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Research Article

Simple Spectrophotometric Methods for the Determination of an anti HIV drug lamivudine in raw and tablet dosage form using two different solvent-blends

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Abstract: A simple, rapid, precise and accurate UV Spectrophotometric method was developed and validated for the estimation of Lamivudine in commercial tablets using two different solvent blends viz. Methanol: Water: 0.1N HCl (3:1:1) and Methanol: Water: 0.1N NaOH (3:1:1) . Absorption maxima were determined and found 282 nm and 272 nm in each respective solvent blends followed by linearity range determination which was found within the range of 1-22 µg/ml. From the linearity range, calibration curve is prepared in the range of 2-10 µg/ml and validated the various parameters in bulk and formulations. All the experiments were carried out in triplicate, for each parameter relative standard deviation and % recoveries was calculated and the data were statistically interpreted and were found to be significant and within the limit. The proposed methods are further evaluated for precision, robustness studies. The results indicate the methods were precise and robust.
Keywords : UV spectroscopic method, Lamivudine, Absorption maxima, Validation.

INTRODUCTION

Lamivudine is a synthetic nucleoside having molecular formula of C8H11N3O3S with molecular weight of 229.3.It has pronoun activity against HIV-1 and HBV [1, 2]. The chemical name of lamivudine is (2R, cis)-4- amino-l-(2- hydroxymethyl-l, 3-oxathiolan-5-yl)-(lH)-pyrimidin-2- one. Lamivudine is the (-) enantiomer of a dideoxy analogue of cytidine. Lamivudine is a white to off-white crystalline solid with a good solubility in water and methanol. The drug is officially listed in Martindale, the Extra Pharmacopoeia. Several analytical methods that have been reported for the estimation of Lamivudine in biological fluids or pharmaceutical formulations include HPLC, Titrimetry and UV-visible spectrophotometry[3-10].

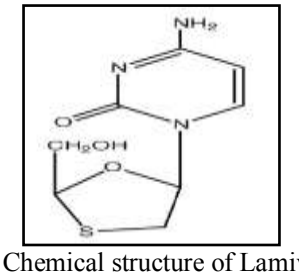

Fig 1: Chemical structure of Lamivudine

In view of the above fact, a simple analytical method is in need for its quantitative estimation. The work is aimed to develop and validate simple, rapid, economic and reproducible UV-spectrophotometric methods for the estimation of zidovudine in bulk and formulations.

MATERIAL AND METHODS

Chemicals and methodology:

A Shimadzu UV/VIS spectrophotometer model 1700 Pharma spec with 1 cm matched quartz cells was used for spectral and absorbance measurements and UV-2000 Spectrophotometer of Hitachi was used for ruggedness study. All the chemicals used in the investigation were of analytical grade including Methanol, 0.1N HCl, NaOH.

Preparation of standard stock solution:

Standard stock solution containing 1000 µg/ml of Lamivudine was prepared in two solvent blend including Methanol: Water: 0.1N HCl (3:1:1) solvent blend-1 and Methanol: Water: 0.1N NaOH (3:1:1) solvent blend-2 by weighing accurately about 10 mg of lamivudine working standard and transferred to a 100 ml volumetric flask and adding 90 ml of respective solvent blend and shake for 5 minutes to dissolve and dilute to volume with respective solventblend-1 and solventblend-2. The standard sample solution was prepared by transferred aliquots of standard stock solution into a series of 10 ml volumetric flask and dilute with solvent blend 1 and 2 differently to get desired concentrations. The method was extended for determination of lamivudine in tablet dosage form. The solutions were scanned on spectrophotometer in the UV range 200-380 nm and absorption maxima were calculated.

Sample preparation for analysis (method A and B):

The method was extended for determination of lamivudine in tablet dosage form. The tablet containing 100 and 150 mg strength were taken. Twenty tablets of each formulation T1 and T2 containing 100 and 150 mg of Lamivudine were accurately weighed and powdered. Transfer equivalent to 50 mg of lamivudine into a 50 ml volumetric flask,

add 40 ml of solvent blend 1 for Method A and kept for ultrasonication for 1 hour, Dilute to volume with solvent blend 1 mix the contents and filter through 0.45 µm membrane filter. The final concentration of lamivudine was brought to100 µg/ml with respective solvent. Similarly sample preparations were prepared in next solvent blends only the sonication hour is 40 min for method B.

Analytical procedure for method-A:

For method A an aliquots of lamivudine ranging from 0.2-1.0 ml of standard solution were transferred into a series of 10ml volumetric flasks. The absorbance were measured at 282 nm against the reagent blank prepared simultaneously. The amount of the drug in a sample was calculated from the calibration graph.

Analytical procedure for method-B:

Pipette out 0.1 to 1 ml of working standard drug solution (100 µg/ml) into a series of 10 ml volumetric flask and adjusted to volume with solvent blend-2. The absorbance of solution was measured at 272 nm against the reagent blank. Each sample preparation of T1 and T2 was taken into 10 ml volumetric flask (final concentration is 10µg/ml) and the above procedure was subsequently followed. The amount of the drug in a sample was calculated from the calibration graph.

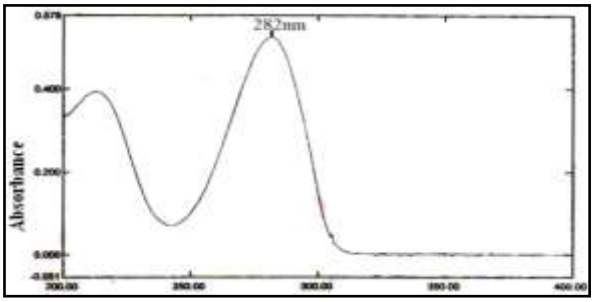

Fig 2: Absorption maxima of lamivudine in methanol: water: 0.1 N HCl (3:1:1)

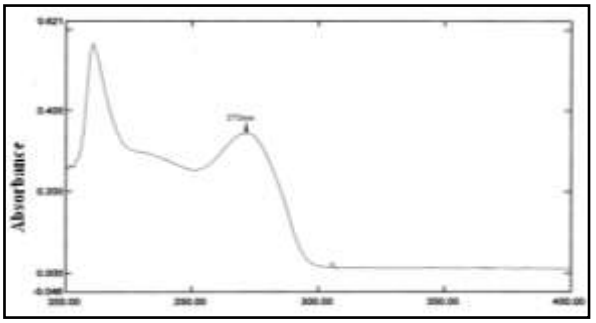

Fig 3: Absorption maxima of lamivudine in methanol: water: 0.1 N NaOH (3:1:1)

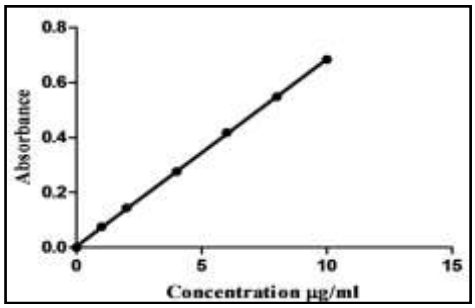

Fig 4 : Calibration curve of Lamivudine in 282 nm

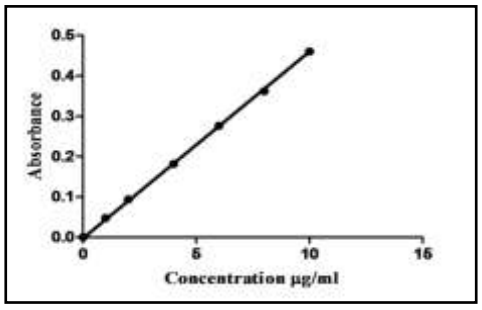

Fig 5: Calibration curve of Lamivudine in 272 nm

Table -1: **Statistical data of Lamivudine calibration Curve in both 272 nm and 282 nm**

λ_{max}(nm)	272	λ_{max}(nm)	282
Beer's law limits (µg / ml)	1-22	Beer's law limits (µg / ml)	1-20
Molar Absorptivity ($mol^{-1}cm^{-1}$)	15.5 x 10^3	Molar Absorptivity ($mol^{-1}cm^{-1}$)	10.4 x 10^3
Sandell's sensitivity	0.014	Sandell's sensitivity	0.022
Best-fit values		Best-fit values	
Slope	0.06785 ± 0.0003720	Slope	0.04490 ± 0.0001195
Y-intercept when X=0.0	0.004078 ± 0.002090	Y-intercept when X=0.0	0.001311 ± 0.0006716
X-intercept when Y=0.0	-0.06011	X-intercept when Y=0.0	-0.02919
1/slope	14.74	1/slope	22.27
95% Confidence Intervals		95% Confidence Intervals	
Slope	0.06690 to 0.06881	Slope	0.04459 to 0.04520
Y-intercept when X=0.0	-0.001295 to 0.009452	Y-intercept when X=0.0	-0.0004160 to 0.003037
X-intercept when Y=0.0	-0.1409 to 0.01887	X-intercept when Y=0.0	-0.06802 to 0.009217
Goodness of Fit		Goodness of Fit	
R square	0.9998	R square	0.9998
P value	< 0.0001	P value	< 0.0001

RESULTS AND DISCUSSION:

The absorption spectral analysis shows the λmax of Lamivudine was found to be 282 nm for method A and 272 nm for method B shown in Fig. 2 and Fig. 3.The calibration curve was obtained for a series of concentration in the range of 2-10 mcg/ml for both the methods (Fig. 4 and Fig. 5). They were found to be linear and hence, suitable for the estimation of the drug. The slope, intercept, correlation coefficient and optical characteristics are summarized in Table 1. Regression analysis of Beer's law plot revealed a good correlation. The proposed methods were validated as per the ICH guidelines [11-13]. The recovery technique was performed to study the accuracy and reproducibility of the proposed methods. For this, known quantities of the Lamivudine solution were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed. The total amount of Lamivudine was determined by using the proposed methods and the amount of added drug was calculated by the difference. This showed that the recoveries studies of Lamivudine by the proposed methods are satisfactory and the results are shown in Table 2. The precision was measured in terms of inter day and intraday, which was determined by sufficient number of aliquots of a homogenous sample. The % RSD was found and lying within the range of 0.471**,** to 1.195. Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by the proposed methods.

Table 2: Data showing Validation results

PARAMETER	METHOD A		METHOD B	
Label claim (tablet- mg)	150 mg	100 mg	150 mg	100 mg
Amount found ± SD[3]	150.1±0.24	100.3±0.09	150.2±0.19	99.5±0.27
Limit of detection µg/ml	0.59		0.66	
Limit of Quantification µg/ml	2.12		2.19	
Intraday precision (% RSD[3])	0.471	1.029	1.195	1.277
Interday precision (% RSD[3])	0.967	0.998	1.003	1.678
% Mean Recovery ± SD[3]	99.2 ± 0.750	99.1 ± 0.850	99.7 ± 1.513	99.6 ± 1.721
Mean Recovery (% RSD[3])	0.756	0.857	1.517	1.727

3 Mean of three determinations, SD indicates standard deviation, RSD indicates relative standard deviation

CONCLUSION:

The developed UV Spectrophotometric methods for the estimation of Lamivudine were found to be simple with high accuracy, precision. Sample recoveries were in good agreement with their respective label claim, suggesting validity of the method. The developed methods were stability specific and validated as per ICH guidelines.

REFERENCE:

1. Bertram G katzung. Basic and clinical Pharmacology, 7th edition, The McGraw hill companies, New York, 1998: 797.
2. Indian Drug Review. 2004; 10: 494-495.
3. Desman J. Validation of analytical methods from the perspective of an analytical chemist in the pharmaceutical industry. J Pharm Biomed Anal 1996; 14:867-869.
4. Srikar A, Ashok S, Venu T, Latheeshjlal, Madhusudhan K, Naveen B. Validated spectrophotometric estimation of Lamivudine in pure and tablet dosage form. Int J Pharm Tech 2009; 1:1: 26-32.
5. Patro SK, Swain SR, Patro VJ, Choudhury NSK. Development and Validation of High Performance Liquid Chromatographic Method for Determination of Lamivudine from Pharmaceutical Preparation. E-J Chem 2010; 7(1): 117-122.
6. Gnana Babu CJ, Vijay KG. Validated spectrophotometric estimation of Lamivudine in pure and tablet dosage form. Int J ChemTech Res 2009; 1(4): 1372-1375.
7. Basavaiah K, Somashekar BC. Sensitive Titrimetric and two spectrophotometric methods for the assay of Lamivudine in pharmaceuticals. J Sci Ind Res 2006; 65: 349-354.
8. Bengi U, Sibel AO. Determination of Lamivudine and Zidovudine in binary mixtures using first derivative spectrophotometric, first derivative of the ratio-spectra and high-performance liquid chromatography–UV methods. Anal Chim Acta 2002; 466: 175-185.
9. Nijamdeen J, Jayalakshmi B, Senthilkumar N, Vijayamirtharaj, Saravanan C. Method development and validation of RP-HPLC method for simultaneous determination of Lamivudine and Zidovudine. J Chem Pharm Res 2010; 2(3): 92-96.
10. Mahua S, Sateesh K, Ramesh P. Development and validation of RP-HPLC and ultraviolet spectrophotometric methods of analysis for the quantitative estimation of antiretroviral drugs in pharmaceutical dosage forms. J Chromat B 2006; 830: 349-354.

11. Quality assurance of pharmaceuticals, (A compendium of guidelines and related materials), (1997), Volume I, WHO, Geneva, 119-124.
12. ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories, 2005
13. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of analytical procedures: definitions and terminology, Geneva (1996)