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ORIGINAL ARTICLE

Analytical Method Development and Validation for the Simultaneous Estimation of Abacavir and Lamivudine by Reversed-phase High-performance Liquid Chromatography in Bulk and Tablet Dosage Forms

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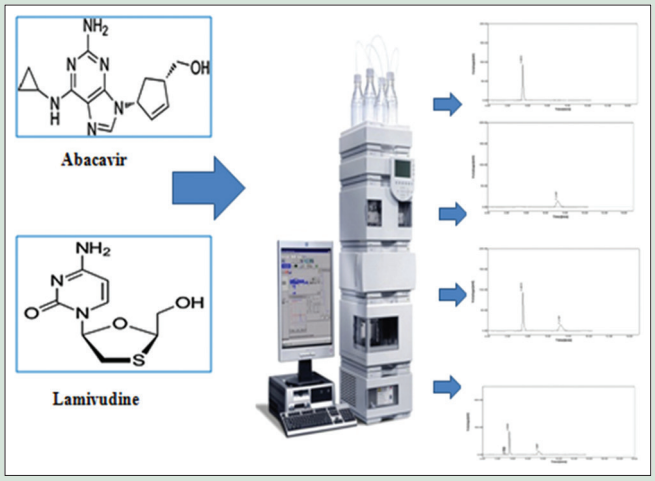
ABSTRACT

Objective: A simple rapid, accurate, precise, and reproducible validated reverse phase high performance liquid chromatography (HPLC) method was developed for the determination of Abacavir (ABAC) and Lamivudine (LAMI) in bulk and tablet dosage forms. **Methods:** The quantification was carried out using Symmetry Premsil C18 (250 mm × 4.6 mm, 5 µm) column run in isocratic way using mobile phase comprising methanol: water (0.05% orthophosphoric acid with pH 3) 83:17 v/v and a detection wavelength of 245 nm and injection volume of 20 µl, with a flow rate of 1 ml/min. **Results:** In the developed method, the retention times of ABAC and LAMI were found to be 3.5 min and 7.4 min, respectively. The method was validated in terms of linearity, precision, accuracy, limits of detection, limits of quantitation, and robustness in accordance with the International Conference on Harmonization guidelines. **Conclusion:** The assay of the proposed method was found to be 99% – 101%. The recovery studies were also carried out and mean % recovery was found to be 99% – 101%. The % relative standard deviation from reproducibility was found to be <2%. The proposed method was statistically evaluated and can be applied for routine quality control analysis of ABAC and LAMI in bulk and in tablet dosage form.

Key words: Abacavir, dosage forms, lamivudine, method development, reverse phase high performance liquid chromatography, validation

SUMMARY

- Attempts were made to develop RP-HPLC method for simultaneous estimation of Abacavir and Lamivudine for the RP-HPLC method. The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness were within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible. So the proposed methods can be used for the routine quality control analysis of Abacavir and Lamivudine in bulk drug as well as in formulations.



Abbreviations Used: HPLC: High-performance liquid chromatography, UV: Ultraviolet, ICH: International Conference on Harmonization, ABAC: Abacavir, LAMI: Lamivudine, HIV: Human immunodeficiency virus, AIDS: Acquired immunodeficiency syndrome, NRTI: Nucleoside reverse transcriptase inhibitors, ARV: Antiretroviral, RSD: Relative standard deviation, RT: Retention time, SD: Standard deviation.

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INTRODUCTION

Abacavir (ABAC) and lamivudine (LAMI) are synthetic nucleoside analogs that show a potent and synergistic effect on the inhibition of human immunodeficiency virus-1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS).[1] HIV encodes at least three enzymes: protease, reverse transcriptase, and endonuclease. ABAC and LAMI belong to the class of nucleoside reverse transcriptase inhibitors (NRTIs). New therapeutic strategy of AIDS treatment requires the combination of these antiretroviral (ARV) drugs. The introduction of highly effective combination regimens of ARV drugs has led to substantial improvements in morbidity and mortality. ABAC tablets in combination with other ARV agents in tablet form are indicated for the treatment of HIV-1 infection. ABAC should not be added as a single agent when ARV regimens are changed due to loss of virologic response.

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Intracellularly, ABAC is converted by cellular enzymes to the active metabolite, carbovir triphosphate,[2] an analog of deoxyguanosine-5' triphosphate. Intracellularly, LAMI is phosphorylated to its active 5'-triphosphate metabolite, lamivudine triphosphate. Chemically, ABAC sulfate is (1S, cis)-4-[2-amino-6-(cyclopropyl amino)-9H-purin-9-yl]-2-cyclopentene-1-methanol sulfate, and LAMI is (2R, cis)-4-amino-1-(2-hydroxymethyl-1, 3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one. Figures 1 and 2 show the structures of ABAC and LAMI, respectively. Numerous analytical methods have been employed for the quantitative determination of single- or multi-component NRTIs in pharmaceutical dosage forms. These methods include ultraviolet (UV)-visible spectrophotometric high-performance thin-layer chromatography and high-performance liquid chromatography

Table 1: Details of chromatogram of standard mixture abacavir and lamivudine

Name of drug	RT (min)	Area	Plates	Tailing factor
Abacavir	3.48	66.22	4943.3	1.4286
Lamivudine	7.21	33.76	3663.5	1.3182

RT: Retention time

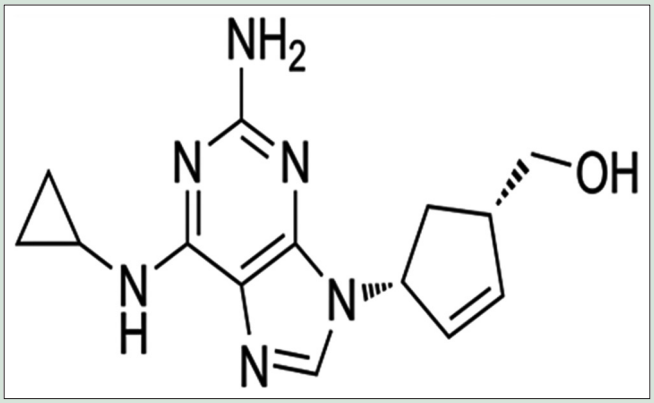

Figure 1: Structure of abacavir

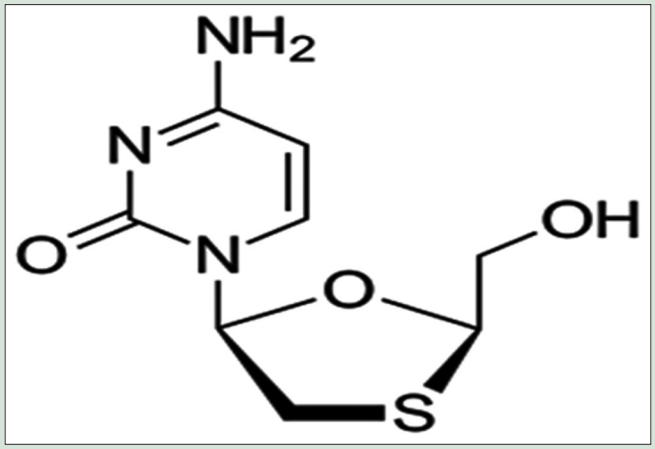

Figure 2: Structure of lamivudine

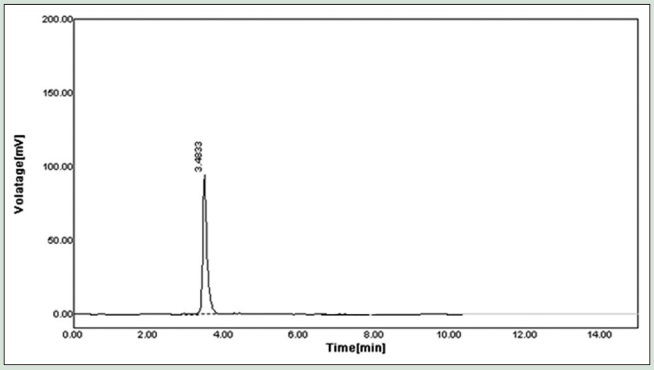

Figure 3: Chromatogram of standard abacavir

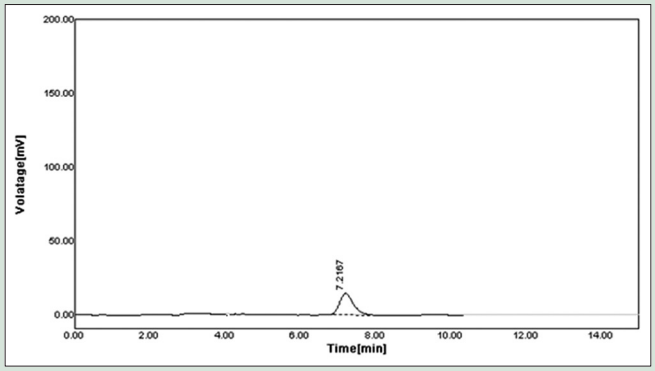

Figure 4: Chromatogram of standard lamivudine

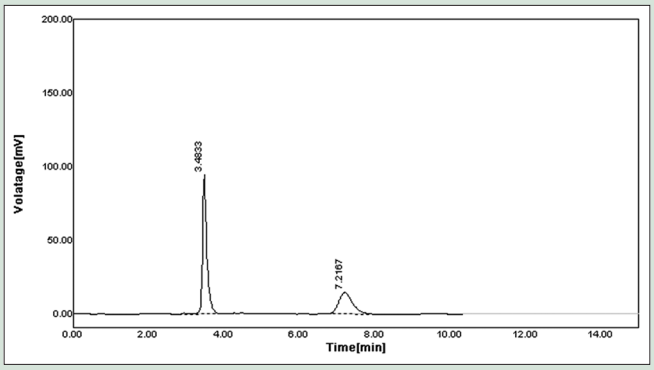

Figure 5: Representative chromatogram of abacavir and lamivudine using methanol + water (acetic acid 0.05% [orthophosphoric acid]) (83% +17%) v/vas mobile phase, showing retention time 3.5 min and 7.4 min

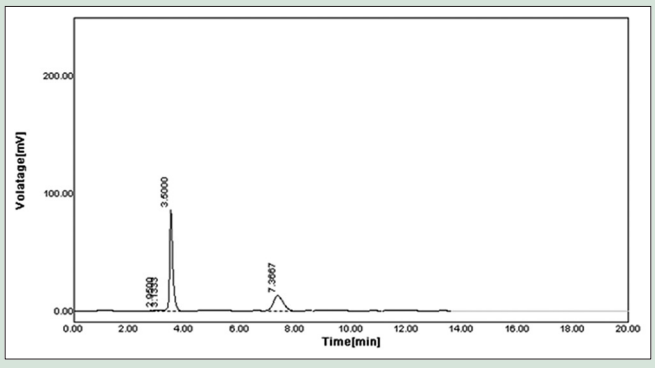

Figure 6: Chromatogram of abacavir and lamivudine in tablet formulation

(HPLC).[3-15] HPLC was considered the best method of assay since this method is the most accurate of all chromatographic and other separation methods. The reported method differs with respect to extraction procedure, eluent used for reverse-phase HPLC (RP-HPLC), and UV detection wavelength. The development and validation of a simple, rapid, accurate, and precise method of assay for ABAC and LAMI in tablet formulations are now reported in this work using RP-HPLC with UV detection at 245 nm.[16]

MATERIALS AND METHODS

Materials and reagents

The analysis of the drug was carried out on Youngline (S.K.) Gradient System UV Detector. This study was equipped with reverse phase (Grace) C18 column (4.6 mm × 250 mm; 5 µm), a SP930D pump, a 20 µl injection loop, UV730D Absorbance detector, and running autochro-3000 software. ABAC and LAMI were procured from CIPLA. Orthophosphoric acid (OPA), methanol, acetonitrile (HPLC Grade Merck Specialties Pvt. Ltd. Shiv Sager Estate "A" Worli, Mumbai.), water, 0.45 µm filter (Millipore, Bangalore) were also used. A combination of ABAC (600 mg) and LAMI (300 mg) in tablet formulation was procured from local pharmacy (ABAMUNE-L, Cipla).

Chromatographic conditions

Column C18 (250 mm × 4.6 mm); particle size packing 5 µm; detection wavelength of 245 nm; flow rate 1.00 ml/min; temperature ambient; sample size 20 µl; mobile phase methanol: water (OPA 0.05%) (83:17); run time of 10 min.

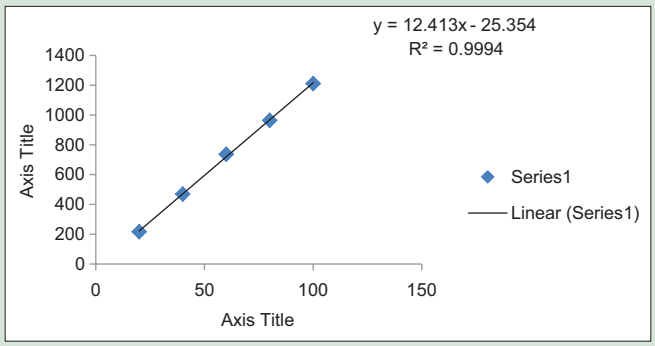

Figure 7: Calibration curve of abacavir

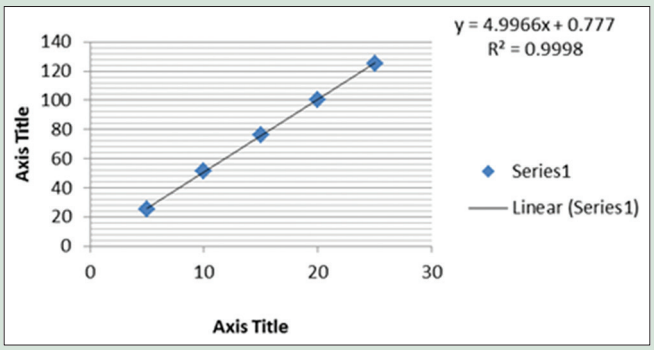

Figure 8: Calibration curve of lamivudine

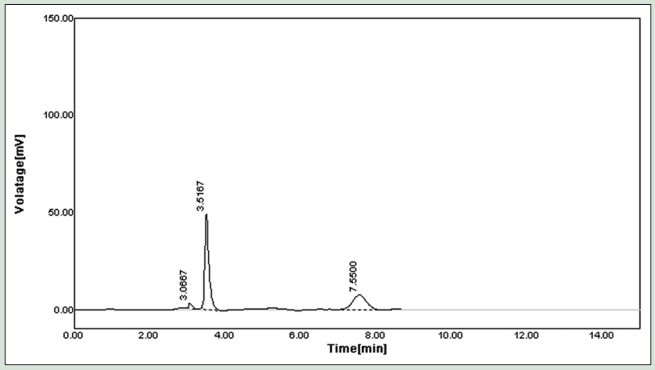

Figure 9: Chromatogram of accuracy 80%

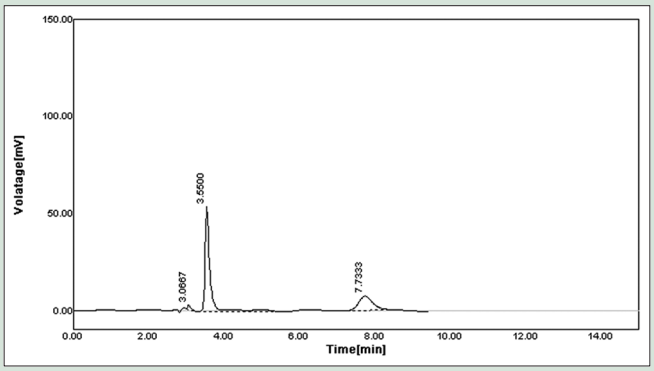

Figure 10: Chromatogram of accuracy 100%

Table 2: Analysis of marketed formulation

Serial number	Amount present in mg		Amount found in mg		Percentage label claim	
	Abacavir	Lamivudine	Abacavir	Lamivudine	Abacavir	Lamivudine
1	60	30	60.96	30.43	101.60	101.45
2	60	30	60.83	30.80	101.38	102.67
Mean±SD	-	-	-	-	101.49±0.27	103.67±0.27
%RSD	-	-	-	-	0.27	0.26

SD: Standard deviation; RSD: Relative standard deviation

Table 3: Details of chromatogram of abacavir and lamivudine in tablet formulation

Name of drug	RT (min)	Area (%)	Theoretical plates	Tailing factor
Abacavir	3.5	66.82	4990.7	1.5833
Lamivudine	7.3	30.72	2047.9	1.1364

RT: Retention time

Preparation of standard stock solution

20 mg of ABAC and 10 mg of LAMI were weighed accurately and transferred to a 10-ml volumetric flask dissolved in methanol and diluted to 10 ml with the mobile phase (methanol**:** water, 83:17 v/v) to give a stock solution of 2000 µg/ml ABAC and 1000 µg/ml LAMI Table 1 shows the details of chromatogram of standard mixture ABAC and LAMI and Figures 3 and 4 show the chromatogram of standard ABAC and LAMI. Figure 5 shows the chromatogram of standard mixture of ABAC and LAMI.

Method development and validation

Serial dilutions were done to prepared various concentration stock working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

Assay preparation for commercial formulation

For analysis of the tablet dosage form, 20 tablets were weighed individually and their average weight was determined. After that, they were crushed to fine powders and powder equivalent to 1 mg was taken and transferred to 10 ml volumetric flask and diluted with 10 ml methanol; from the above solution, 0.2 ml was taken and diluted to 10 ml methanol. The solutions were shaken vigorously for 10 min and filtered through 0.45 µg nylon membrane filters. Then, volume was made up to

Table 4: Linearity study

Concentration (µg/ml)		Area	
Abacavir	Lamivudine	Abacavir	lamivudine
20	10	214.62	146.535
40	20	465.89	255.03
60	30	731.9146	380.505
80	40	970.18	483.445
100	50	1216.433	592.5555

Table 5: Linearity of abacavir

Concentration	Average peak area
20	216.689
40	469.3872
60	736.5661
80	963.5581
100	1210.895

Table 6: Linearity of lamivudine

Concentration	Average peak area
10	146.535
20	255.03
30	380.505
40	483.445
50	592.5555

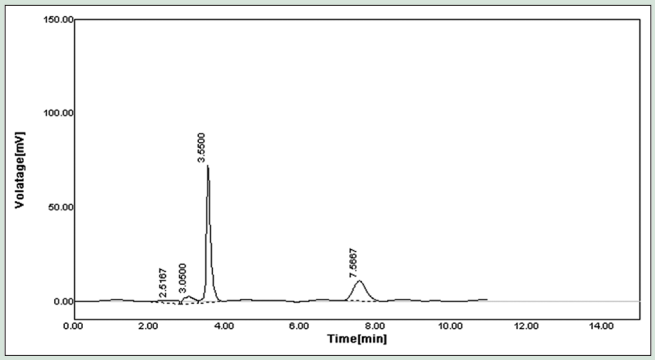

Figure 11: Chromatogram of accuracy 120%

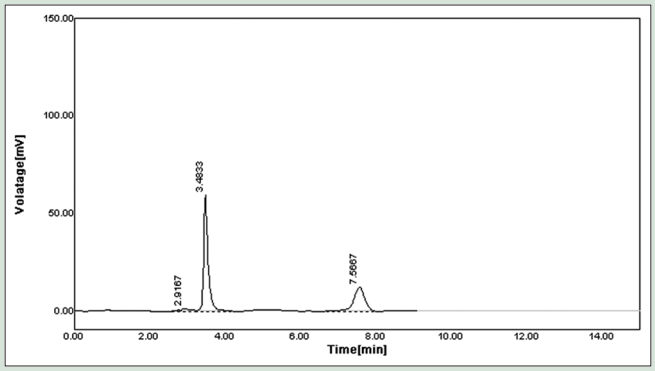

Figure 12: Chromatogram of system suitability studies

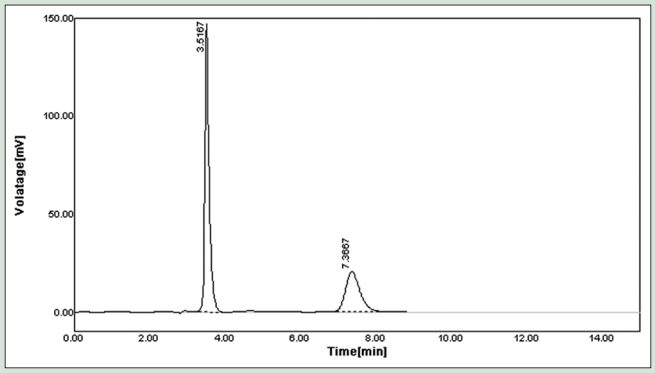

Figure 13: Chromatogram of precision

Table 7: Recovery studies of abacavir and lamivudine

Level of recovery (%)	80		100		120	
	Abacavir	Lamivudine	Abacavir	Lamivudine	Abacavir	Lamivudine
Amount present (mg)	20	10	20	10	10	20
	20	10	20	10	10	20
Amount of standard added (mg)	16	8	20	10	12	24
	16	8	20	10	12	24
Percentage recovery	97.25	98.41	99.45	101.64	99.26	99.62
	100.00	101.06	100.40	99.58	100.94	100.63

the mark with methanol: water (83:17); the amounts of ABAC and LAMI per tablet were calculated from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Results are shown in Tables 2 and 3 that show the analysis of marketed formulation and details of chromatogram of ABAC and LAMI in tablet formulation. Figure 6 shows the chromatogram of ABAC and LAMI in tablet formulation.

RESULTS

Linearity and range

From ABAC and LAMI standard stock solution, different working standard solutions (20–100 µg/ml) were prepared in the mobile phase. Likewise from ABAC and LAMI standard stock solution, different working standard solutions (10–50 µg/ml) were prepared in the mobile phase. 20 µl of sample solution was injected onto the column using fixed volume loop injector. Chromatograms were recorded. The area for each concentration was recorded in Tables 4-6 that show linearity study. Figures 7 and 8 show the calibration curve of ABAC and LAMI, respectively.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To a preanalyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed [Table 7]. Statistical validation of recovery studies is shown in Table 8 and Figures 9-11.

Table 8: Statistical validation of recovery studies

Level of recovery (%)	Drug	Mean percentage recovery±SD*	%RSD
80	Abacavir	35.78±0.31	0.87
	Lamivudine	17.98±0.15	0.80
100	Abacavir	39.99±0.13	0.34
	Lamivudine	20±0.15	0.74
120	Abacavir	44.03±0.17	0.39
	Lamivudine	22.01±0.14	0.64

*Denotes average of three determinations. SD: Standard deviation; RSD: Relative standard deviation

Table 9: System suitability parameters

System suitability parameters	Proposed method	
	Abacavir	Lamivudine
RT	3.4833	7.5667
Area	502.5529	256.7151
Theoretical plate number	4943.3	3527.6
Tailing factor	1.5000	0.8387

RT: Retention time

System suitability parameters

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of ABAC (600 mg) and LAMI (300 mg), system suitability parameters were studied. The results are shown in Figure 12 and Table 9.

Precision

The method was established by analyzing various standards of ABAC and LAMI. All the solutions were analyzed thrice to record any intraday and interday variation in the result. The results obtained for interday and intraday variation are shown in Table 10 and Figure 13.

Robustness

The robustness is a measure of its capacity to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability during normal usage; hence, the following are performed by slight variations in parameters. The assay content of the sample was measured by change in the flow rate of 0.90–1.10 ml/min. The results indicate that less variability in retention time and tailing factor were observed [Tables 11 and 12].

DISCUSSION

The proposed methods for simultaneous estimation of ABAC and LAMI in tablet dosage forms were found to be simple, accurate, economical, and rapid. The method was validated as per the International Conference on Harmonization Q2 (R1) guidelines. Standard calibration yielded correlation coefficient (r^2) 0.999 for both ABAC and LAMI at all the selected wavelengths. The values of % relative standard deviation are within the prescribed limit of 2%, showing high precision of methods, and recovery was close to 100% for both drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for their simultaneous determination, with virtually no interference of any additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of ABAC and LAMI in formulations.

CONCLUSION

The developed HPLC methods in that linearity, precision, range, and robustness were found to be more accurate, precise, and reproducible. The methods were found to be simple and time saving. All proposed methods could be applied for routine analysis in quality control laboratories.

Acknowledgments

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Table 10: Intra- and inter-day precision studies on high-performance liquid chromatography method for abacavir and lamivudine

Method	Drug	Concentration (µg/ml)	Intraday precision		Interday precision	
			Mean±SD	Percentage amount found	Mean±SD	Percentage amount found
RP-HPLC method	Abacavir	20	223.80	100.35	218.34	98.18
		60	725.68	100.85	740.74	102.88
		100	1179.89	97.11	1245.07	102.30
	Lamivudine	10	142.80	100.20	150.89	102.04
		30	380.18	102.57	377.38	101.73
		50	589.57	98.94	592.33	99.46

Mean of each 3 reading for HPLC method. HPLC: High-performance liquid chromatography; RP-HPLC: Reverse phase-HPLC; SD: Standard deviation

Table 11: Robustness study of abacavir

Parameters	Concentration	Amount of detected (mean±SD)	%RSD
Mobile phase composition			
84:16	60	6.38±1.01	0.88
82:18	60	10.77±1.5	1.48
Wavelength change (nm)			
244	60	4.70±0.89	0.59
246	60	9.17±1.4	1.28
Flow rate change (ml)			
0.90	60	2.85±0.41	0.38
1.10	60	5.70±0.74	0.80

SD: Standard deviation; RSD: Relative standard deviation

Table 12: Robustness study of lamivudine

Parameters	Concentration	Amount of detected (mean±SD)	%RSD
Mobile phase composition			
84:16	30	4.8±0.89	1.29
82:18	30	4.95±0.97	1.36
Wavelength change (nm)			
244	30	2.87±0.38	0.76
246	30	0.18±0.09	0.21
Flow rate change (ml)			
0.90	30	0.88±0.14	0.22
1.10	30	2.01±0.77	0.52

SD: Standard deviation; RSD: Relative standard deviation

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Nil.

Conflicts of interest

There are no conflicts of interest.

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