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Development and ICH Compliant Validation of a Spectrometric Method for the Determination of Lamivudine in Pharmaceutical Formulations

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Abstract: *The present study aimed at enhancing a straightforward UV-visible spectrophotometric technique to determine lamivudine in pharmaceutical preparations based on the guidance of the International Conference on harmonisation (ICH). Lamivudine is an antiretroviral drug, which is a nucleoside analogue used in a large number in treating HIV infection and therefore needs quality control analysis methods that are reliable. This research paper measured the analytical wavelength (λmax) of lamivudine with a UV-Visible spectrophotometer at 280 nm. It was performed by the BeerLambert law within the range of concentration of 218ug/mL, having a regression equation of A = 0.048C + 0.068 and correlation coefficient at 0.999 which indicated that it was quite linear. The accuracy, precision, detection limit, quantification limit and robustness was the parameter used to prove the developed method as per the ICH guidelines. The detection and quantification limit were also found within the range of 0.0033 ug/mL and 0.10043 ug/mL respectively. Recovery studies proved to be accurate between 99.70% to 100.34%,, whereas assay of a commercial tablet formulation (Lamivir-HBV) gave a recovery of 99.98% which showed that excipients did not interfere. The findings validated that the developed spectrophotometric technique is easy, correct, exact, cost-effective, and fit to the habitual quality control investigation of lamivudine in drug preparation.*

Keywords: *Lamivudine; UV–Visible spectrophotometry; Method validation; Pharmaceutical analysis; ICH guidelines.*

I. Introduction

Pharmaceutical analysis is important in quality, safety, and efficacy of the drug products. Quantitative analysis of active pharmaceutical ingredients in bulk drugs, as well as in finished pharmaceutical preparations, requires reliable analysis procedures. Spectrophotometric methods are one of the most common methods of analysis due to their simplicity, speed of analysis, low cost, and acceptable accuracy among other available methods of analysis. The validation and analytical techniques can be developed and verified by following internationally recognised guidelines (including those of the International Conference on Harmonisation (ICH)). This is to ensure that the method is appropriate to the purpose it is intended and yields reproducible and reliable results in normal quality control of pharmaceuticals.

1.1 Overview of Lamivudine

Lamivudine is an antiretroviral drug which is mainly used in the treatment of Human Immunodeficiency Virus (HIV) that is a nucleoside analogue drug. It is a nucleoside reverse transcriptase inhibitor (NRTIs), which is an essential companion of antiretroviral therapy. Chemically, lamivudine is known as 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one, with a molecular formula $C_8\ H_{1\ 1}\ N_3\ O_3\ S$ and molecular weight 229.3 g/mol.

Lamivudine is a crystalline solid, white to off-white in color and it is well soluble in water. Its absorption through the gastrointestinal tract is efficient with an estimated bioavailability of about 86%, and it is mostly absorbed orally. Lamivudine has a plasma half-life of 5-7 hours; pharmacokinetically, this drug can be used to maintain good levels of the therapeutic drug in the body. These pharmacophysiological and pharmacokinetic activities render lamivudine as a significant part of combination antiretroviral therapy.

1.2 Mechanism of Action and Therapeutic Importance

Lamivudine can be administered and phosphorylated intracellularly to lamivudine triphosphate, the active metabolite. This is an active metabolite that works by inhibition of this enzyme HIV reverse transcriptase, which is involved in the conversion of viral RNA into DNA during the viral replication process. Lamivudine triphosphate competes with natural nucleosides and inserts into the viral DNA chain leading to the premature end of DNA elongation, thus preventing viral replication.

As a result of this process, lamivudine is one of the most common ingredients of HAART, in the treatment of HIV infection. The correct assessment of the lamivudine concentration in the pharmaceutical preparations is thus required to ascertain the quality of drugs, the effectiveness of therapy, and the regulations.

1.3 Need for Analytical Method Development

Several methods of analysis have been reported to determine lamivudine in drug dosage form such as HPLC, HPTLC, and UV spectrophotometry. The chromatographic methods are usually highly sensitive and specific but need costly instrumentation, multifaceted sample preparation methods, and laborious analysis.

Conversely, UV spectrophotometrics methods have a number of advantages including simplicity, low cost, speed of analysis and low volumes of solvents. Such attributes render spectrophotometric methods especially appropriate in quality control analysis regularly in pharmaceutical laboratories.

1.4. Research Objectives

The present study paper will design and establish a UV spectrophotometric procedure to determine lamivudine in pharmaceutical preparations in line with International Conference on Harmonisation (ICH) regulations. The aim of the study is as follows:

1. To come up with a bare and straightforward UV spectrophotometric technique of the quantification of lamivudine in pharmaceutical preparations.
2. To determine the analysis parameters of the developed method such as the wavelength to be used, calibration curve preparation, and the linearity range.
3. To test the developed analytical method against ICH guidelines by assessing the parameters, accuracy, precision, detecting limit, and quantifying limit, and robust to routine pharmaceutical analysis.

II. Review Of Literature

A variety of analytical methods had been established to the determination of lamivudine either as a separate or conjugated agent with other antiretroviral agents in pharmaceutical preparations and in biological samples. The main approach of these studies was to use chromatographic and spectrophotometric methods in order to obtain precise quantification and credible quality control.

Koppisetty et al. (2023) developed a reverse phase high-performance liquid chromatography (RP-HPLC) system with a Quality by Design (QbD) strategy in quantification of lamivudine and efavirenz in combined pharmaceutical preparations. The focus of their study was on systematic method development with the focus on the experimental design and optimization of the chromatographic conditions to have the reliable separation and accurate quantification of the drugs. The created method showed acceptable levels of analytical performance and emphasized the practicality of the QbD methodology in enhancing the high levels of robustness and reliability in the chromatographic methods.

Kumar et al. (2025) described an HPLC-based stability-indicating Analytical Quality by Design (AQbD) analysis of the three drugs lamivudine, tenofovir disoproxil fumarate, and efavirenz in plasma. The technique was created to assess the steadiness and quantitative estimation of these antiretroviral medications under different circumstances. Their work showed that the principles of AQbD could be applicable to the optimization of the method and provide guaranteed detection and quantification of the drugs in the complex biological samples**.**

Mohammed et al. (2023) developed a UV spectrophotometric assay of the dialysis of abacavir and lamivudine in pharmaceutical dosage forms. The research was aimed at developing an easy and affordable analytical method of regular pharmaceutical examination. The proposed spectrophotometric technique demonstrated good accuracy and precision and was deemed appropriate in the determination of these drugs in mixed formulations.

Raj et al. (2023) designed and tested a stability-indicating analytical procedure based on UV spectrophotometric techniques to determine ritonavir in bulk and pharmaceutical dosage forms. It was aimed at creating a simple and dependable analysis process that would help to trace the drug as well in the context of degradation products. The devised technique proved to have good validation parameters including linearity, precision, accuracy, and robustness which showed that UV spectroscopic methods would be effective to use in regular analysis of pharmaceutical quality control.

Somkuwar et al. (2024) carried out the comparative analysis of UV spectroscopy, reverse phase high-performance liquid chromatography (RP-HPLC), and high-performance thin layer chromatography (HPTLC) in quantifying the antiviral drug lamivudine in tablet formulations. They evaluated the performance of these three methods in their study in terms of sensitivity, accuracy and reliability. The study found that despite the increased sensitivity and selectivity of chromatographic methods, the UV spectrophotometric methods offered a more convenient, fast and cheaper method of pharmaceutical analysis of lamivudine routinely.

III. Materials And Methods

The present study project entailed the construction and verification of a UV-Visible spectrophotometric assay of lamivudine in pharmaceutical preparations. In the analysis process, standard and sample solutions were prepared, analytical wavelength had to be determined, the calibration curve had to be prepared and the developed method had to be validated, in accordance with the ICH, recommendations. All the experiments were conducted under reliable and controlled laboratory conditions using properly calibrated instruments and analytical grade reagents to guarantee reliability and accuracy of the results.

3.1 Instruments

The spectrophotometric study was conducted by a double beam UV- Visible spectrophotometer (UV-3092) using UV Win software to measure absorbance. The determination of pH in the course of solution preparation was done using a digital pH meter. The weigh of the sample powder of the drug and tablets was done using an analytical balance.

3.2 Chemicals and Reagents

Chemicals and reagents in the study were of analytical grade. The analysis was done using the following reagents:

- Cerium (IV) ammonium sulphate (0.1%)
- Perchloric acid (4 M)
- Methanol
- Distilled water

An oxidizing agent was cerium (IV) ammonium sulphate solution and perchloric acid served as the necessary acidic medium in the reaction. Drug dissolution and extraction was done using methanol and dilution of solutions was carried out using distilled water.

3.3 Preparation of Standard Solution

Lamivudine was accurately measured as 10mg in a 100 mL volumetric flask. In order to prepare the standard stock solution a quantity of the drug was dissolved in methanol and the volume was brought to the mark with a distilled water. Based on this stock solution, pertinent dilution of the stock solution was done to develop working standard solutions within the range of 2-18 ug/mL

3.4 Sample Preparation

To prepare the sample solution, twenty lamivudine tablets were weighed correctly and crushed to fine powder using mortar of pestle. One-tenth of the powdered tablet with lamivudine equal to 10 mg was moved into a volumetric flask. Methanol was added to the drug and thoroughly mixed with it before the solution was filtered to give a solution to take off the insoluble excipients. Distilled water was added to the filtrate to have a final solution of 10 µg/mL, which was analyzed.

3.5 Determination of Analytical Wavelength

The lamivudine solution was prepared, and the solution was scanned with the help of UVvis spectrophotometer with the purpose to identify the longest wavelength of maximum absorption. Lamivudine had its maximum absorbance (λmax) at 280 nm, and that was used as the wavelength of the quantitative analysis.

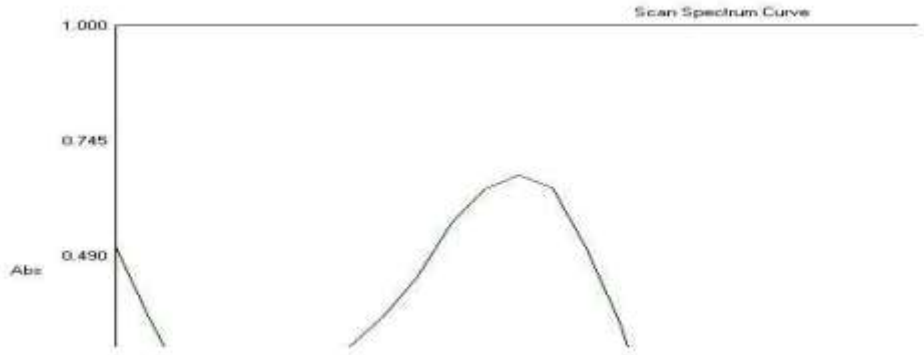

Figure 1: UV–Visible absorption spectrum of lamivudine showing maximum absorbance (λmax) at 280 nm

The UV absorption spectrum revealed that the lamivudine had a clear maximum absorbance peak with a value of 280 nm. This wavelength was subsequently chosen as the wavelength of analysis to be used to further determine lamivudine quantitatively because it gives optimum sensitivity and accuracy in the spectrophotometric analysis.

3.6 Construction of Calibration Curve

Volumetric aliquots of the standard lamivudine solution with the insoluble concentration in the range of 2-18 ug/mL were transferred into a series of volumetric flasks. Each flask was added 1 mL of solution of perchloric acid of 4 M and cerium (IV) ammonium sulphate. The final volume was put in using distilled water and the solutions were left to react in 5 minutes.

Absorbance of individual solutions at 280 nm using the UV -Visible spectrophotometer. This was followed by a calibration curve that was plotted as absorbance vs. lamivudine concentration.

3.7 Method Validation

The spectrophotometric procedure that has resulted has been proven to be valid according to the International Conference on harmonisation (ICH) guidelines. The parameters of validation reflected included the linearity, accuracy, precision, detection limit, quantifying and the robustness in order to establish the reliability and the appropriateness of the method to complete the routine pharmaceutical analysis.

IV. Result And Discussion

The generated UV -Visible spectrophotometric procedure to determine lamivudine in pharmaceutical formulations was assessed with respect to a number of parameters of analysis in line with the ICH guidelines. The results of the determination of wavelength, the construction of a calibration curve and the validation studies including linearity, accuracy, precision, detection limit, quantification limit, robustness, and assay of tablet formulation were critically reviewed. These parameters were evaluated to identify the reliability, sensitivity, and applicability of the devised analytical method in monitoring the analytical quality of the pharmaceuticals under regular quality supervision of lamivudine.

4.1 Optical Characteristics of Lamivudine

In acidic medium lamivudine had the maximum absorbance at 280 nm. The devised spectrophotometric procedure followed the law of BeerLambert at the range of 2-18 μg/mL, implying that the relationship between concentration and absorbance was linear.

The tables below (Table 1) show the optical properties and regression coefficients of lamivudine after the UV spectrophotometric analysis. The table will give a summary of the analysis wavelength, the range of law concentration Beer, and regression parameters obtained by using the calibration curve. Moreover, the key validation parameters to be used, which include the LOD, LOQ, correlation coefficient, slope, and intercept values are also provided.

Table 1: Optical Characteristics and Regression Parameters

Parameter	Value
Color	Colorless
λmax (nm)	280
Beer's law range (μg/mL)	2–18
Limit of detection (μg/mL)	0.0033
Limit of quantitation (μg/mL)	0.10043
Correlation coefficient	0.999
Slope	0.048
Intercept	0.068

Table 1 has indicated that lamivudine had optimum absorbance at 280 nm that was chosen as an analytical wavelength during its quantitative analysis. The procedure adhered to the law of Beer in the concentration range of 2-18 ug/mL which means that there is a high degree of linearity between the concentration and the absorbance. The value of the correlation coefficient was high (0.999) to assure high linearity of the calibration curve. The sensitivity of the developed spectrophotometric method was observed in the LOD (0.0033 μg/mL) and LOQ (0.10043 μg/mL). Moreover, the slope and intercept values showed that there was a good proportionality of the concentration and absorbance.

4.2 Calibration Curve of Lamivudine

Figure 2 shows the calibration curve that has been developed on lamivudine in the developed UV spectrophotometric method. The plot of absorbance versus corresponding concentrations of lamivudine in the range of 218 2 ug/mL at an analytical wavelength of 280 nm was used to construct the curve. The regression

equation obtained off the calibration data is A = 0.048C + 0.068 where A is the absorbance and C is the concentration of lamivudine.

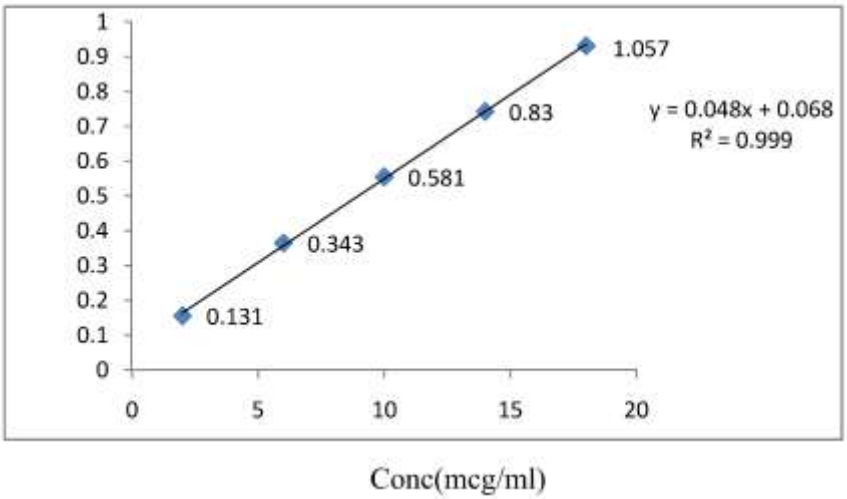

Figure 2: Calibration Curve of Lamivudine

The calibration curve showed the existence of a linear relationship between the absorbance and the concentration of lamivudine at the range studied. Regression equation A = 0.048C + 0.068 found that the proportionality between concentration and absorbance was good, which proved suitability of the Beer law. The linear nature of the calibration curve indicates the accuracy of the elaborated UV spectrophotometric technique of quantitative determination of lamivudine in pharmaceutical preparations.

4.3 Linearity

Table 2 indicates the absorbance values of the lamivudine through various concentrations applied to determine the linearity of the developed spectrophotometric method. The concentrations were 2-18 ug/mL and the values of the absorbance were obtained at 280nm.

Table 2: Linearity Data

Concentration (µg/mL)	Absorbance
2	0.131
6	0.343
10	0.581
14	0.830
18	1.057

Table 2 shows that the absorbance value rose in direct proportion to the concentration of lamivudine. This confirms the developed process obeys the law of BeerLambert in the range of 2-18 ug/mL of the concentration. The linearity and reliability of the suggested spectrophotometric procedure are also supported by the stable growth in the absorbance values.

4.4 Accuracy

Table 3 shows the outcomes of the recovery experiment that was conducted to test the validity of the designed spectrophotometric method. The recovery studies run with three different concentration levels namely low concentration, intermediate concentration and high concentration.

Table 3: Accuracy Data

Level	Concentration (µg/mL)	%Recovery
LC	2	99.70
IC	10	100.34
HC	18	100.09

The recovery values given in Table 3 were between 99.70% and 100.34% and this shows that the developed method has high accuracy. The findings can affirm that the technique can be used to reliably measure lamivudine with a minimal error in analysis**.**

4.5 Precision

Table 4 shows the results of precision study that was conducted to verify repeatability of the developed spectrophotometric method. Three days were used to determine the accuracy of the method by measuring the absorbance of different concentrations.

Table 4: Precision Data

Concentration (µg/mL)	Day 1	Day 2	Day 3
4	0.242	0.245	0.247
8	0.477	0.471	0.479
16	1.002	1.005	1.010

Table 4 shows that the obtained absorbance values on the various days were consistent which shows that there was good precision of the method of analysis. The fact that the standard deviation of the values of absorbance is low proves the repeatability and reliability of the designed spectrophotometric procedure.

4.6 Detection Limit and Quantitation Limit

Table 5 summarizes the summary of the limits of detection and quantification (DL and QL) of lamivudine using the developed method of UV spectrophotometry.

Table 5: DL and QL

Parameter	Value
Detection Limit (DL)	0.0033 µg/mL
Quantitation Limit (QL)	0.10043 µg/mL

Table 5 shows that the developed analytical method is sensitive enough to detect and quantify lamivudine at low concentrations because of the low values of DL and QL.

4.7 Robustness

Table 6 shows the findings of the robustness experiment carried out to test the integrity of the devised spectrophotometric method in the face of minimal changes in the conditions of analysis.

Table 6: Robustness Study

Concentration (µg/mL)	Mean Absorbance	%Recovery
4	0.245	101.24
8	0.476	99.79
16	1.006	100.40

Table 6 shows that minor changes in the experimental conditions did not have a significant impact on the analytical findings. The values of percentage recovery were very near to 100, which is a sign to suggest that the developed method is strong and stable.

4.8 Assay of Tablet Formulation

Table 7 indicates the assay outcomes of the lamivudine in a commercial tablet preparation through the developed UV spectrophotometric method.

Table 7: Analysis of Lamivudine Tablet

Brand	Drug Added (µg/mL)	Drug Found (µg/mL)	%Recovery
Lamivir-HBV	10	9.998	99.98

Table 7 indicates that the assay outcomes indicate that the designed spectrophotometric assay was effective in the quantification of lamivudine in the tablet formulation. The recovery of 99.98% means that it is very accurate and can be able to state that the method is not interfered with by tablet excipients.

V. Conclusion And Recommendations

The present study was able to design and establish an UV-Visible spectrophotometric technique to determine lamivudine in pharmaceutical preparations in the framework of ICH guidelines. The procedure exhibited peak absorbance at 280 nm and obeyed BeerLamberts law at 2-18 ug/mL concentration with 0.999 correlation coefficient. The validation parameters that were used to prove the reliability of the method include accuracy, precision, detection limit, quantification limit and robustness. There were also high recovery values (between 99.70% and 100.34%), and the commercial tablet formulation assay produced a recovery of 99.98%.

The results reveal that the method formulated is convenient, correct, and precise and suitable in the standard analysis of quality control of lamivudine in drug formulations.

- The constructed UV spectrophotometric method is applicable in the routine quality-control analysis of lamivudine in pharmaceutical preparations.
- Further studies can develop the technique of simultaneous determination of lamivudine and other antiretroviral medications in combination preparations.
- The procedure can also be used in the upcoming research to study the stability and degradation of lamivudine.

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