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Development and Validation of a HPLC Method for Simultaneous Estimation of Lamivudine and Zidovudine in Tablet Dosage Forms

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ABSTRACT

A simple, precise and accurate HPLC method has been developed for the simultaneous estimation of lamivudine and zidovudine in tablet formulations. The chromatographic separation was achieved on a Hypersil SS C18 (250 mm × 4.6 mm, 5µm) analytical column. Methanol was used as the mobile phase, at a flow rate of 1 ml/min and detector wavelength at 270 nm. The validation of the method was accomplished for specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness. The linear dynamic ranges were from 0.1–2.0µg/ml for lamivudine and zidovudine. The percentage recovery obtained for lamivudine and zidovudine were 100.36 and 100.46 %, respectively. Limit of detection and quantification for lamivudine were 0.042 and 0.12 µg/ml, for zidovudine 0.039 and 0.12 µg/ml, respectively. The developed method can be used for routine quality control analysis of lamivudine and zidovudine in combination in tablet formulation.

Key words: Lamivudine, zidovudine, HPLC, validation, formulation.

INTRODUCTION

Lamivudine and zidovudine are synthetic nucleoside analogues with activity against human immunodeficiency virus (HIV) [1] and form one of the first line regimens in HIV treatment as fixed dose combination [2]. Fixed dose combinations (FDCs) become the mainstay in clinical management of HIV-1 infection as they offer several advantages over single products with respect to storage, prescribing, dispensing, patient use, consumption and disease management. Lamivudine was initially developed for the treatment of HIV infection [3, 4]. The chemical name

of lamivudine is (2R, cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one. Lamivudine is the (−) enantiomer of 2□- deoxy-3□-thiacytidine, which is a nucleoside analog. The (−) enantiomer of the racemic mixture shows much less cytotoxicity than the positive enantiomer. Lamivudine (Figure 1) has very low cellular cytotoxicity and generally less potent than zidovudine (Figure 2) in inhibiting HIV-1 and HIV-2 replication in vitro [1, 5, 6]. It is rapidly absorbed with bioavailability of approximately 80%.

Zidovudine is also a nucleoside analogue which is structurally similar to thymidine and used in the management of AIDS and AIDS-related complex [6]. It may be given to patients with early HIV infection or to symptomatic patients. In patients with HIV-1 infected MT-4 cells, lamivudine in combination with zidovudine had synergistic antiretroviral activity [4, 5]. It is rapidly absorbed from the gastro-intestinal tract with a bioavailability of about 60–70%. It crosses the blood–brain barrier. The plasma half-life is about 1 h [1, 5, 7].

There have been several high-performance liquid chromatographic (HPLC) method reports of the determination of zidovudine in biological samples and tablet dosage forms. Very few methods appeared in the literature for the determination of lamivudine based on HPLC in biological fluids [3]. Only three HPLC-tandem mass spectrometric methods have been described in the literature for the simultaneous determination of lamivudine and zidovudine in biological samples as well as tablet dosage forms [8, 9]. But these methods are very costly. Therefore in this present study a simple method was developed and validated for simultaneous determination of lamivudine and zidovudine in tablet dosage forms.

MATERIALS AND METHODS

Chemicals

Lamivudine (99.69 %) and Zidovudine (99.58%) were obtained from GlaxoSmithKline, India. Dipotassium hydrogen phosphates (AR Grade), acetonitrile (HPLC Grade) were purchased from E. Merck (India) Ltd. Worli, Mumbai, India. The 0.45 µm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd. Chandigarh, India. Double distilled water was used throughout the experiment. In house film coated tablets containing Lamivudine 150 mg and Zidovudine 300 mg per tablet, were used for the study.

Equipments

Analysis was performed on a chromatographic system of Waters, Alliance System, UV/VIS Detector (Waters 2487), System Software (Waters Empower). Another chromatographic instrument of Shimadzu LC -2010, CHT UV/VIS Detector, System Software (LC Solutions) was used in this present study. Chromatographic separation was achieved on Hypersil SS C18 (250 mm × 4.6 mm, 5µm) analytical column. Data acquisition was made with Empower software. The peak purity was checked with the photodiode array detector.

Standard solutions and calibration graphs

Weighed and transferred accurately about 30.0 mg of Lamivudine working standard and about 60.0 mg of Zidovudine working standard to a 100 mL volumetric flask. Dissolved in 10 ml of methanol and make up the volume with diluent and mixed. The diluent was a mixture of methanol and water (50:50, v/v). To study the linearity range of each component, serial

dilutions were made by adding this standard stock solution in the different weights of Lamivudine in the range of 0.1–2.0µg/ml of Lamivudine and 0.1–2.0µg/ml of Zidovudine. A graph was plotted as concentration of drugs versus peak area response. A mixed standard solution was prepared for the system suitability test. For the same, about 75.0 mg of Lamivudine working standard and about 150.0 mg of Zidovudine working standard were weighed and transferred accurately to a 50 mL volumetric flask and then added 25 mL of methanol and sonicated to dissolve. The system suitability test was performed from five replicate injections of mixed standard solution.

Sample preparation

Sample was prepared, by selecting twenty tablets randomly and weighed and finely powdered. The average weight of the tablets was determined from the weight of 20 tablets. From the prepared sample, a portion of powder equivalent to the weight of one tablet was accurately weighed into 200 ml volumetric flask and 20 ml diluent was added to it. The volumetric flask was sonicated for 15 min with intermittent shaking for complete dissolution; the solution was then made up to volume with diluent. The resulting solution was then filtered through 0.45 µm membrane filter (Vinyl type). The first 2 mL of the filtrate was discarded, then 5 ml of the filtrate was diluted to 25 ml with diluent. Further 5 ml of the resulting solution was diluted to 50 ml with diluent and mixed. Finally, 20 microlitres of the prepared test solution was injected and chromatogram was recorded for the same, and the amounts of the drugs were calculated.

Method validation

The HPLC method was validated according to ICH guidelines in terms of precision, accuracy and linearity [10]. Six independent test solutions were taken for determination of assay method precision. Evaluation of the accuracy of the assay method was done with the recovery of the standards. The LOD and LOQ for analyte were estimated by injecting a series of dilute solutions with known concentration. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

Peak tailing is one of the well known problems in pharmaceutical industry during the analysis of basic drugs like Zidovudine. This is so because basic compounds strongly interact with polar ends of HPLC column packing materials, causing severe peak asymmetry and low separation efficiencies. The problem could be overcome to a great extent by using high purity silica backbone and advancement in bonding technology. The present method was optimized by testing different columns and two organic solvents (acetonitrile and methanol). The chromatographic conditions were also optimized by using different buffers like phosphate, acetate and citrate for mobile phase preparation. After a series of screening experiments, it was concluded that phosphate buffers gave better peak shapes than their acetate and citrate counterparts. With acetonitrile as solvent both the peaks shows less theoretical plates and more retention time compared to methanol. The chromatographic separation was achieved on a Hypersil SS C18 (250 mm × 4.6 mm, 5µm) analytical column, by using a mixture of methanol–phosphate buffer (30mM) (65:35, v/v) as mobile phase. For the mobile phase, methanol was considered as organic solvent instead of acetonitrile. In addition to this, pH was adjusted to 2.5 ± 0.05.

Validation of method

Specificity

Figure 3 shows the specificity of the HPLC method which illustrates the complete separation of Lamivudine and Zidovudine in presence of tablet excipients. There were no interferences at the retention time of Lamivudine and Zidovudine in the chromatogram of the placebo solution. The peak purity was analyzed with photo diode detector and purity angle was less than purity threshold for both the analyte. This clearly indicates that the peak of analyte was pure and excipients in the formulation did not interfere the analyte.

Accuracy

Accuracy of the method was calculated by recovery studies at three levels by standard addition method (Table 1). The mean percentage recoveries obtained for Lamivudine and Zidovudine were 100.36 and 100.46 %, respectively

Precision

The precision of an analytical procedure may be defined as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The system precision is a measure of the method variability that can be expected for a given analyst performing the analysis and was determined by performing five replicate analyses of the same working solution. The relative standard deviation (R.S.D.) obtained for Lamivudine and Zidovudine was 0.2 and 0.3 %, respectively (Table 2). The intra- and inter-day variability or precision data are given in Table 3. The intra-day precision of the developed HPLC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each. In order to evaluate the method precision, the R.S.D. of the assay results (expressed as a percentage of the label claim) was used. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days. The results clearly indicated a good precision of the developed method (Table 2).

Linearity

Linearity of Lamivudine and Zidovudine were in the range of 0.1–2.0 µg/ml; and, 0.1–2.0 µg/ml respectively. The correlation coefficient ('r^2') values for both the drugs were >0.99. Typically, the regression equation for the calibration curve was found to be y = 18691.93x + 1138.41 for Lamivudine and y = 14550.02x + 2177.59 for Zidovudine.

Limit of detection (LOD) and limit of quantization (LOQ)

Calibration curve method was used for the determination of LOD and LOQ of Lamivudine and Zidovudine [21]. Solutions of both Lamivudine and Zidovudine were prepared in the range of 0.1–2.0 µg/ml and 0.1–2.0 µg/ml respectively and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations.

$$LOD = \frac{3.3 \times \sigma}{b}$$

$$LOQ = \frac{10 \times \sigma}{b}$$

Where σ is standard deviation; *b* is slope. LOD and LOQ for Lamivudine were 0.042 and 0.12 µg/ml respectively and for Zidovudine were 0.039 and 0.12 µg/ml, respectively.

Figure 1. Structure of Lamivudine

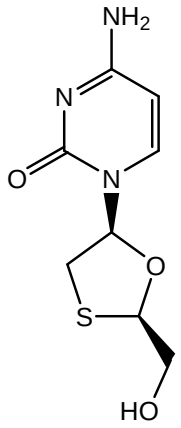


Figure 2. Structure of Zidovudine

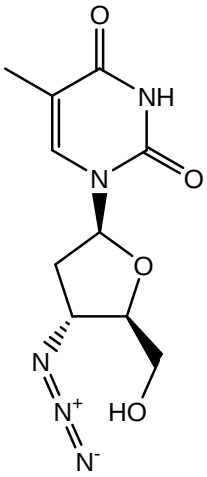
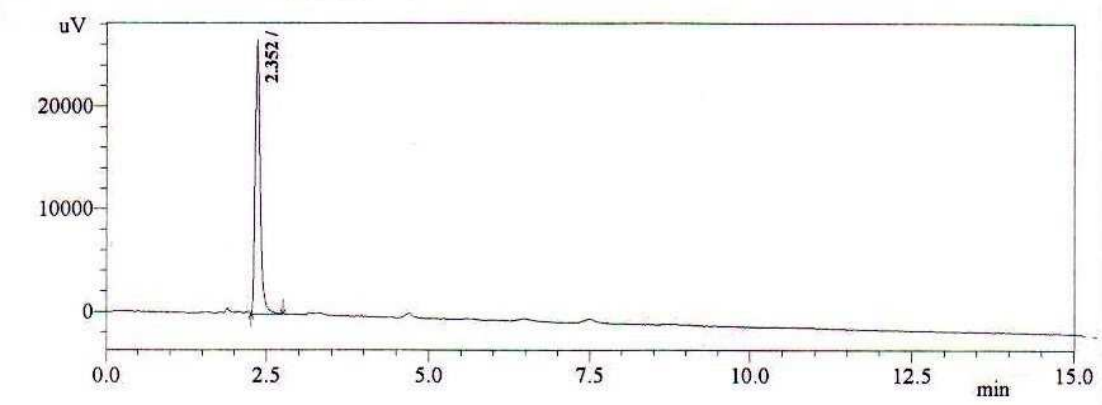
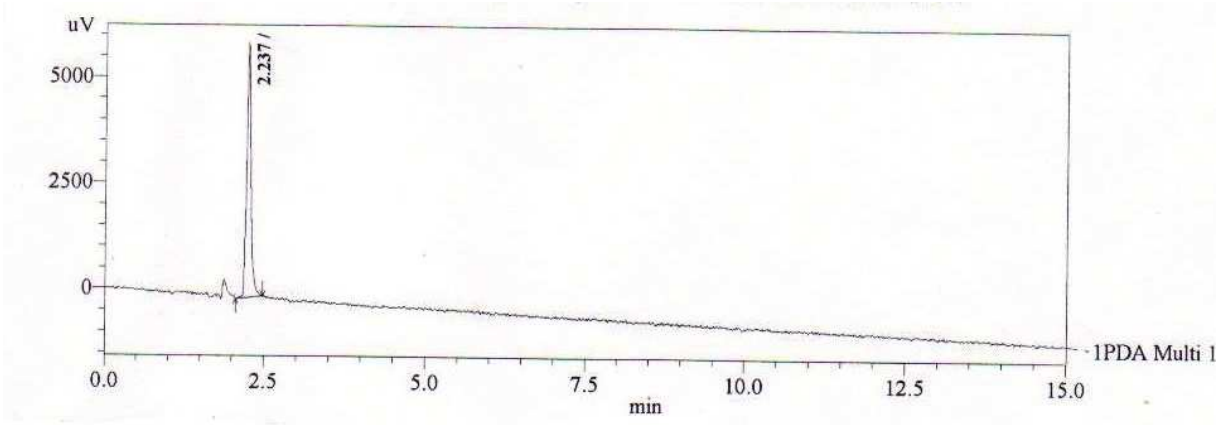


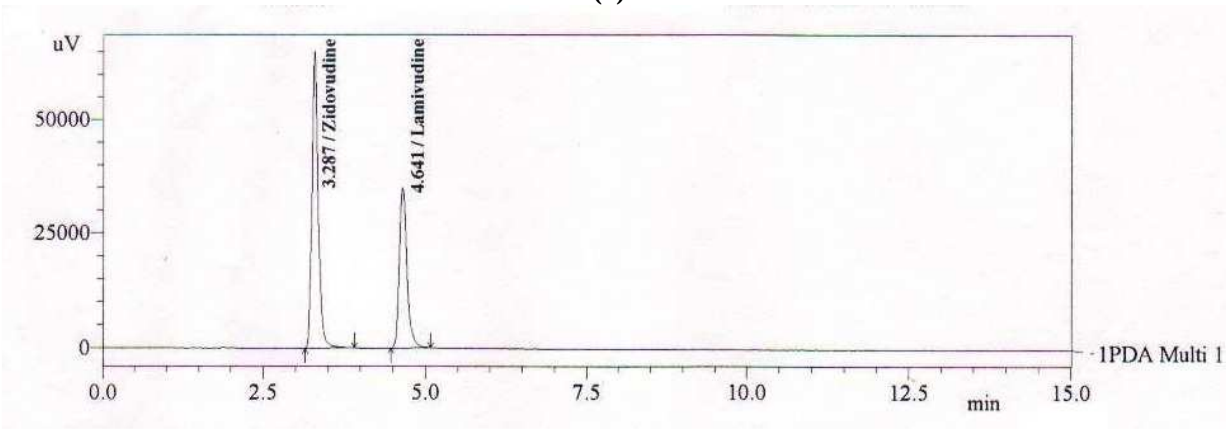
Figure 3. A typical chromatogram of (a) Blank, (b) Placebo, (c) Standard and (d) Test solution



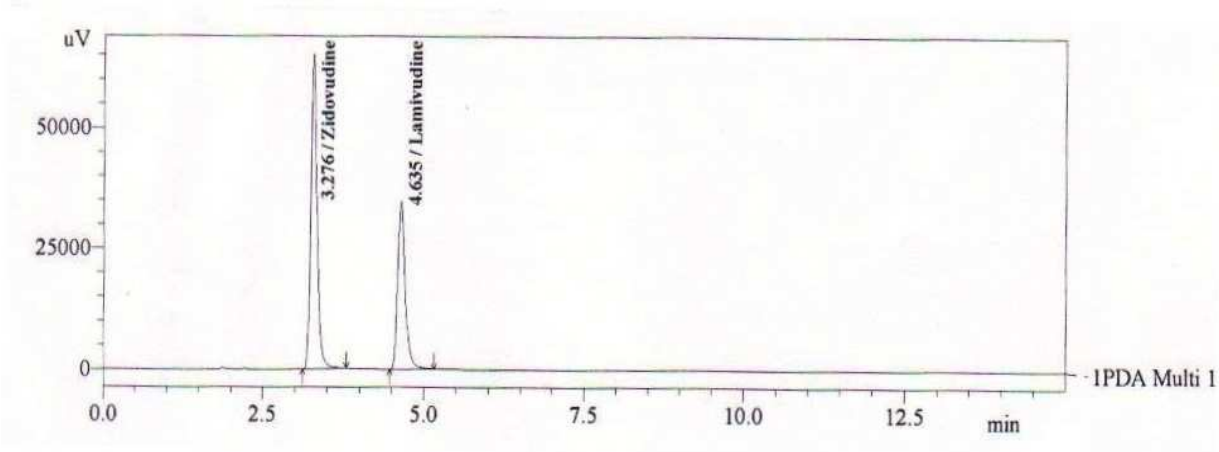
(a)



(b)



(c)



(d)

Robustness

The robustness of an analytical procedure gives its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the proposed assay method was studied by analyzing aliquot of a homogenous test sample by deliberately changing the parameters of the method. The results obtained with each parameter are shown below. The degree of reproducibility of the results

obtained as a result of small deliberate variations in the method parameters has proven that the method is robust (Table 3).

Table 1. Results of the recovery analysis of Lamivudine and Zidovudine

Compound	Wt spiked	Wt recovered	Recovery (%)	R.S.D. (%)
Lamivudine	83.04	84.17	100.6	0.95
	150.63	153.30	101.8	1.88
	222.59	219.58	98.7	0.91
Zidovudine	166.39	168.84	101.5	0.92
	303.56	305.84	100.7	1.33
	443.76	440.10	99.2	0.50

R.S.D.: Relative standard deviation. Wt: weight

Table 2. Results of precision study

Actual concentration	Measured concentration (µg/ml), R.S.D. (%)	
	Intra-day	Inter-day
Lamivudine (µg/ml)		
50	50.12, 0.22	50.03, 1.24
140	140.36, 0.51	140.42, 0.88
200	200.24, 0.48	200.37, 1.37
Zidovudine (µg/ml)		
50	50.48, 0.68	50.11, 1.03
140	140.84, 0.27	140.38, 1.54
200	200.41, 0.78	200.17, 0.96

Data expressed as mean for 'measured concentration' values.

Table 3. Results of robustness study

Factor	Level	Mean Area		% Variation of results	
		Lamivudine	Zidovudine	Lamivudine	Zidovudine
Filter type	Vinyl Type	264048	413680	0.36	0.340
	Disc Type	264757	414583	0.63	0.56
Stability of solution	0 hour	35629206	32126830	-	-
	6 hour	35625966	32912880	0.01	2.45
	12 hour	35362340	33027792	0.75	2.80

CONCLUSION

The HPLC method that has been developed and validated for quantitative determination of Lamivudine and Zidovudine in a new tablet formulation is found to be simple, specific, linear, precise, and accurate. The method can be regarded as simple and specific as both peaks are well separated from its impurities and excipients peaks with total runtime of 20 min. Therefore the method can be successfully used for routine quality control analysis work.

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