sci, 2026; 13(11).*

1. INTRODUCTION:

Lamivudine (LMD) **(Figure 1)** is chemically designated as *4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one* and belongs to the class of the nucleoside reverse transcriptase of inhibitors [1]. It is used popularly in the treatment of HIV infection and chronic infection of the hepatitis B virus [2]. LMD is more often administered along with other antiretroviral therapy drugs also referred to as the Highly Active Antiretroviral Therapy to complement the effect of the therapeutic action and reduce the viral resistance [3]. LMD remains an important component of the initial antiretroviral regimens owing to its established safety, efficacy and excellent pharmacokinetic activity.

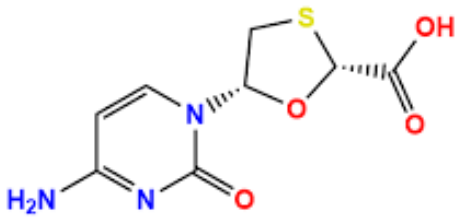

Figure 1: Chemical structure of Lamivudine.

Literature has reported the quantitative determination of LMD in bulk pharmaceutical and drug dosage form to have been determined using various methods of analysis including UV - spectrophotometry [4-6], RP-HPLC [7-8], HPTLC [9-10], and LC–MS techniques [11]. However, the approaches whose reports are most common were calculated by traditional trial-and-error techniques and lack the systematic optimization, strength, and stability-indicating feature as a whole. Moreover, such traditional procedures fail to indicate the interactive and combined value of the essential variables of experiment with respect to the way such methods work. The practices are time-consuming, expensive and a large number of experimental trials must be conducted which may instigate sometimes variable analytical performance in the direction of analysing quality control in normal condition.

An Analytical Quality by Design (AQbD) approach is one of the ways by which these limitations can be solved. Nonetheless in the past years, AQbD has also taken a central role in the pharmaceutical analysis due to its ability to bring strength, consistency as well as control of a technique. AQbD presents a risk and scientific based framework that can enable one to understand analytical processes in depth, minimize the possibility of method failure, increase the control strategies, and ensure that the outcomes of the method repeat themselves throughout the method. The other advantages of AQbD include: the transferability of the methods used in the methodology is enhanced, the post approval changes are diminished, and the increment of regulatory behaviour and continuous improvement of methods [12]. AQbD has an approximate starting point where the Analytical Target Profile (ATP) is defined, and the risk measurement, knowledge of the methodology, the design space design creation, the implementation of the proper control strategy and continuous improvement. In that regard, this allows obtaining the ATP on the basis of the deep understanding of the relationship between critical analytical attributes (CAAs) and critical method parameters (CMPs). CMPs and CAAs are identified and selected using quality risk assessment tools such as the risk screening tools or the techniques of the experimental design [13].

The Q8 (R2) of the ICH principles is dedicated to the application of the principles of QbD to give quality into the pharmaceutical product and method of analysis based on the good scientific knowledge and control of the risk. Besides, the formulation of ICH Q14 guideline emphasizes the AQbD importance in establishing an analytical method that facilitates its further broadening acceptance by regulatory bodies in all parts of the world [14]. Although a few studies have described the analytical processes, little has been done on the AQbD development of LMD to identify it using the stability indicating RP-HPLC procedure. The experimental design methods on understanding the influence of chromatographic variables on performance of methods have not been well researched. Accordingly, there is a high degree of need to establish a selective, robust and stability-reporting RP-HPLC procedure on AQbD paradigm. The AQbD methodological systematic approach was used in the present work to design and develop a stability indicating RP-HPLC method. The 3^2 full factorial design was used to investigate how the variables of the selected chromatograph values adding a percentage of organic modifier, and flow rate affected the most important analysis characteristics retention time, theoretical plates and tailing factor [15]. As a way of enhancing the performance of the analytical method, optimization of the desired design space was used. To a greater extent, the developed method was not only tested as per ICH Q2(R1), it was also compelled to experience forced degradation experiments as per ICH Q1A (R2) in order to assure it of its capability to express stability [16-17]. The present study aims to develop and validate a stability-indicating RP-HPLC method for LMD using a QbD-based 32 full factorial design, thereby establishing a robust and regulatory-compliant analytical method.

2. Materials and Methods

2.1 Materials

Lamivudine pure drug was kindly supplied by the Cipla Ltd., Mumbai. All the reagents and methanol and ammonium acetate were of analysis and high-performance liquid chromatography grade. Pure water was used to conduct this study.

2.2 Instrumentation

The RP-HPLC analysis for the LMD was being performed using an Agilent 1220 Infinity LC system equipped with a proper gradient pump, online degasser, as well as a variable wavelength UV detector. The chromatographic results were obtained and made decisions over through the OpenLAB CDS ChemStation software. Statistica graphs and analysis were done using STATISTICA software (version 8.0.360.0, English, StatSoft, Inc., Tulsa, OK, USA) and Minitab Statistical Software. A reversed-phase C18 column of the analysis was acted (4.6 mm x 250 mm) in the reversed phase. There was sample introduction done with a Rheodyne loop injection injector with a 20.0 mL injection loop attached. The ultrasonication of samples and mobile phase degassing consisted of the Metrex ultrasonic bath sonicator. Basis pH scale: The pH was measured and pH adjusted using a digital pH meter.
All the solutions were filtered with the help of a 0.45 mm Millipore membrane filter (Merck Ltd., Germany) to ensure that no particles remained in the mobile phase and sample solutions before the chromatographic analysis. The LMD and other reagents were determined on high precision weighing balance. In the development of the method, wavelength was selected using UV-Visible spectrophotometer (Shimadzu) which was utilized.

2.3. Analytical Target Profile and Critical Analytical Attributes

Defining the Analytical Target Profile (ATP) is the main and the first step in the Quality by Design (QbD) approach. The ATP presents the objective of the analysis process and gives the relevant and reliable estimation of LMD in bulk drug and tablet dosage form. CMAs and CAs were discovered to access the ATP. CMPs can be different and impact the performance of methods in different ways hence the effects of CMPs in the CAAs should be evaluated and regulated. The discovery of the key CAAs which influence the quality of methods was made based on the background information and experimental learning. The CAAs in the present study were retention time, theoretical plates, and tailing factor because they directly influence the chromatographic efficiency, the form of the peaks, and reproducibility **(Table 1)**.

Table 1: Critical Method Parameters (CMPs) and Critical Analytical Attributes (CAAs).

Category	Code	Parameter	Description / Unit
CMPs	X_1	Organic modifier concentration	% methanol in the mobile phase (% v/v)
	X_2	Flow rate	Flow rate of the mobile phase (mL/min)
CAAs	Y_1	Retention time	Time required for elution of lamivudine peak (min)
	Y_2	Theoretical plates	Measure of column efficiency (number of plates)
	Y_3	Tailing factor	Measure of peak symmetry

**These responses were selected as they directly affect peak resolution, efficiency, and symmetry.*

2.4. Quality by Design-Based Method Development

An AQbD approach was mainly being employed to systematically develop as well as optimize the RP-HPLC method for the LMD. The critical methods parameters were estimated, using the risk evaluation and with the assistance of a factorial design they were adjusted to achieve the preferable analysis performance. This plan rendered it dependable, repeatable and reproducibility of the method using the established design space.

2.4.1. Selection of Detection Wavelength
The selection of the optimal detection wavelength is a critical undertaking when developing an effective technique of RP-HPLC since it is the one that directly interferes with the sensitivity, specificity and accuracy of the process of analysis. In the provided study, the absorption characteristics of the ultraviolet (UV) were used to determine the wavelength at which LMD would be registered. A typical solution of LMD was properly made in the proper mobile phase and UV spectrophotometer scanned in UV in the range of 200-400 nm. The UV absorption spectrum of LMD exhibited a prominent absorption maximum (λmax) at 271 nm, which corresponds to the characteristic chromophoric groups present in the molecule. with the value being related to the common chromophoric groups in the molecule. In the case of HPLC, the wavelength was 271 nm since this wavelength resulted in maximum absorbance and maximum baseline stability, and additionally one with minimum interference of the mobile phase components. At this wavelength LMD

responded very finely making LMD sensitive and offered reproducibility to the peak area reporting. In addition, the selected wavelength also represented an adequate amount of selectivity in forced degradation analysis as the LMD peak was distinguished clearly, and it was feasible to differentiate it among the potential degradation products.

2.4.2. Experimental Design

The central aspect of the AQbD strategy was to determine and sieve the CMPs that can have a significant impact on the CAAs of the LMD RP-HPLC technique. The selected CAAs were the retention time, theoretical plates, and tailing factor which are a direct measure of chromatographic performance. A design of experiments (DOE) design was employed in the achievement of this objective by the systematic variation of the chromatographic parameters. The percentage of organic modifier (methanol) and flow rate were found to be the most affected CMPs that influenced the method performances according to a preliminary experiment and risk assessment research. These parameters were varied on a given parameter of the experiment to produce optimal peak, sensible retention time and increase the efficiency of the column. The main impact of the selected CMPs on the CAAs was examined by the usage of 3^2 full factorial design with the minimum of experimental runs. The experimental trials were carried out in accordance with the design matrix (nine trials were planned to be conducted). The acquired data regarding the experimental findings in each of the runs have been statistically worked on in accordance with the STATISTICA software. Association of CMPs and CAAs, was studied to be aware of how they affected the Analytical Target Profile (ATP) and to ascertain the optimum in chromatography work. DoE methodology aided in the systematic comprehension of methods, reduced the effort invested in experiments, ensured that a powerful and reproducible method was studied.

2.4.3. Method Development Process

Agilent 1220 Infinity LC system was used to perform the chromatographic analysis with the use of gradient, pump and degasser. The separations were carried out in C18 column with a scale of (4.6 mm x 250 mm) and in isocratic conditions. A UV detector was used to perform the process of detection at 271 nm and chromatographic software was used to gather the data. The mobile stage consisted of optimized ratio (methanol and ammonium acetate buffer pH 3.5). Rheodyne injector was maintained at the flow rate of 0.9 mL/min and 20 mL was injected. The mobile stage was initially ultrasonicated then filtered using a 0.45 mm membrane filter. Chromatographic runs were done at ambient temperature and each run had 10 minutes. The ideal chromatographic conditions provided a focused yet symmetrical LMD peak who had a reasonable retention time and unreliable analytical outcomes, which enabled application of that method in daily quality control and stability research.

2.5. Selection of Critical Method Parameters and Analytical Attributes

Based on preliminary experiments and risk assessment, two chromatographic variables were identified as critical method parameters (CMPs), as presented in **Table 2**.

Table 2: Failure Mode and Effects Analysis (FMEA).

Step / Factor	Potential Failure Mode	Possible Effect on CAAs	S	O	D	RPN	Risk Level
Mobile phase composition (% methanol)	Incorrect organic modifier ratio	Change in retention time, peak tailing	4	4	3	48	High
Buffer pH	Improper pH adjustment	Poor peak symmetry, reduced efficiency	4	3	3	36	Medium
Flow rate	Variation in flow rate	Shift in retention time, loss of resolution	4	4	2	32	Medium
Column condition	Column aging or damage	Reduced theoretical plates	3	3	3	27	Medium
Detector wavelength	Incorrect wavelength selection	Reduced sensitivity, inaccurate peak area	3	2	2	12	Low
Injection volume	Inconsistent injection	Peak area variability	3	2	2	12	Low
Sample preparation	Improper dilution or filtration	Peak distortion, assay error	4	2	2	16	Low
Instrument performance	Pump or detector instability	Poor reproducibility	3	2	3	18	Low

** Severity (S): Impact of failure mode on critical analytical attributes (1 = low, 5 = high); Occurrence (O): Likelihood of failure occurrence during routine analysis (1 = rare, 5 = frequent); Detection (D): Ability to detect failure before affecting results (1 = easily detectable, 5 = difficult to detect)., Risk Priority Number (RPN): Calculated as RPN = S × O × D.*

2.5.1. Optimization and Statistical Analysis

Optimization of the method for LMD was carried out using a systematic Analytical Quality by Design (AQbD) approach. The maximization of the approaches and statistical processing were performed using the STATISTICA software. The chromatographic responses of the nine experimental runs generated were studied with the assistance of the 32 full factorial design were examined using the multiple linear regression analysis (MLRA) to determine the effect of the critical method parameters on the critical analytical attributes. The determination of the factors or statistical significance of the model was done through study Analysis of variance (ANOVA). The differences were measured statistically significant at $P < 0.05$, confirming a meaningful effect of method parameters on chromatographic performance. The statistical analysis made it possible to decide the magnitude and the nature of the effect that the independent variables have on the responses.

A polynomial equation describing the relationship between critical method parameters and analytical responses was generated in coded form as:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{12} x_1 x_2 + \beta_{22} x_2^2$$

Where Y = chromatographic response; β_0 is the intercept β_1 and β_2 are the coefficients of the main effects, β_{11}, β_{12}, β_{22} are the inter-action coefficients; and X_1 and X_2 represent the coded values of the critical method parameters.

Graphical optimization was performed to describe the contribution of each chromatographic variable to the performance of the developed method by establishing appropriate minimum and maximum values of the responses but numerical optimization was performed to recapitulate their contributions. The best chromatographic conditions were achieved using the desirability exercise technique that had the required retention time, theoretical plates, and tailing factor.

2.5.2. Risk Assessment

It is possible to adopt a risk-based approach as the guidelines of ICH Q8 and ICH Q9 prescribe to come up with strong and effective analytical techniques. In the presented study, risk assessment (RA) was performed to obtain the values of the method that could possibly have significant effects on the analytical performance and determination of the Analytical Target Profile (ATP) of LMD. The RA study was carried out to minimize the experimental error and as well identify the high-risk factors on the critical analytical attributes (CAAs) of RP-HPLC method of testing LMD. An effect in the centre was drawn on a fishbone (Ishikawa) diagram that included variations in the Analytical Results of LMD and the potential cause(s) of the variation were categorized into six larger groups of variations namely, Method, Materials, Instrument, Personnel, Environment and Quality Control. The parameters considered under the Method category were a percentage methanol in ammonium acetate buffer (pH 3.5), flow rate (0.5-0.9 mL/min), detection wavelength (271 nm) and injection volume (20 uL), isocratic mode of elution and column temperature (25°C) whereas Materials was LMD standard, HPLC-grade solvents, buffer, water and C18 column quality. Agilent 1220 Infinity LC system parts were viewed as Instrument related factors and Personnel, Environment and Quality Control encompassed the study of analyses, lab conditions and system calibration, adequacy of technique and validity and forced degradation. Potential sources of variation affecting the analytical performance of lamivudine were identified using a fishbone diagram **(Figure 2)**.

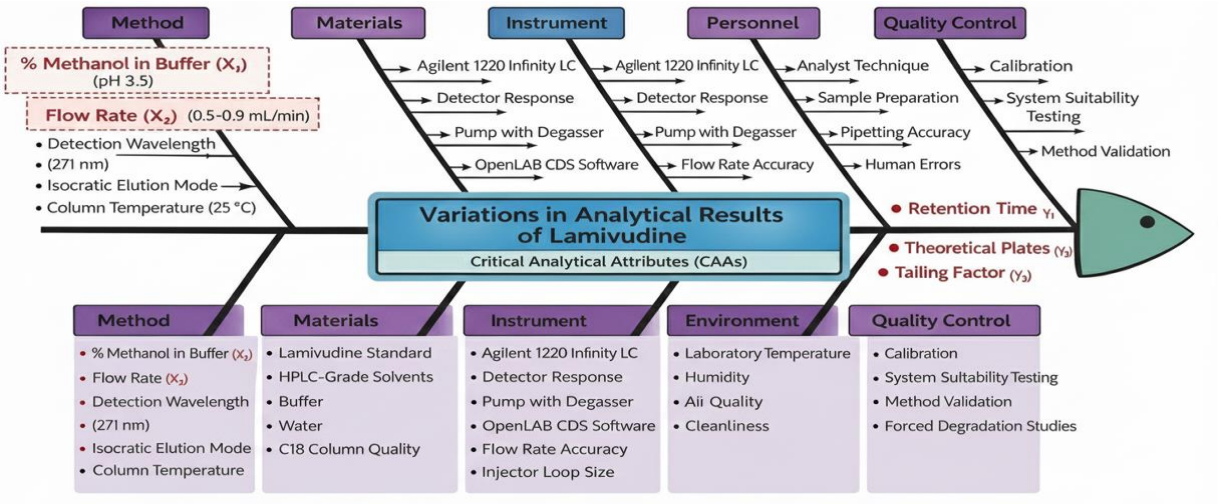

Figure 2: Fishbone diagram illustrating potential factors affecting the analytical performance of the lamivudine RP-HPLC method.

2.5.3. Data Analysis and Method Optimization

The data analysis on the achieved experiment results was statistically done using the statistical program in an effort to determine the size as well as the significance of the effects of the CMPs on CAAs. In order to determine the interaction of the variables in chromatography and the methods performance we have performed analysis of main effects plot and response trends. The results of the method were optimised using desirability criteria to get a suitable retention
time, maximum theoretical plates and minimum tailing factor. The reproduction and strength of the methods of experiment was verified by optimizing the chromatographic conditions that were experimentally validated using the obtained statistical model. The understanding of systematic methods enabled by the application of the STATISTICA software, reduced the practical work, and offered the opportunity to create a robust AQbD-based RP-HPLC methodology of LMD that enables the daily quality control and stability studies. The fishbone risk assessment was performed to identify and prioritize the most likely risks that may disrupt the performance of the analytical ability of the LMD RP-HPLC technique through the use of the failure Mode and Effects Analysis (FMEA) [18]. It was scored on relative severity, occurrence and detection and Risk Priority Number (RPN) was created. The mobile phase composition and rate of flow upsurged RPN values therefore indicated as the critical method parameter (CMPs) to go extra mile and optimize experimental design **(Table 2)**.

2.6. Design of Experiments

Few experimental studies were used to find out the impact of the selected chromatographic variables on the performance of the method using 32 full factorial design. This design is helpful in establishing those factors that are significant in the outcomes of analysis. The experiential design matrix and random sequence of experiment and the statistical analysis were built based on the application of STATISTICA software. Experiments were investigated on all the method parameters in conditions, low (-1) and high (+1) within a selected range. There were nine experimentations run according to the design matrix. In the specified chromatographic conditions per run LMD samples were tested and compared the observed chromatographic reaction in the chart of retention time, theoretical plates, and tailing factor.

2.6.1. Preparation of Solution (Stock and Working) and Linearity Study

Accurate weighing of the 100 mg of LMD was done and the solvent was transferred in 100 mL volumetric flask, sonicated at 20 min and the rest of the solvent up to the mark taken of the same solvent to obtain a primary stock solution of 1000 ug/mL. About 10 mL of this solution was further diluted to 100 mL with distilled water to make a working stock solution of 100 ug/mL. The working stock solution was subsequently pipetted into a set of 10 mL volumetric flasks, and diluted with distilled water to obtain a set of calibration standards of measurement, which included 0-5 ug/mL. Each solution was injected in triplicate (n 3) into chromatography under optimum conditions. The linearity was checked using least squares linear regression method in which the peak area was against the concentration of LMD and the method linearity was good with correlation coefficient (r2) of 0.999.

2.7. Assay of Marketed Tablet

It was assayed with Epivir tablet that were commercially produced containing 150mg LMD. The counted number of twenty hand weighed and crushed the tablets to a fine powder. An amount of the powder which was 100 mg of LMD in weight was weighed correctly and transferred into a 100ml LMD volumetric flask. It was then mixed with 30 mL of distilled water and sonication of the mixture was carried out (30 min) till the drug dissolved completely. Distilled water was subsequently put into the volume until it reached the mark to achieve concentration of 1000 ug/mL. Another 20 mL was diluted to 100 mL based upon this solution of 20 mL using distilled water to achieve a 200 ug/mL solution. After this, 10 mL of this solution was again diluted to 100 mL with the same solvent to obtain the final concentration of 20 ug/mL and then 10 mL was further diluted to 100 mL in order to get the final concentration which is 2 ug/mL. The solution was then filtered using the Whatman filter paper (0.45 um) and 20 µL of the solution was injected into the optimized conditions into the HPLC system six times in total in order to ascertain the amount of drug.

2.8. Validation of Method

After establishing and improving RP-HPLC procedure to determine LMD concentration, the procedure was evaluated to meet parameters of accuracy, precision, robustness, limit of identification and limit of quantification parameters as demanded by ICH Q2 (R1) guideline.

2.8.1. System Suitability

System suitability test is one of the components of the system method development and this test was undertaken to offer proper solution and repeatability of the system. Six times of the injected LMD were injected under the optimum chromatographic conditions in which there was 2 ug/mL solution of LMD. All the parameters such as the retention time, tailing factor and theoretical plates, were documented and ascertained that the system was of pertinent analysis.

Accuracy

Overcoming the studies required recovery studies which were used to identify the accuracy of the method using the standard addition method. The sample solution was loaded with LMD in 80, 100 and 120 percent of the concentration of the sample in analysis (2 ug/mL). The levels (n = 6) were analysed in six replicates each. The percentage recovery was calculated and mean recovery of LMD was determined to be within the acceptable range.

Precision

Precision of the method was tested as concerned intra-day and inter-day precision. Intra-day accuracy was done using 3 concentrations of LMD i.e., 1.6, 2.0 and 2.4 ug/mL on the same day, and rest on three days respectively, inter-day accuracy. The findings were in mean +- SD and percentage relative standard-deviation. The outcomes of the values of RSD were found to be less than 2, and it indicates that the method is specific.

Robustness

Assessment of the integrity of the formulated RP-HPLC method of LMD quantification was done by making deliberate adjustments of the key chromatographic parameters around the optimal conditions, in accordance with ICH Q2(R1) guideline using a pre-analysed tablet formulation. The other parameters that were studied were variation of flow rate of 11:89, 10:90, 9:91 with mobile phases of both methanol and ammonium acetate buffer (pH 3.5) and different ratios of 270 nm, 271 nm and 272 nm of detection wavelength held constant in other chromatographic parameters. The column strength to column variability was also experimented on three types of C18 columns, viz. Thermo C18, ZORBAX C18 and Luna C18. The strength of the RP-HPLC method that has been developed was to be determined by conducting LMD -assay procedure in triplicate, with the perception of percentage content of LMD in the tablet formulation under deliberate variations in the chromatographic circumstances.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) of LMD was determined following the signalto noise (S/N) ratio practice, and based on the statistical parameters of calibration curve as well. Using the standard deviation of response (Sa) and slope (b) of the calibration curve it is done. LOD and LOQ are computed on the basis of the following equations:

$$\text{LOD} = \frac{3 \times S_a}{b}$$

$$\text{LOQ} = \frac{10 \times S_a}{b}$$

Where Sa is standard deviation of response and b is slope of the calibration curve. This way, it was established that LOD and LOQ values of LMD were 0.07 to 0.18 ug/mL respectively.

2.8.2. Forced degradation studies

The forced degradation experimental researches were done to identify the stability indicating and the specific property of the developed RP-HPLC technique of LMD complying with the ICH Q1A (R2) guidelines. LMD also underwent diverse inputs of stress i.e. acidic, alkaline and oxidative degradation and the samples of the subject to the stress were evaluated to achieve the maximum purity and separation of the degradation products relative to the parent drug.

Acid Degradation

Under acid degradation conditions 2 mL of LMD stock solution was mixed with 2 mL of 0.01 N hydrochloric acid and incubated by holding in 60 minutes in at room temperature. The solution was then diluted, accordingly, with distilled water with time as the diluting agent. The 20 µL of HPLC system was injected and the chromatogram was observed.

Alkaline Degradation

When it was degraded alkali-wise 2 mL LMD stock solution was chatted and 2 mL of 0.01 N sodium hydroxide were added and left to incubate at room temperature in 60 minutes. Stressed solution was diluted with the help of distilled water, till the solution contained the desired final concentration of 2 ug/mL, 20 µL of a stressed solution was injected into the HPLC system to be chromatographed.

Oxidative Degradation

In the instance of oxidative degradation, LMD stock solution (2 mL) was mixed with 1-percent hydrogen peroxide (2 mL) and was allowed to stand at room temperature over a period of 60 min. The sample was diluted up to a concentration of 2 ug/mL after which the 2020 µL of solution was injected to HPLC system to obtain the chromatogram.

3. Results and Discussion

Quality by Designed (QbD) paradigm is a scientific system methodology that embraces the application of numerous statistical, risk analysis, planning and

optimization applications in the design space formulation, control strategy specification, and achievement of constant improvement. QbD has had wide application in pharmaceutical industry to provide increased strength of method, quality disjuncture as well as minimize method failure. Therefore, in the present study, the use of the Analytical Quality by Design (AQbD) methodology led to the design and development of a stability-indicating RP-HPLC technique to determine LMD in bulk drug and tablet dosage form.

3.1. Analytical Target Profile (ATP)

The Quality by Design (QbD) system introduced Analytical Target Profile (ATP) as the initial and the most significant of them, which was utilized during the entire analysis design process to achieve the intended set of performance requirements. A decision was made in the current work to develop a potent, precise and stability-incriminating RP-HPLC procedure in determination of LMD in quantitative form in bulk drug and tablet kind of dosage. ATP was a methodological selection of critical attributes (CAAs) such as retention time, peak symmetry and theoretical plates, which is significant in a quality quantification of LMD. The various considerations of the analysis method included the composition of mobile phase (methanol and ammonium acetate buffer pH 3.5), the needs of the instruments (Agilent 1220 Infinity LC system with C-18 column) and the wavelength at which the detection was to occur (271 nm), the quantity of ample sample injected (20 µL), and the sample preparation procedures, solubility of LMD in water, or the pH of the analyte itself, respectively. The ATP along with justification for the selected parameters was summarized to guide the method development strategy **(Table 3)**.

Table 3: Analytical Target Profile (ATP) Assigned for Pure Lamivudine and Marketed Tablet.

ATP Target	Assigned Attributes	Justification
Target sample	Lamivudine (API) and tablet dosage form (Epivir 150 mg)	The method is intended for quantification of lamivudine in bulk drug and finished pharmaceutical product.
Method type	Reverse Phase HPLC	Selected based on the polarity and chemical nature of lamivudine to achieve efficient separation.
Instrument required	HPLC (Agilent 1220 Infinity LC System)	Chosen due to the presence of UV chromophore in lamivudine and suitability for accurate quantification.
Characteristics of sample	Solid API and tablet powder	Defines the need for sonication, filtration and dilution during sample preparation.
Standard and sample preparation	Dilution in distilled water	Justified based on good solubility of lamivudine in water (70 mg/mL) and stability of the drug.
Diluents	Distilled water	Selected considering solubility, stability and compatibility with the mobile phase.
Method application	Assay of API and tablet formulation	The method is applicable for routine quality control analysis of bulk drug and marketed tablet dosage form.

3.2 Risk Assessment (RA) Study

The high-risk variables that sufficiently influenced the CAAs that is, retention time (Y_1), theoretical plates (Y_2) and the tailing factor(Y_3), were considered to be variable to be optimized in 3^2 full factorial analysis with the percent methanol in buffer (X_1) and the flow rate(X_2) as the high-risk variables. Other chromatographic parameters were studied, such as wavelength of detection, inject volume, column temperature and run time, which played an insignificant role and kept constant. This risk prioritization contribution enabled a focused as well as reproducible stability-indicating RP-HPLC of LMD method of reproducibility became achievable.

3.3. Method Development

The objective of this paper was to evaluate and develop a simple, rapid and sensitive RP-HPLC method that would determine the estimation of LMD in bulky dosage form and tablet dosage form. The traditional one factor one factor at a time approach of optimization of methods is laborious, time-consuming and of low cost-efficiency. Therefore, in the study of interest, a paradigm of Analytical Quality by Design (AQbD) was taken into account to mathematically evolve in order to optimize the chromatography technique. The mobile phase was considered the most powerful that influences the chromatographic separation. Different combinations between water, acetone and methanol were experimented on the preliminary trials. The individual ammonium acetate buffer (pH 3.5) did not cause acceptable symmetry of peaks and retention between LMD. Thus, one of the organic modifiers was selected as methanol that improves the chromatographic characteristics. Different

solutions containing methanol and ammonium acetate buffer at different PH have been experimented. It was observed that the phase that underwent the mobile phase was comprised of methanol and ammonium acetate buffer (pH 3.5) and achieved a good chromatography resolution, symmetrical form of the peaks and good resolution time **(Table 4)**.

Table 4: Results of ANOVA for Responses of Experimental Variables.

Response	Term	Reg. Coeff.	Std. Error	t-value	p-value
Y_1	Mean / Intercept	18.8914	1.652600	11.4313	0.001437
	% Methanol (Linear)	0.4275	0.118261	3.6149	0.036378
	% Methanol (Quadratic)	0.0205	0.004991	4.1008	0.026238
	Flow Rate (Linear)	-34.5667	4.469854	-7.7333	0.004496
	Flow Rate (Quadratic)	20.4167	3.119347	6.5452	0.007250
	Interaction ($X_1L \times X_2L$)	-0.6950	0.088228	-7.8773	0.004263
Y_2	Mean / Intercept	-2479.9	16599.59	-0.1494	0.890721
	% Methanol (Linear)	682.1	1187.88	0.5742	0.606074
	% Methanol (Quadratic)	-26.5	50.13	-0.5278	0.634180
	Flow Rate (Linear)	27148.3	44897.59	0.6047	0.588068
	Flow Rate (Quadratic)	-20362.5	31332.38	-0.6499	0.562092
	Interaction ($X_1L \times X_2L$)	127.5	886.21	0.1439	0.894723
Y_3	Mean / Intercept	0.2900	0.144902	6.9742	0.019946
	% Methanol (Linear)	0.0200	0.234524	-0.2778	0.807227
	% Methanol (Quadratic)	1.8400	0.054832	-0.9691	0.434746
	Flow Rate (Linear)	-0.0009	0.358870	-0.0281	0.980172
	Flow Rate (Quadratic)	-0.0100	0.068528	-1.6512	0.240502
	Interaction ($X_1L \times X_2L$)	-1.1700	0.574121	-0.0336	0.976235

Y_1 - Retention time (min); Y_2 - Theoretical plates; Y_3 - Tailing factor; X_1 - % Methanol in ammonium acetate buffer (pH 3.5); X_2 - Flow rate; L - Linear; Q - Quadratic.

With the help of the results of the trial and error, the composition of the final mobile phase was further optimized with the use of factorial design retaining the independent variables as a percent variation of methanol content in the buffer and the flow rate. The optimization achievement of this AQbD resulted in an excellent chromatographic process with improved peak symmetry, high hypothetical plates and low retention time, a good indicator that the developed procedure was appropriate in the routine of LMD analysis. The effects of methanol concentration and flow rate on retention time, theoretical plate count, and tailing factor were evaluated using response-surface plots **(Figure 3)**.

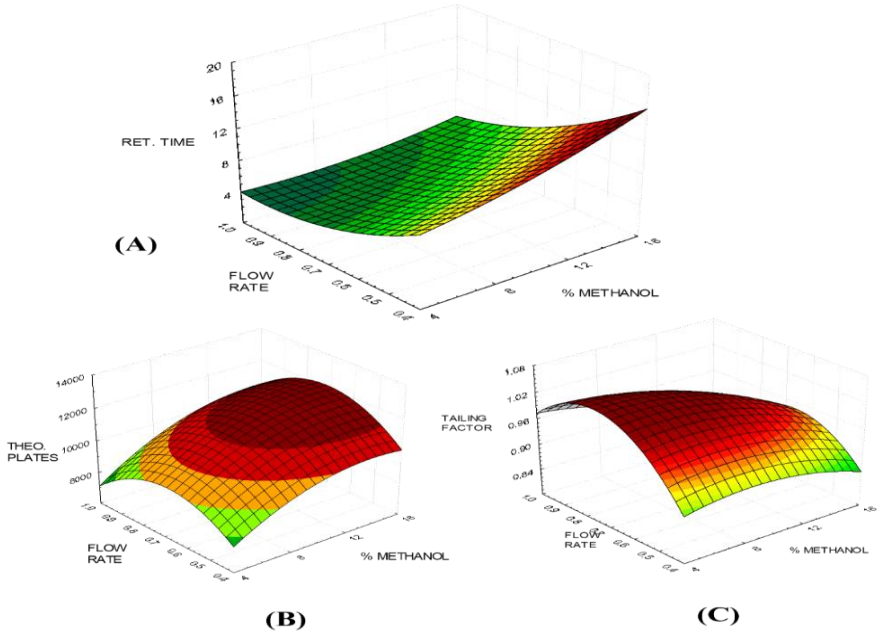

Figure 3: 3D response surface plots showing the effect of % methanol in ammonium acetate buffer and flow rate on (A) retention time, (B) theoretical plates, and (C) tailing factor.

3.4. Data Analysis

Results indicate the outcome of the experiment with the different factorial design runs of LMD. The generated response was then subject to statistical analysis using ANOVA in order to obtain the most appropriate variants of the polynomials. The ANOVA results confirmed that the developed models were statistically significant with $P < 0.05$, indicating the effective models that explained the relationship between the chromatographic variables as well as the analysed responses. The 3D RSM plots illustrates the response surface plots of the interaction of the investigated variables on the chromatographic responses.

Effect of CMPs on CAAs

The Table 1 provides a summary of the various experimental trials that have been carried out through the assistance of a 3^2 full factorial design to determine the main and interaction and the quadratic effects of the selected CMPs on the CAAs. The CMPs in the study were percent methanol in ammonium acetate buffer (X_1) and flow rate (X_2) whereas the CAAs were retention time (RT, Y_1), theoretically obtained plates (TP, Y_2) and tailing factor (TF, Y_3). The factorial design allowed an assessment of the personal and interactive effect of CMPs on the CAAs systematically, and thus it was capable of identifying the most suitable chromatographic parameters in order to consider the use of LMD.

Effect of Chromatographic Variables on Retention Time (RT)

With a 3^2 full factorial design the effect of the chromatographic variables of the retention time (RT) of LMD was determined. The two independent variables were methanol in ammonium acetate buffer (pH 3.5) (X_1) and flow rate (X_2). The correlation of the two variables to the retention time and the retention time of LMD showed a high relevance in the range of 3.73 to 12.65 min.

The poly regression gave the following equation of retention time:

$$Y_1 = 18.8914 + 0.43X_1 - 34.57X_2 + 0.02X_1^2 - 0.70X_1X_2 + 20.47X_2^2$$

Where, X_1 and X_2 are the independent variables i.e. % methanol in buffer (ammonium acetate buffer pH 3.5) (v/v), and flow rate (ml/min), respectively.

It was found that the model was significant ($P < 0.05$). The relationship coefficient of X_1 is positive meaning that the larger the content of methanol is the longer is the retention time and vice versa, the relationship coefficient of X_2 is negative. The negative value of the coefficient of regression of the flow rate is high which proves the fact that flow rate is the strongest factor that is influencing RT. The response surface plots demonstrated that large retention times are associated with low flow rates and extended retention periods using methanol.

Effect on Theoretical Plates (TP)

Column efficiency is also measured to a large extent by use of theoretical plates. The experimental Theoretical Plates were 7729-12479. The second-order linear equation that existed in the existence of chromatographic variables on TP was as follows:

$$Y_2 = -2479.87 + 682.05X_1 + 27148.33X_2 - 26.46X_1^2 + 127.5X_1X_2 - 20362X_2^2$$

Where, X_1 and X_2 are the independent variables i.e. % methanol in buffer (ammonium acetate buffer pH 3.5) (v/v), and flow rate (ml/min), respectively.

The regression equation revealed that two of them (% methanol X_1 and flow rate X_2) had a positive influence on TP, but it has been observed to live a far stronger influence by the flow rate. Theoretical plates with a higher flow rate and optimization of methanol composition multiplication would mean an improvement in the chromatographic efficiency under those conditions.

Effect on Tailing Factor (TF)

The symmetry of the peaks was determined using the tailing factor (TF) which ranged between 0.90 and 1.06 and this value is acceptable in all the experimental conditions. The TF regression equation was a poly equation:

$$Y_3 = 0.29 + 0.02X_1 + 1.84X_2 - 0.0009X_1^2 - 0.017X_1X_2 - 1.17X_2^2$$

Where, X_1 and X_2 are the independent variables i.e. % methanol in buffer (ammonium acetate buffer pH 3.5) (v/v), and flow rate (ml/min), respectively.

The value of the positive X_1 and X_2 coefficients indicates that an increase in the percent of methanol and the flow rate had a slight positive effect on the tailing factor. The quadratic flow rate coefficient (X_2^2) was however negative a fact that shows that even woeful flow rates can be achieved at negative effects on the peak symmetry. Overall, the obtained TF values were decent, which demonstrates that the technique provides symmetric LMD peaks.

3.5. Optimization of Chromatographic Conditions

Under isocratic elution conditions, the optimized mobile phase consisted of methanol and ammonium acetate buffer (pH 3.5) in a ratio of 10:90 (v/v). Chromatographic separation was performed using a C18 column (4.6 × 250 mm) maintained at 25°C, with an injection volume of 20 µL. Based on statistical analysis and the desirability approach, these conditions were selected to achieve a minimum retention time while maintaining satisfactory chromatographic efficiency and peak symmetry. The optimized flow rate was 0.9 mL/min.

3.5.1. Residual Study for Prediction of Responses

The residual analysis was carried out to determine the suitability of the designed models with respect to predicting the retention time, theoretical plates and tailing factor of LMD. The normal probabilities graphs showed that there was random distribution of the residuals in straight line that corresponded to good model fitting. The graphs of the residuals against the predicted response have depicted irreversible graphs that were inconclusive and thus proving the fact that the proposed models were sound and able to provide the prediction of the chromatographic responses within the design space.

3.5.2. Optimization of Method Using Prediction Profile and Desirability Approach

A profile of prediction (PP) was used in development of AQbD-based approach to predict the response surface and to define the optimum combination of critical method parameters (CMPs) to LMD. The numbers were optimized by placing objectives of the critical analytical attributes (CAAs) of the minimum retention time (Y_1), maximum theoretical plates (Y_2) and acceptable tailing factor (Y_3).

The X_1 i.e. the percentage of methanol in ammonium acetate buffer and the X_2 i.e. flow rate were the CMPs selected in the given study. The reply to the CMPs and CMPs concentrations were plotted on X and Y axis respectively. The crossing of the horizontal line of the profiler presented the most desirable combination of CMPs in the estimation of LMD analysis. The products obtained by numerical optimization as the feature of desirability were almost equal to unity, thereby creating the stability of the optimization process. They were determined to be working best under 10 percent methanol in ammonium acetate buffer (pH 3.5) at a flow rate of 0.9 mL/min with LMD eluting at 3.9 min and column efficiency and maximum symmetry satisfactory. Experimental values were found to be quite near to the calculated values and that confirmed the prediction profile and desirability method as the best approach to optimum strategy of RP-HPLC methodology.

3.5.3. Application of AQbD

The recent study clearly demonstrates that Analytical Quality by Design (AQbD) paradigm has been effectively used in the development, optimization and validation of a stability-indicating RP-HPLC procedure of the determination of LMD in bulk drug and tablet dosage form. The first stage, the Analytical Target Profile (ATP), was introduced with an aim at defining the aim and performance requirements of the methodology. Subsequently, the critical retention time, theoretical plates and tailing factor which are the critical attributes (CAAs) of the process was calculated through systematic risk assessment (RA) methodology which assisted in ranking and identifying critical variables of the method.

To screen and optimize the critical method parameters (PMs), percent methanol in ammonium acetate buffer (X_1) and flow rate (X_2) a 3^2 full factorial was embraced. ANOVA statistically was used to determine the influence of the CMPs on the CAAs and second-order poly equations were generated to make the model of the CMPs and chromatographic responses correlation. These results found that the mobile phase composition and flow rate made a significant impact on the retention time of the LMD theoretical plates and tailing factor. In particular, it was discovered that the most important parameter is flow rate that influences retention behaviour. The optimization with AQbD minimized the error with the experimental results and assisted in the observation of a solid design space. Optimal chromatography conditions were as follows: 10 percent of methanol in the ammonium acetate buffer (pH 3.5) and a flow-rate of 0.9 mL/min which provided sharp and symmetrical LMD peak after which the column had a high column efficiency with a retention time of 3.9 min.

The validation of method was done in order to justify that the developed method is selective, specific, precise, and accurate and robust within the ICH guidelines. These forced degradation experiments showed that LMD is susceptible to acidic and oxidative challenge and the devised method is valuable in isolating the degradation items as well as the parent substance in a functional manner, and this demonstrated its capacity to identify the aspect of stability. Further, recovery studies affected the formulation of the marketed tablet proved the usefulness of the process through good precision and consistency. Overall, RP-HPLC process that is created within the current research will enable applying AQbD approach, and the process will be specifically configured to conduct the research on quality control and stability of LMD as a routine task.

3.5.4. Statistical Analysis of ANOVA

The acquired results of ANOVA as developed in the usage of the STATISTICA (Version 8.0.360.0, StatSoft Inc., Tulsa, OK, USA) software package confirm that the developed specimen of the factorial design is statistically viable. The retention time (Y1) model had the F-value of 2.8569 F-value = 0.0001 which means the significant effect on the elution behaviour of LMD is at very high-level owing to the high essence of the concentration of the methanol and the flow rate. The analysed variables also significantly influenced the theoretical plates (Y_2) and demonstrated the fact that the efficiency of a column is omnivorous to the changes in the chromatography conditions ($P = 0.048$). Nonetheless, the tailing factor (Y_3) used relative to the result derived by pharmacology had no

significant value (P > 0.05) since there was no change in symmetry of the levels in the design space. Overall, the results indicate the appropriateness of the developed AQbD-based RP-HPLC process to perform good and reliable analysis **(Table 6)**.

3.6. Assay of marketed formulation

In-marketed tablet formulation (label claim: 150 mg), the quantitative analysis of LMD was performed through the suggested RP-HPLC. The assay results indicated that LMD in the percentage makeup of the tablet formulation had a value of 99.78 +- 0.62 of the labelled product with a percentage ratio standard deviation of 0.16 which are accurate and precise parameters of the procedure developed. The low values of the percentage standard deviation show that the process is also reproducible and there are no excipients contained in the formulation to have caused an interference to the phenomenon. Results indicated that the method was RP-HPLC which was ready to be used in the routine quality management study of LMD in solid dosages.

3.7. Validation of Method

The developed RP-HPLC procedure of AQbD performed well in the functioning of the process and ICH validation was realized. It was established that adequate chromatographic efficiency was obtained as seen by system appropriateness criteria through retention time (3.90 +- 0.33min), high theoretical plate count (10533 +- 1600) and a tailing factor of nearly unity (0.98 +- 0.06) which indicates sharp symmetrical shape of peaks. The experiment revealed a good linearity in the sample concentration of 0-5 ug/mL with the regression equation Y =312.7x +0 with the high correlation coefficient (r2=0.999) that guaranteed proportional detector response. The calibration curve was constructed by plotting the peak area against lamivudine concentration over the investigated range of 0–5 µg/mL **(Figure 4)**.

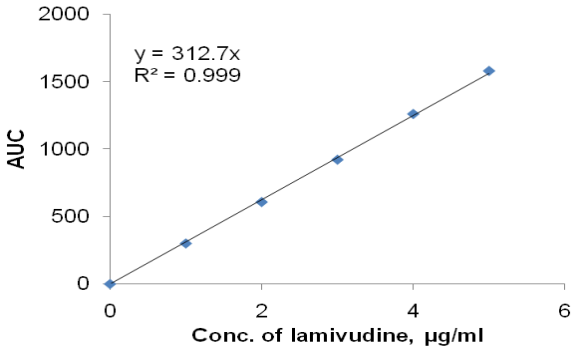

Figure 4: Calibration curve of lamivudine obtained using the developed RP-HPLC method.

The method was sensitive at low LOD (0.07 ug/mL) and LOQ (0.18 ug/mL), and this means that the method can be adopted to detect traces at trace level. Accuracy confirmed the truth of the method with the following recoveries on 80, 100 percent and 120 percent levels: 99.21, 100.84. More results concerning strengthening assessments by performing purposeful alterations in flow-rate, mobile phase proportion, detection wavelength and column type led to the values of the assays within the range of 99.15-100.92 % signifying the stability of the procedure with slight remodels of operations. Precision studies showed that the RSD percentage values of the day and inter-day analysis of the samples were less than 2 and were consistent at all the different concentrations tested therefore showing the method to be repeatable and with an intermediate level of precision. Generally, the validated RP-HPLC method is precise, specific, sensitive, linear and powerful to directly carry out estimations of LMD tablets doses **(Table 5)**.

Table 5: Study of variables on dependent by full factorial design.

Batch	Coded variables		Natural condition		RT (Y_1)		TP (Y_2)		TF (Y_3)	
	X_1	X_2	X_1	X_2	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
L-1	-1	-1	5	0.5	7.63	7.62	9613	9071	0.96	0.97
L-2	-1	0	5	0.7	5.02	4.92	10253	9741	1.06	1.04
L-3	-1	+1	5	0.9	3.73	3.84	7729	8783	1.00	1.02
L-4	0	-1	10	0.5	9.42	9.56	9032	10816	0.97	0.97
L-5	0	0	10	0.7	6.20	6.16	11990	11613	1.00	1.02
L-6	0	+1	10	0.9	4.48	4.39	12189	10782	1.00	0.98
L-7	+1	-1	15	0.5	12.65	12.52	12479	11237	0.93	0.93
L-8	+1	0	15	0.7	8.27	8.42	11274	12162	0.96	0.96
L-9	+1	+1	15	0.9	5.97	5.95	11105	11459	0.90	0.90
				Minimum	3.73	3.84	7729	8783	0.9	0.9
				Maximum	12.65	12.52	12479	12162	1.06	1.04
				Mean	7.25	7.25	10533	10600	0.98	0.97

Where: FR = Flow rate of mobile phase (mL/min); X_1 = percentage of methanol in ammonium acetate buffer (pH 3.5); X_2 = Flow rate, mL/min; RT = retention time (min); TP = theoretical plates; TF = tailing factor; Exp. = experimental value; Pred. = predicted value.

The stability-reporting nature of the formed procedure of LMD was assessed through the forced degradation investigations of the study under the influence of acidic, alkaline and oxidative stress. Stability Inhibitors The property of stability of the developed RP-HPLC method was proved by forced degradation studies concerning the drug. LMD was the most vulnerable one to degradation in the presence of acidic conditions (acid, 37.94, 60 min) and had a number of other degradation curves at 1.80, 2.07 and 4.63 which once again indicated that LMD was highly sensitive to the influence of the acid stress. On the other hand, alkaline stress (0.01 N NaOH, 60 min) gave a low degradation rate percentage of 6.64 with two small degradant peaks at 1.85 and 4.72 min to indicate medium stability under alkaline conditions. It was also established that all the stress conditions clearly divided the parent drug peak indicating that the developed method is effective in separating LMD and degradation products thus is stability indicating in nature **(Table 6).** Representative chromatograms obtained under the tested oxidative, acidic, and alkaline stress conditions are presented in **Figure 5.**

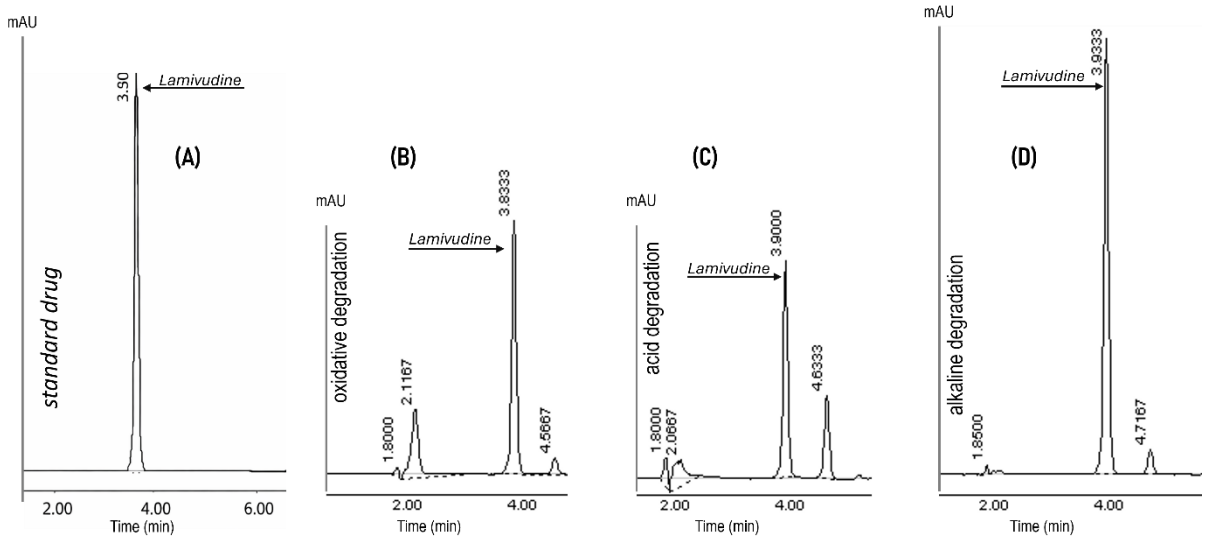

Figure 5. Chromatograms of standard drug (A) and forced degradation studies showing degradation under (B) oxidative (1% H_2O_2) (C) acidic (0.01 N HCl), and (D) alkaline (0.01 N NaOH) stress conditions.

Table 6: Validation, Robustness and Precision Parameters.

Category	Parameter	Condition / Level	Result
Performance	Label claim	Lamivudine tablet	150 mg
System Suitability	Retention time (min)	Optimized	3.90 ± 0.33
	Theoretical plates	Optimized	10533 ± 1600
	Tailing factor	Optimized	0.98 ± 0.06
Linearity	Range (µg/mL)	-	0–5
	Regression equation	-	Y = 312.7x
	Slope	-	312.7
	r^2	-	0.999 ± 0.05
Sensitivity	LOD (µg/mL)	-	0.07
	LOQ (µg/mL)	-	0.18
Accuracy	Recovery (%) #	80% level	99.21
		100% level	100.84
		120% level	99.36
Robustness	Flow rate (mL/min)	0.8	99.47
		0.9 (Optimized)	100.02
		1.0	99.15
	Mobile phase ratio (A: B)	11:89	99.22
		10:90 (Optimized)	100.11
		9:91	99.68
	Detection wavelength (nm)	270	99.35
		271 (Optimized)	100.06
		272	99.74
	Column type	Thermo C18	100.92

		ZORBAX C18	99.18
		Luna C18	99.66
Precision (n = 6)	Conc. (µg/mL)	Inter-day (% Assay)	%RSD
	1.6	100.61	1.51
	2.0	100.15	1.61
	2.4	100.98	1.68
	Mean	100.58	1.60
	Conc. (µg/mL)	Intra-day (% Assay)	%RSD
	1.6	100.05	1.40
	2.0	100.83	1.05
	2.4	100.47	1.82
	Mean	100.45	1.42

Values are expressed as mean ± SD (n = 6) for system suitability and precision, and as mean of three determinations (n = 3) for linearity, sensitivity, accuracy and robustness; # % contents were measured in pre-analysed tablet formulation; A - Methanol, B - Ammonium acetate buffer (pH 3.5); Robustness was assessed by deliberate variation of critical chromatographic parameters, confirming the reliability of the developed RP-HPLC method.

4. CONCLUSION:

A robust, sensitive and stability-indicating RP-HPLC method to measure LMD in the large dose bulky saturation and tablet dosage form was successfully developed in an Analytical Quality by Design (AQbD) methodology. Risk assessment was used to determine the critical parameters of the procedure and through systematic optimization a 3^2 full factorial design was used to optimize the % of methanol in ammonium acetate buffer (pH 3.5) and rate at which the mobile phase passes through. The best specifications of the chromatography which provided the sharp and symmetrical LMD peak with a retention time of 3.9 min were methanol ammonium acetate buffer (10 90 v/v), and flow rate as 0.9 mL min-1 using 271 nm UV. The developed method was found to be highly linear (ICH Q2(R1) guidelines and r^2 = 0.999 in 0-5 µg/mL), very accurate and precise, quite robust, and with low LOD (0.07 µg/mL) and LOQ (0.18 µg/mL). Experiments on forced degradation established that LMD was susceptible to acidic, oxidative and alkaline environment and the process was effective in isolating degradation products with its parent drug that made LMD a measure of stability. Overall, the AQbD-RP-HPLC method discussed in the current paper is reliable, repeatable and can be applied in the quality-control and stability tests of LMD pharmaceutical formulations in a routine fashion.

List of Abbreviations

ANOVA Analysis of Variance
API Active Pharmaceutical Ingredient
AQbD Analytical Quality by Design
ATP Analytical Target Profile
CAAs Critical Analytical Attributes
CMPs Critical Method Parameters
DoE Design of Experiments
FMEA Failure Mode and Effects Analysis
ICH International Conference on Harmonization
LMD Lamivudine
MLRA Multiple Linear Regression Analysis
RA Risk Assessment
RPN Risk Priority Number
λmax Maximum Absorption Wavelength

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Conflict of Interest: None

REFERENCES:

1. Taylor K. Lamivudine. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.
2. Wang Q, Li Z, Smith J, et al. Efficacy and safety of dolutegravir plus lamivudine (DTG + 3TC) in patients with late presentation of HIV infection: a real-world cohort study. *Front Med (Lausanne).* 2025;12:Article ID pending.
3. Dahiya M, Tai T, Hussaini T, Ritchie G, Matic N, Yoshida EM, et al. Virological response to lamivudine and tenofovir treatment in a mono-infected chronic hepatitis B patient with potential tenofovir resistance: a case report. *J Clin Transl Hepatol.* 2025;13(1):84–87.
4. Nagulwar VP, et al. A validated UV spectrophotometric method for the simultaneous estimation of lamivudine, nevirapine and zidovudine in combined tablet dosage form. *J Pharm Res.* 2009;2(4):666–669.
5. Shankar DG, Reddy MVVN, Kumar JMR, Rajendra, Murthy TK. UV spectrophotometric determination of some anti-HIV drugs. *Asian J Chem.* 2002;14:433–436.
6. Garikapati DR. Development and validation of spectrophotometric method for the

determination of anti-HIV drug. *Asian J Chem.* 2004;16:1217–1219.

7. Somkuwar K, Sabale P, Sawale V, Rahangdale P. Comparative study of UV, RP-HPLC and HPTLC methods for quantification of lamivudine in tablet formulation. *Future J Pharm Sci.* 2024;10:81. doi:10.1186/s43094-024-00651-z
8. Shinde GS, Jadhav RS, Godge RK, Kote RB, Tambe VB. RP-HPLC method development and validation of lamivudine, zidovudine and nevirapine in bulk and dosage form using UV detector. *Res J Pharm Technol.* 2024;17(10):5011–5015.
9. Sangeetha RK, Suganya S, Manju AS, Poomalai S. Study on the related substances of lamivudine by a validated HPTLC method in oral dosage forms. *Asian J Pharm Res.* 2024;14(3):213.
10. Wankhede SB, Gupta KR, Tajne MR, Wadodkar SG. A validated HPTLC method for simultaneous estimation of lamivudine and zidovudine in tablets. *Asian J Chem.* 2010;18:2669–2672.
11. Zhao Y, Liu J, Chen R, Zhang Q, Wang W. LC–MS/MS determination of lamivudine in human plasma and its application to pharmacokinetic studies. *J Pharm Biomed Anal.* 2023;215:114789. doi:10.1016/j.jpba.2022.114789
12. Gurumukhi VC, Bari SB. Quantification and validation of a stability-indicating RP-HPLC method for efavirenz in bulk and tablet dosage form using quality by design (QbD): a shifting paradigm. *J Chromatogr Sci.* 2017;55(3):253–263.
13. Manwar JV, Vispute SS, Kumbhar DD, Manmode RS, Bakal RL, et al. Response surface-based optimization of system variables for liquid chromatographic analysis of candesartan cilexetil. *J Taibah Univ Sci.* 2017;11:159–172. doi:10.1016/j.jtusci.2016.04.003
14. Sathuluri K, Bakam RS, Jain R, Dande A, Gajbhiye R, Ravichandiran V, Peraman R. Analytical quality by design (AQbD) in the ICH Q14 guidelines for analytical procedure development. *Accredit Qual Assur.* 2024;30:1–14. doi:10.1007/s00769-024-01587-w
15. Manwar JV, Patil SS, Bhalerao CA, Mandpe SR, Kumbhar DD. Experimental design approach for chromatographic determination of ketorolac tromethamine from bulk drug and tablet formulation. *Glob J Pharm Sci.* 2017;3(2):555609. doi:10.19080/GJPPS.2017.03.555609
16. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Validation of analytical procedures: text and methodology Q2(R1). Geneva: ICH; 1995.
17. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Stability testing of new drug substances and products Q1A(R2). Geneva: ICH; 2003.
18. Prajapati P, Patel HB, Shah S. DoE-based failure mode effect analysis (FMEA) for development of a stability-indicating HPTLC method for estimation of apremilast. *SN Appl Sci.* 2020;2:1371. doi:10.1007/s42452-020-2890-1
19. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH harmonised guideline: Validation of Analytical Procedures Q2(R2). Geneva: ICH; 2023.