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Research Article

DEVELOPMENT OF UV SPECTROPHOTOMETRIC FIRST ORDER DERIVATIVE METHOD FOR THE SIMULTANEOUS ESTIMATION OF ABACAVIR AND LAMIVUDINE IN COMBINED TABLET DOSAGE FORM

VP. Nagulwar* and KP. Bhusari

Sharad Pawar College of Pharmacy, Wanadongri, Hingna Road, Nagpur, Maharashtra, India.

ABSTRACT

Simple, accurate, precise, economical and reproducible analytical method has been developed for the simultaneous estimation of abacavir and lamivudine in pure bulk drug and in combined tablet dosage form by UV spectrophotometric first order derivative method. The stock solutions were prepared in mixture of acetonitrile and methanol followed by the further required dilutions with distilled water. In the first order derivative method, the wavelengths at which abacavir and lamivudine were analyzed were 229.2 nm and 284.8 nm respectively. At 229.2 nm abacavir has absorbance while lamivudine shows zero absorbance. Similarly, at 284.8 nm lamivudine shows absorbance while abacavir has zero absorbance. Thus both the drugs do not interfere in the quantitation of one another. Calibration graphs were obtained by the concentration ranges of 5-25 μg/ ml of both the drugs. In bulk drugs, abacavir was estimated as 100.05%, lamivudine 100.11% whereas in the marketed tablets abacavir was found as 99.01% lamivudine 99.09% respectively. The results of analysis have been validated as per ICH guidelines and were found to be satisfactory. Hence, present study gives excellent methods for the determination of both the drugs in combined tablet formulation.

Key words: ABA, LAM, First order derivative, UV Spectrophotometry, Tablets.

INTRODUCTION

Abacavir (ABA) and Lamivudine (LAM) are Nucleoside Analog of anti HIV drugs. Literature survey has revealed methods for their quantitation alone or in combination by spectrophotometry[1-4], HPLC[5] and HPTLC[6] but no method was found which estimated both the drugs as proposed by first order UV spectrophotometry. Hence the present work has been carried out.

MATERIAL AND METHODS

Materials

Shimadzu 1601 UV –visible spectrophotometer with a matched pair of 10 mm quartz cells was used. ABA and LAM pure drugs (Cipla Ltd. Goa and Patalganga, INDIA), Acetonitrile, Methanol (LOBA, India Ltd) and distilled water were used in the present study. The commercially available tablets containing a combination of ABA-

600mg and LAM -300 mg were procured from pharmacy.

Methods

i) Preparation of standard solutions

An accurately weighed quantity of ABA and LAM (50 mg each) were transferred to two separate (50 mL) volumetric flasks (50 mL). Both drugs were dissolved in acetonitrile (30 mL) and volume was made up to mark with methanol (50 mL) to get the standard stock solutions having concentration as 1mg/mL of ABA and LAM respectively.

ii) Preparation of mixed standard solution

Aliquots of the standard solutions of both the drugs were mixed so as to get the working standards of concentration of ABA and LAM in ratio of 2:1 as 5:2.5, 10:5, 15:7.5, 20:10, and 25:12.5 µg/mL after diluting with distilled water, respectively.

iii) Selection of scanning range and sampling wavelengths

The standard solutions of ABA and LAM were diluted with distilled water individually to get the concentration of 20 and 10 µg/mL, respectively and were scanned in the UV range 400-200 nm. The spectral data was processed to obtain first order derivative spectrum at wavelength interval of 0.1 nm. The two spectra were overlain and are shown in Figure 1 which indicates that ABA shows zero crossing at 284.4 nm while LAM shows zero crossing at 229.2 nm. At zero crossing point of ABA (284.4 nm), LAM shows a measurable $dA/d\lambda$ where as at the zero crossing point of LAM (229.2 nm), ABA shows appreciable $dA/d\lambda$. Hence, the wavelengths 284.4 nm and 229.2 nm were selected as analytical wavelengths for determination of ABA and LAM, respectively.

iv) Analytical concentration range and plotting of calibration curves

For the linearity study, aliquots of the drug solutions were further diluted with distilled water to get the final working standards of concentration range as ABA as 5-25 µg/mL and LAM as 5-25 µg/mL, respectively. Calibration plots were obtained by plotting concentration against derivative absorbance values (Figure 2 and 3) and the following regression equations ($A = \alpha + \beta x C$) and r for both the drugs were obtained.

ABA- A = (-0.000305) + (-0.002555) x C, r = (-0.999815) .... (Eq.1)

LAM- A = 0 + (-0.002195) x C, r = (-1) ....(Eq.2)

where,

α = Intercept

β = Slope

C = concentration in µg/mL,

A = absorbance of the first order derivative curves at 229.2 nm and 284.4 nm for ABA and LAM, respectively

r = correlation coefficient.

v) Estimation of drugs in standard laboratory mixture by proposed method

The mixed standard solutions of ABA and LAM (20:10 µg/mL) were scanned at analytical wavelengths and their derivative absorbance was noted. The concentrations of both the drugs in the mixed standard solution were obtained from the regression equations of ABA and LAM. The results are depicted in Table 1.

vi) Application of proposed method for the estimation of ABA and LAM in tablets

Tablets containing ABA (600 mg) and LAM (300 mg) were weighed and finely powdered. A quantity of powder equivalent to ABA (50 mg) was accurately weighed, transferred to volumetric flask (50 mL) and was dissolved in acetonitrile. The volume was made up to the mark with methanol (50 mL). The solution was filtered through Whatman filter paper No.1. Aliquot of solution was diluted with distilled water to get the working standards of ABA as 20 µg/mL (~ LAM-10 µg/mL). The sample solutions were scanned for their derivative absorbance at 229.2 nm and 284.4 nm. The concentration of both the drugs in the tablet solution was determined by using the regression equation. The results are shown in Table 1.

vii) Recovery studies

To the preanalyzed tablet powder equivalent to ABA (25 mg) in volumetric flask (25 mL), ABA (5 mg) and LAM (10 mg) were added.The mixture was shaken thoroughly with acetonitrile 15 mL and diluted up to 25 mL with methanol.The solution was filtered through Whatman filter paper No.1. Aliquot portions of the resultant solution were appropriately diluted with distilled water to get concentration with in linearity range. The derivative absorbances of the solution was measured at the analytical wavelengths. The concentration of the two drugs in the mixed standard solution was obtained from the standard calibration curves of ABA and LAM respectively by regression equations. The weight of ABA and LAM contributed by tablet powder, calculated earlier was deducted from total ABA and LAM estimated. The remaining amount of drug was assumed to be recovered from that was added. The results of recovery studies on the marketed prepartion are shown in Table 1.

viii)Validation parameters

a) Accuracy: Accuracy of the proposed method was ascertained on the basis of recovery
studies performed by standard addition method. The results of recovery studies are shown in
Table 1.

b) Precision: Precision of the proposed method is expressed as S.D of series of measurement.
The results are shown in Table 1.

c) Specificity: An accurately weighed quantity of tablet powder equivalent to 25 mg of ABA
were taken in different volumetric flasks (25 mL) and were stored for 24 h under the following different conditions.

i. At room temperature (normal)
ii. At 50^0after addition of 1.0 mL of 0.1 M NaOH.
iii. At 50^0after addition of 1.0 mL of 0.1 M HCl.
iv. At 50^0after addition of 1.0 mL of 3% H_2O_2.

The samples were diluted with acetonitrile and then volume was made up to 25 mL with methanol and filtered through Whatman filters. Aliquot of the filtrate was diluted with distilled water so as to get concentration equivalent to 20 µg/mL of ABA. The solutions were analyzed as per the procedure described for the analysis of laboratory mixture. The results of specificity studies are shown in Table 2.

d) Ruggedness: An accurately weighed quantity of tablet powder equivalent to 25 mg of
ABA was taken in volumetric flasks (25 mL). The sample was dissolved in acetonitrile (15 mL) and the volume was made up to the mark i.e.25 mL with methanol. The solution was then and filtered through Whatman filters. Aliquot of the filtrate was diluted with distilled water so as to get concentration equivalent to ABA (20 µg/mL). The solution was analyzed as per the procedure described for the analysis of laboratory mixture. It involves the Performance of the test under two different conditions -different days and different analysts.
The results are shown in Table 3.

RESULTS AND DISCUSSION

The first order derivative technique has determined the percent content of ABA as 100.05 and LAM as 100.11 in bulk drug mixture whereas the analysis of marketed tablet estimated the percent of the label claim as ABA-99.01 and LAM-99.09. The recovery studies done by standard addition method has given satisfactory results as ABA -99.81 and LAM-100.03 respectively. Validation of the proposed method was carried out as per ICH guidelines and the results obtained were found to be satisfactory.

CONCLUSION

The main advantage of the proposed method is its suitability for routine determination of ABA and LAM from the marketed tablet formulations as the results obtained reflects the accuracy, sensitivity, precision of the method.

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Table 1: Estimation of ABA and LAM in standard laboratory mixture, marketed formulation and by recovery studies

Drug	Analytical test	Mean% estimated	±SD	SE	CV
ABA	**Standard**	100.05	0.37	0.17	0.0037
LAM	**laboratory mixture**	100.11	0.76	0.34	0.0076
ABA	**Marketed**	99.01	0.38	0.17	0.0039
LAM	**formulation**	99.09	0.41	0.18	0.0041
ABA	**Recovery**	99.81	0.55	0.25	0.0055
LAM	**studies**	100.03	0.37	0.17	0.0037

Table 2: Specificity studies

S. No.	Sample	% of label claim	
		ABA	LAM
1	Normal	95.15	97.01
2	Alkali	23.2	44.7
3	Acid	28.6	46.3
4	Oxide	30.5	44.1

Table 3: Results of Ruggedness Parameters

i) Different analyst

S. No.	Analyst	% of label claimed	
		ABA	LAM
1	1	99.25	98.81
2	2	99.75	98.79
3	3	98.61	99.15

ii) Different days

S. No.	Days	% of label claim	
		ABA	LAM
1	1	98.56	98.92
2	2	99.62	99.19
3	3	99.18	99.01
	Mean	99.12	99.04

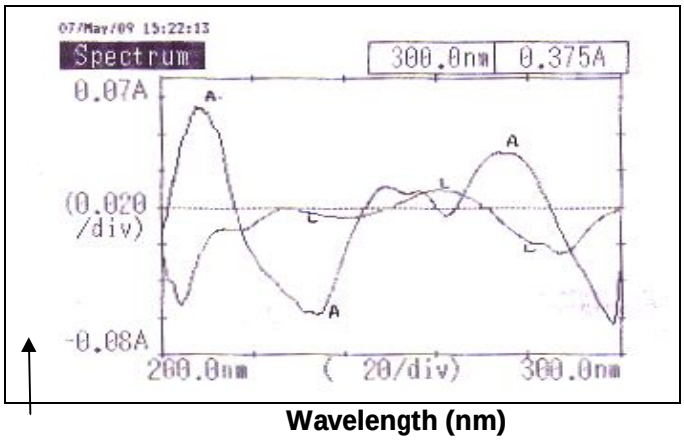

Fig. 1: First order derivative overlain spectra of ABA and LAM (20:10 µg/mL)

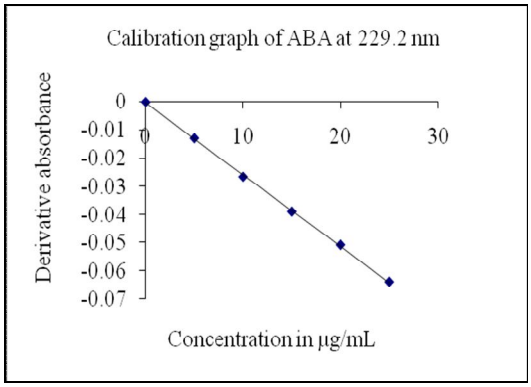

Fig. 2: Calibration curve of ABA at 229.2 nm

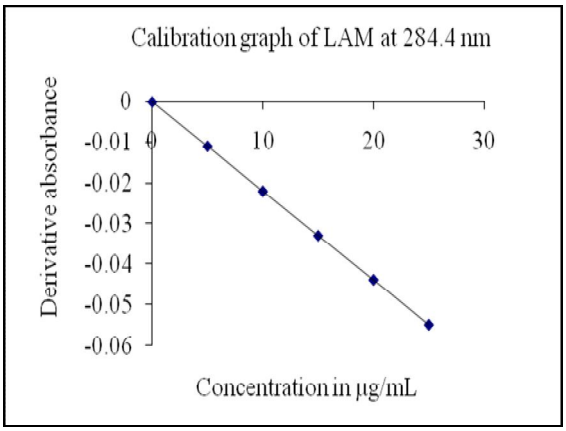

Fig. 3: Calibration curve of LAM at 284.4 nm

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