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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND ZIDOVUDINE IN API AND PHARMACEUTICAL DOSAGE FORM USING RP-HPLC

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ABSTRACT
A rapid, sensitive and specific RP-HPLC [1-5] method involving UV detection was developed and validated for determination and quantification of Lamivudine and Zidovudine. Chromatography was carried out on Thermo Hypersil BDS, C18,(150 x 4..6 mm,5µ) column using filtered and degassed mixture of Buffer : Methanol : Acetonitrile (70:5:25) as mobile phase at a flow rate of 0.8ml/min and effluent was monitored at 267nm. The method was validated in terms of linearity, precision, accuracy, robustness and specificity, limit of quantification and limit of detection. The assay was linear over the concentration range of Lamivudine and Zidovudine was 37.5µg - 225µg/ml and 75µg to 450µg/ml respectively. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the pre analyzed test solution and was found to be 99.50%-100.7% and 99.9%-100.7% within precision RSD of 1.30 and 0.61 for Lamivudine and Zidovudine respectively. The method does require only 10 minutes as run time for analysis which prove the adoptability of the method for the routine quality control of the drug.

Key words: Lamivudine, Zidovudine, Method development, Validation.

INTRODUCTION

Lamivudine is chemically 1[(2R,5S)-2-(Hydroxy methyl)- 1-3 oxathiolan-5yl] cytosine and used as an antiretroviral activity [6,7]. Lamivudine is an analogue of cytidine. It can inhibit Both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B. It needs to be phosphorylated to its triphosphate form before it is active. 3TC triphosphate also inhibits cellular DNA polymerase. Zidovudine is chemically 1-[(2R,4S,5S)- 4azido-5-(hydroxymethyl)tetrahydrofuran-2-yl]- 5-methylprimidine-2,4(1H,3H0-dione and used as an antiretroviral activity [6,7]. There is a plethora of analysis of such formulations without prior separation. For the estimation of multi - component formulation, the instrumental techniques, which are commonly employed, are spectrophotometry, GLC, high performance thin layer chromatography (HPTLC), HPLC etc. These methods are based upon the measurement of specific and nonspecific physical properties of the substances. The literature survey [8-14] reveals that there are some HPLC methods have been reported. But the present study is to develop an accurate and reliable HPLC method for simultaneous estimation of Lamivudine and Zidovudine in solid dosage form. In this paper we describe a simple, inexpensive, sensitive and validated HPLC method for the simultaneous determination of Lamivudine and Zidovudine in pharmaceutical formulation.

MATERIALS AND METHODS

Experimental work: Working standards of Lamivudine and Zidovudine were obtained from well reputed research laboratories. HPLC grade Methanol, Potassium di hydrogen ortho phosphate and Milli-Q

water were procured from the market. The separation was carried out on isocratic HPLC system (WATERS 2695) with Thermo Hypersil BDS, C18,(150 x 4..6 mm,5µ) column using filtered and degassed mixture of Buffer : Methanol : Acetonitrile(70:5:25) as mobile phase.

Standard preparation: Accurately Weighed and transferred 10mg of Lamivudine and 10mg of Zidovudine working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent , sonicated for 5 minutes and make up to the final volume with diluents.(standard stock Lamivudine(1000 ppm) Zidovudine (1000ppm).

Chromatographic conditions: Flow rate 0.8ml/min; Detection wavelength 267nm; Injection volume 10µl; Column used Thermo Hypersil BDS, C18, (150 x 4..6 mm,5µ); Column temperature: 25◦C; mobile phase: Buffer : Methanol : Acetonitrile(70:5:25)

Method development: Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

Assay preparation for commercial formulation: 5 tablets were weighed and crushed into powder, in order to calculate the average weight of each tablet. From that powder weight equivalent to 10mg of Lamivudine and 10mg of Zidovudine were transferred into a 500 mL volumetric flask, 400mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Label Claim: 150mg Lamivudine + 300mg of Zidovudine

Procedure: 10µl of the standard preparation and assay preparation were separately injected and chromatographed.

Validation of method

Accuracy: Accuracy of the method was calculated by recovery studies at three levels by standard addition method (Table 1and 2). The mean percentage recovery for lamivudine and Zidovudine was found to be between 98.95-102.3%, 98.94-102.66 % and 98.98-101.66 % respectively, which are well within the limit and hence the method was found to be accurate.

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 0.1 and 0.2 %, respectively (Table 3). The intra- 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 results clearly indicated a good precision of the developed method.

Linearity: Linearity of Lamivudine and Zidovudine were in the range of 7.5–225 µg/ml; and, 75–450 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 = 14583x + 13947 for Lamivudine and y = 6715x + 5446 for Zidovudine.

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.

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 .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. Where σ is standard deviation; b is slope. LOD and LOQ for Lamivudine were 3.15 and 9.56µg/ml respectively and for Zidovudine were 2.67 and 8.11 µg/ml, respectively.

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.

RESULTS AND DISCUSSION

The Response of Lamivudine and Zidovudine shown is linear from 37.5mcg - 22.5mcg/ml and 75mcg to 450mcg/ml respectively. Coefficient of correlation was 0.9998 and 0.9999.Selectivity experiment showed that there is no interference or overlapping of the peaks either due to excipients or diluents with the main peak of Lamivudine and Zidovudine. The percentage RSD for precision is <2 which confirms that method is sufficiently precise and the total run time required for the method is only 20mins for eluting both Lamivudine and Zidovudine. The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control of Lamivudine and Zidovudine**.**

CONCLUSION

Simple, rapid, reproducible and economic UV spectroscopic determination, analytical method development and validated as per ICH guidelines and USP2000 for two anti-retroviral drugs *viz.*, for Lamivudine and zidovudine. The solvent blends viz., water: ACN (70:30), Buffer: Methanol: Acetonitrile (70:5:25) were used for Lamivudine and zidovudine in bulk and formulations to develop the analytical method. The developed methods were further validated for accuracy, precision, specificity, linearity, robustness, LOD and LOQ with statistical data.

TABLE 1: RESULTS OF RECOVERY STUDIES FOR LAMIVUDINE

% Level	Concentration taken	Recovery (%)	Mean recovery(%)	% RSD
50%	37.5µg/ml	99.74	99.58	0.23
		99.67		
		99.32		
100%	150µg/ml	101.59	100.67	0.83
		100.48		
		99.95		
150%	225µg/ml	99.94	99.74	0.41
		100.01		
		99.26		

TABLE 2: RESULTS OF RECOVERY STUDIES FOR ZIDOVUDINE

% Level	Concentration taken	Recovery(%)	Mean recovery(%)	% RSD
50%	75µg/ml	100.55	100.70	0.32
		100.47		
		101.07		
100%	300µg/ml	98.28	100.44	1.05
		99.91		
		100.24		
150%	450µg/ml	99.51	100.67	1.54
		102.43		
		100.07		

TABLE 3: Linearity report of Lamivudine and Zidovudine:

Parameters	Results(n=6)	
	lamivudine	**zidovudine**
Linearity range	37.5-225 g/ml	75-450g/ml
Slope	14583	6715
Intercept	13947	5446
Correlation coefficient	0.999	0.999

Table 4: RESULTS OF PRECISION

Sample No	Sample (LAM)	%Assay	Sample(ZID)	%Assay
1.	2097415	98.87	1984279	100.67
2.	2097172	98.86	1992624	101.10
3	2121106	99.99	1986962	100.81
4	2149829	101.34	1987831	100.86
5	2159829	101.81	1959311	99.41
6	2149371	101.32	1975166	100.21
AVG	2129120	100.36	1981029	100.51
S.D	27828.45	1.3118	12103.89	0.61411
% RSD	1.30704	1.31	0.61099	0.61

Figure 1: Structure of Lamivudine

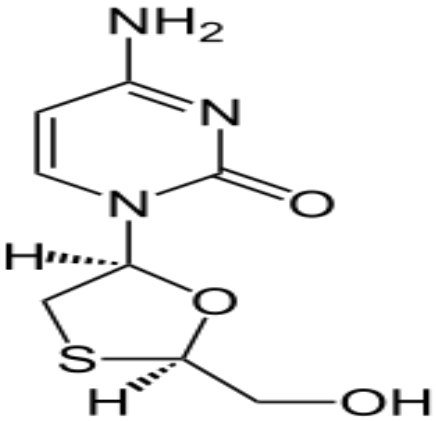


Figure 2. Structure of Zidovudine

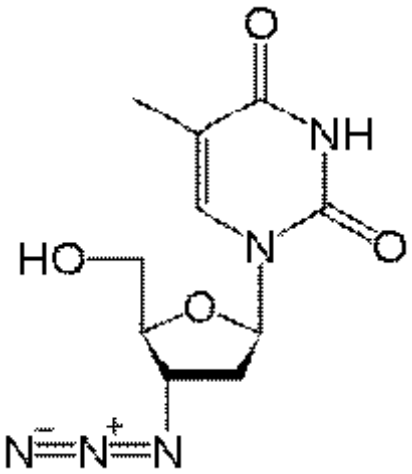
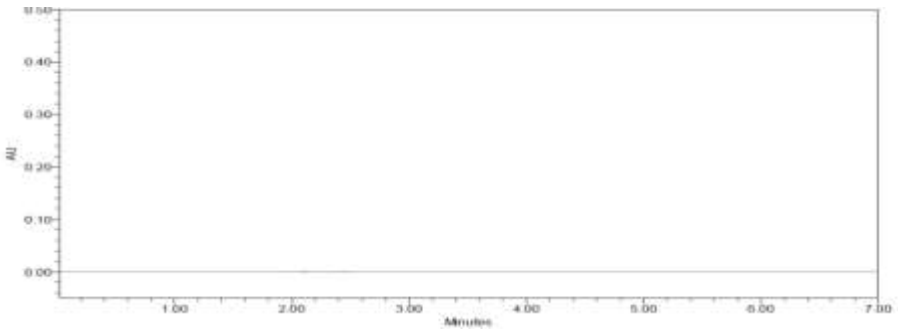
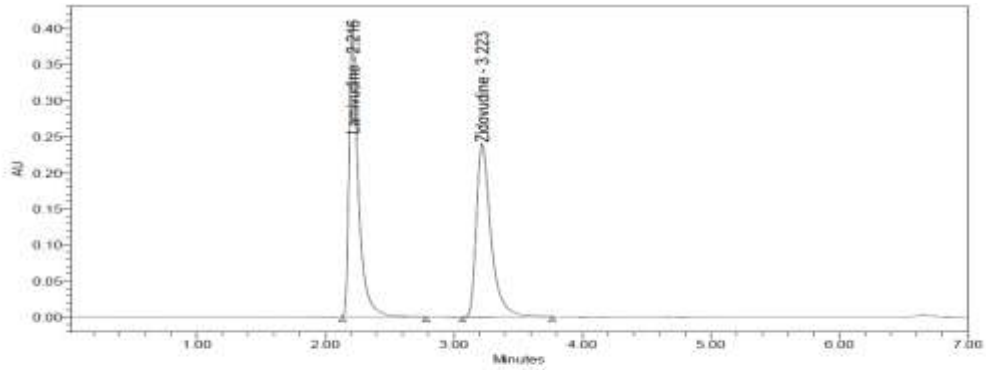


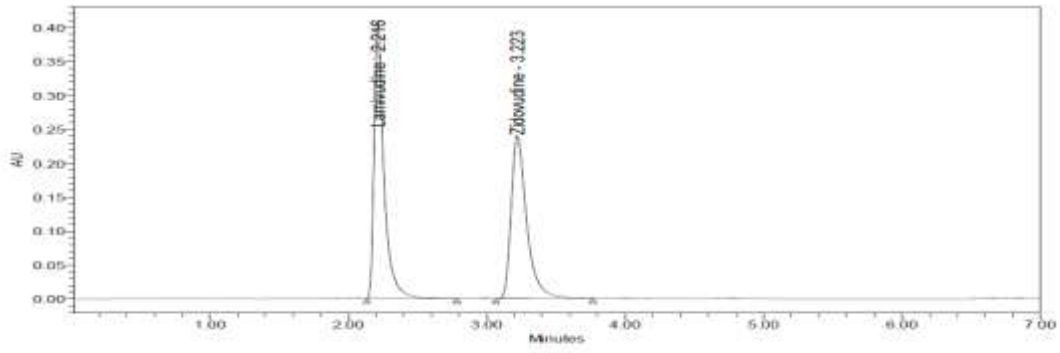
Figure 3. A typical chromatogram of (a) Placebo, (b) Standard and (c)Test solution



(a)



(b)



(c)

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