Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2012, 4 (3):728-733
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Development of uv spectrophotometric vierodt's method for the simultaneous estimation of abacavir and lamivudine in combined tablet dosage form

Vaishali P. Nagulwar* and Kishor P. Bhusari

Sharad Pawar College of Pharmacy, Wanadongri, Hingna Road, Nagpur(MS), India

---

ABSTRACT

Simple, accurate, precise, economical and reproducible analytical method have been developed for the simultaneous estimation of abacavir and lamivudine in pure bulk drug and in combined tablet dosage form by UV spectrophotometric Vierodt's method. The stock solutions were prepared in mixture of acetonitrile and methanol (3:2) followed by the further required dilutions with distilled water. In proposed Vierodt's method, the λmax for the estimation of abacavir and lamivudine were selected at 260 nm and 271 nm respectively. Linearity in concentration range of 5-25 µg/ ml by both the drugs was found. The method has estimated abacavir 100.01%, lamivudine 100.02% in the bulk drug and 99.86%, 99.82% of the both in tablets respectively. The results of analysis have been validated statistically and also by recovery studies. Thus the present study gives excellent method for the determination of both the drugs in combined tablet formulation.

Keywords: ABA, LAM, 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 UV spectrophotometric Vierodt's method. Hence the present work has been carried out.

MATERIALS AND METHODS

Materials:
Shimadzu 1601UV –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) Standard stock solution: The stock solutions having 1 mg/mL solutions of ABA and LAM were prepared by dissolving an accurately weighed quantity of (50 mg) of both the drugs in acetonitrile (30 mL). The volume was made up to mark with methanol (50 mL) in two separate volumetric flasks (50 mL).

ii) Mixed standard solutions: Aliquots of both the stock solutions were mixed and diluted using distilled water so as to get the mixed concentrations as 10:5, 15:7.5, 20:10, 25: 12.5 and 30: 15 µg/mL of ABA and LAM respectively.

iii) Study of wavelengths of peak absorption of the drugs

A solution of ABA (20 µg/mL) and LAM (10 µg/mL) were scanned over the UV range from 400 nm to 200 nm and the λmax of ABA and LAM were found as 260 nm and 271 nm, respectively. An overlain spectrum of both the drugs is shown in Figure-1.

Figure- 1. Overlain spectrum of ABA and LAM (20:10 µg/mL)

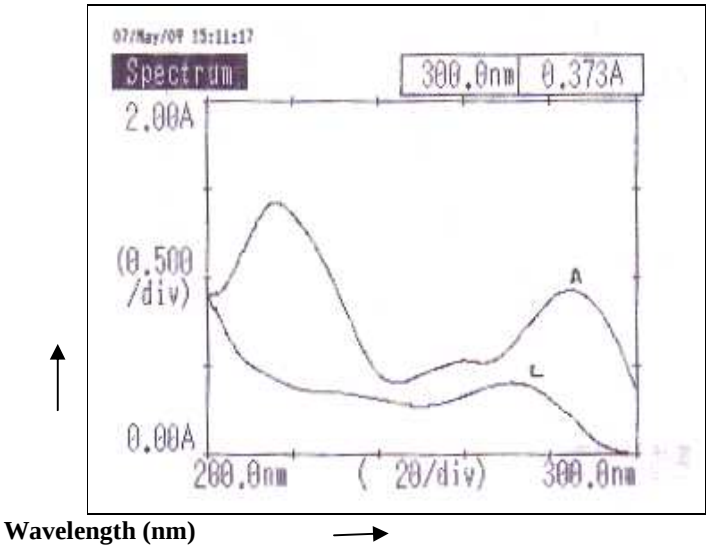
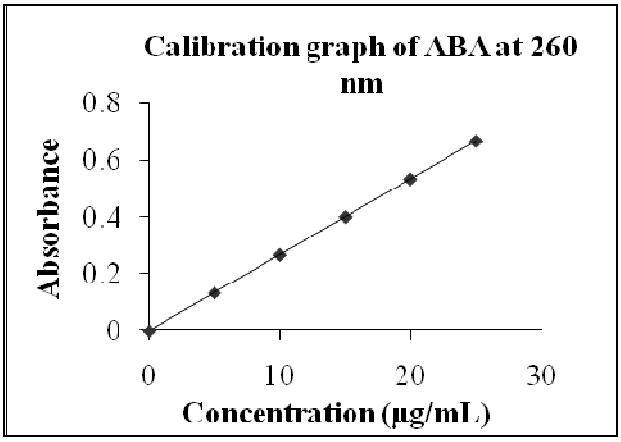


Figure-2. Calibration graph of ABA at 260 nm



Study of Beer-Lambert's law for individual components and their mixture

By appropriate dilutions of both the stock solutions using distilled water, the working standard solutions having concentration of ABA and LAM as 5-25 µg/mL were prepared and scanned for absorbance values. From the absorbance values obtained at 260 nm of ABA and 271 nm of LAM and of mixed standards solutions respectively,

calibration graphs were plotted which were found to be linear over the selected range (Figure-2, Figure-3,Figure- 4 and Figure-5).

Figure-3. Calibration graph of LAM at 271 nm

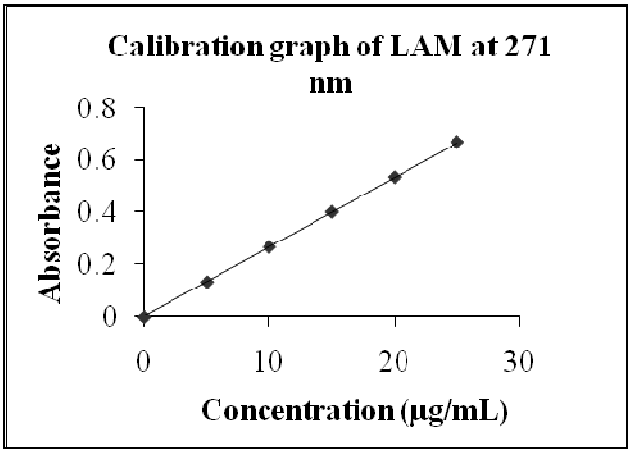


Figure-4.Calibration graph of laboratory mixture at 260 nm

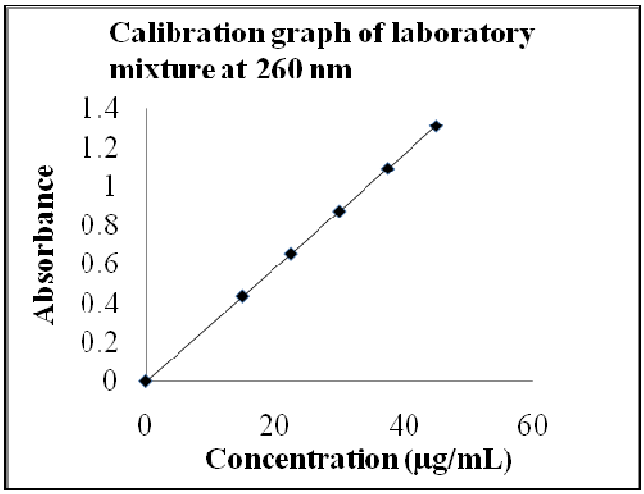
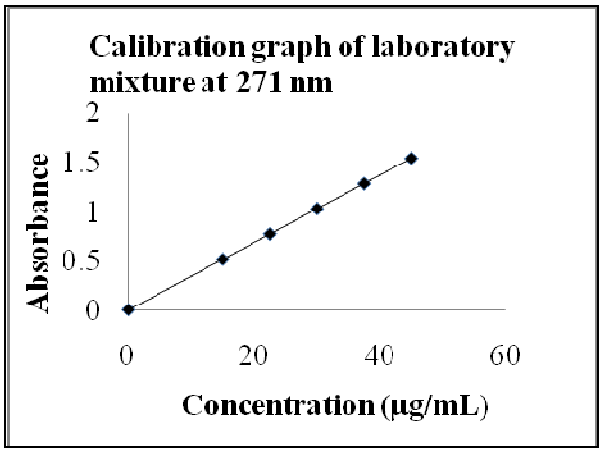


Figure-5. Calibration graph of laboratory mixture at 271 nm



Determination of absorptivity values of drugs at selected wavelengths

In the Abs/%T mode, the absorbances of ABA and LAM drug solutions were measured at analytical wavelengths. These values were divided by the concentration to get their absorptivities by using the following equation-

E 1%,1 cm= Absorbance/Concentration(g per 100 mL)

The calculated absorptivity values of ABA and LAM are shown in Table- 1.

Table- 1. Absorptivity values of ABA and LAM in the proposed method

Absorptivity values	Wavelengths (nm)	
	260	**271**
ax_1	267.5	-
ax_2	-	309.5
ay_1	338	-
ay_2	-	409

ax_1 and ax_2 = absorptivity values of ABA at the respective wavelengths
ay_1 and ay_2 = absorptivity values of LAM at the respective wavelengths

Estimation of drugs in standard laboratory mixture by proposed method

Mixed standard solution of concentration 20:10 µg/mL of ABA and LAM were prepared by diluting appropriate portion of standard stock solution of both drugs with distilled water and was analyzed at the selected wavelengths. From the absorbance values obtained at respective wavelengths, concentrations of both the drugs were calculated using following Vierodt's equation.

$cx = (A_2\ ay_1 - A_1 ay_2)/ (ax_2 ay_1 - ax_1 ay_2)$ …. (Eq.1)
$cy = (A_1 ax_2 - A_2 ax_1)/ (ax_2 ay_1 - ax_1 ay_2)$ …. (Eq.2)

where,
cx and cy = concentration of ABA and LAM
ax_1 and ax_2 = absorptivity values of ABA at 260 nm and 271 nm, respectively
ay_1 and ay_2 = absorptivity values of LAM at 260 nm and 271 nm, respectively
A_1 and A_2 = absorbance of diluted sample at 260 nm and 271 nm, respectively

The results obtained are summarized in Table- 2.

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 and transferred to volumetric flask (50 mL) and dissolved in acetonitrile. The volume was made up to with the methanol (50 mL). The tablet solution was filtered through Whatman filter paper No.1. Aliquots of the resultant solution was diluted with distilled water to get the working standards of ABA as 20 µg/mL (LAM~ 10 µg/mL). The sample solution was scanned for the absorbance of ABA and LAM at 260 nm and 271 nm respectively. The concentration of both the drugs in the tablet solution was determined by using simultaneous equation. The results are shown in Table- 2.

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) for 15 min and then diluted up to 25 mL with methanol. The solution was filtered through Whatman filter paper No.1. Aliquot portion of the resultant solution was appropriately diluted with distilled water to get concentrations within the linearity concentration range of standard solutions. The absorbances of the solution was measured at the analytical wavelengths. The content of both the drugs were calculated using simultaneous equation.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- 2.

Table 2: 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 laboratory mixture**	100.01	0.14	0.063	0.0014
LAM		100.02	0.12	0.054	0.0012
ABA	**Marketed formulation**	99.86	0.69	0.30	0.0069
LAM		99.82	0.74	0.33	0.0074
ABA	**Recovery studies**	100.01	0.3	0.13	0.003
LAM		100.11	0.4	0.18	0.004

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 2 and 3.

Table -3. Results of specificity and ruggedness

Specificity parameters			
S. No.	**Sample**	**% of label claim**	
		ABA	**LAM**
1	Normal	96.17	97.19
2	Alkali	22.5	45.6
3	Acid	28.7	46.7
4	Oxide	31.5	43.6
Ruggedness parameters: i) Different analyst			
S. No.	**Analyst**	**% of label claimed**	
		ABA	**LAM**
1	1	99.13	99.75
2	2	99.82	99.71
3	3	99.76	98.65
ii) Different days			
S. No.	**Days**	**% of label claim**	
		ABA	**LAM**
1	1	99.16	99.32
2	2	99.73	98.62
3	3	98.18	99.17
Mean		99.02	99.04

RESULTS AND DISCUSSION

The present method of estimation for the simultaneous quantitation of ABA and LAM has determined the percent content of ABA as 100.01 and LAM as 100.02 in bulk drug mixture whereas the analysis of marketed tablet estimated the percent of the label claim as ABA-99.86 and LAM-99.82. The recovery studies done by standard addition method has given satisfactory results as ABA -100.01 and LAM-100.11 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.

Acknowledgements

The authors are thankful to the Director, Sharad Pawar College of Pharmacy, Nagpur for providing necessary facilities for research work. They are also grateful to Cipla Ltd. Goa and Patalganga for giving gift samples of pure drugs.

REFERENCES

[1] SK Appalaraju; B Arvind; OS Kamalapurkar; S Sarasambi. *Asian Journal of Chemistry*, **2002**, 14, 815.
[2] S Mirza; PS Palaskar; MHG Dehghan; SN Mokale. *Asian Journal of Chemistry*, **2009**, 2(4), 461.
[3] VP Devmurari. *International Journal of Pharmaceutical Sciences and Research*, **2010**, 1(7), 83.

[4] T Sudha; J Saminathan; A Kondapalli; K Manthena; B Yanamadala; V Ganesan. *Journal of Chemistry and Pharmaceutical Research*, **2010**, 2(5), 45.
[5] T Sudha; VR Ravikumar; PV Hemalatha. *International Journal of Pharmaceutical and Biomedical Research*, **2010**, 1(4), 108.
[6] T Sudha; VR Ravikumar; PV Hemalatha. *International Journal of Pharmaceutical Sciences and Research*, **2010**, 1(11), 107.