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SIMULTANEOUS ESTIMATION OF ABACAVIR, LAMIVUDINE AND ZIDOVUDINE IN COMBINED TABLET DOSAGE FORM BY UV SPECTROPHOTOMETRIC METHOD

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ABSTRACT
The present study deals with the development of an accurate, economical and reproducible UV spectrophotometric method for the simultaneous estimation of abacavir, lamivudine and zidovudine in pure bulk drug and in combined tablet dosage form. The stock solutions were prepared in acetonitrile followed by the further required dilutions with distilled water. The λ max for abacavir, lamivudine, and zidovudine were observed at 295.6, 279.8 and 266.2 nm respectively and linearity was also shown at these wavelengths in concentration range of 5-30 µg/ mL, 5-25µg/ mL and 5-30µg/mL by all the three drugs. Mixtures containing abacavir -30 µg/mL, lamivudine-15µg/mL and zidovudine- 30µg/mL were analyzed at their respective wavelengths and using the simultaneous equations (Cramer's rule), their contents were calculated.
The proposed method has estimated abacavir 99.25%, lamivudine 99.12% and zidovudine 100.16% in standard mixture and 98.55%, 99.70 and 99.92% respectively in the marketed tablets. The results of analysis have been validated statistically and also by recovery studies. Thus the present study gives an excellent method for the determination of all the three drugs in combined dosage formulation without their prior separation.
KEYWORDS: Abacavir (ABA), Lamivudine (LAM), Zidovudine (ZID) and Cramer's rule.

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INTRODUCTION

Abacavir (ABA), Lamivudine (LAM), and Zidovudine (ZID) are Nucleoside analogs of anti HIV drugs. Several methods including spectrophotometric[1,2], HPLC[3,5-8] and HPTLC[4] methods have been reported for their estimation alone or with other drugs. But no method has been found for their simultaneous estimation in selected fixed dose combination by UV spectrophotometric technique.

MATERIALS AND METHODS

Materials

Shimadzu's 1601 UV–visible spectrophotometer with a matched pair of 10mm quartz cells, ABA, LAM and ZID pure drugs (Cipla Ltd. Patalganga and Kurkumbh, INDIA), Acetonitrile (LOBA, India Ltd), distilled water and tablets containing a combination of ABA-300mg, LAM-150mg and ZID-300 mg were used in the present study.

Methods

1mg/mL stock solutions of the drugs were prepared in acetonitrile. 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 -5-30 µg/ mL, LAM -5-25 µg/ mL and ZID-5-30µg/ mL respectively. The λ max for abacavir, lamivudine, and zidovudine were observed at 295.6, 279.8 and 266.2 nm respectively and the calibration graphs were plotted at the respective wavelengths (**Fig.1, 2 and 3**).

For the proposed method, a combination of drugs in concentration as ABA (30µg/ mL), LAM (15µg/ mL) and ZID (30µg/ mL) was selected and the overlain spectrum of the same was obtained (**Fig.4**).Similarly, their mixture containing the same concentration was also scanned and measured at 295.6, 279.8 and 266.2 nm respectively. From the absorbance values obtained of all the three drugs at all the three λ max, absorptivity values were calculated and are shown in **Table 1**.

The content in the mixture was determined by using the following three component equations/ Cramer's rule:

x component = A1 (β2 γ3 - β3 γ2)- A2(β1 γ3- β3 γ1)+ A3(β1 γ2- β2 γ1) /
α1((β2 γ3 - β3γ2)- α2(β1 γ3- β3 γ1)+ α3(β1 γ2- β2 γ1).

Similarly, **y** and **z** component can be estimated.

The content of all the three drugs in tablets were estimated by same procedure after dissolving the appropriate quantity of tablet powder equivalent to 50mg of abacavir in acetonitrile in a 50 mL volumetric flask followed by vigorous shaking and filtering. The volume was made up to the mark and then aliquots diluted with distilled water containing ABA (30µg/ mL), LAM (15µg/ mL) and ZID (30µg/ mL) were taken for analysis. The contents were calculated using the above mentioned formula. The results are shown in **Table 2**.

The validity of the method was done by performing the recovery studies using standard addition method. The validation parameters are depicted in **Table 3**.

RESULT AND DISCUSSION

The proposed method has estimated abacavir 99.25%, lamivudine 99.12% and zidovudine 100.16% in standard mixture and 98.55%, 99.70% and 99.92% respectively in the marketed tablets. The accuracy, selectivity and validity of the method were further assessed by applying the standard addition technique in the recovery studies, wherein sample was spiked with known quantity of standard drug of ABA, LAM and ZID. The percentage recovery was found to be ABA- 99.89 ± 0.05, LAM-99.52± 0.08 and ZID-100.02 ± 0.03 respectively. Validation of the findings was carried out according to the ICH guidelines.

The main advantage of the proposed method is its suitability for routine determination of ABA, LAM and ZID without their prior separation from marketed tablet formulations. The proposed method is economic, simple, most sensitive, precise and reproducible and do not require any expensive or sophisticated apparatus, in contrast with the reported chromatographic methods.

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Table 1: The absorptivity values of Abacavir, Lamivudine and Zidovudine in the proposed method.

Absorptivity values	Wavelength (nm)		
	295.6	**279.8**	**266.2**
ax_1	456.66	-	-
ax_2	-	274.33	-
ax_3	-	-	255.33
ay_1	250	-	-
ay_2	-	546	-
ay_3	-	-	385.33
az_1	18	-	-
az_2	-	232.66	-
az3	-	-	356.66

* ax_1, ax_2 and ax_3 are the absorptivity values of abacavir at the respective wavelengths.
** ay_1, ay_2 and ay_3 are the absorptivity values of lamivudine the respective wavelengths.
*** az_1, az_2 and az_3 are the absorptivity values of zidovudine the respective wavelengths.

Table 2: The results of the analysis of commercial formulations and the recovery studies

Standard mixture	Concentration (µg/mL)	Content estimated (µg/mL)	% amount estimated	Standard deviation (±)	
ABA	30	29.78	99.25	0.204	-
LAM	15	14.87	99.12	0.051	-
ZID	30	30.05	100.16	0.208	
Tablet	**Label claim (mg/tab)**	**Amount found (mg/tab)**	**%label claim**	**Standard deviation (±)**	**% recovery ± SD**
ABA	300	295.65	98.55	0.070	99.88±0.05
LAM	150	149.55	99.70	0.474	99.52±0.08
ZID	300	299.76	99.92	0.218	100.02±0.03

* All the values are the mean of five readings.

Table 3: Regression analysis of Calibration curves and summary of validation parameters

Parameters	ABA	LAM	ZID
Wavelength (nm)	295.6	279.8	266.2
Beer's law limit(µg/mL)	5-30	5-25	5-30
ε-Molar absorptivity (l/mol/cm)	1.307563x 10^4	1.251759x10^4	0.953138 x10^4
Limit of detection (µg/mL)	1	1	2
Limit of quantitation (µg/mL)	3	2	5
Sandell's sensitivity (µg/cm^2)	0.02189813	0.01831502	0.02803791
Regression equation*			
Intercept(α)	0.00428571	-1.11022E-16	0.00025
Slope(β)	0.04571429	0.0546	0.0356786
Correlation coefficient(r)	0.9999	1	0.9999

Where, *y= α + β x, x is the concentration of the analyte and y is the absorbance value.

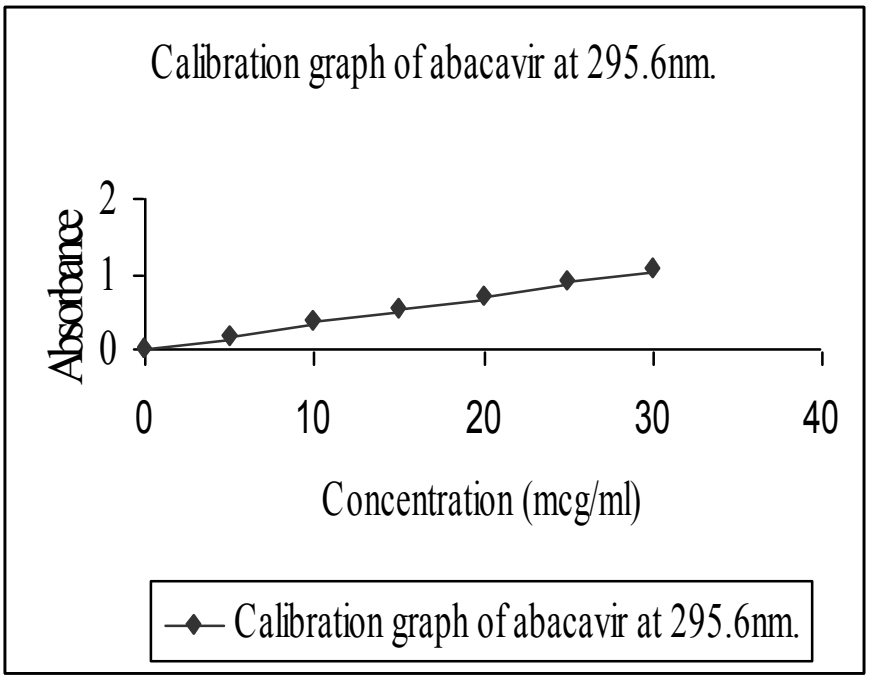

Fig.1: Calibration graph of abacavir at 295.6nm

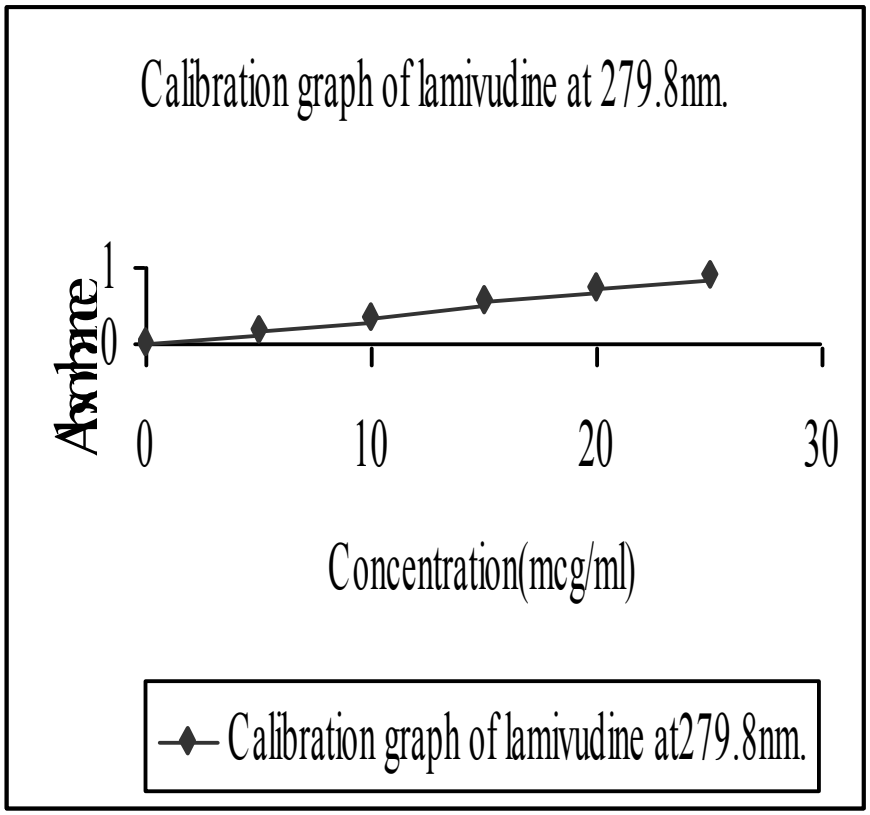

Fig.2: Calibration graph of lamivudine at 279.8nm

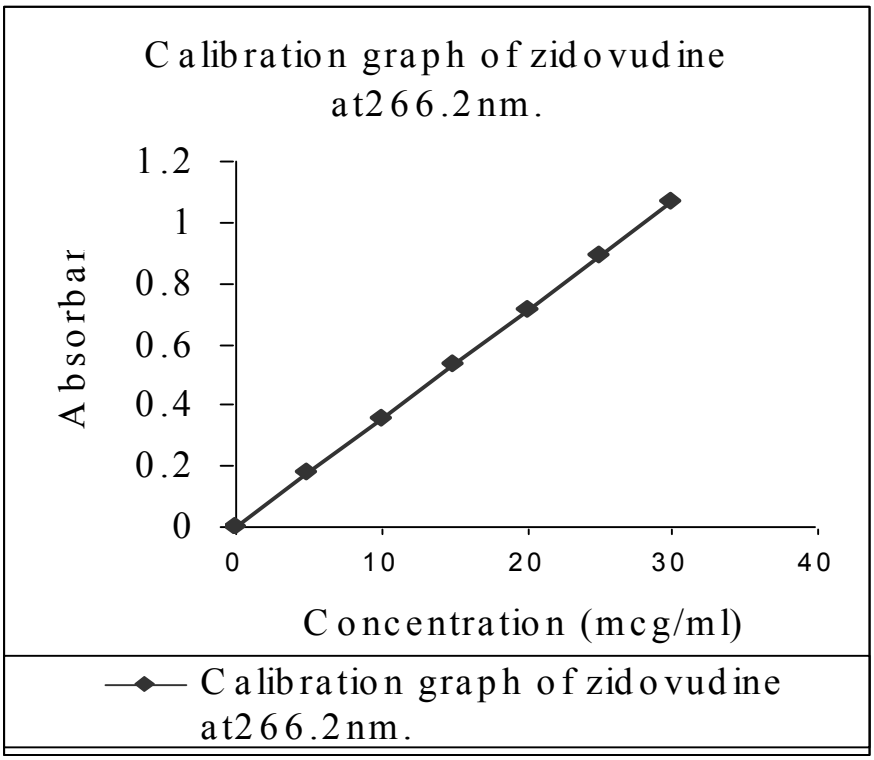

Fig.3: Calibration graph of zidovudine at 266.2 nm

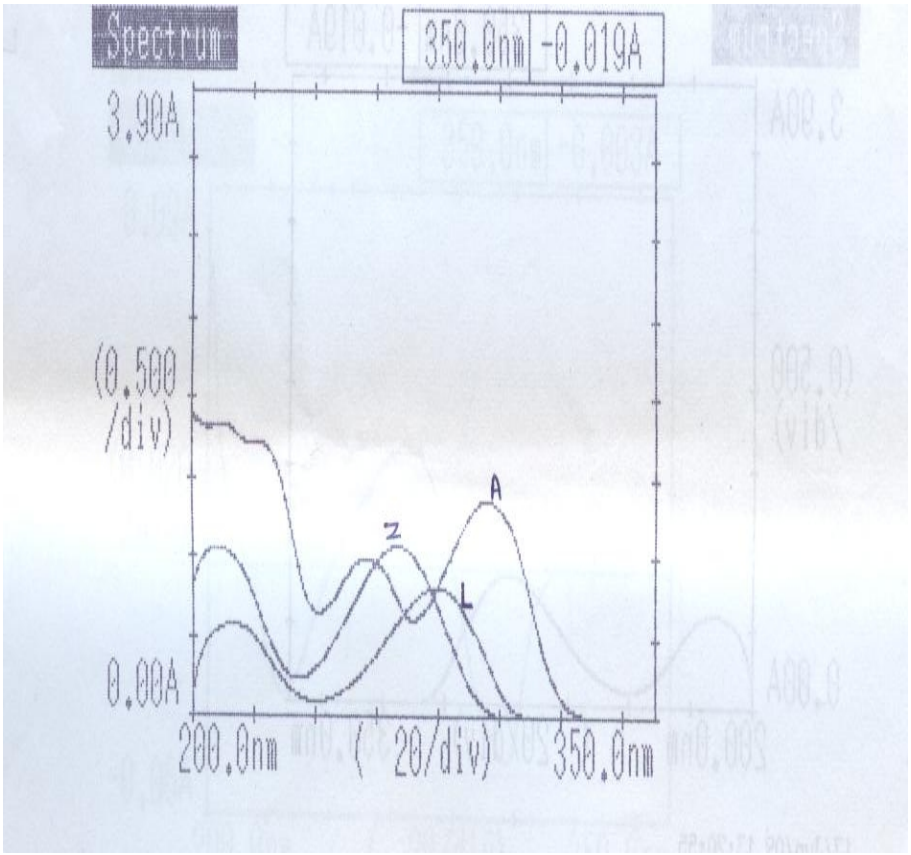

Fig.4. Overlain spectra of abacavir (30 µg/mL), lamivudine (15µg/ mL) and zidovudine (30µg / mL)

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