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DEVELOPMENT OF UV SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ABACAVIR AND LAMIVUDINE IN COMBINED TABLET DOSAGE FORM USING MULTICOMPONENT MODE

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ABSTRACT

A simple, accurate, precise, economical 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 spectrophotometry in multicomponent mode. The standard solutions of abacavir and lamivudine in mixture of acetonitrile and methanol were diluted with distilled water individually to get the concentration of (20 µg/mL and 10 µg/mL) and the scanning range between 200 nm to 350 nm was selected. The sampling wavelengths 224, 241, 257, 280 and 296 nm was selected and the concentrations of individual drugs in five mixed standard solutions were fed to the multicomponent mode of the instrument. 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 with relative ease.

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 UV spectrophotometry using multicomponent mode. Hence the present work has been carried out to develop an economical and simple method by spectrophotometry for the simultaneous estimation of ABA and LAM in bulk and marketed formulation.

MATERIAL AND METHODS:

Materials: Shimadzu 1601 UV-Visible spectro photometer 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:

Standard solutions: An accurately weighed quantity of ABA and LAM (50 mg each) were transferred to two separate volumetric flasks (50 mL). Both drugs were dissolved in acetonitrile and volume was made up to 50 mL with methanol. This gave standard stock solutions having 1 mg/mL (1000 µg/mL) of ABA and LAM, respectively.

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

Selection of Scanning Range and Sampling Wavelengths: The scanning range between 200 nm to 350 nm was selected and sampling wavelengths 224, 241, 257, 280 and 296 nm was selected on trial and error basis. The concentrations of individual drugs in five mixed standard solutions were fed to the multicomponent mode of the instrument. All the five mixed standards were scanned at above selected wavelengths. The graph is depicted in **Figure 1.**

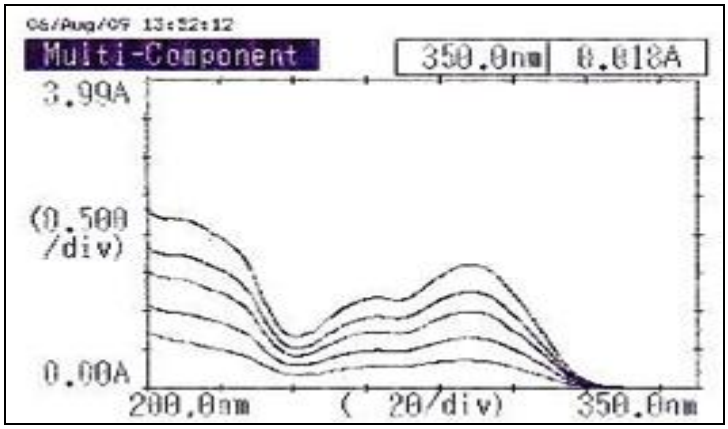
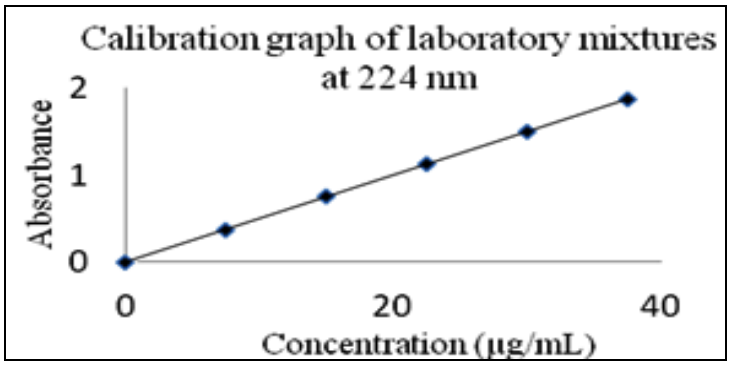
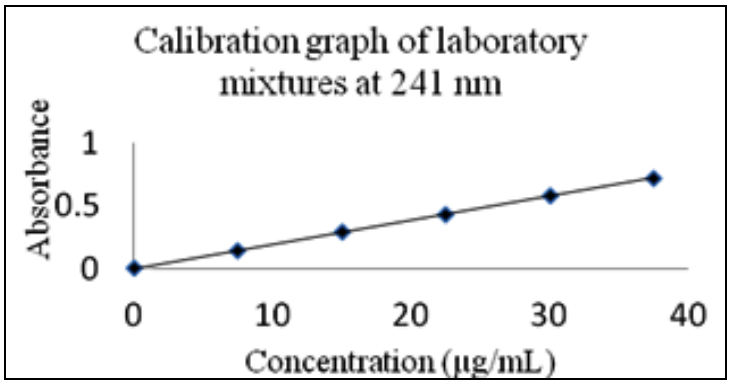
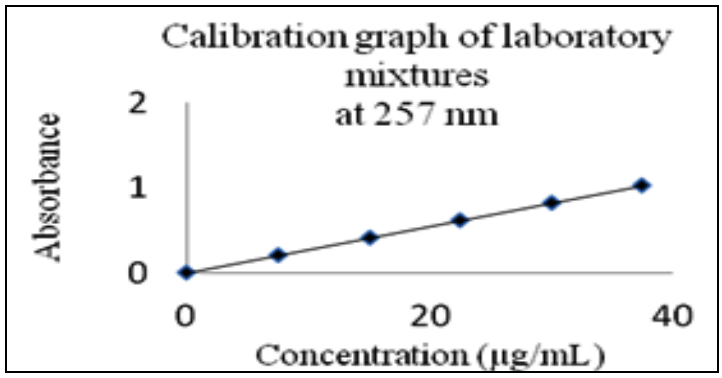


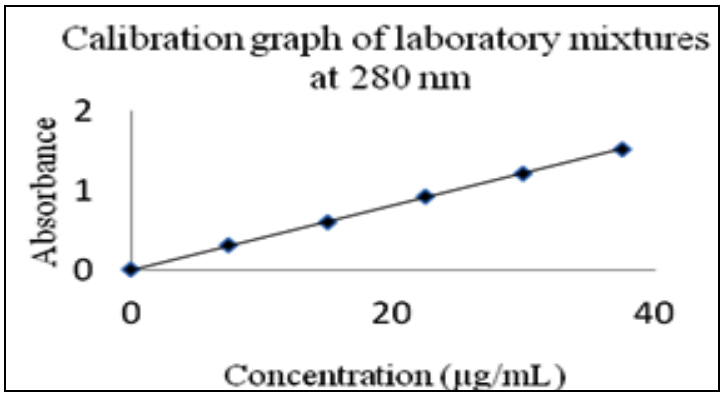
FIGURE 1: OVERLAIN SPECTRA OF MIXED STANDARD SOLUTION AT SAMPLING WAVELENGTHS

Study of Beer's- Lambert's law at selected wavelengths: All the mixed standards followed the Beer's-Lambert's law at the selected wavelengths. The results of the study are represented graphically in **Figure 2.**









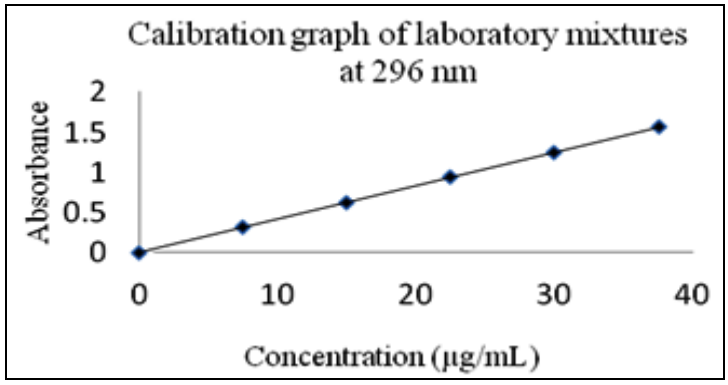


FIGURE 2: STUDY OF BEER-LAMBERT'S LAW AT SELECTED SAMPLING WAVELENGTHS

Application of proposed method for the analysis of laboratory mixture:

Mixed Standard Solutions: Accurately weighed quantities of ABA (50 mg) and LAM (50 mg) were transferred to (50 mL) volumetric flasks and dissolved in acetonitrile. The volume was made up to the mark (50 mL) with methanol. The above solutions were appropriately mixed and diluted with distilled water to obtain final concentration in the range of mixed standards. The five wavelengths (224, 241, 257, 280, 296 nm) were selected on the multicomponent mode of spectrophotometer. The concentrations of both the components of each of the five mixed standards were entered in it.

Then, all the mixed standard solutions were scanned over the range of 200 nm to 350 nm against solvent blank followed by calculation command. The sample solution was scanned over the same wavelength range. The instrument solves the equations by least square method and directly gives the concentration of individual components in μg/mL. The results of the estimation are shown in **Table 1.**

Application of proposed method for the estimation of ABA and LAM in tablets: Commercially available tablets containing ABA (600 mg) and LAM (300 mg) were used. The content of both the drugs in tablets were estimated by same procedure as described in standard laboratory mixture after dissolving the appropriate quantity of tablet powder equivalent to ABA (50 mg) in acetonitrile in volumetric flask (50 mL) followed by vigorous shaking and then the volume was made up to the mark i.e. 50 mL with methanol. Tablet solution was filtered through Whatman filter No. 1 and aliquots were diluted with distilled water to get the solution containing ABA (20 μg/ mL) and LAM (10 μg/ mL).The contents were calculated automatically by the instrument using least square method and directly gives the concentration of individual components in μg/mL The results are shown in **Table 1.**

Recovery studies: An accurately weighed tablet powder equivalent to about ABA (25 mg) was taken in volumetric flask (25 mL) and accurately weighed quantities of ABA and LAM (˜5 mg each) were added to it followed by addition of 15 mL of acetonitrile. The mixture was shaken for 15 min. The volume was adjusted with methanol up to 25 mL. The solution was filtered through Whatman filter No. 1. Aliquots portion of the resultant solution was appropriately diluted with distilled water to get the final concentration within the concentration range of mixed standards.

The content of both the drugs were analyzed by same procedure mentioned for standard laboratory mixture. The weight of ABA and LAM contributed by tablet calculated earlier was deducted from total amount of ABA and LAM. The remaining amount of drug was assumed to be recovered from that of added. The results of recovery studies on the marketed preparation are shown in **Table 1.**

Validation parameters: Study of some validation parameters like accuracy (recovery studies), precision (S.D), specificity and ruggedness were carried out as per ICH guidelines and the results are shown in **Table 1 and 2.**

TABLE 1: ANALYSIS OF STANDARD LABORATORY MIXTURE, MARKETED TABLETS AND RECOVERY STUDIES FOR ABA AND LAM BY PROPOSED METHOD

Standard laboratory mixture	**Drug**	**%Mean**	**±SD**	**SE**	**CV**
	ABA	99.995	1.118	0.5	0.011
	LAM	100.009	1.129	0.5	0.011
Tablets	**ABA**	99.122	0.3482	0.155	0.0035
	LAM	99.118	0.3571	0.159	0.0036
Recovery studies	**ABA**	99.56	0.5335	0.238	0.0053
	LAM	99.558	0.5386	0.240	0.0054

TABLE 2: RESULTS OF SPECIFICITY AND RUGGEDNESS

Specificity parameters			
S. No.	**Sample**	**% of label claimed**	
		ABA	**LAM**
1	Normal	96.27	96.82
2	Alkali	21.8	46.3
3	Acid	28.4	46.6
4	Oxide	32.3	43.6
Ruggedness parameters: **i) Different analyst**			
S. No.	**Analyst**	**% of label claimed**	
		ABA	**LAM**
1	1	98.85	98.92
2	2	99.13	99.17
3	3	99.26	99.03
ii) Different days			
S. No.	**Days**	**% of label claim**	
		ABA	**LAM**
1	1	99.05	99.19
2	2	99.2	98.75
3	3	98.81	99.16
	Mean	99.02	99.03

RESULTS AND DISCUSSION: Thus, the results obtained by the proposed multicomponent mode technique has determined the percent content of ABA as 99.99 and LAM as 100 in bulk drug mixture whereas the analysis of marketed tablet estimated the percent of the label claim as ABA-99.12 and LAM-99.11.

The recovery studies done by standard addition method has given satisfactory results as ABA -99.56 and LAM-99.55 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 ease of estimation 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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