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# METHOD DEVELOPMENT AND VALIDATION OF UV-SPECTROSCOPIC METHOD FOR THE DETERMINATION OF LAMIVUDINE AS AN ACTIVE PHARMACEUTICAL INGREDIANT AND IN TABLET DOSAGE FORM

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## Abstract

The aim of this work was to develop and validate a simple estimation method for Lamivudine in bulk and in tablet dosage form using UV spectroscopic method. The method was developed using distilled water as a solvent and absorbance was measured at 271 nm. Beers law was obeyed the concentration range of 5 – 40 µg/ ml. Calibration curve shows a linear relationship between the absorbance and concentration. The line equation y = 0.04075x + 0.00468 with correlation coefficient (r) of 0.9998 was obtained. The method was validated as per ICH guidelines. The method was validated statistically and by recovery studies. The percentage recovery was found to be in the range between 98.09 and 102.46%. The %RSD value was found to be less than 2. A simple, accurate and cost efficient spectroscopic method has been developed for the estimation of Lamivudine in bulk powder and in tablet dosage form.

**Keywords:** Lamivudine; Distilled water; Validation; ICH Guidelines; UV Spectroscopy.

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## Introduction

Lamivudine is (2R, 5S)-4-amino-1[2(hydroxyl methyl)-1, 3-oxathiolan-5yl]-2(1H)-pyrimidinone [1] were shown in Fig 1.

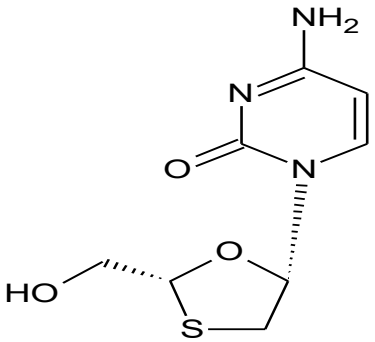


**Fig. No. 01: Structure of Lamivudine**

Lamivudine is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B virus [2]. It is used as an Anti retroviral agent and Nucleoside reverse transcriptase inhibitor [3-4]. Lamivudine is used for the prophylaxis and chronic treatment of HIV in adults and children of 3 months to 12 years. Lamivudine is rapidly absorbed after oral doses and peak plasma concentration occur about in 1 hour. Lamivudine crosses the blood brain barrier with the ratio of cerebrospinal fluid to serum concentration of about 0.12. It crosses the placenta and is distributed into the breast milk. It is metabolized intracellular to the

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active antiviral triphosphate. Hepatic metabolism is low eliminated through renal excretion [5].

Extensive literature survey revealed that only UV spectroscopy [6-12] and RP-HPLC [13-22] methods were reported for the estimation of Lamivudine in combination with other drug but there is no method was reported for the estimation of Lamivudine alone in bulk and in formulation by UV Spectrophotometry. A few HPLC [23-26] and HPTLC [27] methods were also developed for the determination of Lamivudine. So, an attempt was made to develop simple, cost effective and accurate UV spectrophotometric method for the estimation of Lamivudine in bulk and in tablet formulation and to validate the developed method.

## Materials and methods

Lamivudine raw material was procured from Aurobindo pharma limited. Hyderabad, India. Tablet formulation LAMIVIR HBV (Cipla Ltd. Sikkim, India) containing Lamivudine IP 100 mg was purchased from local market. All reagents and solvents used were analytical grade. Double distilled water was obtained from the Millipore unit. UV spectrophotometric method was performed on PERKIN ELMER Double Beam UV-Visible Spectrophotometer with pair of 10 mm matched quartz cell.

**Selection of solvent**

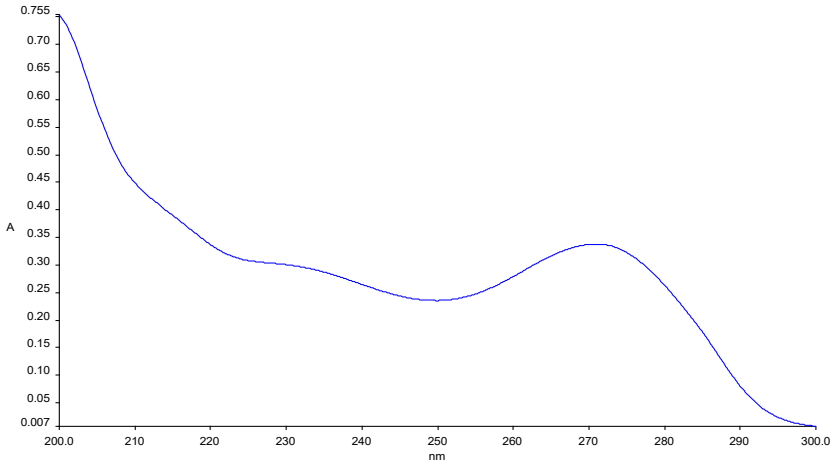
Different solvents such as distilled water, methanol, ethanol, toluene, acetic acid, isopropyl alcohol, N-butanol carbon tetrachloride, benzene, hexane, ethyl alcohol, acetonitrile, chloroform, diethyl ether and acetone were tried for the estimation of Lamivudine in tablet dosage form. Because of easy availability and cost effectiveness distilled water was selected as the solvent for the analysis of Lamivudine.

**Preparation of standard stock solution**

100 mg of Lamivudine raw material was accurately weighed and transferred into the 100 ml volumetric flask. Dissolved in minimum quantity of distilled water and made up to 100 ml with the same. 1 ml of the stock solution was transferred into 100 ml and dilute with distilled water. The dilution was observed to contain 10 µg/ ml.

**Selection of wavelength for estimation and stability studies**

The concentration solution of 10 µg/ ml was scanned between the ranges of 200 - 400 nm using distilled water as blank. From the UV spectra, λmax was found to be 271 nm and was selected as analytical wavelength. The UV spectrum of Lamivudine was shown in Fig 2.




**Fig, No. 02: UV Spectrum of Lamivudine in distilled water (10 µg/ ml)**

The stability was performed by measuring the same solution at different time intervals. It was observed that Lamivudine in distilled water was stable up to 24 hours at the selected wavelength.

**Preparation of calibration graph**

In this method, 1-8 ml of the aliquots of stock solution of Lamivudine containing 500 µg/ ml were transferred into a series of eight 100 ml volumetric flasks (5 - 40 µg/ml) and made up to the volume with distilled water. The absorbance of different concentration solutions

were measured at 271 nm against blank. The calibration curve was constructed by plotting concentration against absorbance. The sample was found to be linear with the concentration range of 5 - 40 µg/ ml at 271 nm.

**Quantification of raw material**

3 ml of working standard solution (15 µg/ ml) was taken into a series of six 100 ml volumetric flasks and the volume was made up to mark with distilled water. The absorbance of these solutions was measured at 271 nm. The amount of Lamivudine present in the raw material was determined by using slope and intercept values from calibration graph.

**Quantification of formulation**

Ten tablets of formulation (LAMIVIR containing 100 mg of Lamivudine) were weighed accurately and the average weight of each tablet was found. The tablets were ground to a fine powder. The tablet powder equivalent to 50 mg of Lamivudine was weighed and transferred into 100 ml volumetric flask. Added about 20 ml of distilled water to dissolve the substance and the solution was sonicated for 10 minutes. Then it was made up to the volume to 100 ml with Distilled water and centrifuged for 15 minutes. The supernatant liquid was filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 3 ml into 100 ml with distilled water in six replicates to get the concentration of 15 µg/ ml of Lamivudine, theoretically. The absorbances were measured at 271 nm and the amount of Lamivudine present in formulation was determined by using slope and intercept values from equation. This procedure was repeated for six times. The percentage label claim of Lamivudine present in each tablet formulation was found to be 98.09% ± 0.4400%. The % RSD value was found to be 0.4486 indicates that the method has good precision.

**Recovery studies**

The recovery experiment was done by adding known concentrations of Lamivudine raw material to the pre analyzed formulation. 50 mg equivalent of Lamivudine formulation was taken into a series of six 100 ml standard flasks. To that 2 ml, 2.5 ml and 3 ml of raw material stock solution (500 µg/ ml) were added into a series of each six 100ml volumetric flasks. Dissolved and made up to the volume with distilled water. The solutions were sonicated for 10 minutes. After sonication, the solution was centrifuged at 100 rpm for 15 minutes. The solutions were filtered through Whatmann filter paper No. 41. From each series, 3 ml of the clear filtrate was transferred into a series of six 100 ml standard flasks and made up to volume with distilled water. The absorbance of the resulting solutions was measured at 271 nm against blank and the amount of drug recovered from the formulation was calculated by using slope and intercept values. The procedure was repeated for three times for each concentration.

## Validation of developed UV Spectrophotometric method

**Linearity and range**

A calibration graph was plotted between concentration and absorbance. Lamivudine was linear with the concentration range of 5 – 40 µg/ ml at 271 nm.

**Precision**

The repeatability of the method was confirmed by the analysis of formulation was repeated for 6 times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The %RSD was calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated six times in the same day and on three successive days. The amount of drug were determined, % RSD also calculated.

**Accuracy (Recovery studies)**

It means the concordance between it and true or most probable value. To the pre analyzed formulation, a known quantity of Lamivudine raw material solution was added and the procedure was followed as per the analysis of formulation. The amount of each drug recovered was calculated. This procedure was repeated for six times for each concentration.

**LOD and LOQ**

The LOD and LOQ were estimated from the set of 6 calibration curves used to determine method linearity. LOD=3.3σ/S and LOQ=10σ/S

Where, σ = the standard deviation of y-intercepts of regression lines S = the slope of the calibration curve

**Ruggedness**

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not considered necessary to study these effects individually. The use of an experimental design (matrix) is encouraged.

**Robustness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variation in method parameters and provides an indication of its reliability during normal usage.

## Results and Discussion

**Linearity**

The linearity of Lamivudine was found to be in the range of 5 - 40 µg/ ml at 271 nm. With correlation coefficient 0.9984. Calibration curve were shown in Fig.3. Optical characteristics were shown in Table 1. The Correlation coefficient value indicates the method was found to be linear.

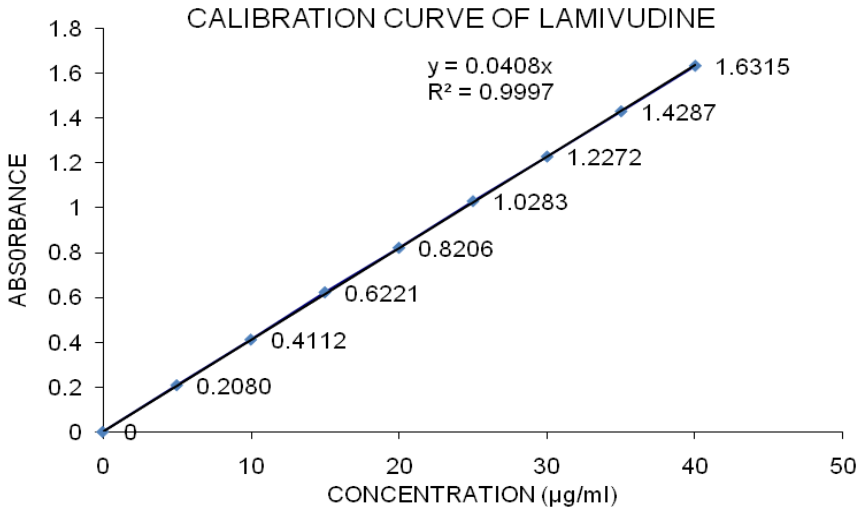



**Fig. No. 03: Calibration curve of Lamivudine in distilled water**

**Table No. 01: Optical characteristics of Lamivudine**

| Parameters | Results |
|---|---|
| λ max (nm) | 271 |
| Beer's Law Limit (µg/ ml) | 5 – 40 |
| Sandell's sensitivity (µg/cm$^2$/0.001 A.U) | 0.024539 |
| Molar absorptivity (L mol$^{-1}$ cm$^{-1}$) | 9.450 ×10$^3$ |
| Correlation coefficient (r) | 0.9998 |
| Regression equation (y=mx+c) | Y = 0.04075x + 0.00468 |
| Slope (m) | 0.04075 |
| Intercept (c) | 0.00468 |
| LOD ( µg/ ml) | 0.0323 µg/ml |
| LOQ ( µg/ ml) | 0.0978 µg/ml |
| Standard error of mean of Regression line | 0.01099 |

**Precision**

The percentage amount of Lamivudine present in the raw material was found to be 101.53% ± 0.7515 were shown in Table 2.

**Table No. 02: Quantification of raw material by UV spectroscopic method**

| S.No | Concentration (µg/ ml) | Absorbance | Amount found (µg) | Percentage recovery* | Average *(%) | S.D | %RSD |
|---|---|---|---|---|---|---|---|
| 1 | 15 | 0.6251 | 15.23 | 101.53 | | | |
| 2 | 15 | 0.6168 | 15.02 | 100.13 | | | |
| 3 | 15 | 0.6170 | 15.03 | 100.20 | 101.53 | 0.7515 | 0.7402 |
| 4 | 15 | 0.6262 | 15.25 | 101.66 | | | |
| 5 | 15 | 0.6175 | 15.04 | 100.26 | | | |
| 6 | 15 | 0.6488 | 15.81 | 105.40 | | | |

*Mean of six observations

The percentage label claim present in the tablet formulation lamivir was found to be 98.09% ± 0.4400 of Lamivudine. The precision of the method was confirmed by the repeated analysis of formulation. The %RSD was found to be 0.4486 were shown in Table 3.

**Table No. 03: Quantification of formulation by UV spectroscopic method**

| S.No | Formulation | Expected amount (µg/ ml) | Amount found (µg/ ml) | Percentage obtained (%) | Average (%) | S.D | % RSD |
|---|---|---|---|---|---|---|---|
| 1 | | 15 | 14.99 | 100.09 | | | |
| 2 | | 15 | 14.66 | 98.82 | | | |
| 3 | LAMIVIR | 15 | 14.5889 | 97.98 | 98.09 | 0.4400 | 0.4486 |
| 4 | | 15 | 14.69 | 97.47 | | | |
| 5 | | 15 | 14.82 | 97.06 | | | |
| 6 | | 15 | 14.73 | 97.11 | | | |

*Mean of six observations

Precision of the method was confirmed by intraday and inter day analysis. The percentage RSD value of the intraday and inter day analysis of Lamivudine was found to be 0.4460 % and 0.3722 %, respectively were shown in Table 4. The low % RSD values indicated that the method was found to be precise.

**Table No. 04: Intraday and Inter day analysis of formulation – UV spectroscopic method**

| S.No | Concentration (µg/ ml) | Percentage obtained* | | S.D | | %RSD | |
|---|---|---|---|---|---|---|---|
| | | INTRA DAY | INTER DAY | INTRA DAY | INTER DAY | INTRA DAY | INTER DAY |
| 1 | 15 | 98.16 | 99.10 | | | | |
| **2** | 15 | 98.69 | 98.10 | 0.4413 | 0.3683 | 0.3722 | 0.4460 |
| 3 | 15 | 99.98 | 99.64 | | | | |
| | Mean | 98.94 | 98.95 | | | | |

*Mean of six observations

**Accuracy**

Accuracy of the method was confirmed by recovery study from marketed formulation at three levels of standard addition viz., 80%, 100% and 120%. The percentage recovery for Lamivudine was found to be in the range of 98.09 – 102.46%. The % RSD was found to be 0.4277 were shown in Table 5. This indicates that there is no interference due to the excipients used in formulation. Hence the method was found to accurate.

**Table No. 05: Recovery analysis of formulation by UV spectroscopic method**

| S.No | Amount present (µg/ ml) | Amount added (µg/ ml) | Amount found (µg/ ml) | Amount recovered (µg/ ml) | Percentage recovery (%) | SD | %RSD |
|---|---|---|---|---|---|---|---|
| 1. | 14.75 | 12.5860 | 27.1467 | 12.3967 | 98.49 | 0.4247 | 0.4277 |
| | | | 27.6546 | 12.9046 | 102.46 | | |
| | | | 27.1025 | 12.3525 | 98.09 | | |
| 2. | 14.75 | 15.5060 | 30.0593 | 15.3093 | 98.71 | | |
| | | | 30.1109 | 15.3609 | 99.03 | | |
| | | | 30.2998 | 15.5498 | 100.26 | | |
| 3. | 14.75 | 18.0211 | 33.5254 | 17.7754 | 98.61 | | |
| | | | 32.5032 | 17.7533 | 98.50 | | |
| | | | 32.6824 | 17.9324 | 99.50 | | |
| | | | **Mean** | 15.2594 | 99.29 | | |

*Mean of six observations

**Limit of detection and limit of quantification**

LOD and LOQ were found to be 0.0323 µg/ ml and 0.0978 µg/ ml, respectively were shown in Table 1.

**Ruggedness**

The ruggedness of the method was confirmed by the analysis of formulation was done by using different analysts. The %RSD was found to be 0.1474, 0.2815 and 0.4501 were shown in Table 6.

**Table No. 06: Ruggedness**

| S.No | Category | % Label claim | SD | %RSD |
|---|---|---|---|---|
| 1 | ANALYST I | 98.68 | 0.1454 | 0.1474 |
| 2 | ANALYST II | 99.39 | 0.2798 | 0.2815 |
| 3 | ANALYST III | 99.28 | 0.4469 | 0.4501 |

*Mean of six observations

**Robustness**

The robustness of the method was confirmed by a small deliberate change in the analytical wavelength. The wavelength change applied as ±2 nm. At the selected variable wavelength, the amount of formulation was found. The % RSD was found to be 0.0897, 0.0770, 0.2102, 0.2167 and 0.08625 to variables of 1, 2, 3, 4 and 5, respectively were shown in Table 7.

**Table No. 07: Robustness**

| Parameter | Variables | Percentage Purity (%)* | SD | %RSD |
|---|---|---|---|---|
| WAVELENGTH | 269nm | 98.77 | 0.0886 | 0.0897 |
| | 270nm | 99.07 | 0.0763 | 0.0770 |
| | 271nm | 99.26 | 0.2087 | 0.2102 |
| | 272nm | 99.71 | 0.2161 | 0.2167 |
| | 273nm | 99.83 | 0.0863 | 0.0865 |

*Mean of six observations

## Conclusion

The proposed method for the determination of Lamivudine in solid dosage form was found to be precise, selective, rapid and economical. Lamivudine exhibited maximum absorption at 271 nm and obeyed Beer's law in the concentration range of 5 - 40 µg/ ml. The proposed method for the determination of Lamivudine showed linear regression Y = 0.04075x + 0.00468 with correlation coefficient (R2) of 0.9998.

The % RSD for analysis of formulation was found to be within the limit. Our studies revealed a recovery percentage of 98.09 – 102.46%, which indicates that the developed method was found to be accurate. The proposed method can be used for the drug analysis in routine quality control and method proves to be more economical. Hence the proposed method can be effectively applied for the routine quality control analysis of Lamivudine in bulk and in tablet dosage form.

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