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DEVELOPMENT AND PARTIAL VALIDATION OF THE LAMIVUDINE DRUG IN BULK AND SOLID DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, specific, accurate and precise RP HPLC method has been developed for the determination of Lamivudine from Semisolid Dosage Form by reverse phase HPLC. C_{18} column (shimadzu C_{18} , 250 x 4.6 mm, 5 µm). The sample was analyzed using acetate buffer and methanol in the ratio of 90:10 (v/v) and glacial acetic acid was added to adjust the pH 3.8 as a mobile phase at a flow rate of 1.0ml/min and detection at 277nm. The retention time for Lamivudine was found to be 6.37 min. The stability assay was performed and was validated for accuracy, precision, linearity, specificity and sensitivity in accordance with ICH guidelines. Validation revealed the method is specific, rapid, precise, reliable, and reproducible. Calibration plots were linear over the 25 to 100 mcg/ml of concentration ranges for drug . The method can be used for estimation of Lamivudine drug in solid dosage form.

Keywords: HPLC method, Estimation of Lamivudine, Lamivudine Tablet.

INTRODUCTION

Lamivudine is a potent nucleoside analog reverse transcriptase inhibitor (nRTI) used as an antiretroviral agent that inhibits replication of some retroviruses in combination with zidovudine in the management of HIV (human immunodeficiency virus). Lamivudine (2',3'-dideoxy-3'-thiacytidine, commonly called 3TC) is a potent nucleoside analog reverse transcriptase inhibitor (nRTI). It is marketed by GlaxoSmithKline with the brand names Zeffix, Heptovir, Epivir, and Epivir-HBV. Lamivudine has been used for treatment of chronic hepatitis B at a lower dose than for treatment of HIV. It improves the seroconversion of e-antigen positive hepatitis B and also improves histology staging of the liver. Long term use of lamivudine unfortunately leads to emergence of a resistant hepatitis B virus (YMDD) mutant. Despite this, lamivudine is still used widely as it is well tolerated. Literature survey revals only few analytical methods that have been developed for its determination of Lamivudine in human plasma has been mainly determined using liquid or gas chromatography with mass spectrometry, following a liquid–liquid extraction. Hence it was thought worthwhile to develop simple spectrophotometric method for the same (Ramana Rao G *et al*., 1986; Snyder LR *et al*., 1997; IP 1996).

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MECHANISM OF ACTION

Lamivudine is a synthetic nucleoside analogue and is phosphorylated intracellularly to its active 5'-triphosphate metabolite, Lamivudine triphosphate (L-TP). This nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination (Beckett AH *et al*., 1997).

MATERIALS AND METHODS

Instrumentation:

An isocratic high performance liquid chromatography (SHIMADZU, HPLC) with LC-10ATVP, HPLC-pump K-501, with soft ware N4000 version 1.7 and UV-Vis detector SPD-10A (SHIMADZU) was used. Column used was C_{18} (250 x 4.6 mm, 5 µm).

Reagents:

Pure drug sample of Lamivudine was received as a gift sample from Hetero drugs Limited Hyderabad, India and was used as such. The water, methanol and ammonium acetate used were of HPLC grade from Qualigens, Mumbai and glacial acetic acid was of analytical grade from Fischer Scientific, Mumbai. The mobile phase consists of acetate buffer and methanol in the ratio of 90:10 (v/v) and glacial acetic acid was added to adjust the pH 3.8.

Preparation of Working Stock Solution of Lamivudine:

About 100 mg of Lamivudine was weighed accurately and dissolved in 100 ml of mobile phase in a 100 ml volumetric flask and diluted up to the mark with mobile phase to get the concentration of 1 mg/ml. From this, a working standard stock solution containing 100 mcg/ml was prepared with mobile phase for RP-HPLC method.

Chromatographic Conditions:

Chromatographic separation was achieved at ambient temperature on a RP- HPLC by using a mobile phase consisting of acetate buffer and methanol in the ratio of 90:10 (v/v) and glacial acetic acid was added to adjust the pH 3.8. The mobile phase was pumped at a rate of 1.0 ml/min. The detector wavelength was set at 277 nm.

Assay Procedure:

Working standard solutions containing 25 to 100 mcg/ml Lamivudine were prepared by appropriate dilution of the stock solution with the mobile phase. Twenty µl aliquot of each solution was injected automatically onto the column five times and the chromatograms (Fig. 2) were recorded. The retention time was found to be 6.37 min. Calibration graph was constructed by plotting the mean peak area as a function of Lamivudine concentration (Fig. 3).

Analysis of Formulation:

Twenty tablets of Lamivudine each containing 100 mg were accurately weighed, average weight was determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 50 mg of Lamivudine was transferred into 50 ml volumetric flask and dissolved in 25 ml of mobile phase and sonicated for 5 mins. The solution was filtered through Whatmann filter paper no.41. The residue was washed with 5 ml portions of mobile phase three times and the total volume of the filtrate was made up to 50 ml with methanol (1 mg/ml). The final concentration was brought 100 mcg/ml with mobile phase. The solution was then analyzed after dilution by RP-HPLC method. This solution was further diluted stepwise with mobile phase in such a way that, various aliquots contain 25 to 100 mcg/ml and was filtered through a 0.45 µm membrane filter. All determinations were conducted five times.

RESULTS & DISCUSSION

The absorption spectra were recorded in the wavelength region of 200-400 nm in UV method. The absorption maxima (λ max) were observed at 270nm for Lamivudine.The quantitative estimation was carried out on formulation by taking a concentration range of 5-25µg/ml for Lamivudine. The quantitative results obtained were subjected to statistical analysis to find out standard deviation and standard error values. The relative standard deviation values are given below 2% indicating the precision of the methodology and low standard error values shown the accuracy of the method. The validation of the proposed method was further conformed by recovery studies.

The percentage recovery values vary from 98 to101% for Lamivudine formulation. This serves as a good index of accuracy and reproducibility of the studies. The results obtained in repeatability test expresses the precision of the method. The percentage recovery greater than 98% shows that the method was free from the interference of excipients used in the formulation.

RECOVERY STUDIES

Results obtained with proposed methods confirm the suitability of these methods for pharmaceutical dosage forms. The other active ingredients and excipients usually present in the pharmaceutical dosage forms did not interfere in the estimation when some commercial dosage forms were analyzed by these methods. The accuracy of the methods is confirmed by the recovery studies.

INTERFERENCE STUDIES

The other active ingredients and excipients present in the dosage forms of Lamivudine in did not interfere, when added in the above concentration range to the drug and estimated by the proposed method.

Table 1. DRUG PROFILE (Sharma BK, 2004; Kasture AV *et al*., 2006; Devala Rao G, 2005; Ravi Sankar S, 2005)

S.NO.	CHARACTER	DETAILS
1.	CHEMICAL FORMULA	$C_8H_{11}N_3O_3S$
2.	PHYSICAL APPEARANCE	It is a white to off-white crystalline powder
3.	MOLECULAR WEIGHT	229.26 g/mol
4.	IUPAC NAME	4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1H-pyrimidin-2-one
5.	SOLUBILITY	Soluble in water
6.	pKa	10.2
7.	MELTING POINT	186-188 ^{o}C
8.	STORAGE CONDITION	Stable under normal conditions. Store in air tight container at room temperature

Table 2. Optimized Chromatographic conditions

Parameter	Optimized condition
Instrument	SHIMADZU-HPLC
Column	(Shimadzu C_{18} 250 X 4.6 Mm, 5 µm Particle Size)
Mobile phase*	Acetate buffer and methanol in the ratio of 90:10 (v/v) and glacial acetic acid was added to adjust the pH 3.8.
Flow rate	1.0ml/min
Detection	277nm
Injection volume	20µl
Temperature	Ambient

*Filtered through a 0.45µ membrane filter (Millipore), degassed and sonicated.

Table 3. Evaluation of Lamivudine in pharmaceutical formulations

Brand used	Label claimed(mg)	Amount found by proposed method(mg)	% label claim	% RSD*
Tab-a	100	99.80	99.80	0.415

*Average of five determinations.

Table 4. Results of recovery studies of Lamivudine estimation by RP-HPLC method

Brand used	Label claimed (mg)	Mean assay value	Known amount of Nevirapine added	Mean % recovery ±%RSD*
Tab-a	100	99.80	10mg	100.14±0.78
			20mg	101.58±0.42

*Average of five determinations.

Table 5. Results of intra day precision studies of Lamivudine estimation by RP-HPLC method

Brand used	Label claimed(mg)	Amount found by proposed method(mg)	% label claim	% RSD*
Tab-a	100	99.76	99.76	0.515

*Average of five determinations.

Table 6. Results of inter day precision studies of Lamivudine estimation by RP-HPLC method

Brand used	Label claimed(mg)	Amount found by proposed method(mg)	% label claim	% RSD*
Tab-a	100	99.62	99.62	0.64

*Average of five determinations.

Table 7. Effect of different pH on chromatogram of Lamivudine

pH	Observation
3.0	Fronting
3.8	Good symmetrical peak
5.0	Asymmetrical peak

Table 8. Effect of flow rate on chromatogram of Lamivudine

Flow Rate (ml/min)	Observation
0.4	Tailing
0.8	Fronting
1.0	Good peak

Fig 1. STRUCTURE OF LAMIVUDINE

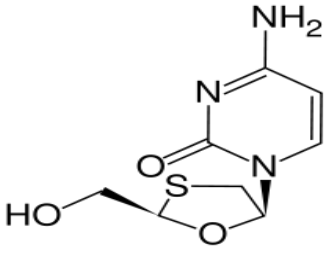


Fig. 2. Chromatogram of Lamivudine in RP-HPLC

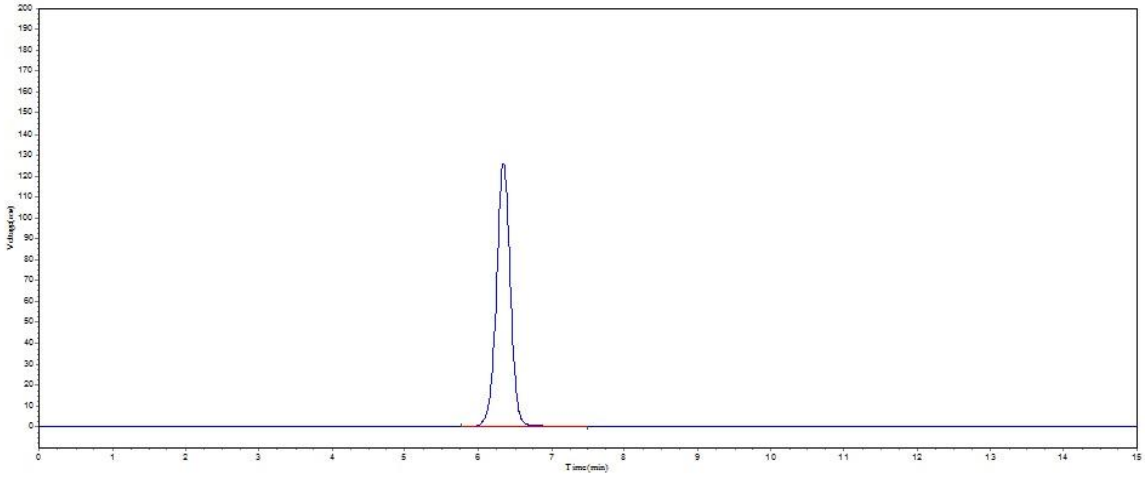
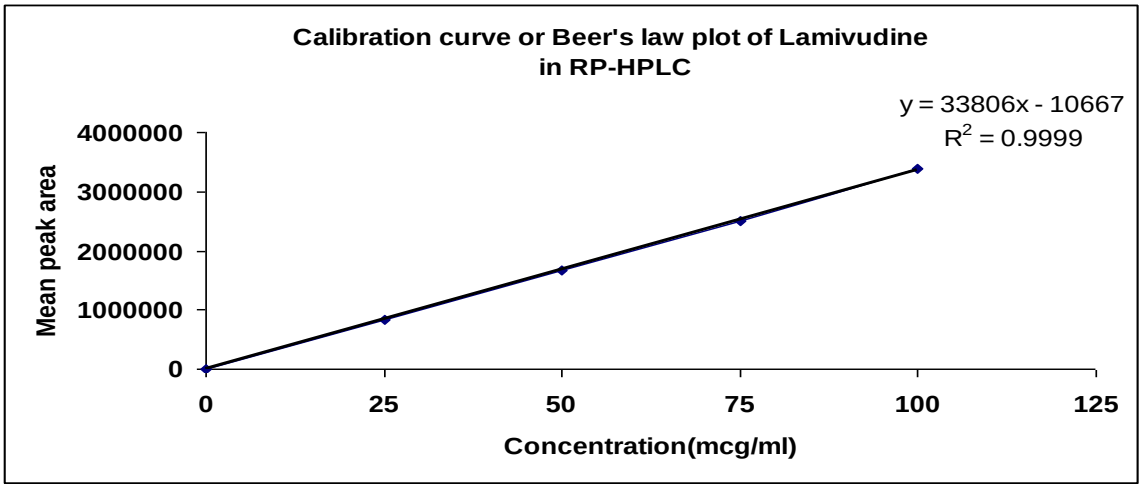


Fig. 3: Calibration curve of Lamivudine in RP-HPLC



CONCLUSION

Hence, the chromatographic method developed for Lamivudine Were Found to be Simple, Precise, Accurate and Cost Effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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