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# METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF LAMIVUDINE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM BY FT-IR SPECTROSCOPY

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**ABSTRACT**

New, simple, precise method has been developed for the estimation of Lamivudine using FT-IR spectroscopy in bulk and tablet dosage form. The quantitative analysis of Lamivudine was carried out using a pressed pellet KBr method. The method involve the measurement of peak of carbonyl group at 1671cm$^{-1}$. Linearity was observed at the range of 10-18mg. The regression coefficient for the calibration data was y =0.0733x + 0.0058 with correlation coefficient of 0.9982 for Lamivudine. The value of the standard deviation did not exceed 2%. The respective method was successfully applied for the estimation of Lamivudine. The developed method was found to be precise and accurate and also simple, economical and time consuming other than available method. This technique does not require any organic solvent which is considered as the chief advantage.

**KEYWORDS**

Lamivudine, KBr, Fourier transform infrared spectroscopy and Method validation.

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## INTRODUCTION[1,2]

Lamivudine commonly called as 3TC, is an antiretroviral drug used to prevent and treat HIV/AIDS. It is also used to treat Hepatitis B. Lamivudine is (4 amino 1-[2R, 5S)-2-hydroxymethyl)-1, 3 oxathiolan 5 -y]-1, 2 dihydroxy pyrimidn-2-one [C8H12N3O3S]. It is a synthetic nucleotide analogue and is phosphorylated intracellularly to its active 5 – triphosphatemetabolate. The nucleoside analogue is incorporated into viral DNA by HIV reverse

transcriptase and HBr polymerase resulting in DNA chain termination. Literature survey reveals that many by analytical works have been done in Lamivudine using UV, RP – HPLC etc. Hence, a simple, rapid and accurate FTIR method was developed for the estimation of Lamivudine.

## MATERIAL AND METHODS[3-5]

### Chemicals and Reagents

Lamivudine pure drug was provided as gift sample (Emcure Pharmaceutical Ltd, Hyderabad), KBr used to formulate standard and sample pellets was of spectroscopic grade.

### Instrumentation and analytical condition Equipment

The Shimadzu model IR Affinity 1 FTIR spectrophotometer was used for the spectrophotometric analysis. IR region lies in between 4000 to 400cm$^{-1}$. The obtained values were interpretated using IR solution software.

### Obtaining of Analytical curve

50mg of Lamivudine (LAMI) was taken and diluted with sufficient amount of potassium bromide to obtain 300mg mixture. From this 50mg was taken and again add 50mg potassium bromide to obtain 100mg pellets. From this further dilution was done by taking 10, 12, 14, 16, and 18mg of LAMI (pure) were taken and diluted with sufficient amount of potassium bromide to obtain 100mg pellets. The powder were mixed and ground until obtaining a homogeneous mixture. Thus, this mixture was compressed in a mechanical die press with 10 ton pressure for 2min to obtain translucent pellets, through which the beam of the spectrometer can pass, after obtaining the FT-IR spectrum and with the assistance of the IR solution software. Quantitative analysis was carried out in the spectral region 1651cm$^{-1}$ and bands had its height analyzed in terms of absorbance. Absorbance was measured in the spectral region 1651cm$^{-1}$.

### Determination of Lamivudine in dosage form Preparation of standard pellets

14mg standard LAMI was taken and diluted with sufficient amount of potassium bromide to obtain100mg pellet. Absorbance was measured in the spectral region 1651cm$^{-1}$.

### Preparation of sample pellets

14mg of sample was taken and diluted with sufficient amount of potassium bromide. The powder were mixed and ground until obtaining a homogeneous mixture. Thus, this mixture was compressed in a mechanical die press with 10 ton pressure for 2min to obtain translucent pellets, through which the beam of the spectrometer can pass after obtaining the FT-IR spectrum and with the assistance of the IR solution software. Quantitative analysis was carried out in the spectral region 1651cm$^{-1}$ and bands had its height analyzed in terms of absorbance. Absorbance was measured in the spectral region 1651cm$^{-1}$.

$C_{sample}$= (A sample/ A standard) × C standard

### Method Validation[6-8]

The method was validated by determining the following parameters: linearity, accuracy, precision, limit ofdetection and limit of quantification.

### Linearity

Linearity with the intension of validate the method, five concentration of standard LAMI 10, 12, 14, 16 and 18mg were used. Linearity was evaluated by linear regression analysis.

### Accuracy

Accuracy was attained via the recovery assay, in which known quantity of pure drugs was added to known quantity of the sample. The recovery was performed in the three levels, 80%, 100% and 120% and the pellets were prepared in three replicate.

### Precision

The precision of the method was evaluated in two requisites: Repeatability and intermediate precision. Repeatability (intra-day) was studied by the performance of three determinations of the sample in a concentration 14mg per pellet, all in the same day and identical working conditions. Intermediate precision (inter-assay) was assessed by performing the assay in three different days under the same experimental conditions. At the end of test, the percentage relative standard deviation (%RSD) values of the determinations were analysed.

**Detection and Quantification limit**

The detection (LOD) and quantification (LOQ) limits were calculated based on the intercept standard deviation and the curve slope.

LOD=3.3$\sigma$/$S$ LOQ=10$\sigma$/$S$

Where, σ the standard deviation and S is the slope of the curve.

## RESULTS AND DISCUSSION[9]

FTIR was developed for the estimation of Lamivudine by making pellets of tablet powder by pressed pellet technique. The standard and sample pellets were prepared and spectrums were recorded. The recorded spectrums were given as Figure No.1 and Figure No.2. After obtaining the IR spectrum and with the assistance of the IR solution software, quantitative analysis is carried outin the spectral region 1651cm-$^{1}$ related to a C=O Stretch respectively and these bands had its height analysed in terms of absorbance. Calibration curve were constructed by plotting absorbance Vs Concentration and the regression equations were calculated. The response of the LAMI werefound to be linear in the investigational concentration range and the linear regression equation for LAMI was,

y = 0.073x+0.005 with a regression coefficient of 0.998.

The assay procedure was repeated in three replicate and the percentage of individual drugs found in formulation, percentage relative standard deviation were calculated and presented in the Table No.1.

**Method Validation**

**Linearity**

Linearity with the intension of validate the method, five concentration of standard LAMI 10, 12, 14, 16 and 18mg were used. Linearity was evaluated by linear regression analysis. Absorbances obtained in the above concentrationare given in the Table No.2. The calibration curve was shown in Figure No.3 for Lamivudine respectively.

**Accuracy**

The accuracy of the method was determined at three percentage level 80%, 100% and 120% levels. The recoverystudies were carried out three times and the percentage recovery and percentage relative standard deviation was found to be less than 2 and given in Table No.3.

**Precision**

The precision of the method was evaluated in two requisites: repeatability and intermediate precision. Repeatability (intra-day) was studied by the performance of three determinations of randomly selected samples in a concentration of 14mg per pellet, all in the same day and identical working conditions. Intermediate precision (inter-assay) was assessed by performing the assay in three different days under the same experimental conditions. At the end of test, the percentage relative standard deviation (%RSD) values found to be less than 2. The values are given in the Table No.4.

**Table No.1: Estimation of formulation by FT-IR**

| S.No | Drug | Amount of drug inone tablet (mg) | % Purity | %RSD |
|---|---|---|---|---|
| 1 | LAMI | 1.687 | 102.0 | 0.521 |
| | | 1.662 | 101.0 | |
| | | 1.665 | 101.2 | |

**Table No.2: Linearity values for standard Lamivudine**

| S.No | Lamivudine | |
|---|---|---|
| | Concentration (mg) | Absorbance |
| 1 | 10 | 0.764 |
| 2 | 12 | 0.892 |
| 3 | 14 | 0.998 |
| 4 | 16 | 1.184 |
| 5 | 18 | 1.331 |

**Table No.3: Accuracy results**

| S.No | Drug | Theoretical % Target level | Amount added (mg) | Amount recovered (mg) | % Recovery | % RSD |
|---|---|---|---|---|---|---|
| 1 | LAMI | 80 | 11 | 102.0 | 102.0 | 0.20 |
| | | | | 101.9 | 101.9 | |
| | | | | 101.6 | 101.6 | |
| | | 100 | 14 | 101.3 | 101.3 | 0.15 |
| | | | | 101.1 | 101.1 | |
| | | | | 101.0 | 101.0 | |
| | | 120 | 17 | 100.0 | 100.0 | 0.82 |
| | | | | 99.5 | 99.5 | |
| | | | | 98.4 | 98.4 | |

**Table No.4: Precision results**

| S.No | Drug | Amount (mg) | Intraday | | Interday | |
|---|---|---|---|---|---|---|
| | | | % content | % RSD | %content | % RSD |
| 1 | LAMI | 14 | 100.6 | 0.52 | 99.3 | 1.23 |
| | | | 99.8 | | 100.0 | |
| | | | 100.5 | | 101.7 | |

**Detection and Quantification limit**

**Table No.5: LOD and LOQ results**

| S.No | Lamivudine | |
|---|---|---|
| | LOD (mg) | LOQ (mg) |
| 1 | 0.94 | 2.87 |

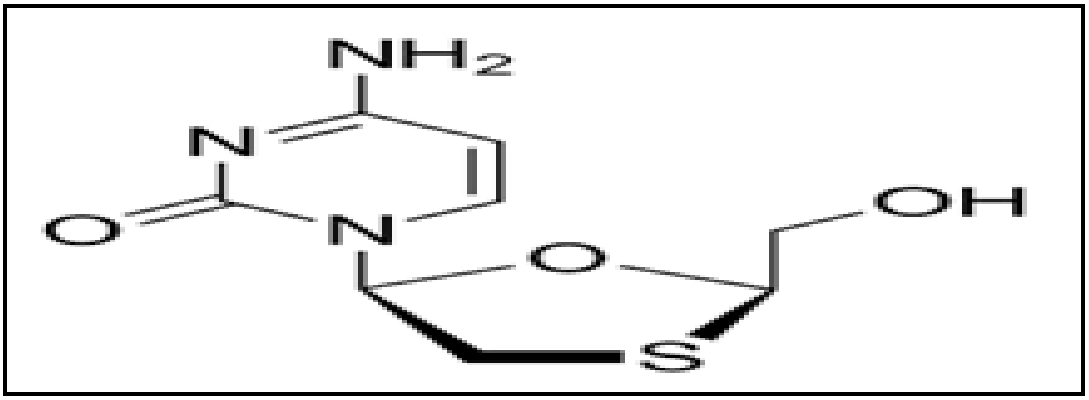


**Figure No.1: Structure of Lamivudine**

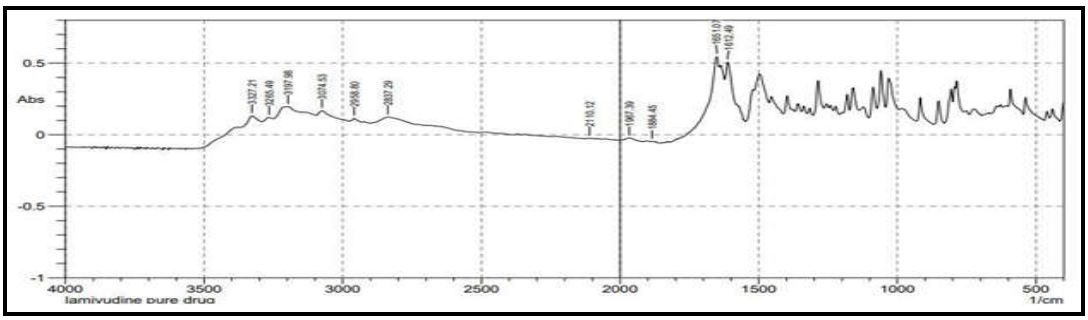


**Figure No.1: FT-IR Spectrum of Lamivudine standard drug**

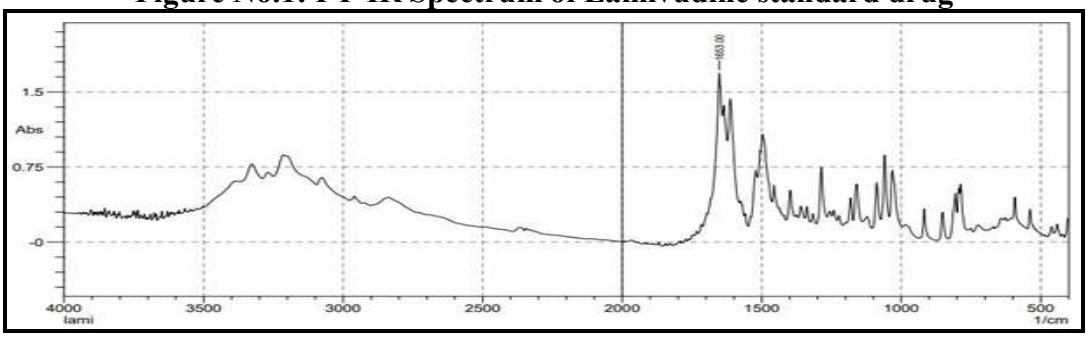


**Figure No.2: FT-IR Spectrum of formulation**

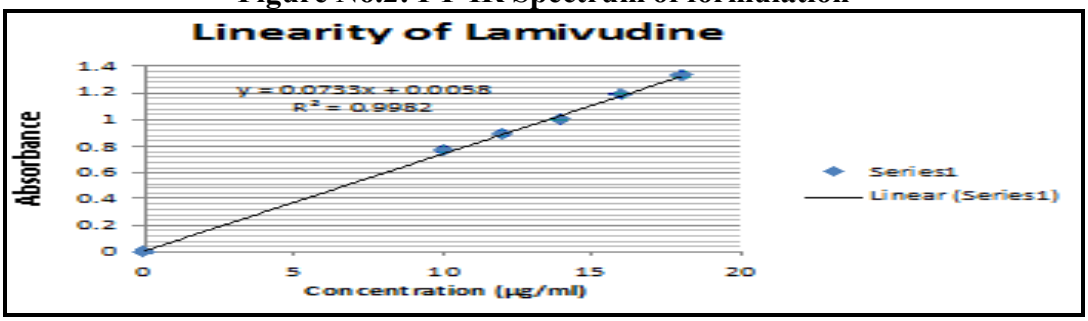


**Figure No.3: Calibration curve for standard lamivudine**

## CONCLUSION

In FT-IR method for the determination of the KBr pellets containing known amount of standards and samples were used for acquisition of the FT-IR spectra. The method involves the measurements of peak of C=O groups at 1651cm$^{-1}$. Linearity was observed in the range of 10-18mg for Lamivudine. The regression equation for the calibration data was y = 0.073x+0.005 with correlation coefficient of 0.998. The limits of detection were 0.03mg and the limit of quantitation were 0.5mg respectively. The precision of the method was good. The values of the relative standard deviation did not exceed 2%. The proposed method was successfully applied for the estimation of Lamivudine in pure and its pharmaceutical dosage form with good accuracy and precision. The developed method was found to be precise and accurate for the estimation of Lamivudine in its dosage forms. This technique does not use organic solvents, which is one great advantage over the most common analytical methods.

The developed methods proved to be simple in procedure and it produced precise and accurate results. FT-IR method was more sensitive. However all the developed methods can be applied for the routine analysis of Lamivudine in dosage form.

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**CONFLICT OF INTEREST**

We declare that we have no conflict of interest.

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