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HPLC METHOD FOR THE ESTIMATION OF LAMIVUDINE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A reverse phase high performance liquid chromatographic (HPLC) method has been developed for the estimation of lamivudine in its tablet dosage forms. The quantification was carried out using a RP C–18 column in isocratic mode, with mobile phase consisting of methanol and water (containing 0.4% triethylamine adjusted to pH 4.5 with 5% orthophosphoric acid) in the raio of 35 : 65 (v/v). Zidovudine was used as internal standard. The detection was carried out at 272 nm and the linearity was found to be in the range of 0.2–30 µg/mL. The proposed method was found to be simple, precise, accurate, less time consuming and reproducible for the estimation of lamivudine in pharmaceutical dosage forms (i.e. tablets).

Key words : HPLC, Lamivudine, Pharmaceutical dosage forms.

INTRODUCTION

Lamivudine[1] (LMD) is an aniti–HIV agent. Chemically, it is (3TC; 2'R,5'S)–1–[2–(hydroxymethyl)–1, 3–(oxathiolan– 5–yl) cytosine]. It belongs to the class of anti–HIV agents called the nucleoside reverse transcriptase inhibitors. LMD is also currently under investigation for the treatment of hepatitis infection. Literature survey reveals that few HPLC[2–5] methods were reported for the estimation of LMD in human plasma. The present investigation has been undertaken to develop a simple method for the estimation of lamivudine in tablet dosage forms.

EXPERIMENTAL

Instrumentation: An isocratic high performance liquid chromatograph (Shimadzu HPLC class VP series) with two LC–AT VP pumps, variable wavelength programmable UV–visible detector SPD–10A VP, CT0–10 AS VP column oven, SCL–10A VP system controller (Shimadzu) and RP C–18 column (250 mm x 4.6 mm i.d; particle size 5 µm) was used.

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Chemicals and reagents: Lamivudine and zidovudine were the gift samples from Cipla Limited, Mumbai. HPLC grade methanol was purchased from E. Merck (India) LTD., Mumbai. All other reagents (orthophosphoric acid and triethylamine) used in the study were of AR quality (Qualigens). Triple distilled water was used.

Chromatographic conditions: The chromatographic column used was a 250 x 4.6 mm Partisil C–18 with 5 µm particles. Both methanol and water (conrtaining 0.4% triethylamine adjusted to pH 4.5 with 5% orthophosphoric acid) were filtered through 0.4 µm membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1 mL/min in the ratio of 35 : 65 (methanol : water consisting of 0.4% triethylamine and pH adjusted to 4.5 with 5% orthophosphoric acid). The column was maintained at 30^{o} C and the pressure was around 165 kgf/cm^2. Detection was carried out by UV detector at 272 nm and the injection volume was 20 µL.

Procedure: About 50 mg of pure sample of LMD was weighed accurately and transferred to a 50 mL volumetric flask and dissolved in 25 mL of the mobile phase. The solution was sonicated for 15 min and then the volume made up with a further quantity of the mobile phase to get 1mg/mL solution. Subsequent dilutions of this solution were made after addition of zidovudine (20 µg/mL) as an internal standard (IS) to get concentrations of 0.2–30 µg/mL of LMD and 2 µg/mL of IS in each dilution. The solutions prepared as above were filtered through 0.4 µm membrane filter and then 20 µL of filtrate was injected five times in to the column at a flow rate of 1mL/min. The ratio of drug peak area to that of internal standard for each of the drug concentration was calculated. The regression of the drug concentration over the ratio of drug peak area to that of internal standard was obtained. This regression equation was used to estimate the amount of LMD in tablet dosage forms.

Estimation of LMD in tablet dosage forms: Two commercial brands of tablets (Lamivir of Cipla and Lamidac of Zydus) were chosen for testing suitability of the proposed method to estimate LMD in tablet dosage forms. Twenty tablets were weighed and powdered, powder equivalent to 100 mg was taken in 100 mL volumetric flask and 50 mL mobile phase was added. The solution was sonicated for complete solubility of the drug, made up to the mark with the mobile phase and filtered through a 0.4 µm membrane filter. From the filtrate, different aliquots were taken in separate 10 mL volumetric flasks. These solutions were spiked with suitable volume of the internal standard solution, such that concentration of the internal standard in each was 2 µg/mL. The contents of the flask were made up to the volume with the mobile phase and mixed well. Each of these solutions (20 µL) was then injected five times in to the column. The mean peak area ratios of the drug to the internal standard of five such determinations were calculated and the drug content in the tablets was quantified using the regression equation obtained from the pure sample.

RESULTS AND DISCUSSION

The present study was carried out to develop a simple, fast, accurate and precise HPLC method for the analysis of LMD in pharmaceutical dosage forms. A typical chromatogram is shown in Fig.1. The retention times for LMD and internal standard (Zidovudine) were 3.2 min

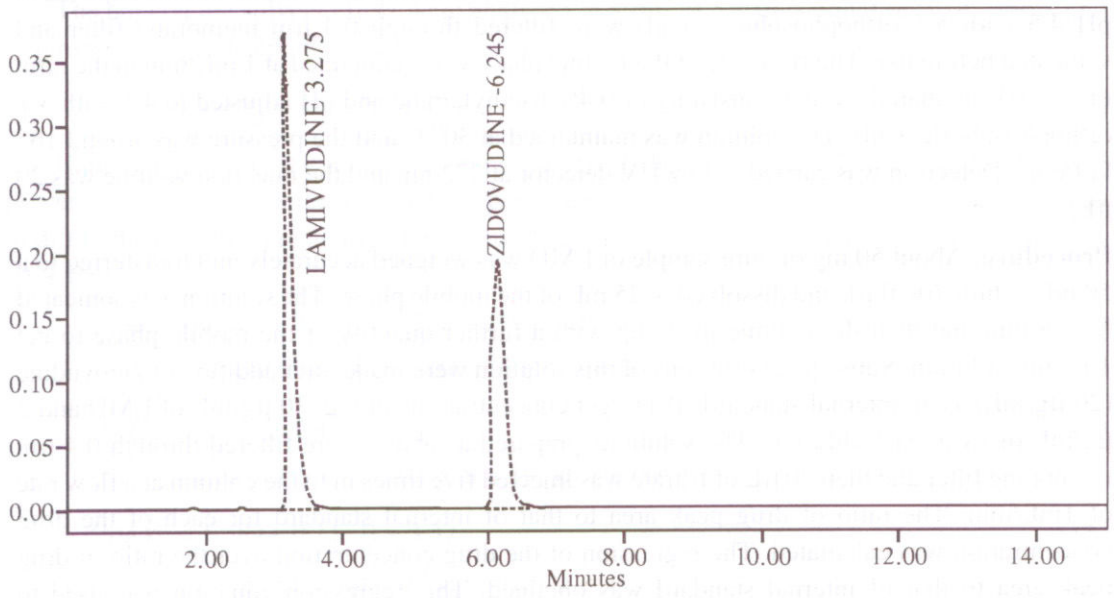

Fig. 1. Model Chromatogram for Lamivudine

and 6.2 min. Each of the samples was injected five times and the same retention times were observed in all cases. The ratio of the peak area of the LMD to peak area of internal standard for different concentrations set up as above were calculated and the average values for five such determinations are shown in Table 1. The peak areas of both the drug and internal standard were reproducible as indicated by low coefficient of variation (1.77).

A good linear relationship (r = 0.9999) was observed between the concentration of the LMD and the respective ratio of peak areas. The calibration equation was found to be Y = – 0.005539 + 0.13080 X (where Y is the ratio of peak area of drug to that of internal standard and X = concentration of LMD). The intra–day and inter–day variations of the method were determined using five replicate injections of three different concentrations, which were prepared and analyzed on the same day and three different days over a period of two weeks, a low coefficient of variation was observed (Table 2). This shows that the present HPLC method is highly precise.

Table 1. Calibration of the proposed method

Drug concentration (μg/mL)	Mean peak area ratio (n = 5)	Coefficient of variance (%) CV
0.2	0.019	1.23
0.5	0.064	0.96
1.0	0.127	0.68
2.0	0.271	0.28
4.0	0.519	1.03
6.0	0.789	1.15
8.0	1.016	0.24
10	1.289	1.53
20	2.611	0.92
30	3.926	1.77

Regression equation (from 0.2 to 30 μg/mL); $Y = -0.00553 + 0.13080\ X$ ($r = 0.9999$)

Table 2. Precision of the proposed method

Concentration of LMD (μg/mL)	Observed concentration of LMD (μg/mL)			
	Intra–day		Inter–day	
	Mean (n = 5)	% CV	Mean (n = 5)	% CV
10	10.06	0.38	9.99	0.53
15	14.98	0.27	14.87	1.22
20	20.01	0.64	19.98	0.32

To ensure the reliability and accuracy of the method, recovery studies were carried out by mixing a known quantity of drug with preanalyzed sample and contents were reanalyzed by the proposed method. The values are shown in Table 3. About 99.8% of LMD could be recovered from the preanalyzed samples indicating the high accuracy of the proposed HPLC method.

Table 3. Results of recovery study

Amount of drug added (μg)	Recovery from drug solution		Recovery from tablet formulation	
	Mean amount found (n = 5)	Mean % recovery	Mean amount found (n = 5)	Mean % recovery
10.0	10.02	100.2	9.98	99.8
20.0	19.97	99.85	20.01	100.05
30.0	29.95	99.84	29.99	99.96

Table 4. Assay of LMD in tablet dosage forms

Brand name of the Tablet	Labeled amount of drug (mg)	Mean (± s.d.) amount (mg) found by the proposed method (n = 5)	Mean (± s.d.) % labeled amount (n = 5)
LAMIVIR	150	149 ± 0.31	99.92 ± 0.15
LAMIDAC	100	99.97 ± 0.01	99.97 ± 0.02

The drug content in the tablets was quantified using the proposed analytical method. The mean amount of LMD in two different brands of tablets dosage forms is shown in Table 4. The absence of additional peaks in the chromatogram indicates the non–interference of the common expecients used in the tablets. The tablets were found to contain 99.92 to 99.97% of the drug. It can be concluded that the proposed HPLC method is sufficiently sensitive and reproducible for the analysis of LMD in pharmaceutical dosage forms within a short analysis time. The method was duly validated by evaluation of the required parameters.

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