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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND ZIDOVUDINE IN TABLET BY REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: The objective of the study was to develop and validate reverse-phase high-performance liquid chromatography (RP-HPLC) method and apply method to tablet dosage form.

Methods: A simple, rapid, economical, precise, and accurate RP-HPLC method for simultaneous estimation of lamivudine and zidovudine in their combined dosage form has been developed.

Results: A RP-HPLC method was developed for the simultaneous estimation of lamivudine and zidovudine. In their combined dosage form has been developed. The separation was achieved by LC-C_{18} column (150 mm ×4.6 mm, 5 µm) and water: methanol (65:35v/v) as mobile phase, at a flow rate of 0.8 ml/min. Detection was carried out at 272 nm. Retention time of lamivudine and zidovudine was found to be 3.007 min and 4.647, respectively. The method has been validated for linearity, accuracy, and precision. The assay method was found to be linear from 50% to 150% for lamivudine and zidovudine.

Conclusion: Developed method was found to be accurate, precise, and rapid for simultaneous estimation of lamivudine and zidovudine in their combined dosage form.

Keywords: Infrared spectroscopy method, Lamivudine and zidovudine, Reverse-phase high-performance liquid chromatography method, Validation.

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INTRODUCTION

Lamivudine designated chemically as 4-Amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one (Fig. 1) is a compound of the pyrimidine class and is used as antiviral agent [1,2]. It acts by inhibiting the HIV reverse transcriptase as well as hepatitis B virus. Its incorporation into DNA results in chain termination. Most human DNA polymerases are not affected and systemic toxicity of lamivudine is low. Various analytical methods have been reported for the estimation of lamivudine as alone as well as in combination with other drugs. They include thin-layer chromatography (TLC) [3], high-performance liquid chromatography (HPLC) [4], and reverse-phase (RP)-HPLC methods with other drugs [4-6].

Zidovudine designated chemically as 1-[(2R,4S,5S)-4-Azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dion (Fig. 2) is a compound of pyrimidine class and is used as antiviral [1,7,8]. Zidovudine (prototype of NRTI) is a thymidine analog. After phosphorylation in host cell, zidovudine triphosphate selectively inhibits viral reverse transcriptase (RNA-dependent DNA polymerase) in the presence of cellular DNA polymerase. It prevents conversion of single-stranded viral RNA to double-stranded viral DNA. They include TLC [9,10], RP-HPLC [9], and RP-HPLC methods with other drugs [11-13].
However, an extensive literature search did not reveal any estimation method for both the drugs in their combined dosage form. Therefore, attempt was made to develop and validate simple, precise, and accurate, stability-indicating RP-HPLC method for simultaneous determination of both the drugs in their combined dosage form. The guideline on drug analytical method validation FDA [14].

METHODS

Reagents and Chemicals

Lamivudine and zidovudine were obtained as gift samples from Cipla Pharmaceuticals Ltd., combined dosage form tablet was purchased from local market. HPLC grade water and methanol of analytical grade were obtained from SD Fine Chem Ltd.

Instruments and chromatographic conditions

Young Lin HPLC system was used for method development, degradation studies, and validation. Data acquisition was performed on YL 9100 HPLC software. The separation was achieved on C_{18} column (150 mm ×4.6 mm, 5 µm). The column was maintained at room temperature and the eluent was monitored at 272 nm using PDA detector. The mixture of water: methanol (65:35v/v) at a flow rate of 0.8 ml/min was used as a mobile phase. The injection volume was 20 µl.

Preparation of solutions

Preparation of lamivudine standard stock solutions (100 µg/ml)

Weigh accurately 10 mg of lamivudine and transfer it to 100 ml volumetric flask. Add 50 ml of methanol, shake well to dissolve and dilute up to the mark with methanol, and mix them thoroughly.

Preparation of zidovudine standard stock solutions (250 µg/ml)

Weigh accurately 25 mg of zidovudine and transfer it to 100 ml volumetric flask. Add 50 ml of methanol, shake well to dissolve and dilute up to the mark with methanol, and mix them thoroughly.

Working standard solution of lamivudine and zidovudine (10:25 µg/ml)

Pipette out 1 ml of lamivudine standard stock solution and 1 ml of zidovudine standard stock solution into 10 ml volumetric flask and dilute up to the mark with diluents and mixed thoroughly.

Preparation of mobile phase

Accurately measure 650 ml of 0.1% TFA in HPLC grade H_2O and 350 ml of methanol, mix thoroughly and degassed by sonication to make 65:35 %v/v.

System suitability parameters

System suitability tests were performed to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters monitored for system suitability include retention time, theoretical plate number, peak area, tailing factor, and resolution. The repeatability of these parameters was checked by injecting 3 times the test solution of lamivudine 10 µg/mL and zidovudine 25 µg/mL. The results shown in Table 1 were within acceptable limits.

Method validation

Specificity

Specificity of method can be termed as absence of any interference at retention times of samples. Specificity was performed by injecting blank and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for the identification of analytes.

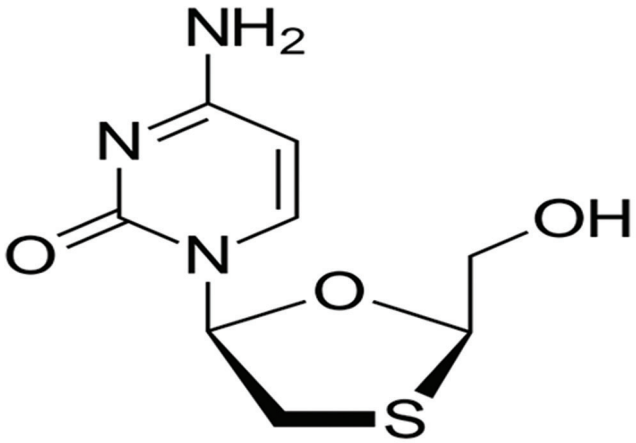

Fig. 1: Chemical structure of lamivudine

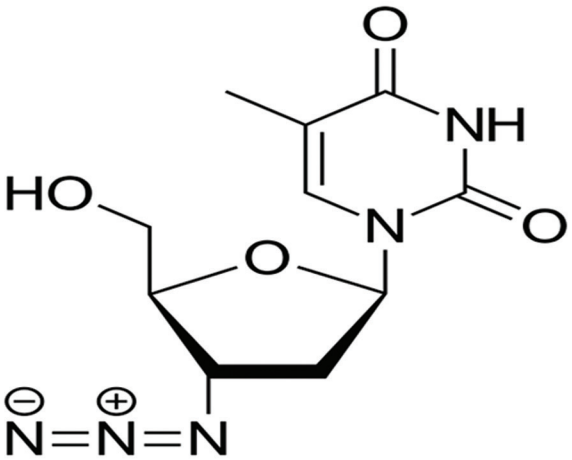

Fig. 2: Chemical structure of zidovudine

Table 1: Results for system suitability parameters

Parameter	Lamivudine (Average±SD)	Zidovudine (Average±SD)
Retention time (min) (n=3)	3.007±0.040	4.660±0.073
Theoretical plate (n=3)	4395±78.234	4427±76.567
Asymmetry (n=3)	1.17±0.15	1.10±0.012
Resolution (n=3)	7.18±0.337	

Linearity and range

A series of standard solutions 5–15 µg/ml of lamivudine and 15–35 µg/ml of zidovudine were prepared. An aliquot of 20 µl of each solution was injected 3 times for each standard solutions and peak area was observed. Plot of average peak area versus the concentration is plotted and from this, the correlation coefficient and regression equation were generated. The calibration data of lamivudine and zidovudine are given in Table 2a and b, while Fig. 3a and b represents linearity graphs of three drugs, respectively.

Precision

The method was validated in terms of intraday and interday precision. The solution containing lamivudine – 10 µg/ml and zidovudine – 25 µg/ml was injected 6 times for repeatability study. Interday and intraday study was performed by injecting 5, 10, and 15 µg/ml of lamivudine and 15, 25, and 35 µg/ml of zidovudine solutions 3 times for each aliquot. The %RSD for precision study was found to be <2%, as shown in Table 3a-c.

Accuracy

Accuracy was determined by calculating recovery of lamivudine and zidovudine by the standard addition method. Known amounts of standard solutions of lamivudine (5, 10, and 15 µg/ml) and zidovudine (15, 25, and 35 µg/ml) were added to a pre-quantified test solutions

Table 2a: Linearity study lamivudine

S. No.	Lamivudine			
	Conc. in µg/ml	Average area (n=3)	SD	RSD
1	5	1,425,631	5765.35264	0.374352
2	7.5	2,013,254	7273.82154	0.312863
3	10	2,523,568	13,758.416	0.508546
4	12.5	3,253,647	98,251.2259	0.308521
5	15	3,752,136	8185.74361	0.22536

Table 2b: Linearity study zidovudine

S. No.	Zidovudine			
	Conc. in µg/ml	Average area (n=3)	SD	RSD
1	15	7,012,583	21,451.01	0.308258
2	20	9,154,236	46,213.14	0.421586
3	25	10,826,086	21,239.24	0.112569
4	30	12,958,902	28,512.21	0.201258
5	35	15,782,169	51,452.42	0.325896

Table 3a: Repeatability data for lamivudine and zidovudine

Lamivudine		Zidovudine	
Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
10	2,568,124	25	10,666,320
10	2,578,084	25	10,661,582
10	2,554,452	25	10,624,256
10	2,582,441	25	10,682,784
10	2,574,532	25	10,654,321
10	2,578,486	25	10,697,869
Mean	2,573,236	Mean	10,662,356
SD	10,060.23	SD	22,310.272
RSD	0.355256	RSD	0.2022413

of lamivudine (10 µg/ml) and zidovudine (25 µg/ml). Each solution was injected in triplicate and the recovery was calculated by measuring peak areas. Results obtained are shown in Table 4a and b.

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

LOD and LOQ for SC and EV were calculated as suggested by ICH guidelines using equations LOD =3.3 σ/s and LOQ =10 σ/s, respectively. Where, σ is the SD of the response and S is the slope of the calibration curve.

Robustness

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by making two small changes. The mobile ration was changed by ±2 ml and flow rate was changed by ±0.02 ml/min and pH was changed by ±0.2. After each changes, sample solution was injected and system suitability parameters were observed. The results are shown in Table 5.

RESULTS AND DISCUSSION

System suitability study

The detection was carried out in the ultraviolet region at 272 nm. The different composition of mobile phase was testing and the composition giving retention time of 3.007 min for lamivudine and 4.660 min for zidovudine with good resolution and theoretical plates was selected that optimized mobile phase was water: methanol (65:35v/v). A chromatogram of the mixture in optimized conditions is shown in Fig. 4 and the system suitability parameters are shown in Table 1.

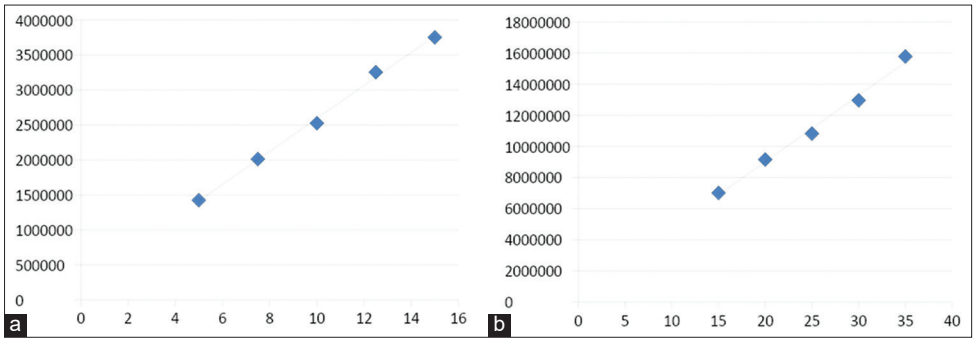

Fig. 3: (a) Linearity graph for lamivudine. (b) Linearity graph for zidovudine

Table 3b: Intraday data for lamivudine and zidovudine

Lamivudine			Zidovudine		
Conc. (µg/mL)	Area mean ±SD (n=3)	% RSD	Conc. (µg/mL)	Area mean ±SD (n=3)	% RSD
5	2,123,615.2±11,616.02	0.54422	15	95,002,431±364,628.5	0.374721
10	2,676,544±12,445.54	0.57443	25	10,231,574±57,091.72	0.479944
15	3,244,259.7±21,523.22	0.61427	35	13,258,586±74,249.93	0.54263

Table 3c: Interday data for lamivudine and zidovudine

Lamivudine			Zidovudine		
Conc. (µg/mL)	Area mean ±SD (n=3)	% RSD	Conc. (µg/mL)	Area mean ±SD (n=3)	% RSD
5	2,114,251.4±4467.526	0.205896	15	95,235,896±18,619.11	0.011258
10	2,652,866.1±14,123.45	0.524021	25	10,251,342±95,642.44	0.802413
15	3,243,725.2±21,268.14	0.605823	35	13,421,842±50,241.14	0.351242

Table 4a: The accuracy study results of lamivudine

% accuracy level	Amount of lamivudine taken (µg/ml)	Amount of standard lamivudine added (µg/ml)	Total amount of lamivudine (µg/ml)	Amount of lamivudine found (µg/ml) ±SD (n=3)	Mean % recovered± SD (n=3)
50	10	5	15	15.20±0.05	99.82±0.11
100	10	10	20	19.98±0.10	99.75±0.21
150	10	15	25	24.95±0.04	99.72±0.19

Table 4b: The accuracy study results of zidovudine

% accuracy level	Amount of zidovudine taken (µg/ml)	Amount of standard zidovudine added (µg/ml)	Total amount of zidovudine (µg/ml)	Amount of zidovudine found (µg/ml) ±SD (n=3)	Mean % recovered±SD (n=3)
50	25	12.5	37.5	37.21±0.10	99.74±0.10
100	25	25	50	50.17±0.20	99.71±0.20
150	25	37.5	62.5	62.16±0.11	99.68±0.19

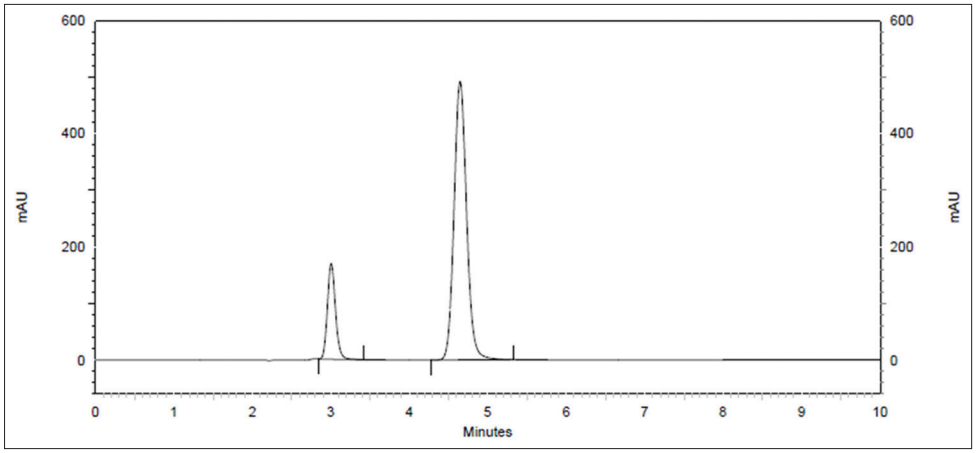

Fig. 4: Optimized condition chromatogram of lamivudine and zidovudine

Table 5: Robustness study results for lamivudine and zidovudine

Parameter		Area (n=3)	
		Lamivudine	Zidovudine
	0.78 ml	2,631,115.31	11,491,258
	0.8 ml	2,637,453.31	10,256,983
	0.82 ml	2,605,215	10,256,893
Flow rate (±0.02 ml/min)	Mean±SD	2,624,795.448±15,512.54809	10,963,525±95,586.412
	% RSD	0.60152368	0.8100642
	63:37%	2,635,569.67	10,934,223
Mobile phase composition	65:35%	2,624,163	10,904,136
	67:33%	2,598,010.67	10,959,475
Water:methanol (±2 ml)	Mean±SD	2,619,247.778±18,233.88682	10,931,277±28,780.471
	% RSD	0.608132851	0.2396
	270	2,635,584.33	10,934,112
	272	2,624,130	10,913,467
	274	2,598,010.67	11,125,675
Wavelength (±2nm)	Mean±SD	2,619,241.667±18,257.9064	10,991,085±107,015.14
	%RSD	0.698208712	0.8458512

Method validation

Specificity

The method was found to be specific as there was no interference observed in any of the parameters under observation.

Linearity and range

The linearity of lamivudine and zidovudine was found between 5–15 µg/ml and 15–35 µg/ml, respectively. The results are shown in Table 2a and b.

Precision

The %RSD for repeatability study for lamivudine and zidovudine was found to be 0.355 and 0.202, respectively. The interday and intraday study also shows %RSD value for lamivudine and zidovudine within the acceptable limit. Results for precision study are shown in Table 3a-c.

Accuracy

Accuracy of the method was confirmed by recovery study at three levels (50%, 100%, and 150%) of standard addition. Percentage recovery for lamivudine was found to be 99.72–99.82% and zidovudine was found to be 99.68–99.74%, as shown in Table 4a and b.

LOD and LOQ

The LOD was found to be 0.285 µg/ml for lamivudine and 0.225 µg/ml for zidovudine, while the LOQ was found to be 0.889 µg/ml for lamivudine and 0.723 µg/ml for zidovudine.

Robustness

The typical variations studied under this parameter were mobile phase composition, pH, and flow rate. Overall %RSD was found to be <2% for all the variations which indicates that the proposed method is robust. Robustness data are shown in Table 5.

Analysis of marketed formulation by proposed method

Applicability of the proposed method was tested by analyzing the commercially available marketed formulation. The percentage of lamivudine and zidovudine was found to be 100.02% for lamivudine and 99.86% for zidovudine, respectively.

CONCLUSION

From the above discussion, it can be concluded that the proposed method is specific, precise, and accurate. Results are in good agreement with label claim which indicates that there is no interference of excipients. Therefore, the proposed method can be used for routine analysis of lamivudine and zidovudine in combined tablet formulation.

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