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Research Article

SIMULTANEOUS EVALUATION OF ABACAVIR SULFATE AS WELL AS LAMIVUDINE IN MEDICAL FORMULATIONS BY GRADIENT REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TECHNIQUE

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ABSTRACT

Objective: A precise, accurate, simple, and gradient reversed-phase high-performance liquid chromatography (HPLC) method was adapted for the determination of abacavir sulfate (ABV) in combination with lamivudine (LMV) having tablet formulations simultaneously. This method developed has been validated as per the guidelines of ICH.

Method: Waters HPLC has been used in the method with a column named Zorbax C_{18} with the dimensions as 4.6 nm×150 mm, 3.5 µm. Phosphate buffer (P^H - 3.9) was used as Eluent - A, Eluent - B was methanol, and water and methanol (50:50 v/v) were utilized as diluents. The rate of flow was 1.5 ml/min.

Results: The wavelength of detection has been detected at about 270 nm. Linearity ranges of ABV and LMV were 88–266 µg/ml and 38–116 µg/ml, respectively. Retention times of ABV (3.66 min) and LMV (10.71 min) were determined. The values of the study of percentage recovery of ABV and LMV were determined to be within 98.3–99.2%.

Conclusion: The estimation of ABV and LMV in all pharmaceutical dosage forms could be performed successfully by employing this method.

Keywords: Abacavir sulfate, Lamivudine, Reversed-phase high-performance liquid chromatography, Validation, Simultaneous estimation, ICH guidelines, Pharmaceutical dosage forms.

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INTRODUCTION

Chemically, abacavir sulfate (ABV) is (1S-cis)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]cyclopent-2-en-1-yl}methanol sulfate [8-11]. It acts as a nucleoside reverse transcriptase inhibitor. It is converted into a metabolite which is active called as carbovir triphosphate (CBV-TP) equivalent to deoxyguanosine - 5' - triphosphate (dGTP). CBV-TP acts by hindering the reverse transcriptase activity of HIV-1. It competes with a natural substrate called dGTP which leads to DNA growth termination.

Chemically, lamivudine (LMV) is 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one [8-10]. LMV is an antiretroviral drug which acts by getting incorporated within viral DNA. HIV reverse transcriptase enzyme is inhibited competitively by LMV. This drug serves as a chain terminator for the synthesis of DNA.

Literature survey explains that several ultraviolet (UV) spectroscopy [1,2], reversed-phase high-performance liquid chromatography (RP-HPLC) [3-6] and high-performance thin-layer chromatography [7] methods are available for the estimation of ABR and LMV. The purpose of the current research is to establish an innovative method which gives advanced analytical techniques for identification, detection, resolution, accuracy, and precision.

METHODS

Instrumentation

HPLC (make-waters) was used for this method. It has a quaternary pump. The column used here was the column named Zorbax luna C_{18} with the dimensions as 4.6 mm×150 mm, 3.5 µm. The drugs were discovered using UV Detector (Empower - 2 Software). The weighing balance used here was Sartorius make. Sonicator (spectra lab) and pH meter (polmon) were also used during the method development and validation of these drugs.

Reagents and chemicals

HPLC grade Methanol, Milli - Q grade water, analytical reagent grade ammonium dihydrogen phosphate, Analytical Reagent Grade diammonium hydrogen phosphate, and Analytical Reagent Grade trifluoroacetic acid were used. Pure samples of ABR and LMV were procured from Hetero labs limited, Hyderabad.

Chromatographic conditions

The analysis was performed using waters HPLC. The method involves a column named Zorbax C_{18} with the dimensions as 4.6 mm×150 mm, 3.5 µm being used as a stationary phase. The resolution for the drugs ABR and LMV was achieved using the mobile phase, phosphate buffer (pH -3.9) and methanol and water in 50:50 combination in gradient mode. The rate of flow of the eluent was 1.5 ml/min. The runtime was 20 min. 10 µl was the injection volume and 270 nm was the detection wavelength reported. The gradient chromatography conditions were as shown in Table 1.

Solution preparation

Phosphate buffer (pH - 3.9)

Ammonium dihydrogen phosphate (2.3 g) and diammonium hydrogen phosphate (1.32 g) were dissolved in 1 L of distilled water. The pH was maintained at 3.9±0.05 using 50% V/V trifluoro acetic acid (10 ml of TFA was transferred into a volumetric flask of 200 ml capacity). The volume of the flask was made up to the mark using distilled water. The above solution was passed through a filter named membrane having dimensions 0.22 µm.

Preparation of the diluent

Methanol and water were degassed and mixed in the ratio of 50:50. This solution was called the diluent.

Preparation of the Standard Solution

The drugs ABV (88 mg) and LMV (38 mg) were transferred into a 50 ml standard flask. The flask was sonicated after adding 30 ml of the diluent until the drugs dissolve. The volume was made up to the mark with the diluent.

Calibration curves

Linearity for the drugs was observed as 100–300 ppm for ABV and 40–120 ppm for LMV. The values were in a linear range. The linearity plot for ABV and LMV was plotted as response factor versus concentration (Figs. 1 and 2 and Tables 2 and 3).

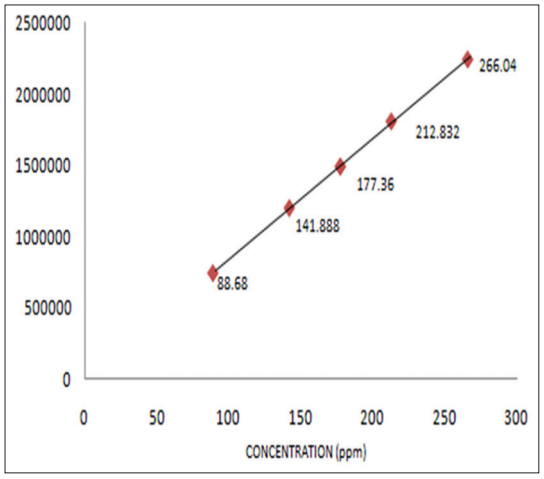

Fig. 1: Linearity curve of abacavir sulfate

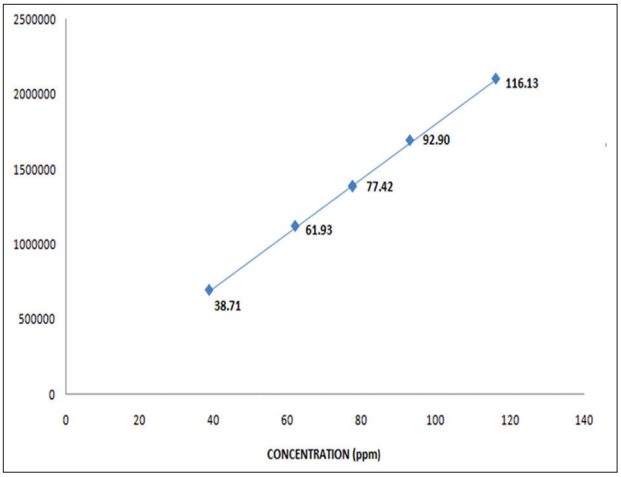

Fig. 2: Linearity curve of lamivudine

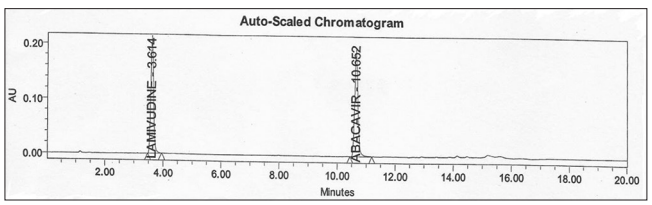

Fig. 3: Typical chromatogram of abacavir sulfate and lamivudine

Analysis of tablet formulation

A total of 20 tablets were collected, powdered, and weighed. LMV (300 mg) was added to a 100 ml standard flask, 60 ml of diluent was then added and kept in a water bath for 30 min at a temperature of 20–25°C. The flask was diluted with the diluent until it reaches the mark. The solution of the sample was centrifuged for 5 min at a speed of 5000 rpm. 5 ml of the above solution was added to 200 ml of the standard flask and diluted until it reaches the mark using the diluent. This was passed through a filter called membrane with 0.45 µm dimension. Initially, some ml was discarded.

Procedure

The column was equilibrated for half an hour at 1.5 ml/min flow rate. 10 µl of the blank solution (diluent) was injected into the system first, followed by standard solution (5 injections) and then the sample solution. The chromatograms were recorded and the peak responses were computed. The typical chromatogram is shown in Fig. 3.

Validation of HPLC method

Specificity

The chromatograms of the mixed standard and sample solutions were compared. The specificity of the RP-HPLC method was determined. The retention time, tailing factor, and resolution were also evaluated. The correlation coefficient was found to be good, and the results were shown in Table 1a.

Linearity

A graph was plot between concentration and response for the drugs to establish linearity of detector response. At about 50–150% of test concentration, the detector responses were linear. The correlation coefficient was within the acceptable level. The reports were as reported in Tables 2 and 3. The linearity plot was obtained as shown in Figs. 1 and 2.

Table 1: Gradient program of the optimized process

Time (min)	Rate of flow	Eluent A %	Eluent B %
0 min	1.5 ml/min	95	5
10 min	1.5 ml/min	65	35
14 min	1.5 ml/min	20	80
15 min	1.5 ml/min	95	5
20 min	1.5 ml/min	95	5

Table 1a: System suitability

Parameter	ABV	LMV	Acceptance criteria
Tailing factor	1.16	1.16	NMT 2
Resolution (RS)	6.5		>2
Plate number (N)	41549	6089	>2000
Capacity factor	4.54		K>2
Peak summery factor	1.006	0.956	0.9–1.1

ABV: Abacavir sulfate, LMV: Lamivudine

Table 2: Linearity of detection response for ABV

Levels	Concentration (ppm)	Mean area	%RSD
Level 1	88.68	748,537	0.24
Level 2	141.888	1,203,886	
Level 3	177.36	1,494,144	
Level 4	177.36	1,494,144	
Level 5	212.832	1,810,109	
Level 6	266.04	2,243,040	0.71
		Slope	8442.76
		Y-intercept	1569.6
		% 0F Y-intercept	0.11
		Correlation coefficient	0.99991

ABV: Abacavir sulfate, RSD: Relative standard deviation

Precision

The value of the precision for the proposed method was 0.43% relative standard deviation (RSD) ABV and 0.64% RSD LMV. This indicates good precision value for the sample being analyzed which is shown in Tables 4 and 5.

Accuracy

Recovery studies were used to determine how accurate the developed method is at different measures of the test solution usually three which were analyzed previously. The standard solution was added to the solution containing the drugs (ABR and LMV) within the concentration range. The quantity of the recovered drug at each level (n=6) was determined, and its percentage recovery was also estimated. The recovery studies of ABR and LMV were evaluated to be 98.73 and 99.06, respectively. The values are given in Tables 5 and 6. The recovery studies prove that the current method had no interference with the other things which were there in the formulation. The reports were shown in Tables 6 and 7.

Table 3: Linearity of detection response for LMV

Levels	Concentration (ppm)	Mean area	% RSD
Level 1	38.71	696,053	0.59
Level 2	61.936	1,123,050	
Level 3	77.42	1,389,538	
Level 4	77.42	1,389,538	
Level 5	92.904	1,695,335	
Level 6	116.13	2,106,171	0.97
		Slope	18250.56
		Y-intercept	-1301.12
		% 0F Y-intercept	-0.94
		Correlation coefficient	0.99982

LMV: Lamivudine, RSD: Relative standard deviation

Table 4: Method precision for ABV

Sample no.	Peak area	% Assay
1	1,537,605	102.5
2	1,530,101	102
3	1,533,166	102.2
4	1,542,077	102.9
5	1,522,358	101.5
6	1,533,156	102.2
	Mean	102.2
	SD	0.47
	% RSD	0.46

ABV: Abacavir sulfate, RSD: Relative standard deviation

Table 5: Method precision for LMV

Sample no.	Peak area	% Assay
1	1,347,989	100
2	1,336,374	99.1
3	1,339,601	99.3
4	1,353,322	100.4
5	1,334,982	99
6	1,354,089	100.4
	Mean	99.7
	SD	0.64
	% RSD	0.64

LMV: Lamivudine, RSD: Relative standard deviation

Robustness

By changing the temperature, flow rate and p^H of the given method robustness were determined. The method was found to be robust and the results was represented in Tables 8 and 9.

System suitability parameters

The system suitability parameters of this method were calculated. The values of percentage RSD were found to be within the limits for 5 replicate injections of ABV and LMV and were found to be 0.48 and 0.65. The tailing factors for the drug peaks ABV and LMV were 1.16 and 1.16, respectively. The number of theoretical plates for abacavir and LMV was 41,549 and 68,109 which was found to be within limits. The system suitability parameters were calculated. The values were found to be within limits and are represented in Table 1.

RESULTS AND DISCUSSION

The current method employs a RP-HPLC procedure using a column named Zorbax luna C_{18} with dimensions 4.6 mm×150 mm and 3.5 µ. The eluent used here was phosphate buffer (pH - 3.9) and methanol and water (50:50). To select the wavelength for sampling, the drugs were measured using UV-visible spectrophotometer in 200–400 nm wavelength range. After scanning the spectrum of each one of the drugs, 270 nm was finally selected as a wavelength suitable for the estimation of the drugs of our interest. The resolution for ABR and LMV was achieved in 20 min (runtime) with a given flow rate (1.5 ml/min) and was found to the best.

The linearity values for ABV and LMV were found to be within the range (100–300 ppm and 40–120 ppm, respectively). The method of interest was evaluated for accuracy by addition of a standard drug solution containing the drugs within the concentration range of ABV and for LMV, and it was added to the previously analyzed test solution. The recovery studies for ABV and LMV were 98.73% and 99.06%, respectively. The method of interest was evaluated for precision and the percentage RSD values were reported as 0.43 for ABV and 0.64 for LMV. This indicates that a good precision value for the sample was reported. By changing the temperature, flow rate or pH of the given method, the suitability parameters of the system were observed to be within limits. Hence, the method is said to be robust. The validation parameters such as selectivity and sensitivity of our method of interest were proved to be acceptable because the parameters such as tailing factor, the number of theoretical plates, separation factor, resolution, and statistical parameters and results of the analysis were found be within limits.

Table 6: Accuracy data for ABV

Concentration of spiked level(%)	Sample no	Amount added	Amount found	% Recovery	Mean	SD	% RSD
50	1	84.8	83.25	98.2	98.9	1.04	1.05
	2	85.03	85.08	100.1			
	3	84.86	83.47	98.4			
100	1	172.83	171.37	99.2	99.0	0.62	0.63
	2	172.8	171.88	99.5			
	3	173.14	170.17	98.3			
150	1	255.15	255.15	98.1	98.3	0.40	0.41
	2	259.71	256.69	98.8			
	3	259	254.15	98.1			

ABV: Abacavir sulfate, RSD: Relative standard deviation, SD: Standard deviation

Table 7: Accuracy data for LMV

Concentration of spiked level (%)	sample no	Amount of the drug added (mg)	Amount of the drug found (mg)	Percentage recovery	Mean value	SD	% RSD
50	1	38.2	37.45	98.0	99.2	1.11	1.12
	2	38.06	38.13	100.2			
	3	38.18	37.97	99.4			
100	1	75.97	75.64	99.6	99.2	0.55	0.55
	2	76.19	75.79	99.5			
	3	75.96	74.92	98.6			
150	1	113.94	112.46	98.7	98.8	0.40	0.40
	2	113.36	112.44	99.2			
	3	113.71	111.9	98.4			

LMV: Lamivudine, RSD: Relative standard deviation, SD: Standard deviation

Table 8: ABV robustness

Parameters	Results	RT (min)	Mean peak area (n=5) of abacavir	% RSD
Flow rate	1.5 ml/min	10.701	1,543,762	0.04
Flow rate	1.4 ml/min	12.359	1,623,612	0.34
Flow rate	1.6 ml/min	9.281	1,183,133	0.07
Column temperature	45°C	10.452	1,482,427	0.06
Column temperature	55°C	10.679	1,468,953	0.13
Buffer Ph	3.7	11.249	1,543,198	0.10
Buffer pH	4.2	10.129	1,574,944	0.39

ABV: Abacavir sulfate, RT: Retention time, RSD: Relative standard deviation, SD: Standard deviation

Table 9: Results of robustness (LMV)

Parameters	Results	RT (min)	Mean peak area (n=5) of LMV	% RSD
Flow rate	(1.5 ml/min, 50°C)	3.675	1,352,313	0.50
Flow rate	1.4 ml/min	4.592	1,554,365	0.42
Flow rate	1.6 ml/min	2.973	1,096,323	0.12
Column temperature	45°C	3.842	1,474,128	0.04
Column temperature	55°C	3.290	1,496,459	0.07
Buffer pH	3.7	4.702	1,387,432	0.14
Buffer pH	4.2	3.482	1,453,421	0.37

LMV: Lamivudine, RT: Retention time, RSD: Relative standard deviation, SD: Standard deviation

CONCLUSION

The current work explains a simple, robust, economical, and non-interfering simultaneous method for the evaluation of ABV and LMV using RP-HPLC technique. The present procedure was observed as a simple, accurate, economic, reproducible, and precise while performing the analysis of drug formulations having both the drugs.

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