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Development and Validation of RP-HPLC Method in Simultaneous Estimationof Lamivudine and Abacavir as Tablet Dosage Form

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ABSTRACT:

For a concurrent evaluation of lamivudine and abacavir drugs in a tablet dosage form, a primary, explicit method has been brought about in this study. In this work, an THERMOSIL C18 150X4.6mm,.5µ was utilized for chromatogram, and as a mobile phase, Water: acetonitrile: buffer 2.5 (45:15:40) ratio was opted to pump at 1ml/min flow rate via column. The buffer pH was maintained at 2.5, and the temperature was made ambient for the evaluation. Eventually, the wavelength for lamivudine and abacavir was noticed at 232nm. Further, the retention time for both the opted drug was discerned at 2.087 mins and 6.067 min respectively, Even the percentage purity of these drugs was observed at 101.1% & 99.9% correspondingly. Later the system suitability criteria i.e., tailing factor, theoretical plates for lamivudine were revealed at 1.0 and 2566.5, whereas abacavir was at 1.1 and 2357.2. Finally, the validation of the method was determined such that the linearity range was observed well at 300-700 ppm and 600-1400 ppm concentration series, the correlation coefficient (r^2) was noticed at 0.998 and 0.999 for both, and then the %mean recovery was at 101.7% and 99.9 %, for %RSD repeatability it was 0.38 and 1.29, whereas for %RSD in intermediate precision it was at 0.58 and 1.76 respectively. The LOD values were obtained as 2.97 and 3.04, and LOQ values were obtained as 9.98 and 9.94. Eventually, the method validation was discovered to be precise, robust, & repeatable, allowing the study to proceed on RP-HPLC an approachable technique for the evaluation of lamivudine and abacavir as pharmaceutical dosage form on a daily basis, due to their rapidity, clear-cut results, durability, sturdiness, and reproducibility.

Keywords:lamivudine, abacavir,RP- HPLC, Simultaneous estimation.

1. INTRODUCTION

Lamivudine (3TC) is a cytosine analog with potent activity against hepatitis B viruses (HBV) and human immunodeficiency (HIV) through inhibition of reverse transcriptase activity. Lamivudine is used in the treatment of HBV infections and it has strongly been recommended for the treatment of HIV infections in combination with other antiviral drugs [1]. Abacavir is a nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency virus Type 1 (HIV-1). Abacavir is phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HOV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis [2,3,4].

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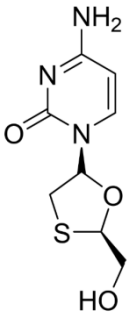

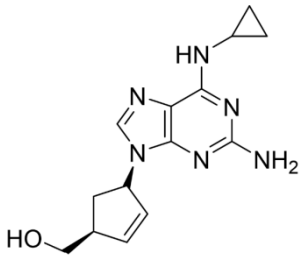

Fig. 1: lamivudine StructureFig. 2: abacavir Structure

The literature review was carried out to enumerate the reported analytical methods for the selected drugs in individually or in combination with other drugssuch as UV, RP-HPLC, HPTLC, LC-MS-MS, MALDI-TOF/TOF, spectrophotometric methods are reported for the determination of Lamivudine alone and Abacavir alone[5-17]also methods such as LC-MS-MS by Lamivudine and abacavir combinationand few RP-HPLC methods for lamivudine and abacavir combination with other drugs like Zidovudineand HPTLC methods for only Abacavir and HPTLC methods for Lamivudine and abacavir have been reported for the determination of lamivudine and Abacavir[18-38]. As far as we know, up to now no reversed high-performance chromatographic liquid methods for Lamivudine and Abacavir have been published. The present study aimed to build, optimize and validate, for the concurrent determination of Lamivudine and abacavir, a simple, quick RP-HPLC process.

2. MATERIALS AND INSTRUMENTS

2.1. Chemicals and Reagents:

Lamivudine &Abacavir standards and tablets were bought from the market, Water HPLC Grade, Orthophosphoric acid, Acetonitrile HPLC grade, potassium dihydrogen orthophosphate, etc.

2.2. Instruments specification:

A WATERS YL9160 High-performance liquid chromatograph system was used to perform chromatography that contains PDA DETECTOR, ADWA AD120 PH meter, Soltec ultra sonicator, Labindia-3000, UV-visible spectrophotometer, AFCOSET electronic balance, Ultrasonic water bath, and a software application named empower 2. the analysis was performed with the column THERMOSIL C18 150X4.6mm,.5µ column, and mobile phase by using water: acetonitrile: phosphate buffer with PH 2.5 in the ratio of 45:15:40 with the flow rate of 1.0µl/min at wavelength 232nmand the runtime was 14 min. lamivudine retention time is 2.087 and abacavir retention time is 6.067 minutes shown in fig 4.

2.3. Preparation of solutions:

2.3.1. Preparation of Phosphate buffer

Weighed 7.0grams of potassium dihydrogen orthophosphate into a 1000ml beaker, dissolved, and diluted to 1000ml with HPLC water. Adjusted the pH to 2.5 with Orthophosphoric acid

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2.3.2. Preparation of mobile phase

Mixed a mixture of above buffer 400 ml (40%) and 450 ml of Acetonitrile HPLC (15%) and 150 ml of HPLC Water (45%) and degas in an ultrasonic water bath for 5 minutes. Filtered through 0.45µ filter under vacuum filtration.

2.3.3. Diluent Preparation

The mobile phase used as Diluent, shown in fig 3.

2.4. Preparation of the Lamivudine &Abacavir Standard Solution:

weighed accurately and transferred 100 mg of Lamivudine and Abacavir working standard into a 10ml clean dry volumetric flask & about 7ml of Diluent was added and sonicated to dissolved it completely and make volume up to the mark with the same solvent.

2.4.1. Stock solution

Further pipetted 0.5ml &1.0ml of Lamivudine &Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

2.5. Preparation of the Lamivudine &Abacavir Sample Solution Preparation:

weighed accurately and transferred 100 mg of Lamivudine and Abacavir tablet powder into a 10ml clean dry volumetric flask & about 7mL of Diluent was added and sonicated to dissolved it completely and filtered with 0.45µ filter paper and make volume up to the mark with the same solvent.

2.5.1. Stock solution

Further pipetted 0.5ml&1.0ml of Lamivudine &Abacavir the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

2.6. Procedure:

Inject 20 µl of the standard, sample into the chromatographic system and measure the areas for the Lamivudine and Abacavir peaks and calculate the % Assay by using the formulae.

3.RESULTS AND DISCUSSION

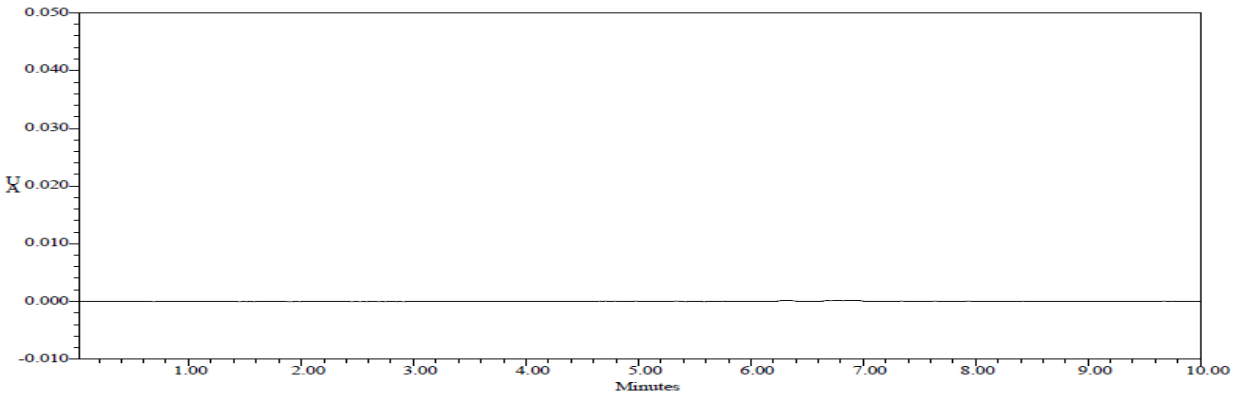

Fig. 3. The chromatogram of blank reading.

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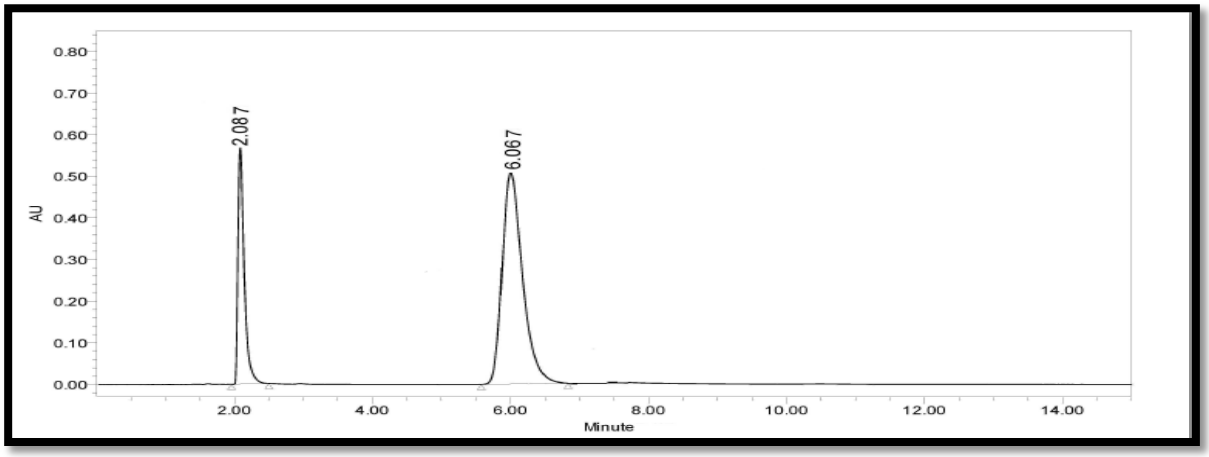

Fig. 4. The chromatogram for assay of standard.

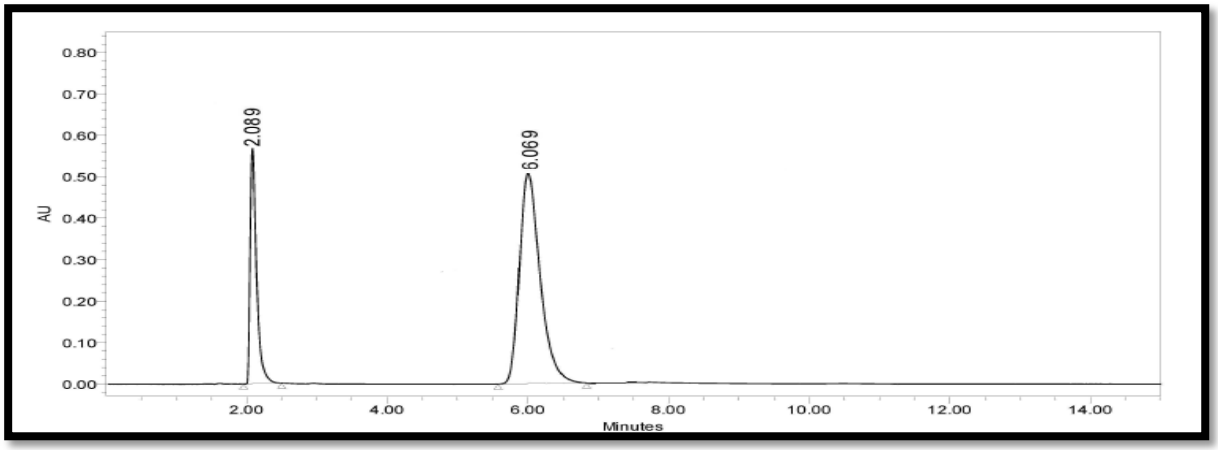

Fig. 5. Chromatogram for assay of sample.

3.1. Validation of the analytical method:

The created chromatographic approach was checked for linearity, suitability with system, precision, accuracy, durability as well as effectiveness based on ICH standards.

3.2. System Suitability:

In order to assess the system viable criteria like tailing factors, retention time, 'USP' theoretical plate count, then with the help of the column at a circulation rate of 1.0 ml / 1 min for about 14 min the mobile phase is passed, in order to equilibrate column at required temperature level as shown in the Table-1. By injecting about 20 µl of criterion right into THERMOSIL C18 150X4.6mm,.5µthe chromatographic splitting is achieved, then the mobile Phase of composition Water: acetonitrile: buffer2.5 (45:15:40) was allowed to pass along with column at circulation flow of 1.0 ml per minute. Retention time, tailing variable as well as 'USP' theoretical plate count of the particular technique as depicted in table 1.

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Table 1:Parameters of System Suitability.

Parameters	lamivudine	Abacavir
Retention time	2.087	6.067
USP Plate count	2566.5	2357.2
USP Tailing	1.0	1.1

3.3.Assay of pharmaceutical formulation:

The recommended verified technique was efficiently put on figuring out Lamivudine and Abacavir in their tablet dosage type. The obtained result Lamivudine and also Abacavir that was comparable with the respective labeled quantities as shown in Table-2.

Table 2: Results of Lamivudine and Abacavir Assay.

	Label Claim (mg)	% Assay
Lamivudine	300	101.1
Abacavir	600	99.9

3.4. Linearity:

The linearity research work was carried out to have a focus on levels of 300 ppm – 700 ppm and 600 ppm – 1400 ppm. Each degree was directly infused into the chromatographic system. At each level, the area is used in calculating the relationship coefficient. The injection of each level into the chromatographic system is completed, and the peak area is measured. A chart was plotted with peak area (on the Y-axis) vs. concentration (on the X-axis), and the correlation coefficient was calculated. outcomes are depicted in table 3,4 and graphs in figure 6,7.

Table 3:Linearity Results (for Lamivudine).

S.No	Linearity Level	Concentration	Area
1	I	300ppm	2310252
2	II	400ppm	2849374
3	III	500ppm	3572706
4	IV	600ppm	4121068
5	V	700ppm	4752813
Correlation Coefficient			0.999

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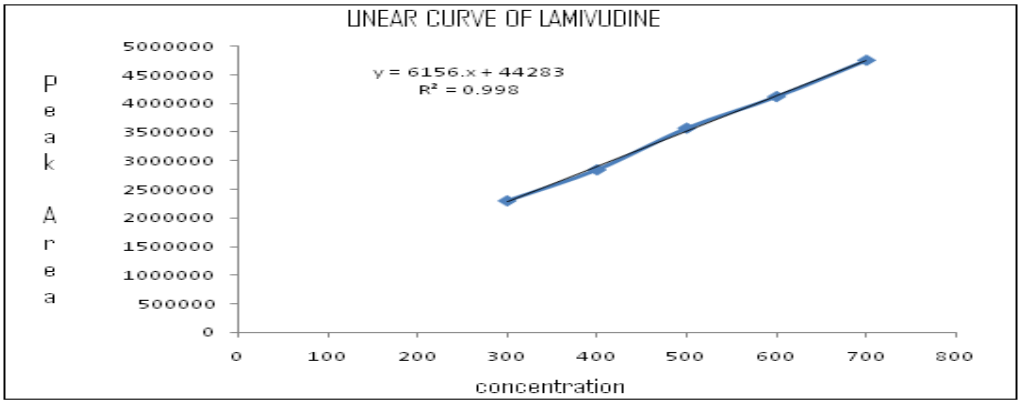

Fig. 6: The linearity curve of lamivudine.

Table 4: Observation table for linearity of abacavir.

S.No	Linearity Level	Concentration	Area
1	I	600ppm	10050807
2	II	800ppm	16318417
3	III	1000ppm	22287985
4	IV	1200ppm	28913928
5	V	1400ppm	34584741
	Correlation Coefficient		0.999

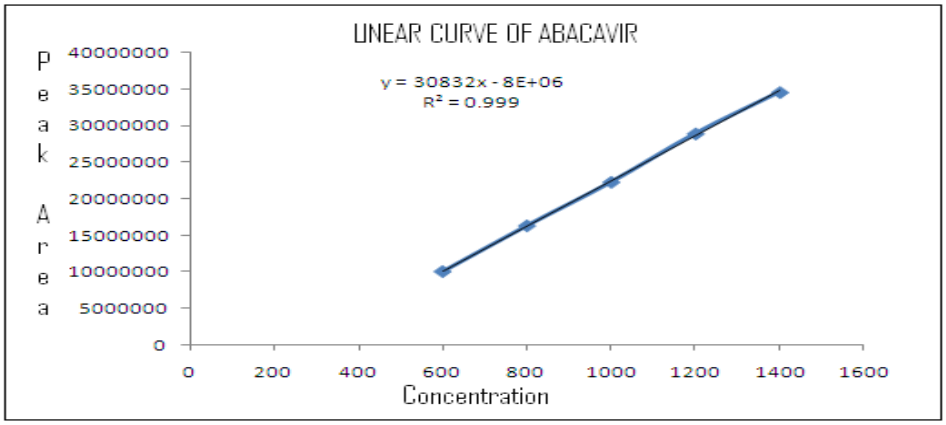

Fig. 7: The linearity curve of abacavir.

3.5. Accuracy:

The accuracy was determined with the help of a recovery study. The recovery technique is executed at three levels such as 50%, 100%, 150%. Later, the standard solution is injected into the chromatographic system. Calculation of quantity required and the amount added

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for lamivudine and also Abacavir has done as well as determine the individual recovery and mean recovery values. The outcomes as depicted in the table 5,6.

Table 5: Observation table for accuracy of lamivudine.

%Concentration(at specificationLevel)	Area	AmountAdded(mg)	AmountFound(mg)	%Recovery	MeanRecovery
50%	1743792	50.0	50.8	101.7%	
100%	3568345	102.0	104.0	102.0%	101.7%
150%	5224766	150.0	152.3	101.5%	

Table 6: Observation table for accuracy of Abacavir.

%Concentration (At specification Level)	Area	Amount added(mg)	AmountFound(mg)	% Recovery	Mean Recovery
50%	8241086	50.0	50.6	101.2%	
100%	16264786	100.0	99.8	99.8%	99.9%
150%	24124456	150.0	148.1	98.7%	

3.6. Precision:

Evaluation of precision was done with coefficient variance for six replicate injections depending on the requirement. For five times, the injection of the standard solution was done & gauged the location for all five Injections in HPLC. The %RSD for the area of five reproduce shots were noticed. The results are displayed in table7,8.

Table 7: Observation table for Precision of lamivudine.

Injection	Area
Injection-1	3582694
Injection-2	3586491
Injection-3	3598154
Injection-4	3564125
Injection-5	3569412
Average	3580175
StandardDeviation	13628.34

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%RSD	0.380661

Table 8: Observation table for Precision of Abacavir.

Injection	Area
Injection-1	16254781
Injection-2	16259478
Injection-3	16248751
Injection-4	16254232
Injection-5	16345781
Average	3184696
Standard Deviation	41083.2098
%RSD	1.29001983

3.7. Intermediate Precision/Ruggedness:

The precision was carried out on different days to review the approach's intermediate accuracy. The standard solution was injected five times, and the area for all five injections was measured in HPLC. The percentage RSD for the area of five replicate injections was discovered. Tables 9 and 10 show the results.

Table 9: Observation table for Intermediate Precision/Ruggedness of lamivudine.

Injection	Area
Injection-1	3481579
Injection-2	3458121
Injection-3	3426581
Injection-4	3465712
Injection-5	3451476
Average	3456694
Standard Deviation	20227.73
%RSD	0.585176

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Table 10: Observation table for Intermediate Precision/Ruggedness of abacavir.

Injection	Area
Injection-1	15264987
Injection-2	15369852
Injection-3	15248454
Injection-4	15874692
Injection-5	15236547
Average	15398906.4
Standard Deviation	271176.60
%RSD	1.76

3.8. Robustness:

Because of the robustness, deliberate changes in the circulation rate, mobile phase composition, and temperature variation were made to assess the impact on the method. The flow rate ranged from 0.9 ml/min to 1.1 ml/min. Tables 11,12,13, and 14 show the results.On evaluation of the results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.The method is robust only in less flow condition.On evaluation of the results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

Table 11: Observation table of robustness flow condition for lamivudine.

Sl. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2921.2	1.2
2	1.0	2566.5	1.0
3	1.1	2911.1	1.2

Table 12: Observation table of robustness flow condition for abacavir.

Sl. No	Flow Rate(ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2669.0	0.9

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2	1.0	2357.2	1.0
3	1.1	2399.2	1.1

Table 13: Observation table of robustness organic composition condition forlamivudine.

Sl. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2922.2	1.2
2	*Actual	2566.5	1.2
3	10% more	2868.8	1.2

Table 14: Observation table of robustness organic composition condition for abacavir.

Sl. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2999.1	1.2
2	*Actual	2357.2	1.0
3	10% more	2989.2	1.1

3.9. LOD and LOQ:

In the order to estimate the RP-HPLC sensitivity LOD as well as LOQ has to be considered, which were calculated from the calibration curves by the formulas below based on ICH standards. The results are as depicted in table15.

LOD = 3.3σ/S and LOQ = 10 σ/S

Where, σ= Standard deviation of the y-intercept of the regression line

S = Slope of the calibration curve

Table 15: LOD, LOQ of lamivudine and abacavir.

Drug	LOD	LOQ
lamivudine	2.97	9.98
abacavir	3.04	9.94

CONCLUSION

The HPLC technique recommended in this technique is considered clear-cut, exact, precise, and even sensitive for the estimation of lamivudine and abacavir in pharmaceutical dose types. For this reason, this approach can comfortably be taken for regular quality assurance evaluation of pure as well as in their pharmaceutical dosage forms.

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