International Journal of Pharmaceutical Applications ISSN 0976-2639.
Vol 3, Issue 1, 2012, pp 293-296 http://www.bipublication.com

DEVELOPMMENT OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ABACAVIR AND LAMIVUDINE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT
A validated simple and accurate RP-HPLC method has been developed for the simultaneous estimation of abacavir (ABA) and lamivudine (LAM) in pure bulk drug and in tablet dosage form. The stock solutions were prepared in mixture of acetonitrile and methanol followed by the further required dilutions with distilled water. The mobile phase composition was Methanol: phosphate buffer- pH 3.0 (35:65 v/v). The retention time for abacavir and lamivudine were observed at 9.05 and 5.192 min, respectively with flow rate of 0.6 ml/min at 270 nm. The proposed method has estimated abacavir 98.6 % and lamivudine 98.75 % in marketed tablets. The results of analysis have been validated statistically and also by recovery studies.
Keywords: ABA, LAM, RP-HPLC, Methanol-buffer, Tablets, Validation.

[I] 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 so economically as proposed. Hence the present work has been carried out.

[II]MATERIAL AND METHODS

2.1. Materials:

Chemito LC 6600 equipped with Knauer HPLC pump K-501 and Chemito UV-visible detector connected to Chemito's Chemitochrom C2000 data module. Column used was Eurosphere 100-5 C_{18} (250 mm x 4.6 mm) with precolumn was used in the present study. HPLC grade Methanol, Water (Loba, India Ltd.) and other reagents were utilized. ABA and LAM pure drugs were generously donated as gift samples by Cipla Ltd. Goa and Patalganga, INDIA. The commercially available tablets containing a combination of ABA-600mg and LAM -300 mg were procured from pharmacy.

2.2. Methods:

Standard drug solutions

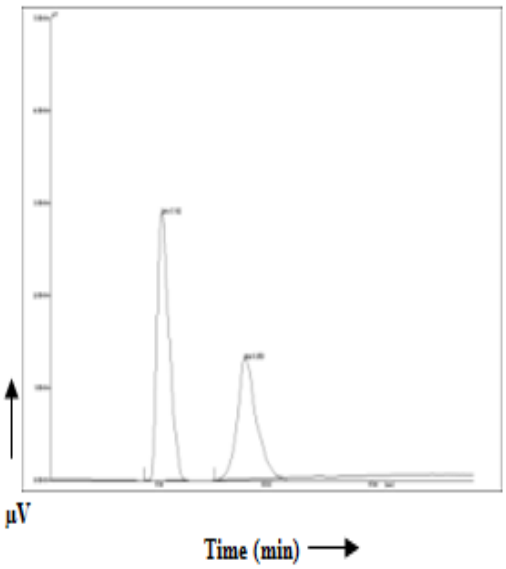
Standard stock solutions of 1 mg/ ml of ABA and LAM were prepared by dissolving appropriate amount of both in mixture of acetonitrile and methanol (3:2).

Chromatographic parameters

Column	:	Eurosphere 100-5 C_{18} (250 x 4.6mm) with precolumn
Detection wavelength	:	270 nm
Flow rate	:	0.6 mL/min
Temp	:	Ambient
Injection volume	:	20 µL
Mobile phase	:	Methanol: buffer pH 3.0 (35:65v/v)

The mobile phase containing methanol: buffer pH 3.0 (35:65v/v) was selected due to high resolution, sensitivity and suitability for the determination of ABA and LAM in combined dosage form. The chromatogram is shown in **[Figure-1].**

Fig: 1. Chromatogram of ABA and LAM



Study of linearity range

The aliquot portions of stock solution of ABA and LAM were diluted with distilled water to get concentration range of ABA and LAM between 10-50 µg/mL and 5-25 µg/mL, respectively. The mobile phase was allowed to equilibrate with stationary phase. Each of the standard solution was injected separately. The chromatograms were recorded. The linearity graph was plotted as concentration against detector response (peak area) and is depicted in **[Figure-2]** and **[Figure- 3].**

Fig: 2. Calibration graph of ABA

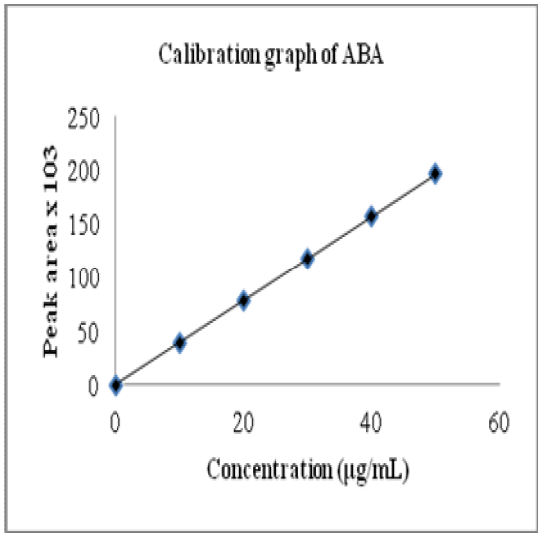
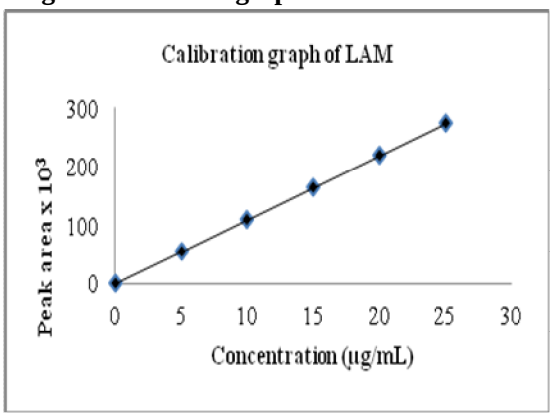


Fig: 3.Calibration graph of LAM



The data of verification of linearity and system suitability of proposed method for ABA and LAM is given below.

Table: 1. Verification of linearity

Drug	Regression parameters		r
	Slope	Intercept	
ABA	3.93134	-0.00033	0.998
LAM	10.97145	-0.00014	0.994

Table: 2. Data of system suitability tests

Drug	Retention time (min)	Resolution	Capacity factor	Column efficiency Theoretical plates	Asymmetric factor
				Per column (30 cm)	
ABA	9.050	0.000	1	650.49	1.23
LAM	5.192	3.1620	1	409.07	1.40

Analysis of laboratory mixture by proposed method

Standard stock solutions of ABA and LAM were mixed in the ratio 2:1, respectively. The resulting solution was diluted with distilled water to get different concentrations ranging from ABA and Lam as 10:5, 20:10, 30:15, 40:20 and 50:25 µg/mL. Standard laboratory mixture was injected separately and the chromatograms were recorded. The concentration of each drug was estimated by comparing peak area with standard. The results of estimation of standard laboratory mixture are shown in [Table- 3]. Analysis of tablets by proposed method

Tablets containing ABA (600 mg) and LAM (300 mg) were finely powdered. An accurately weighed quantity of tablet powder equivalent to

about ABA (25 mg) was dissolved in acetonitrile (15 mL) in volumetric flask (25 mL). The volume was made up to 25 mL with methanol. The solution was filtered. The filtrate was diluted with distilled water to get the concentration of ABA equivalent to 20 µg/mL. The mobile phase was allowed to equilibrate with stationary phase. The equal volume of standard and sample solution was injected separately. The chromatograms were recorded and the contents of ABA and LAM were calculated. The results are shown in [Table-3].

Recovery studies

To an accurately weighed quantity of preanalysed tablet powder equivalent to ABA (25 mg) in volumetric flask (25 mL), pure drugs of ABA and LAM (5 mg each) were added. The content was shaken for 15 min with acetonitrile and then the volume was made up to the mark with methanol. The solution was filtered. An aliquot portion of the resultant solution was appropriately diluted with distilled water to get final concentration within the range of mixed standard. The results of recovery study are shown in **[Table- 3].**

Table: 3. 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**	99.302	0.66	0.295	0.0066
LAM		98.984	0.47	0.210	0.0047
ABA	**Marketed formulation**	98.60	0.51	0.23	0.0051
LAM		98.75	0.43	0.19	0.0043
ABA	**Recovery studies**	99.45	0.60	0.27	0.0060
LAM		99.04	0.73	0.33	0.0073

Validation parameters

Validation of the proposed RP-HPLC method was carried out as per ICH guidelines. The results of various parameters like accuracy (recovery studies), precision (±SD), specificity and ruggedness are shown in **[Table-3 and 4].**

Table: 4. Results of Specificity and Ruggedness

Specificity parameters

S. No.	Sample	% of label claim	
		ABA	**LAM**
1	Normal	96.3	97.16
2	Alkali	22.8	45.2
3	Acid	28.6	46.5
4	Oxide	31.6	43.4

Ruggedness parameters:

i) Different analyst

S. No.	Analyst	% of label claimed	
		ABA	**LAM**
1	1	99.02	98.91
2	2	99.20	98.95
3	3	98.79	98.92

ii) Different days

S. No.	Days	% of label claim	
		ABA	**LAM**
1	1	99.23	99.16
2	2	99.11	99.72
3	3	98.82	99.91
	Mean	99.05	99.60

[III]RESULTS

The present RP-HPLC method has determined the percent content of ABA as 99.3 and LAM as 98.98 in bulk drug mixture whereas the analysis of marketed tablet estimated the percent of the label claim as ABA-98.6 and LAM-98.75. The recovery studies done by standard addition method has given satisfactory results as ABA-99.45 and LAM-99.04 respectively. Validation of the proposed method was carried out as per ICH guidelines and the results obtained were found to be satisfactory.

[IV]DISCUSSION

The proposed reversed phase chromatographic method for the simultaneous estimation of ABA and LAM was found to be very simple and accurate. The chromatographic conditions reflecting the flow rate of 0.6 ml/min and the mobile phase composition indicates that the developed method is economical too for routine analysis of both the drugs in combined tablet dosage form.

[V]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 that the developed method is more accurate, sensitive, precise and economical.

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.

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