Original Article

Method Development and Validation of a Reverse phase Liquid Chromatographic Method for Simultaneous Determination of Lamivudine and Abacavir Sulphate in Tablets

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ABSTRACT

A reverse phase liquid chromatographic method for the simultaneous determination of lamivudine and abacavir in pure and tablet formulation was developed and validated. The method was found to simple, precise and accurate. The separation was carried out using Phenomenex C_{18} (250 x 4.6 mm, 5 µm particle size) column, with a mobile phase consisting of phosphate buffer (pH 7.8) and methanol in the ratio of 50:50 % v/v. The flow rate was set at 1.0 mL/min and detection was monitored at 216 nm. The retention times of lamivudine and abacavir were found to be 3.147 and 6.367 min, respectively. The linearity was found in the concentration range of and 80-280 µg/mL and 75-450 µg/mL for lamivudine and abacavir, respectively. The liquid chromatography method was extensively validated for linearity, accuracy, precision, and robustness. All these analytical validation parameters were found satisfactory and the % RSD was determined which indicates the usefulness of method for determination of lamivudine and abacavir in bulk drug and tablet formulation.

Keywords: Simultaneous LC, Lamivudine (LMV), Abacavir (ABA), Method development, Validation

INTRODUCTION

Lamivudine and abacavir are nucleoside analog reverse transcriptase inhibitors (NRTIs). NRTIs are a class of antiretroviral drugs that are used to treat HIV infected patients. NRTIs, developed in 1987, were the first drugs designed to treat HIV. They act by preventing HIV virus from replicating inside a cell. Lamivudine[1], (4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-

one) is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B. It's phosphorylated active metabolite competes for incorporation into viral DNA. The (-) enantiomer has less cytotoxicity and greater antiviral activity than the (+) enantiomer. It is rapidly absorbed with a bio-availability of over 80%. Lamivudine shows no evidence of carcinogenicity and mutagenicity.

Abacavir[2], ({(1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]cyclopent-2-en-1-yl}methanol) is an analog of guanosine. Its active metabolite carbovir triphosphate, an analogue of deoxyguanosine-5'-triphosphate (d GTP), inhibits the activity of HIV-1 reverse transcriptase. It has high bioavailability of 83%. It is metabolised primarily through alcohol dehydrogenase or glucuronyl transferase.

Literature survey of these drugs revealed that there are methods for the determination of lamivudine and abacavir by spectrophotometry[3-6], LC[7-13] and HPTLC[14]. As there is a necessity for estimation of these drugs in combination, the authors here proposed a new, accurate and precise simultaneous RP-LC method for the determination of lamivudine and abacavir in bulk and tablet dosage forms.

MATERIALS AND METHODS

Equipment

SHIMADZU LC 20AD LC system with SPD-20A UV/VIS detector equipped with spinchrom software was used for method development, Double-beam Perkin Elmer (LAMBDA 25) UV-VIS spectrophotometer was used for spectral measurements, ELICO pH meter was used for pH measurements.

Reagents and standards

Reference standard samples of lamivudine and abacavir were procured from Aurobindo Pharma Ltd., Hyderabad. Water and methanol (HPLC grade, Rankem); potassium dihydrogen orthophosphate (AR grade, Rankem) were used in the analysis. A commercial sample Abamune-L (cipla) tablets containing abacavir sulphate (600 mg) and lamivudine (300 mg) were purchased from the local market.

PROCEDURE

Chromatographic conditions

The separation was achieved on a Phenomenex C_{18} (250 mm x 4.6 mm, 5 µ) column, with a mobile phase of methanol:buffer of pH 7.8 in the ratio of 50:50 %v/v and at a flow rate of 1.0 mL/min. The detection was monitored at 216 nm and at ambient temperatures.

Preparation of buffer of pH 7.8

To 125 mL of 0.2 M potassium dihydrogen orthophosphate in 500 mL volumetric flask was added 111 mL of 0.2 M sodium hydroxide and the volume was made up to 500 mL with water. The pH was adjusted to 7.8 with 0.2 M sodium hydroxide, filtered through 0.45µm membrane filter and sonicated prior for 30 min to use.

Preparation of diluent

Equal volumes of methanol and buffer of pH 7.8 were mixed, filtered and deggased.

Preparation of stock and working standard solutions of LMV and ABA

Stock solutions of lamivudine and abacavir were prepared by dissolving 25 mg of each drug taken in a separate volumetric flask in specified diluent and volume is made to 25 mL with the same, sonicated for about 15 min. From the individual stock solutions, working standard solutions of 400 µg/mL of lamivudine and 750 µg/mL of Abacavir were prepared .

Chromatographic procedure

The standard solutions are injected in to chromatograph under optimized conditions and chromatograms were recorded.

Estimation of the drugs from tablet dosage forms

Twenty tablets of abamune-L, containing abacavir sulphate (600 mg) and lamivudine (300 mg) were weighed and finely powdered. A quantity of the powder equivalent to 600 mg of ABA and 300 mg of LMV was weighed, transferred into 50 mL volumetric flask and dissolved in the diluent by sonication for about 15 min. These solutions were filtered through 0.22 µm membrane filter and sonicated for 30 min. From the filtrate 6.25 mL was transferred to a 50 mL flask and made up to the volume with the diluent. Then these samples were injected and peaks were recorded.

RESULTS & DISCUSSION

Method development

The suitable parameters were chosen after several trails with buffers of several pH and various compositions of methanol and buffer. However the final parameters were adjusted to achieve good resolution. The trails revealed that with the increase in methanol concentration, the peaks obtained were broad with less symmetry. The peak obtained with a composition of buffer and methanol at 50:50% v/v was proved to be most suitable of all the combinations since the peaks obtained were better defined and resolved. To determine the effect of flow rate, the method was performed at different flow rates 0.5 mL/min, 0.7 mL/min, 1.1 mL/min and 1.2 mL/min. The optimum flow rate was 1.0 mL/min with retention times of 3.147 and 6.367, for lamivudine and abacavir respectively. A typical chromatogram obtained by using the above parameters was shown in Fig-1. System suitability parameters were shown in Table-1.

Method validation

The method was statistically validated as per ICH guidelines. The linearity was obtained in the range of 80-280 µg/mL and 75-450 µg/mL for lamivudine and abacavir, respectively where the area obtained was found to be linear with concentration of drug. The values were depicted in Table-2. The % RSD values of Intra-day and inter-day precision studies were found to be satisfactory and the results were depicted in Table-3. Ruggednesss of the method was determined by carrying out the method by several analysts under optimized chromatographic conditions. The % RSD was calculated and found to be less than 2%. The method was found to be robust by the studies performed on small change in flow rate, mobile phase composition and wavelength of absorption. The results were shown in Table-4. Accuracy of the method was studied by spiking the sample solution with known concentration of standard solution at various levels (50%, 100%, and 150%). The mean recovery and % RSD were calculated and depicted in Table-5.

CONCLUSION

It is evident from the present study that the RP-HPLC method for the simultaneous determination of lamivudine and abacavir in pure and tablets formulation was simple, accurate, specific and precise. The method was validated statistically. The results of recovery studies were in good agreement with the respective label claim of the formulation. There was no interference from the excipients present in the formulation. The retention times of the mentioned drugs were less than ten minutes. Thus the method is less time consuming and can be employed for routine analysis in laboratories.

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Table 1. System suitability parameters of the proposed method

Parameter	Lamivudine	Abacavir
Linearity (µg/mL)	80-280	75-450
Correlation coefficient	0.9998	0.9999
Retention time (min)	3.147	6.367
Theoretical plates	3808	8771
Tailing factor	0.53	0.47
LOD (µg/mL)	4.14	6.51

Table 2. Results of the linearity studies

Concentration of LMV (µg/mL)	Area	Concentration of ABA (µg/mL)	Area
80	1452	75	1618
120	2469	150	2597
160	3385	225	3657
200	4301	300	4589
240	5235	375	5608
280	6185	450	6612

Table 3. Results of intra-day and inter-day precision

Concentration (µg/mL)	Intra-day precision			Inter-day precision		
	Mean amount found	Percent amount found	Percent RSD	Mean amount found	Percent amount found	Percent RSD
LMV-200	200.03	100.02	0.05	199.95	99.97	0.03
ABA-300	299.99	99.99	0.03	299.94	99.98	0.03

*Mean of six determinations

Table 4. Results of robustness studies

Result	Flow rate				Organic proportion			
	0.9		1.1		45		55	
	LMV	ABA	LMV	ABA	LMV	ABA	ABA	LMV
Tailing factor	0.65	0.62	0.75	0.71	0.76	0.66	0.60	0.59
Theoretical plates	3452	7965	3651	8311	3755	7726	3211	7691

Table 5. Results of Accuracy studies

Spiking level %	*Recovery		% R.S.D	
	LMV	ABA	LMV	ABA
50	101.03	100.1	0.302	0.584
100	100.5	100.1	1.108	0.207
150	100.5	100.2	0.772	0.434

*Mean of three determinations

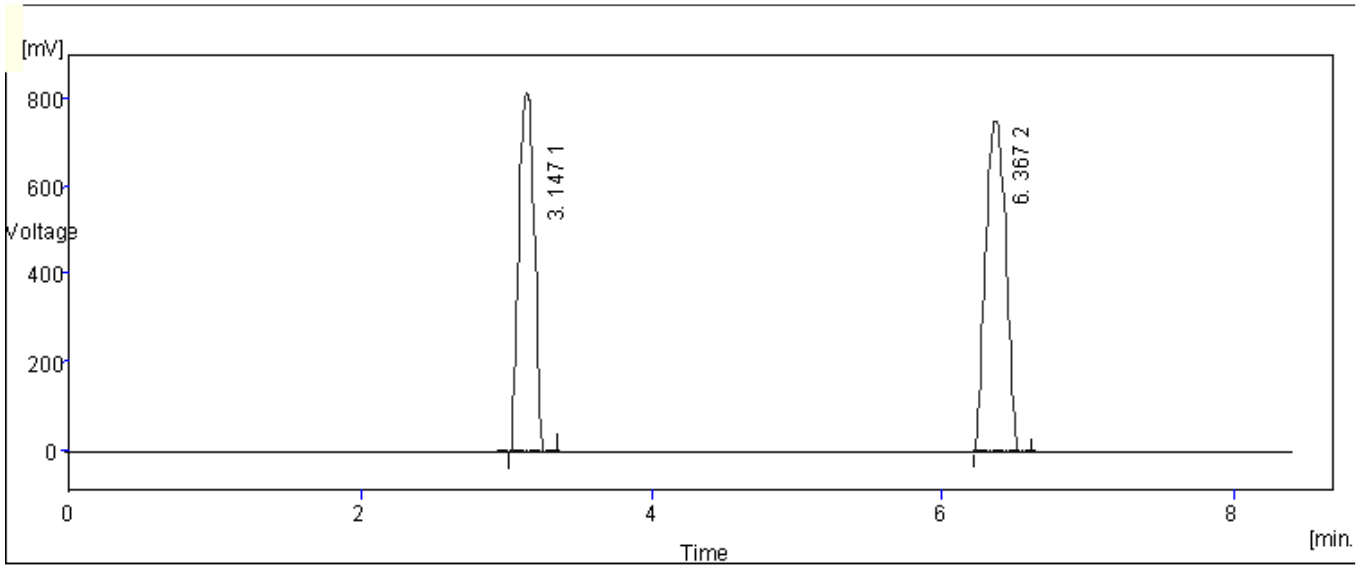

Figure.1. Typical chromatogram of lamivudine and abavavir.