IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

# Review article of UV Spectroscopy Method and RP-HPLC Method oflamivudine alone and combination drug.

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**Abstract :**

Lamivudine, a first-generation nucleoside reverse transcriptase inhibitor (NRTI), is a pivotal antiviral medication used in the treatment of HIV-1 and hepatitis B. This paper provides an overview of lamivudine's chemical structure, IUPAC designation, and mechanism of action, emphasizing its therapeutic applications both as a monotherapy for hepatitis B and in combination with other antiretroviral agents for HIV-1.

Analytical techniques for the quantification and quality control of lamivudine are critically examined, focusing on ultraviolet (UV) spectroscopy and high-performance liquid chromatography (HPLC). UV spectroscopy is highlighted for its cost-effectiveness and ease of application, demonstrating maximum absorbance at approximately 271 nm, which facilitates accurate measurement of lamivudine concentration in various formulations.

Conversely, HPLC is recognized for its precision and reliability in separating and quantifying lamivudine alongside other compounds in complex mixtures. This technique is integral to drug development, stability testing, and regulatory compliance. The validation parameters for both UV and HPLC methods, such as accuracy, linearity, precision, and robustness, are also discussed, ensuring their applicability in pharmaceutical quality control.

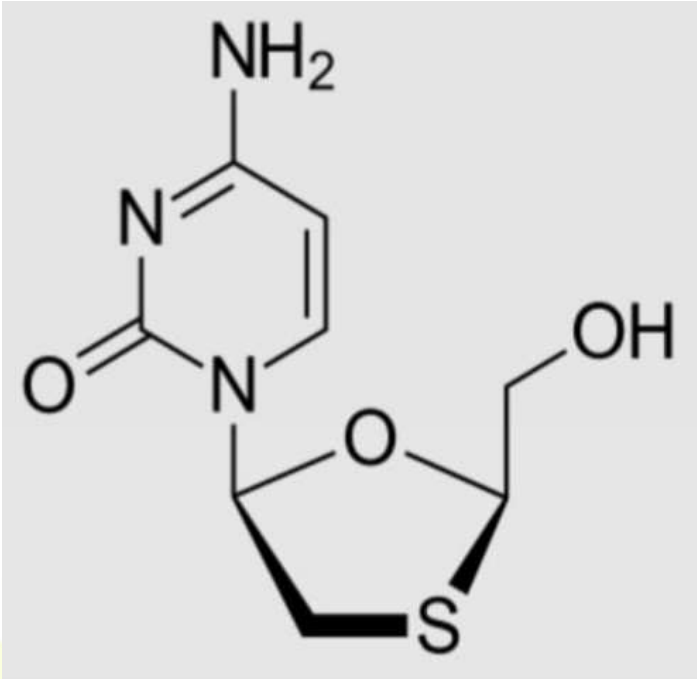
In conclusion, the integration of these analytical methods is essential for ensuring the safety, efficacy, and quality of lamivudine, thereby supporting its crucial role in the clinical management of HIV and hepatitis B.

**Keyword:**

Lamivudine,Zidovudine, Tenofovir, Disoproxil Fumarate, Efavirez, Dolutegravir,Emtricitabine, UV Spectroscopy System ,RP-HPLC Method,HIV ,Hepatitis B.

**Introduction**

Structure: Lamivudine




The antiviral medication lamivudine is useful in both treating and preventing AIDS. When all other treatments for hepatitis B are not working, this treatment can be utilized. Lamivudine is known by its IUPAC designation, 2',3'-dihyroxy3'thiacytidine 4-Amino-1- [(2R,5S)-2- (hydroxymethyl)-1,3-oxathiolan-5-yl].1,2-dihydropyrimidinone. The molecular formula is C8H11N3O3S.[1,2]

Lamivudine (2′-deoxy-3′-thiacytidine, 3TC) is a first-generation nucleoside reverse transcriptase inhibitor (NRTI) and antiretroviral agent[3,4,5] that was authorized in 1995 to treat HIV-1 infection and in 1998 to treat hepatitis B virus (HBV) infection[6,7] .

An antiviral medication was the original purpose of the pyrimidine nucleoside lamivudine. Lamivudine triphosphate, which includes HIV reverse transcriptase and hepatitis B DNA polymerase, is created intracellularly from simple cytidine. A prescription medication called lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) that is used as a monotherapy for the hepatitis B virus (HBV) and in conjunction with other medications as an antiviral treatment for man-against HIV-1[8] .Lamivudine's extended half-life (5–7 hours) and good oral bioavailability allow for once-daily dosage increases in hepatitis B patients[9].Brand Name of lamivudine is cimdus, Combivir, Delstiga Dovata, Epivir, Epivir Hby, Epizicom...

**UV spectroscopy :**

The measurement of light absorption in the visible and ultraviolet (UV) parts of the electromagnetic spectrum is the focus of ultraviolet (UV) spectroscopy, which also includes UV-VIS spectrophotometry. The affordability and ease of application of this technology make it frequently used in a variety of sectors. Chromophores, or materials that absorb light in the UV and visible spectrum, are essential components of samples. When detecting and measuring molecules within various samples, UV-VIS spectrophotometry is especially helpful. A light beam is passed through the sample in accordance with the basic principle, and the absorbance at various wavelengths is measured. In a sample, the concentration of the absorbing

species directly relates to the degree of light absorption. Fluorescence spectroscopy is enhanced by this method, which measures vital factors including absorbance (A) [10,11,12,13].

Max Absorption Wavelength (λmax) of Lamivudine typically exhibits maximum absorbance around 271 nm[14]and Category of lamivudine is antiviral and hepatitis B [15]. Reagent,Chemical and Solvent that Involved in UV Spectroscopy method of the lamivudine drug 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.[16]

Method of validation of UV Method

After method optimization,it was validated for accuracy,linearity as per ICH guidelines ,which detailed below. [17,18,19]

1. Accuracy
2. Linearity
3. Specificity
4. Precision
5. LOD
6. LQD
7. Ruggedness

Reported method

There are several methods for determining the effectiveness of lamivudine both by itself and in combination with other drugs.

| Method | description | Wavelength (λmax) | Combination Drug | Application | Reference |
|---|---|---|---|---|---|
| UV Spectroscopy (Lamivudine Alone) | Simple UV method for determining lamivudine in bulk and dosage forms. | 270-280nm | None | Qualification in pharmaceutical formulation | 20 |
| UV Spectroscopy<br><br>Lamivudine | Derivative UV method for selective determination of lamivudine in the presence of excipients | 272 nm | None | Quality control in tablets and bulk drugs | 21 |
| UV Spectroscopy (Lamivudine and Zidovudine | Simultaneous estimation of lamivudine and zidovudine using zero-order and first-order derivative UV spectroscopy | 270 nm (Lamivudine), 266 nm (Zidovudine) | Zidovudine | Simultaneous quantification in fixed-dose combinations | 22 |
| UV Spectroscopy (Lamivudine and Tenofovir) | Dual-wavelength UV method for simultaneous determination of lamivudine and tenofovir in tablet dosage form | 270 nm (Lamivudine), 260 nm (Tenofovir) | Tenofovir | Simultaneous quantification in combined dosage forms | 23 |
| UV Spectroscopy (Lamivudine and Efavirenz) | Method for the simultaneous quantification of lamivudine and efavirenz using zero-crossing derivative technique | 272 nm (Lamivudine), 247 nm (Efavirenz) | Efavirenz | Analysis of fixed-dose combination therapies | 24 |

| | | | | | |
|---|---|---|---|---|---|
| UV Spectroscopy (Lamivudine and Dolutegravir) | Simultaneous estimation of lamivudine and dolutegravir using UV spectrophotometry. | 270 nm (Lamivudine), 260 nm (Dolutegravir) | Dolutegravir | Simultaneous analysis in fixed-dose combinations | 25 |
| UV Spectroscopy (Lamivudine, Zidovudine, and Nevirapine) | Simultaneous estimation using multi-component UV spectroscopy for triple-drug combination. | 271 nm (Lamivudine), 266 nm (Zidovudine), 315 nm (Nevirapine) \| Zidovudine, Nevirapine | Zidovudine, Nevirapine | Quantification of triple combination antiretroviral therapy | 26 |
| UV Spectroscopy (Lamivudine and Emtricitabine) | UV method developed for simultaneous determination of lamivudine and emtricitabine. | 270 nm (Lamivudine), 254 nm (Emtricitabine) | Emtricitabine | Quantification in combination dosage forms | 27 |

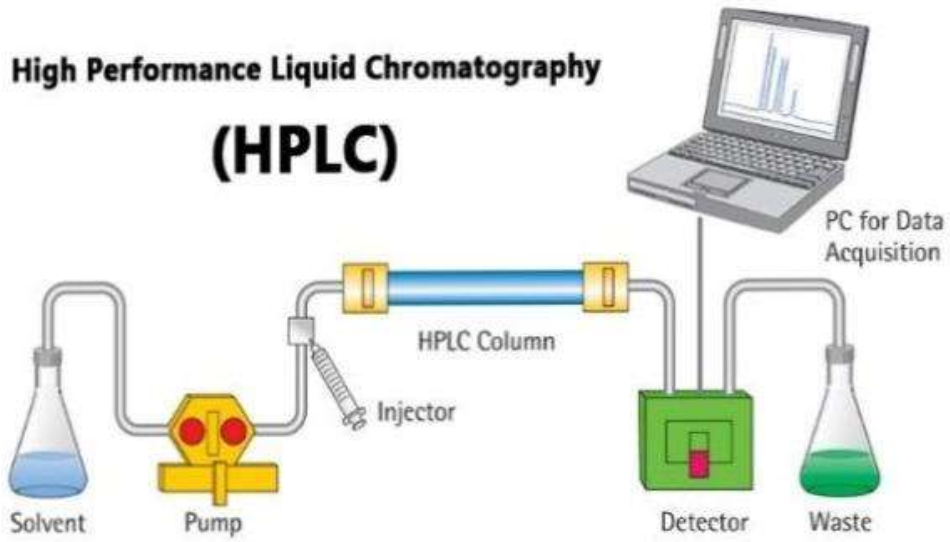
**HPLC METHOD**

HPLC method is a very common analytical method globally used across various industries for accurate and efficient analysis. In the pharmaceutical industry, HPLC is an essential analytical instrument utilized in every phase of drug development, manufacture, and discovery. [28] It is designed to use the HPLC method to separate and quantify the primary drug, reaction contaminants, produced intermediates, and degradants. High-performance liquid chromatography (HPLC) is a valuable analytical technique for evaluating the stability of drug products, and it may be used to collect data with great accuracy and scope [29]. sample is inserted into a stationary phase and the mobile phase is pushed through the column at hiThe gh pressure in accordance with the HPLC concept. Based on adsorption, the separation principle is based on the solute's affinity for the stationary phase. [30]

The In many fields, such as the biological, food, chemical, pharmaceutical, and medical sciences, RP-HPLC is the method most frequently used for the analytical separation of complex compounds. This process uses silica gel as the stationary phase and a nonpolar hydrophobic packing with an octyl or octadecyl functional group attached as the mobile phase. The polar solvent is used in this approach. The majority of drugs and medications elute quickly in the polar environment and are not maintained for long.[31]The In RP-HPLC, a non-polar stationary phase and a polar or moderately polar mobile phase are utilized.[32]The stationary phases used may include C8, C18 (ODS), phenyl, and Trimethyl Silane (TMS) columns.[33] The The column oven keeps the column at a consistent temperature. For technique development, the ambient temperature is typically maintained constant and adjusted as needed.[34 ,35 ,36 ]

The aim of an analytical technique is to determine whether it is appropriate for the intended use. For each process, method, piece of machinery, substance, or activity, validation is crucial to proving compliance under certain circumstances. Guidance for validating analytical procedures can be found in the ICH harmonised guideline Q2(R1). The objective is to guarantee the precision, dependability, and uniformity of the techniques employed for evaluating pharmaceutical items. [37]. The process of creating a method can be difficult and time-consuming. The right mobile phase, temperature, pH, column, and gradient must be estimated for each task in method development labs in order to achieve precise and separation. [38].

Fig 2 :simple instrumention OF HPLC [39]




The analytical methods' validation parameters of HPLC are listed below. [40]

1.Detection limit,

2.quantitation limit,

3.robustness,

4.range,

5.accuracy,

6.linearity,

7.precision,

8.robustness

**Reported method:**

There are several methods for determining the effectiveness of lamivudine both by itself and in combination with other drugs.

| Drug | HPLC Make | Method | Column | Mobile phase | Wavelength | Flow rate | Detectors | Temperatures | References |
|---|---|---|---|---|---|---|---|---|---|
| Lamivudine | HPLC Shimadzu LC 2010 CHT series | RP-HPLC | Thermo BDS hypersil C18 column | 500 mL of 0.01 M ammonium dihydrogen orthophosphate buffer 500 mL of methanol in 50:50 v/v | 264 nm. | 0.6 mL/min | Photodiode Array Detector | 25°C | 41 |
| Lamivudine, Tenofovir Disoproxil Fumarate and Efavirenz | Shimadzu HPLC system | RP-HPLC | Phenomenex Luna C18 | acetonitrile : methanol : water (30 : 45 : 25, v/v/v) | 258 nm | 0.5 mL/min. | UV Visible detector) | ambient | 42 |
| tenofovir disoproxil fumarate, lamivudine, and efavirenz | Shimadzu (LC-2010CHT ) | RP - HPLC | Kromasil C18 column | methanol and phosphate buffer of pH 5.0 (adjusted with 10% solution of orthophosphoric acid) in a ratio of 70:30 v/v | 254 nm | 1.0 ml/min | UV and Photodiode Array (PDA) detector | 40°C | 43 |
| Lamivudine, Tenofovir, | Shimadzu HPLC system | RP-HPL C | ODS-3VC18 | Water and methanol in | 260nm | 1.0mL/min | photo diode | 35°C | 44 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| and Dolute gravir | | | | the ratio 30:70 v/v | | | array detector | | |
| Lamivudine, Dolute gravir | Waters 2489 | RP-HPLC | Sun fire C8 (150× 4.6mm, 3.5 µm) | Acetonitrile: Potassium Dihydrogen orthophosphate (55:45%v/v) | 260nm | 1.0mL/ min | Uv – visible detector | 35°C | |
| Lamivudine, Tenofovir Alafenamide and Dolute gravir | HPLC Agilent separation module model | RP-HPLC | Agilent C18 (250 × 4.6 mm, i.d., 5 µm) column | 0.05M phosphate buffer pH 6.2 (solvent A) and acetonitrile (solvent B) 60:40 v/v | 260 nm | 1 ml/min | UV Visible detector | ambient | 46 |
| Lamivudine , Dolute gravir and Tenofovir Alafenamide | Agilent | RP-HPLC | Agilent C18 (4.6 X250 mm, 5um) | 0.05 Phosphate Buffer: Acetonitrile (60:40%v/v) | 260nm | 1ml/min | PDA Detector | 30°C | 47 |

Conclusion:

Lamivudine is a critical antiviral medication for treating HIV-1 and hepatitis B, recognized for its effectiveness as a nucleoside reverse transcriptase inhibitor. Analytical methods such as UV spectroscopy and HPLC play vital roles in the quantification and quality control of lamivudine in various formulations. UV spectroscopy is cost-effective and straightforward, allowing for accurate measurement of lamivudine's absorbance around 271 nm. HPLC provides precise separation and quantification, essential for drug development and regulatory compliance. Together, these techniques ensure the reliability and efficacy of lamivudine in clinical settings.

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