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Derivative Spectrophotometric Method for Estimation of Antiretroviral Drugs in Fixed Dose Combinations

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ABSTRACT

Purpose: Lamivudine is cytosine and zidovudine is cytidine and is used as an antiretroviral agents. Both drugs are available in tablet dosage forms with a dose of 150 mg for LAM and 300 mg ZID respectively. ***Method:*** The method employed is based on first order derivative spectroscopy. Wavelengths 279 nm and 300 nm were selected for the estimation of the Lamovudine and Zidovudine respectively by taking the first order derivative spectra. The conc. of both drugs was determined by proposed method. The results of analysis have been validated statistically and by recovery studies as per ICH guidelines. ***Result:*** Both the drugs obey Beer's law in the concentration range 10-50 µg mL^{-1},for LAM and ZID; with regression 0.9998 and 0.9999, intercept – 0.0677 and – 0.0043 and slope 0.0457 and 0.0391 for LAM and ZID, respectively.The accuracy and reproducibility results are close to 100% with 2% RSD. ***Conclusion:*** A simple, accurate, precise, sensitive and economical procedures for simultaneous estimation of Lamovudine and Zidovudine in tablet dosage form have been developed.

Introduction

Lamivudine is chemically, 1[(2R, 5S)-2-(Hydroxy methyl)-1-3 oxathiolan-5yl] cytosine and used as an antiretroviral activity.[1] Lamivudine is an analogue of cytidine. It can inhibit both types (I and II) 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. The chemical structure of Lamivudine is shown in Figure 1.

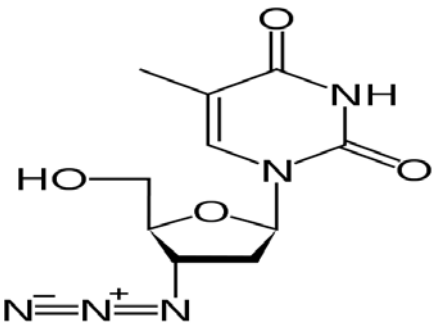

Figure 1. Structure of Lamivudine

Zidovudine is chemically, 1-[(2R, 4S, 5S) -4azido -5-(hydroxy methyl) tetrahydrofuran-2-yl]-5-methylprimidine-2,4(1H,3H)-dione and used as an antiretroviral activity.[2] The chemical structure of Lamivudine is shown in Figure 2 .

Literature survey revealed that several analytical methods including spectrophotometric[1-8], HPLC (High-performance liquid chromatography) [9-11], ,HPTLC (High-performance thin layer chromatography) [12,13] ,RP-HPLC (Reverse phase-high performance liquid chromatography) [14,15] and LC/MS (Liquid chromatography-mass spectrophotometry)[16,17] methods have been reported for its estimation of Lamivudine and Zidovudine in various dosage forms alone or in fixed dose combination with other drugs. Like LAM, ZID is also a Nucleoside Analog. Both the drugs are available in combined dosage form with a dose of 150 mg for LAM and 300 mg for ZID respectively.

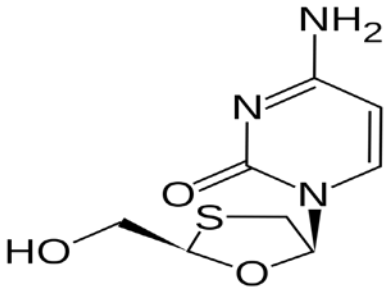

Figure 2. Structure of Zidovudine

Both these drugs are not official in Indian Pharmacopoeia, United States Pharmacopoeia.

Extensive literature survey reveals that derivative spectrophotometric method is yet not reported for simultaneous determination of LAM and ZID in tablet dosage form. In present work an attempt is being made to develop simple, precise, accurate and economical

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derivative spectrophotometric methods for simultaneous determination of binary drug formulation.

Materials and methods

The instrument used in the present study was JASCO double beam UV/Visible Spectrophotometer (Model V-630) with spectral bandwidth of 1 nm and 10 mm a matched quartz cell was used. All weighing was done on electronic balance (Model Shimadzu BL 320-H).

Reagents and chemicals

Analytically pure sample of LAM and ZID was kindly supplied by Cipla Pharmaceuticals Ltd. (Daund, India) and used as such without further purification. The pharmaceutical dosage form used in this study was a Combivir tablets manufactured by Glenmark Pharmaceuticals Ltd. (Sinnar, India) labeled to contain 150 mg of LAM and 300 mg of ZID. All chemicals are of AR grade and were purchased from Qualigens fine Chemicals, Mumbai, India.

First Order Derivative Spectroscopic Method [18,19]

The method is based on first order derivative spectroscopy to overcome spectral interference from other drug. First order derivative spectra of both the drugs were recorded in Figure 3. It was observed that LAM showed dA/dλ zero at 279 nm in contrast to ZID that has considerable dA/dλ at this wavelength. Further, ZID has zero dA/dλ at 300 nm while at this wavelength LAM has significant dA/dλ. Therefore these two wavelengths were employed for the estimation of LAM and ZID without any interference. The calibration curves were plotted at these two wavelengths of concentrations against dA/dλ within the above mentioned range. The equations of line obtained to determine concentrations LAM and ZID are

$$C_{LAM} = DA/D\lambda_{300} - 0.0150 / 0.0168$$

$$C_{ZID} = DA/D\lambda_{279} - 0.0124 / 0.0176$$

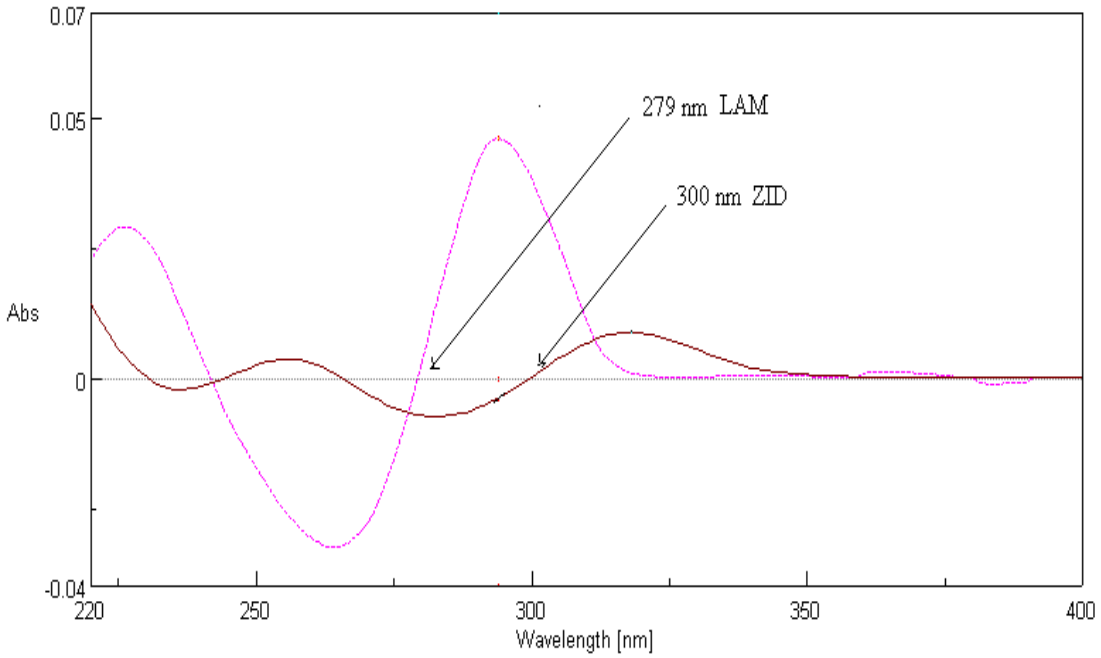

Figure 3. First order derivative overlain spectra of LAM (10 µg mL^{-1}) and ZID (10 µg mL^{-1}) in 0.1M HCl

Preparation of Standard Stock Solutions

Standard stock solutions were prepared by dissolving separately 10 mg of each drug in 100 mL of 0.1 N HCl to get concentration of 0.1 mg mL^{-1}. 1 mL of the stock solution was further diluted to 10 mL with 0.1 N HCl to get a working standard solution of concentration 10 µg mL^{-1} of both LAM and ZID and scanned in the wavelength range of 200-400 nm.

Preparation of Sample Stock Solution

Contents of twenty tablets were weighed accurately and powdered. Powder equivalent to 100 mg of ZID and 50 mg of LAM was weighed and dissolved in 50 mL of 0.1 N HCl with the aid of ultrasonication for 5 min. The solution was filtered through Whatman filter paper no. 41 to a 100 mL volumetric flask. Filter paper was washed with 0.1 N HCl, adding washings to the volumetric flask and volume was made up to the mark with 0.1 N HCl to get sample stock solution which was further diluted with 0.1 N HCl to get final concentration of solution (LAM 10 µg mL^{-1} and ZID 20 µg mL^{-1}) in the linearity range.

Results and Discussions

Linearity and range

A standard stock solution was prepared for both Lamivudine and Zidovudine; they were serially diluted to yield five standard solutions.For UV spectrophotometric method, linearity was obtained in

concentration range of 10 –50 µg .mL^{-1}, for LAM and ZID; with regression 0.9998 and 0.9999, intercept – 0.0677 and – 0.0043 and slope 0.0457 and 0.0391 for LAM and ZID , respectively.The results are depicted in table1.

Accuracy and precision

The accuracy of the proposed methods was checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels within the range of linearity for both the drugs.Results of recovery studies are shown in Table 2. The accuracy and reproducibility is evident from the data as results are close to 100 % and the value of standard deviation and % R.S.D. were found to be < 2 %; shows the high precision of the method. The proposed method is simple, economical, rapid, precise and accurate. Hence it can be used for routine analysis of LAM and ZID in tablet formulation.

Specificity

The proposed method was found to be specific as there is no interference from other excipients.

Results of analysis of tablet formulation

Analysis of tablet formulation combivir was carried out and the amount recovered were expressed as percentage amount of tablet claim.The percentage recovery for LAM is 99.98±0.645 and ZID is 98.56±0.542 respectively.The proposed methods was evaluated by the assay (n = 6) of commercially available tablets containing LAM and ZID. The results of assay are presented in Table3.

The proposed method has an advantage two advantage over earlier developed methds. One is the of use of 0.1N HCl as solvent which is easily available and less costlier as compared to methanol and second is good recovery results. Also the derivative spectrophotometry provides greater selectivity and offers a solution in resolving a overlapping spectra.

Table 1. Linearity Results

Components	Concentration range(µg mL^{-1})	Equation for regression line	r^2
LAM	10-50	Y=0.0457x - 0.0677	0.9998
ZID	10-50	Y=0.0391 x - 0.0043	0.9999

**Mean of six determinations,R.S.D. is relative standard deviation.*

Table 2. Recovery studies of LAM and ZID

Drug	Conc of drug added		%Recovery ±S.D.*	Intra day Precision	%Recovery ±S.D.*	Inter day Precision
	µg mL^{-1}	%Level				
LAM	5	50	100.43±0.54	0.45	99.74±0.34	0.35
	10	100	100.21±0.08	0.54	99,28±0.38	0.44
	15	150	99.85±0.28	0.51	99.55±0.28	0.4
ZID	10	50	98.76±0.34	0.35	99.06±0.54	0.39
	20	100	98.98±0.29	0.46	98.88±0.69	0.43
	30	150	98.65±0.42	0.45	99.65±0.42	0.58

** Mean of three determinations*

Table 3: Results of commercial formulation analysis

Method	Formulation	Label claim (mg/Tablet)	%label claim estimated* (Mean±S.D.)	% R.S.D.
Derivative spectroscopy	Combivir	LAM	99.98±0.645	0.345
		ZID	98.56±0.542	0.412

**Mean of six determinations,R.S.D. is relative standard deviation*

Conclusion

The developed and validated spectrophotometric method employed here proved to be simple, economical, rapid, precise and accurate. Thus it can be used for routine simultaneous determination of LAM and ZID in tablet dosage form instead of processing and analyzing each drug separately.

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Conflict of interest
The authors report no conflicts of interest.

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