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Original Article

Analytical metthod development and validation for the simultaneous estimation of lamivudine and zidovudine by reverse-phase high-performance liquid chromatography in bulk and tablet dosage forms

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ABSTRACT

A simple, rapid, accurate, precise, and reproducible validated reverse-phase high-performance liquid chromatography (HPLC) method was developed for the determination of lamivudine and zidovudine in bulk and tablet dosage forms. The quantification was carried out using symmetry PremsilC_{18} (150 mm × 4.6 mm, 5 µm) column run in isocratic way using mobile phase comprising of acetonitrile: water (0.05% orthophosphoric acid with PH 3) 50:50 v/v and a detection wavelength of 271 nm and injection volume of 20 µL, with a flow rate of 0.5 ml/min. The retention times of lamivudine and zidovudine were found to be 6.200 min and 3.616 min, respectively. The method was validated in terms of linearity, precision, accuracy, limit of detection, limit of quantification, and robustness in accordance with the ICH guidelines. The assay of the proposed method was found to be 99-101%. The recovery studies were also carried out, and mean % recovery was found to be 99-101%. The % RSD from reproducibility was found to be <2%. The proposed method was statistically evaluated and can be applied for routine quality control analysis of lamivudine and zidovudine in bulk and tablet dosage form.

Keywords: Lamivudine and zidovudine, method development, validation, simultaneous estimation, high-performance liquid chromatography

Introduction

Lamivudine (LAMI) is chemically 1[(2R,5S)-2-(Hydroxy methyl)-1-3 oxathiolan-5yl] cytosine and used as an antiretroviral activity.[1] Zidovudine (ZIDO) is chemically 1-[(2R,4S,5S) -4azido-5-(hydroxymethyl) tetrahydrofuran-2-yl]-5-methyl pyrimidine-2, 4(1H,3H0-dione and used as an antiretroviral activity (Figure 1).[2] Reverse transcriptase inhibitors such as LAMI and ZIDO have been used in combination to treat patients with HIV.[3-7] The treatment is used to prevent or prolong the onset of acquired immune deficiency syndrome, which can lead to a variety of fatal complications. LAMI is also used in lower doses to treat patients with chronic hepatitis B, in whom the virus has replicated and caused liver inflammation.[8-12] ZIDO is used along with other antiviral medications to treat patients with HIV. ZIDO is also used in pregnant women to reduce the risk of HIV transmission from a mother to an unborn child. These methods are based on the measurement of specific and non-specific physical properties of the substances. The literature review reveals that few reverse-phase-high-performance liquid chromatography (RP-HPLC) methods for the estimation of LAMI and ZIDO alone and in combination with other drugs.[13-27] However, the present study is to develop an accurate and reliable HPLC method for simultaneous estimation of LAMI and ZIDO in tablet dosage form as per the ICH norm.

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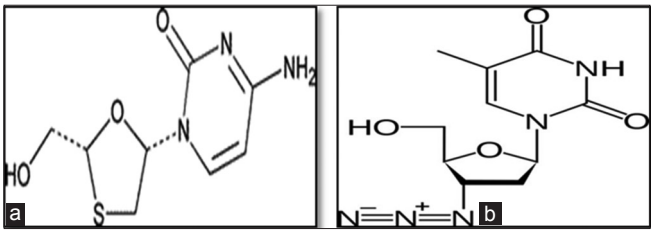

Figure 1: (a and b) Structure of lamivudine and zidovudine

Materials and Methods

Materials and reagents

The analysis of the drug was carried out onYoungline (S. K.) Gradient System ultraviolet (UV) Detector equipped with reverse-phase (Grace) C18 column (4.6 mm × 250 mm; 5 µm), a SP930D pump, a 20 µl injection loop, and UV730D absorbance detector and running autochro-3000 software.

The API of both drugs LAMI and ZIDO procured from R. S. I. T. C, Jalgaon, India, orthophosphoric acid (HPLC grade Avantor Performance material India Ltd., Thane, Maharashtra) Methanol, acetonitrile, and water (HPLC grade Merck Specialties Pvt. Ltd., Shiv Sager Estate "A" Worli, Mumbai.), and 0.45 µm filter (Millipore, Bengaluru). A combination of LAMI (150 mg) and ZIDO (300 mg) in tablet formulation was procured from local pharmacy (Duovir, J.B Chemical Pharmaceutical Pvt. Ltd.).

Chromatographic conditions

The chromatographic conditions are column C18 (250 mm × 4.6 mm); particle size packing 5 µm; detection wavelength 266 nm; flow rate 0.7 ml/min; temperature ambient; sample size 20 µl; mobile phase acetonitrile + ph. buffer KH2po4,10 mm (50+50% v/v); and run time 15 min.

Preparation of standard stock solution

Preparation of standard lamivudine solution: (Stock I)

From the freshly prepared standard stock solution (1000 µg/ml), 0.1 ml stock solution was pipette out in 10 ml of volumetric flask, and volume was made up to 10 ml with mobile phase to get a final concentration of 10 µg/ml (Figure 2).

Preparation of standard zidovudine solution: (Stock II)

From the freshly prepared standard stock solution (1000 µg/ml), 0.1 ml stock solution was pipetted out in 10 ml of volumetric flask, and volume was made up to 10 ml with mobile phase to get final concentration 10 µg/ml (Figure 3).

Preparation of standard lamivudine and zidovudine solution: (Stock III)

From the freshly prepared standard stock solution (1000 µg/ml), 0.1 ml stock solution was pipetted out in 10 ml of volumetric flask, and volume was made up to 10 ml with mobile phase to get final concentration 10 µg/ml (Figure 4).

Method development and validation

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.[28]

Assay preparation for commercial formulation

Weigh 20 lamivudine and zidovudine combination tablets and calculated the average weight, accurately weigh and transfer the

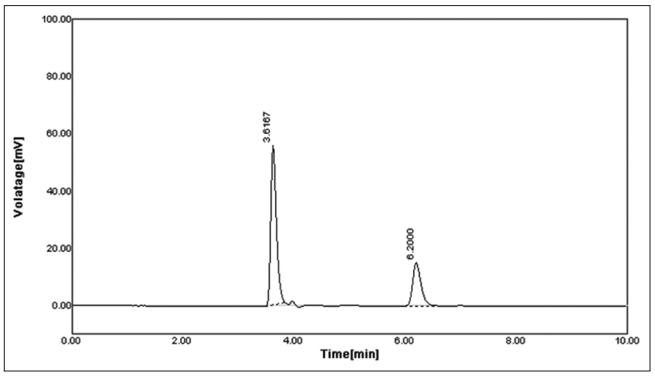

Figure 2: Representative chromatogram of LAMI and ZIDO using acetonitrile + water (acetic acid 0.05% [orthophosphoric acid]) (50%+50%) v/vas mobile phase, showing retention time 6.200 min and 3.616 min

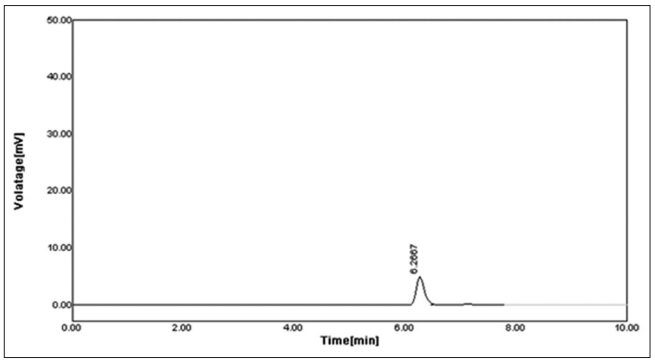

Figure 3: Chromatogram of standard lamivudine

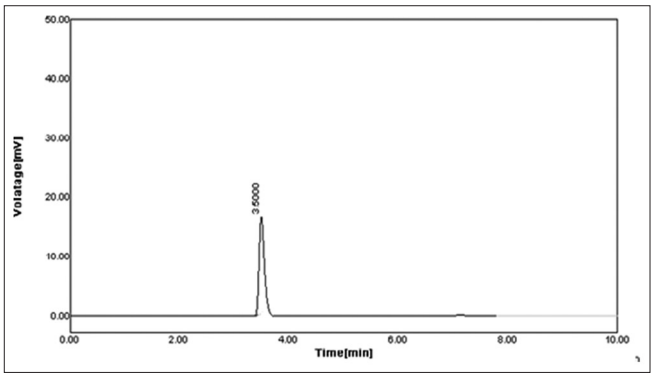

Figure 4: Chromatogram of standard zidovudine

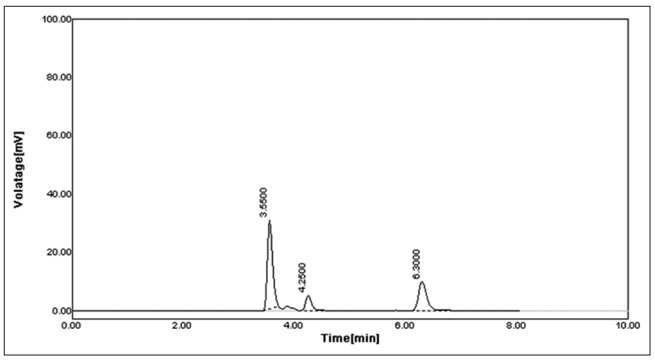

Figure 5: Chromatogram for marketed formulation

sample equivalent to 49.26 mg LAMI and ZIDO into 10 ml volumetric flask. Add about 10 ml ACN of diluents and sonicate to dissolve it completely and make the volume up to the mark with diluents. Mix well and filter through 0.45 µm filter. Further, pipette 0.1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents (10 µg/ml). The simple chromatogram of test LAMI and ZIDO was shown in Figure 5. The amounts of LAMI and ZIDO per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Analysis of marketed formulation were also % label claim was found to be 99-101% satisfactory were concluded (Table 1).

Results

Linearity and range

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 5-25 µg/ml for lamivudine and 10-50 µg/ml for zidovudine. Tables 2 and 3 depict the calibration data of LAMI and ZIDO. The respective linear equation for lamivudine was $y = 4.996\times + 0.777$ and zidovudine equation was $y = 6.152\times + 6.882$, where x is the concentration, and y is an area of peak. The correlation coefficient was 0.999. The calibration curve of LAMI and ZIDO is depicted in Figures 6 and 7.

Table 1: Analysis of marketed formulation

Assay	Drug	Label claimed	Amount found	% label claim	SD	% RSD
RP-HPLC method	LAMI	20	19.92	99.60	0.06	0.28
	ZIDO	40	39.61	99.03	0.54	1.35
	LAMI	20	20.00	100.00	0.28	0.28
	ZIDO	40	40.37	100.93	0.27	0.27

RP-HPLC: Reverse-phase high-performance liquid chromatography, SD: Standard deviation

Accuracy

It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. The accuracy of the methods was determined at three different concentration levels, i.e., 80%, 100%, and 120% (Figures 8-10) in triplicate for each drug as per the ICH guidelines. From the total amount of drug found, the percentage recovery was found in range of 99-101% (Tables 4 and 5).

Precision

Precision was studied to find out intra- and inter-day variations in the test method of LAMI and ZIDO. Intraday precision was determined by analyzing three concentrations in three replicate measurements of within linearity range of drugs on three different times in the same day. Interday precision was conducted during routine operation of the system over a period of 3 consecutive days. Intra- and inter-day precision studies on RP-HPLC method for LAMI and ZIDO which show the high precision % amount in between 98% and 100% indicates to analytical method that concluded (Table 6).

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions.

Table 2: Linearity data for lamivudine

Method	Concentration µg/ml	Peak area (µV.sec)		Average peak area (µV.sec)	SD of peak area	% RSD of peak area
		1	2			
RP-HPLC method	5	25.02	25.01	25.015	0.01	0.03
	10	49.92	50.46	50.19	0.38	0.76
	15	78.39	80.18	79.285	1.27	1.60
	20	100.6	100.3	100.45	0.21	0.21
	25	126.17	124.69	125.43	1.05	0.83
	Equation		$y = 4.996\times + 0.777$			
	R^2		0.999			

RP-HPLC: Reverse-phase high-performance liquid chromatography, SD: Standard deviation

Table 3: Linearity data for zidovudine

Method	Concentration µg/ml	Peak area (µV.sec)		Average peak area (µV.sec)	SD of peak area	% RSD of peak area
		1	2			
RP-HPLC method	10	60.84	61.56	61.200	0.51	083
	20	117.14	115.32	116.23	1.29	1.11
	30	173.44	175.54	174.49	1.48	0.85
	40	242.38	241.21	241.75	0.83	0.34
	50	299.17	301.66	300.41	1.76	0.59
	Equation		$y = 6.152\times-6.882$			
	R^2		0.999			

RP-HPLC: Reverse-phase high-performance liquid chromatography, SD: Standard deviation

Table 4: Result of recovery data for lamivudine and zidovudine

Method	Drug	Level (%)	Amount taken (µg/ml)	Amount added (µg/ml)	Absorbance mean*±SD	Amount recovered mean *±SD	% Recovery mean *±SD
RP-HPLC method	LAM	80	10	8	18.10±0.02	8.10±0.02	101.22±0.30
		100	10	10	20.14±0.14	20.58±0.14	101.41±1.43
		120	10	12	21.98±0.06	11.98±0.06	101.58±0.44
	ZIDO	80	20	16	36.19±0.08	48.70±46.05	101.19±0.54
		100	20	20	39.90±0.22	20.58±0.22	99.48±1.10
		120	20	24	44.06±0.30	24.06±0.30	101.58±1.32

*Mean of each three reading for RP-HPLC method. RP-HPLC: Reverse-phase high-performance liquid chromatography, SD: Standard deviation

Table 5: Statistical validation of recovery studies lamivudine and zidovudine

Method	Level of recovery (%)	Drug	Mean % recovery	SD*	% RSD
RP-HPLC method	80	LAMI	101.22	0.30	0.29
		ZIDO	101.19	0.54	0.53
	100	LAMI	101.41	1.43	1.41
		ZIDO	99.48	1.10	1.10
	120	LAMI	101.58	0.44	0.43
		ZIDO	101.58	1.32	1.29

*Denotes average of three determinations for RP-HPLC. RP-HPLC: Reverse-phase high-performance liquid chromatography, SD: Standard deviation

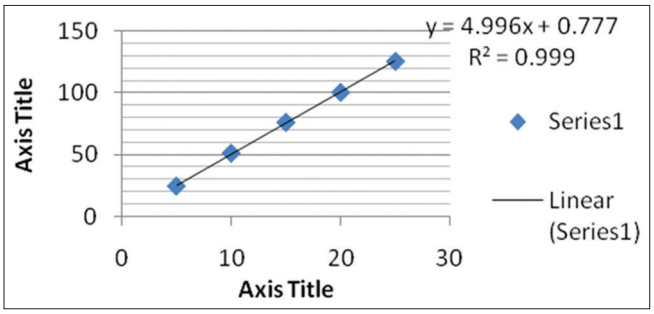

Figure 6: Calibration curve of lamivudine

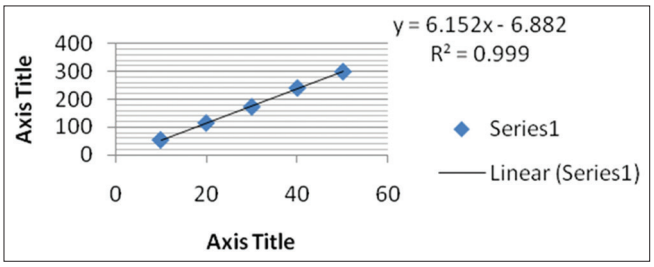

Figure 7: Calibration curve of zidovudine

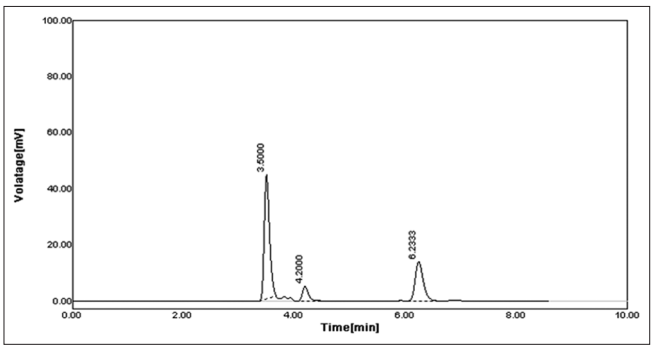

Figure 8: Chromatogram of accuracy 80%

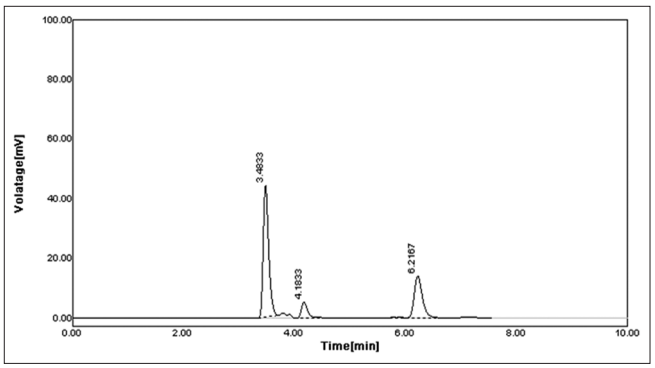

Figure 9: Chromatogram of accuracy 100%

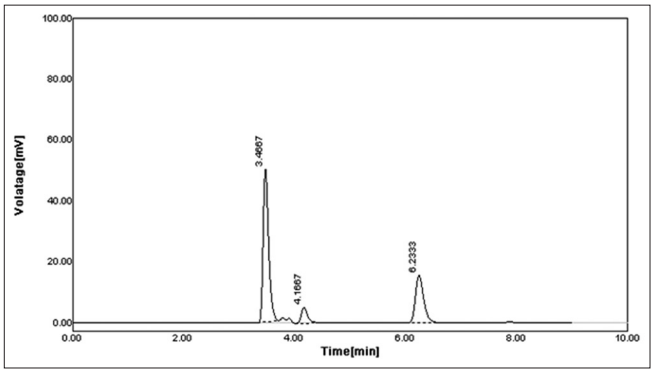

Figure 10: Chromatogram of accuracy 120%

Robustness

The robustness is measure of its capacity to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability during normal usage, and hence, the following is performed by slight variations in parameters. The assay content of the sample was measured by change in the flow rate of 0.6-0.8 ml/min.

Repeatability

Repeatability studies on RP-HPLC method for LAMI and ZIDO were found to be, the % RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded (Table 7).

DISCUSSION

The proposed methods for simultaneous estimation of LAMI and ZIDO in tablet dosage forms were found to be simple, accurate,

Table 6: Result of intra- and inter-day precision studies on HPLC method for LAMI and ZIDO

Method	Drug	Concentration (μg/ml)	Intraday precision		Interday precision	
			Mean±SD	% Amount found	Mean±SD	% Amount found
RP-HPLC method	LAMI	10	50.69±0.38	99.90	51.44±0.17	101.40
		15	74.93±1.24	99.00	75.77±0.46	100.07
		20	102.35±1.46	101.65	102.25±0.41	101.50
	ZIDO	20	115.37±1.37	99.35	115.12±1.09	99.10
		30	179.01±2.80	100.70	178.93±1.02	100.67
		40	241.80±0.98	101.05	240.94±2.42	100.70

Mean of each 3 reading for HPLC method. RP-HPLC: Reverse-phase high-performance liquid chromatography, SD: Standard deviation

Table 7: Repeatability studies on RP-HPLC for lamivudine and zidovudine

Method	Concentration of LAMI and ZIDO (mg/ml)	Peak area	Amount found (mg)	% Amount found
RP-HPLC method for LAMI	15	76.56	15.16	101.07
	15	77.50	15.10	100.02
	15	79.45	16.30	101.00
	15	75.49	15.15	100.10
	15	80.16	16.01	102.02
		Mean	15.34	
		SD	0.78	
		% RSD	1.01	
RP-HPLC method for ZIDO	30	177.90	30.03	100.10
	30	178.10	31.01	101.01
	30	180.12	32.05	102.14
	30	175.09	30.01	100.00
	30	176.15	30.00	99.11
		Mean	30.62	
		SD	2.99	
		% RSD	1.68	

RP-HPLC: Reverse-phase high-performance liquid chromatography

economical, and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration curves for LAMI and ZIDO were linear with correlation coefficients (r2) values in the range of 100.45-116.23 at all the selected wavelengths, and the values were average of three readings. The values of %RSD are within the prescribed limit of 2%, showing the high precision of methods, and recovery was close to 100% for both the drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of LAMI and ZIDO in formulations.

Conclusion

The developed RP-HPLC methods were found to be more accurate, precise, and reproducible. The analysis of tablets containing two drugs gave the satisfactory results. The statistical parameter of these methods showed good results. The recovery studies revealed excellent accuracy and high precision of the method. The methods were found to be simple and time-saving. All proposed methods could be applied for routine analysis in quality control laboratories.

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