International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.1, pp 311-314, Jan-Mar 2012

Validated Spectrophotometric Method for Simultaneous estimation of Zidovudine and Lamivudine in Combined Pharmaceutical dosage form

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Abstract: A simple, accurate, precise and economical procedure for simultaneous estimation of zidovudine and lamivudine in combined tablet dosage form has been developed utilizing concept of Dual WaveLength method. This method involves selection of two wavelengths at which the drug has same absorbance. The method is based upon determination of Zidovudine at 272 and 287.2nm and Lamivudine at 256nm and 275.2nm, in 0.1N HCl. Different analytical performance parameters such as linearity, precision, accuracy, LOD, LOQ and assay were determined according to ICH guidelines. The method was found linear between the range of 50-425µg/mL for Zidovudine and 50-275µg/mL for Lamivudine. The results of formulation given as percentage of label claim were found to be 100.73±0.56 and 101.86±0.68 for zidovudine and lamivudine respectively. Therefore, the proposed methods can be used for the routine analysis of both drugs in quality control laboratories.
Keywords: Zidovudine, Lamivudine, Dual wavelength method.

Introduction

The combination of Zidovudine and Lamivudine are used as anti retroviral agents. Both Zidovudine and Lamivudine are reverse transcriptase inhibitors1, and are chemically 3'-azido-3'-deoxythymidine2 and 2',3'-dideoxy-3'-thia-cytidine3 respectively Methods for the simultaneous determination of lamivudine and zidovudine in biological samples and in pharmaceutical preparations were also reported[1-4]. There is, however, no work reported on combination of these drugs by UV – Spectrophotometric methods. Hence, in the present communication we propose fast, simple, and accurate spectrophotometric method, for the simultaneous estimation of both the drugs in tablet dosage form by Dual Wave Length method.

Experimental

Apparatus:
A Jasco V650 double beam UV-Visible spectrophotometer equipped with 10mm matched quartz cells was used in the present study. Software

used is Spectra manager. A LC-GC AX-220 single pan balance was used.

Chemicals and Reagents.
0.1N HCl (AR grade) and double distilled water was used in the present study. Pure Zidovudine and Lamivudine were obtained as gift sample from Hetero Drugs Pvt. Ltd., Hyderabad; the tablet dosage form COMBIVIR (claim: 300mg Zidovudine and 150mg Lamivudine) was procured from the local market.

Preparation of Standard Stock Solutions:
Standard stock solution containing Zidovudine and Lamivudine was prepared by dissolving 10mg of Zidovudine and Lamivudine separately in 10mL of 0.1N HCl. . These solutions were scanned in the range of 200-400nm in 1cm cell against blank. From the overlain spectra shown in Fig-1, the wavelength selected for the estimation are 256nm and 275.2nm for lamivudine and 272 and 287.2nm for Zidovudine respectively. In this method, Zidovudine was determined by plotting the difference in absorbance at 272nm and 287.2nm (difference is zero for lamivudine) against the concentration of Zidovudine. Similarly for the determination of lamivudine, the difference in absorbance at 256nm and 275.2 nm (difference is zero for Zidovudine) was plotted against the concentration of Lamivudine. The aliquot portions of stock solutions of Zidovudine and Lamivudine were diluted appropriately with 0.1N HCl to obtain concentration of 50µg/mL of Zidovudine and 50µg/mL of lamivudine. The difference in absorbances at 272nm and 287.2nm were plotted against the concentration of Zidovudine and that at 256nm and 275.2nm were plotted against the concentration of Lamivudine to construct two separate calibration curves for Lamivudine and Zidovudine. The method shows good linearity in the concentration range for both Lamivudine and Zidovudine. The concentration of two drugs in mixture was calculated by using the calibration curve equation given in table 2.

Preparation of Synthetic Mixture of Zidovudine and Lamivudine
The standard solutions of Lamivudine were prepared in the concentration range of 50 µg/mL to 275 µg/mL, and that of Zidovudine were prepared in the range of 50 µg/mL to 425 µg/mL in 0.1N HCl. All the mixed standard solutions were scanned over the range of 200-400nm; using two sampling wavelengths 256nm and 275.2nm for Lamivudine and 272 and 287.2nm for Zidovudine respectively. The spectral data from these scans were used to determine the concentration of these drugs in tablet formulation.

Procedure for Analysis of Tablet Formulation
Marketed tablet formulation COMBIVIR containing 300mg Zidovudine and 150mg Lamivudine were analyzed by this method. From the triturated powder of 20 tablets, an amount equivalent to an average weight of the tablet was accurately weighed, transferred to 100ml volumetric flask and sonicated with 0.1 N HCl for 15minutes and filtered through Whatman filter paper . Filtrate was appropriately diluted to get concentration of 75µg/mL of lamivudine and 150µg/mL of Zidovudine. The sample was analyzed in triplicate by Dual Wavelength Method.

Validation of the Method
The following validation parameters; linearity, range, accuracy, precision, LOD and LOQ were studied as per ICH guidelines [5-6]. The accuracy of the method was ascertained by carrying out recovery studies using Dual wave length method. The recovery study was performed to determine if there was any positive or negative interference from excipients present in the formulation. The precision of an analytical method is expressed as standard deviation and relative standard deviation of a series of measurements. It was ascertained by triplicate estimation of drug by the proposed method. LOD and LOQ were calculated by using the formula 3.3S.D/S and 10S.D/S where S.D is the standard deviation of Y-intercept and S is the slope of the calibration curve.

Table-1: Validation Parameters

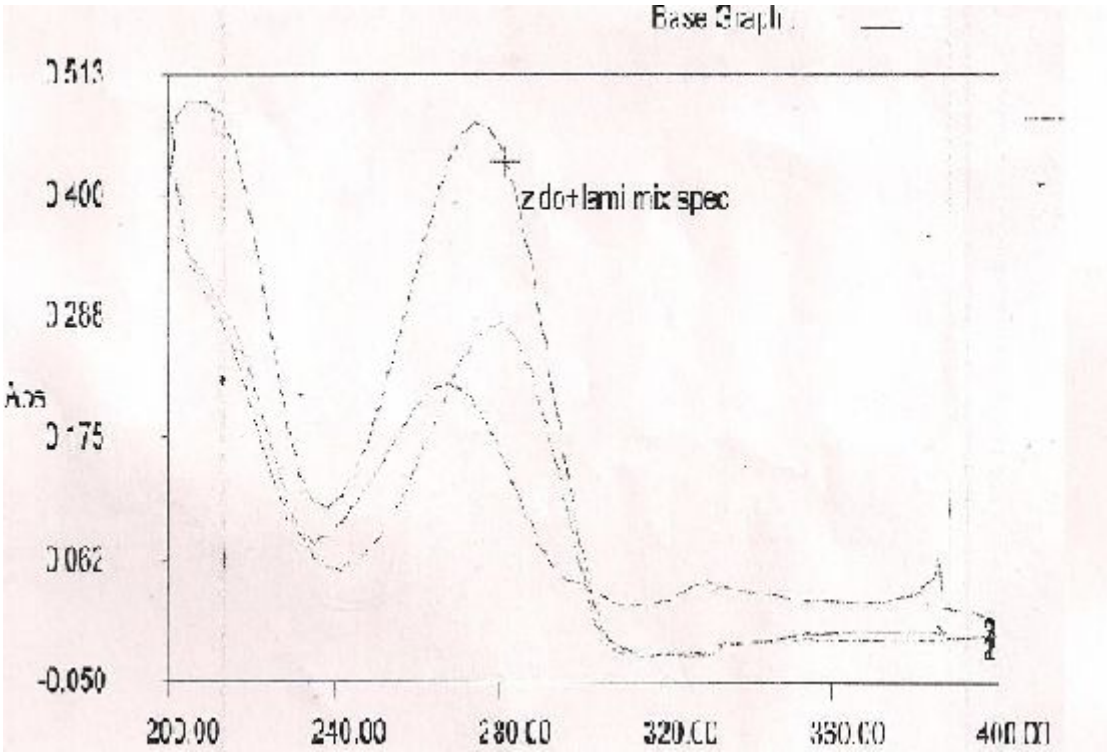
Parameters	Lamivudine	Zidovudine
Beer's law range	50-275 µg/mL	50-425 µg/mL
Wavelength	256nm and 275.2nm	272 and 287.2nm
Correlation Coefficient	0.998	0.999
Linearity Equation y = mx+c		
Slope	0.002	0.002
Intercept	0.017	0.001
LOD	15.74 µg/mL	5.79 µg/mL
LOQ	47.69 µg/mL	17.56 µg/mL

Table-2: Accuracy

Sample Name	Amount present in µg/mL	Amount added in µg/mL	Amount found in µg/mL ±S.D.	%RSD	%Recovery
Zidovudine	150	120	267.5±0.24	0.089	99.07
	150	150	302.8±0.91	0.3	100.93
	150	180	332.1±0.86	0.258	100.63
Lamivudine	75	60	134.1±1.30	0'96	99.33
	75	75	148.1±0.64	0.43	98.73
	75	90	166.2±0.79	0.47	100.72

Table-3: Precision

Drug	Amount added in µg/mL	Amount found±SD;%RSD	
		Intra day	Inter day
Zidovudine	100	98.20±0.45;0.45	99.10±0.63;0.63
	200	201.9±0.84;0.41	202.8±0.38;0.18
	300	302.8±0.77;0.25	301.2±0.58;0.19
Lamivudine	75	74.20±0.87;1.17	74.30±0.427;0.57
	100	102.0±0.55;0.53	102.32±0.63;0.61
	125	302.8±0.45;0.35	126.92±0.45;0.35



Results and Discussion

The proposed method for simultaneous estimation of Zidovudine and Lamivudine was validated as per the ICH guidelines. The Linearity was observed in the concentration range 50-425µg/mL Zidovudine and 50-275µg/mL lamivudine with regression coefficient of 0.999 and 0.998 respectively. Amount of drugs estimated by the proposed method was in good agreement with the label claim. The accuracy of the method was assessed by recovery experiments. Recovery experiment indicated the absence of interference from commonly encountered pharmaceutical additives (Table 2). The precision of the method was studied as repeatability, intra-day and inter-day variations; the % RSD less than 2, indicates proposed method is precise. Recovery was close to 100% for both the drugs.

Conclusion

The present study comprises a UV-spectroscopic, dual wave length method of analysis for the simultaneous determination of Zidovudine and Lamivudine in tablet dosage form. From the study of validation parameters, it was observed that the method is specific, accurate, precise, reproducible and rugged. The proposed method could be applied to routine analysis in quality control laboratories.

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