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RP-HPLC Method Development and Validation for the Estimation of Abacavir and Lamivudine

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ABSTRACT

A new RP-HPLC method is developed and validated for the determination of Abacavir and Lamivudine in combined pharmaceutical tablet dosage form. The HPLC method was developed by using Symmetry C_{18} column; (150×4.6×5µ) column at 281nm, flow rate of 0.6ml/min., Injection volume of 20µl, column oven temperature of 25ºC using an equal volume of Methanol and Water used as mobile phase (50:50v/v). The retention times were found to 4.675 and 2.682mins. The % purity was found to be 99.9 and 99.9% w/w respectively. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The correlation coefficient (r^2) was found to be 0.999 respectively, % recovery was 100.0%, 100.3%, %RSD for precision was found to be 0.2, 0.2 respectively. The HPLC method was found to be accurate, precise, economical and reproducible. The method can be suggested for routine analysis and the method can be recommended for the determination of the substance-related, relative substance of Abacavir and Lamivudine in the combined pharmaceutical dosage form.

INTRODUCTION

Chromatography was originally developed by the Russian botanist Michael Tswett in 1903 for the separation of colored plant pigments by percolating a petroleum ether extract through a glass column packed with powdered calcium carbonate. It is now, in general, the most widely used separation technique in analytical chemistry has developed into several related but quite different forms that enable the components of complex mixtures of organic or inorganic components to be separated and quantified. A chromatographic separation involves the placing of a sample onto a liquid or solid stationary phase and passing a liquid or gaseous mobile phase through or over it, a process known as elution. Sample components or solutes, whose distribution ratios between the two phases differ will migrate (be eluted) at different rates, and this differential rate of migration will lead to their separation over some time and distance. Chromatographic techniques can be classified according to whether the separation takes place on a planar surface or in a column. They can be further subdivided into gas and liquid chromatography, and by the physical form, solid or liquid, of the stationary phase and the nature of the interactions of solutes with it, known as sorption mechanisms. “Chromatography is a physical method of separation in which the compound to be separated are distributed between two-phase, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction".

High-Pressure Liquid Chromatography (HPLC) sometimes called High-Performance Liquid Chromatography is a separation technique that can be used for the analysis of organic molecules and ions. HPLC is based on mechanisms of adsorption, partition, and ion-exchange or size exclusion, depending on the type of stationary phase used. HPLC involves a solid stationary phase, normally packed inside a stainless-steel column, and a liquid mobile phase. Separation of the components of a solution results from the difference in the relative distribution ratios of the solutes between the two phases. The rate of distribution of drugs between stationary and mobile phase is controlled by the diffusion process, if diffusion is minimized, a faster and effective separation can be achieved. The techniques of HPLC are so-called because of their improved performance when compared to classical column chromatography.

Method development and optimization in liquid chromatography are still an attractive field for theoreticians and attracts also a lot of interest from practical analysts. Among all, the liquid chromatographic methods, the reversed-phase systems based on modified silica offers

the highest probability of successful results. However, a large number of (system) variables (parameters) affect the selectivity and the resolution.

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, strength, and quality, for the quantification of the drug substances and drug products. Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products.

MATERIALS AND METHODS

Chemical specifications:

S. No.	Name of the Chemical/Reagent	Manufacturer/Suppliers	Grade
1.	Abacavir working standard	Aurobindo Pharma Ltd	-
2.	Lamivudine working standard	Hetero Health Care Ltd	-
3.	Abamune-L tablets	Cipla Ltd	-
4.	Acetonitrile	Merck	HPLC
5.	Water	Millipore	HPLC
6.	Methanol	Merck	AR
7.	0.45µm Nylon Filter	Millipore	AR

Instruments and specifications:

S. No.	Name of Instrument	Model	Make
1.	Semi micro balance	CPA225D	Sartorius
2.	Micro balance	BSA2245-CW	Sartorius
3.	HPLC	Waters2695series, Empower	Alliance
4.	UV	UV-3000+	Lab India
5.	Column	C18(150X4.6),5µ	BDShypersin
6.	Column	C18(100X4.6),5µ	Waters
7.	Sonicator	UCB 70	Spectra lab
8.	0.45µm GHP membrane filter	NA	Pall

Optimized chromatographic conditions:

Stationary phase: Symmetry C_{18} column (150 x 4.6 mm, 5µm)

Mobile phase : Methanol: Water

Mobile phase ratio : 50:50 (v/v)

Flow rate : 0.6 ml / min

Detector wavelength : 281 nm

Column temperature : Ambient

Injection volume : 20 µl

Run time : 10 min

Preparation of mobile phase:

Mix a mixture of HPLC Grade Water 500 mL (50%), 500 mL of Methanol HPLC (50%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Use the mobile phase as diluents.

Preparation of the Lamivudine & Abacavir Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Lamivudine and 20mg of Abacavir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution:

Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 1442 mg of Lamivudine and Abacavir sample into a 100mL clean dry volumetric flask add about 70mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution:

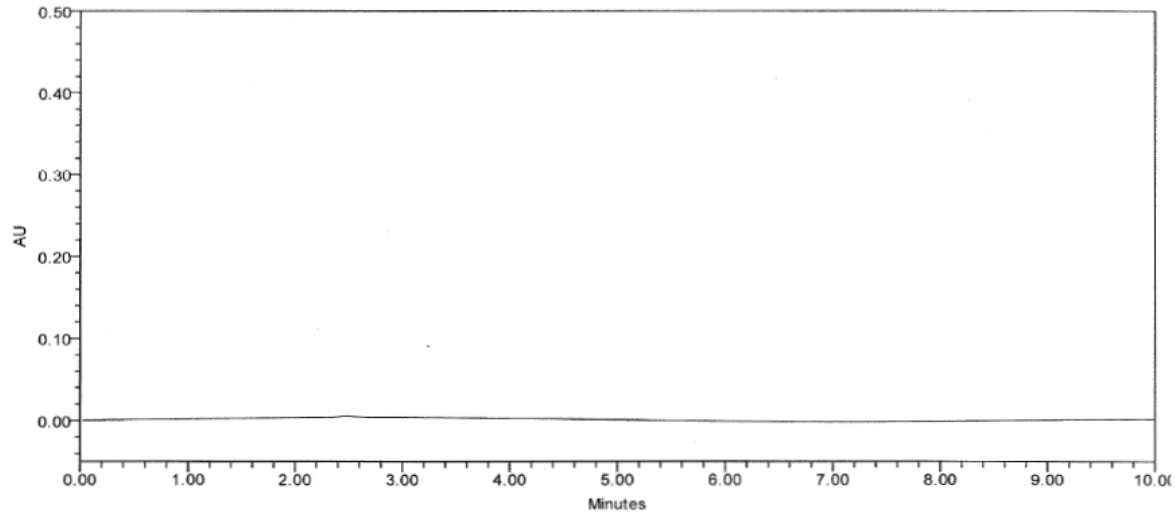
Further pipette 0.1ml of Lamivudine and Abacavir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

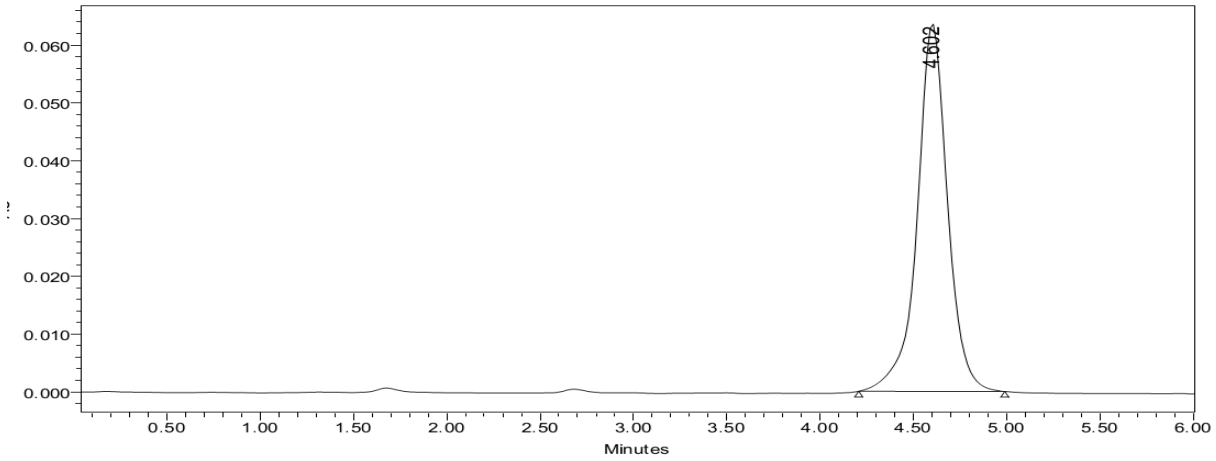
Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Lamivudine and Abacavir peaks and calculate the % Assay by using the formulae.

RESULTS

Chromatogram for Blank:

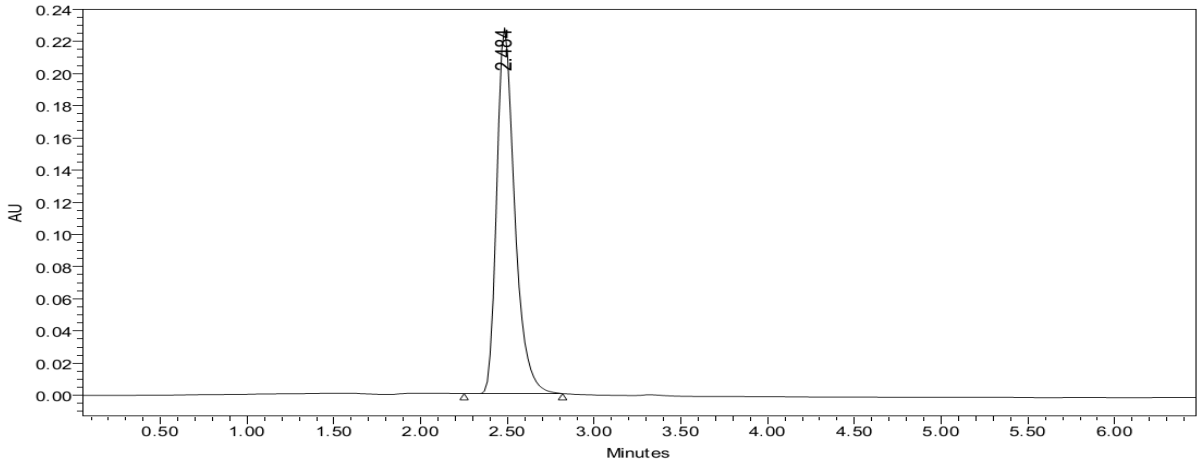


Chromatogram for Abacavir Standard:



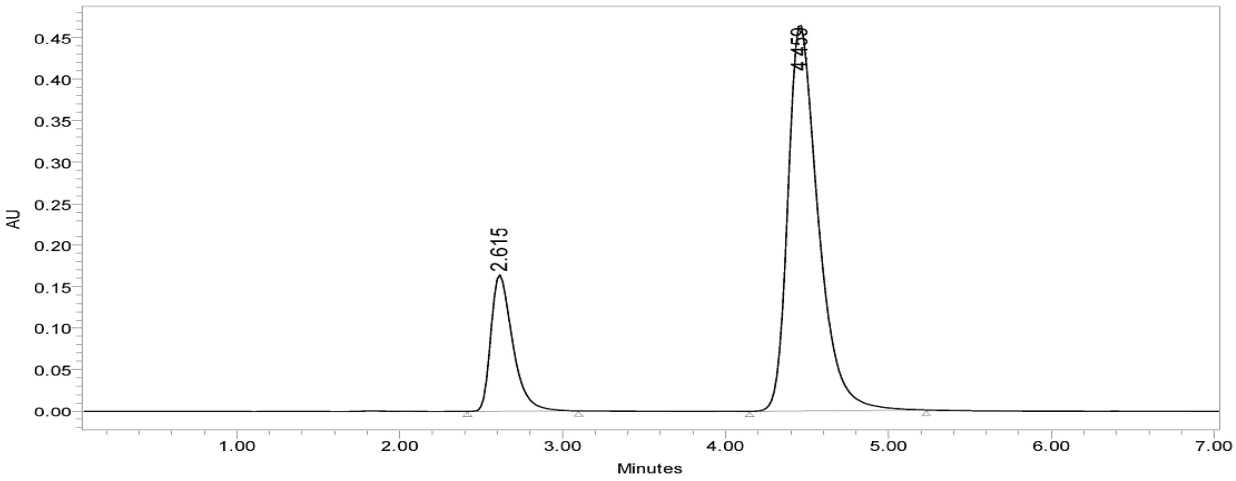
	Peak Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Abacavir	4.602	719003	63650	0.9	4290

Chromatogram for Lamivudine Standard:



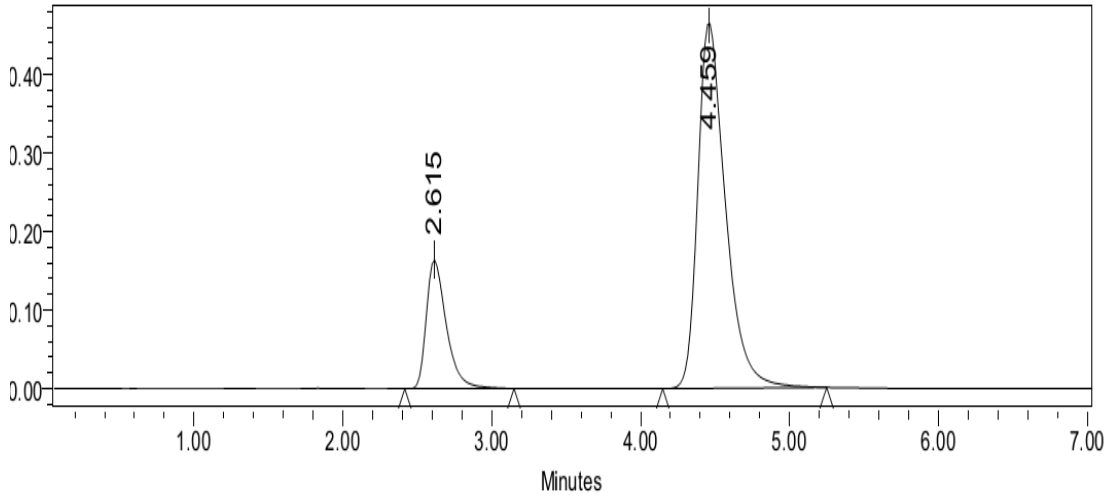
	Peak Name	RT	Area	% Area	Height	USP Tailing	USP Plate Count
1	Lamivudine	2.484	1636077	100.00	226249	1.35	2860

Chromatogram for Standard Solution:



	Peak Name	RT	Area	Height
1	Lamivudine	2.615	1522080	163883
2	Abacavir	4.459	6010690	465161

Chromatogram for Sample Solution:

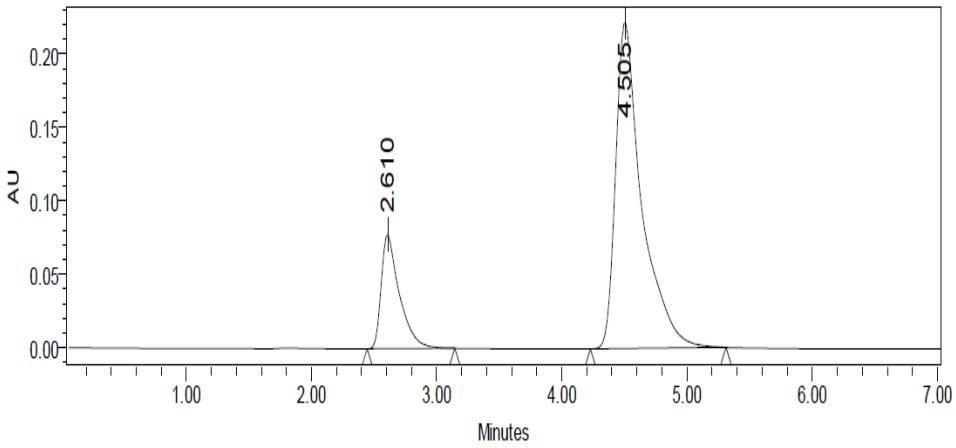


S.NO	PEAK NAME	RT	AREA	HEIGHT
1	LAMIVUDINE	2.615	152045	163922
2	ABACAVIR	4.549	6014491	465194

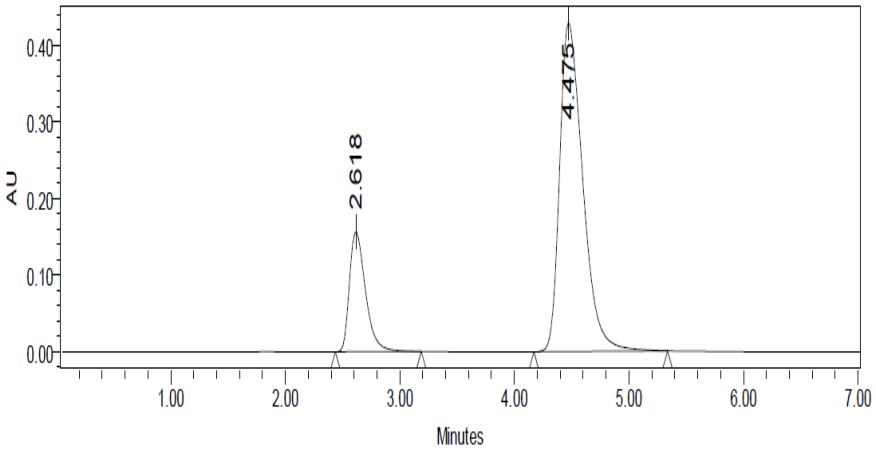
Assay of Abacavir and Lamivudine:

	Abacavir	Lamivudine
Standard area	6008963	1521389
Sample area	6014491	1525045
Standard wt.	20mg	10mg
Sample wt.	1442mg	1442mg
Average Weight	1442mg	1442mg
Label Claim	600mg	300mg
%purity	99.9	99.9

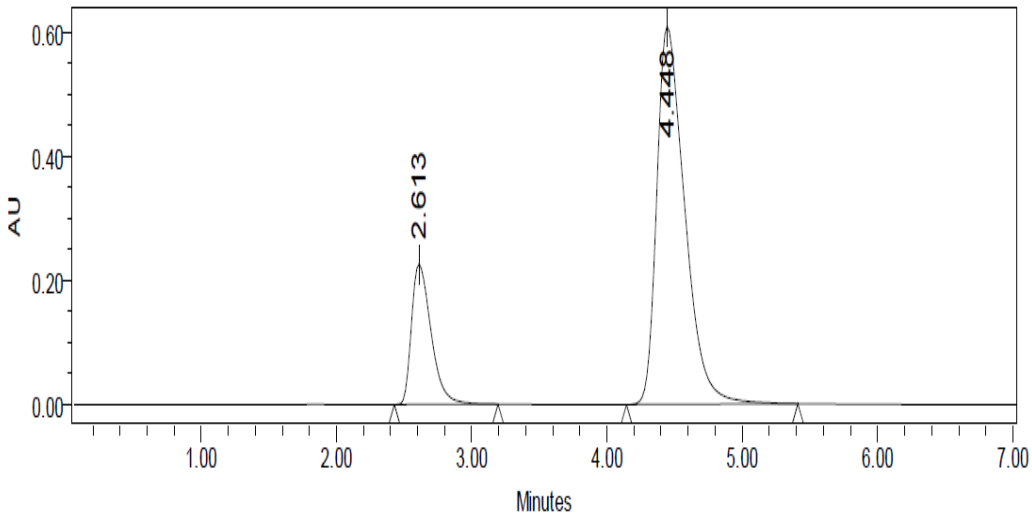
Accuracy-50%:



Accuracy- 100%:



Accuracy-150 %:



Peak results for Accuracy of Abacavir and Lamivudine:

Drug	Conc. of sample (mg)	Area	%recovery	Mean % Recovery
Abacavir	50%	3203.253	101.7%	100.0%
	100%	6006.329	100.9%	
	150%	8741.038	98.1%	
Lamivudine	50%	7794.976	101.8%	100.3%
	100%	1529.534	99.9%	
	150%	2275.818	99.1%	

Peaks results for Precision of Abacavir:

S. No.	Retention Time	Area
1	4.520	6080.912
2	4.526	6082.730
3	4.534	6079.366
4	4.541	6097.425
5	4.548	6064.705
Mean		6081027.5
SD		11629.3
%RSD		0.2

Peaks results for Precision of Lamivudine:

S. No.	Retention Time	Area
1	4.520	6063.814
2	4.526	6068.039
3	4.534	6065.636
4	4.541	6076.404
5	4.548	6042.019
Mean		6063182.6
SD		12774.0
%RSD		0.2

Ruggedness results for Abacavir:

S. No.	Retention Time	Area
1	2.616	1547.033
2	2.619	1545.256
3	2.619	1548.422
4	2.620	1549.235
5	2.621	1541.013
Mean		1546191.9
SD		3265.0
%RSD		0.2

Ruggedness results for Lamivudine:

S. No.	Retention Time	Area
1	2.616	1539.061
2	2.619	1538.419
3	2.619	1537.682
4	2.620	1540.110
5	2.621	1535.091
Mean		1538072.5
SD		1890.4
%RSD		0.1

Peaks results for Precision study:

Parameter	Abacavir	Lamivudine
Precision (%RSD)	0.2	0.2
Intermediate precision (%RSD)	0.2	0.1

DISCUSSION

Abacavir and Lamivudine were found to be soluble in methanol and water. The substance was dissolved in water to get a suitable concentration containing 100µg/ml and the resulting solution was scanned under the UV region. The overlay spectrum of Abacavir and Lamivudine shows the isosbestic point 281nm. Hence 281nm selected was maximum absorbance and the detection wavelength for the proposed method. In the HPLC method, the condition was optimized to obtain an adequate elution of compounds. Initially, various mobile phase compositions were tried to separate the titled ingredients. The mobile phase, column selection, wavelength selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor) and run time. The system with the mobile phase using Methanol: Water in the ratio of 50:50(v/v) and a flow rate of 0.6 ml /min was found to be robust. The optimum wavelength for detection was 281 nm and a run time of 7 min at which better detector for the drug along with no interference was obtained. The standard chromatograms were taken for the proposed method and various system suitable parameters were recorded.

The validated HPLC method was used for the simultaneous determination of Abacavir and Lamivudine in their combined dosage form. In the assay experiment, seven samples were weighed separately and analyzed. The mean assay results expressed as a percentage of the label claim are listed in the table. The results indicate that the amount of each drug in the tablets is within the requirements of 90-110% of the label claim.

The retention time was found to be 4.6 and 2.6 mins and the %purity of the Abacavir and Lamivudine was found to be 99.9 % w/w & 99.9% w/w respectively.

The calibration curve was constructed for Abacavir and Lamivudine standard by plotting the concentration of compound versus peak area response. Standard solutions containing 10, 20, 30, 40, 50 µg/ ml of Abacavir and Lamivudine with respectively were prepared and 20µl was

injected into the HPLC column the linearity was evaluated by linear regression analysis, which was calculated by the least square regression method on the ordinate.

The accuracy study was performed for 50%, 100% and 150 % for Abacavir and Lamivudine. Each level was injected in triplicate into a chromatographic system. The area of each level was used for calculation of % recovery.

The reproducibility of the method was estimated by analyzing samples. Five injections of the standard mixture were analyzed.

The intermediate precision study was performed on a different day by using different make columns of the same dimensions. Each standard injection was injected into the chromatographic system. The area of each standard injection was used for calculation of % RSD.

CONCLUSION

The project summarizes a method development and validation for the determination of Abacavir and Lamivudine in combined pharmaceutical tablet dosage form. An efficient high performance liquid chromatographic method was developed for Abacavir and Lamivudine. The HPLC method was developed by using Symmetry C_{18} column; (150×4.6×5µ) column at 281nm, flow rate of 0.6ml/min., Injection volume of 20µl, column oven temperature of 25^0 C using an equal volume of Methanol and Water used as mobile phase(50:50v/v). The retention times were found to 4.675 and 2.682mins. The % purity was found to be 99.9 and 99.9% w/w respectively. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The correlation coefficient (r^2) was found to be 0.999 respectively,% recovery was 100.0%, 100.3%, %RSD for precision was found to be0.2, 0.2 respectively. The HPLC method was found to be accurate, precise, economical and reproducible. The method can be suggested for routine analysis and the method can be recommended for the determination of substance-related, the relative substance of Abacavir and Lamivudine in the combined pharmaceutical dosage form.

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I humbly present this work to the eternal almighty. Indeed my final work is done with the help of primitive persons at heart. So it is my bounded duty to promulgate them individually.

I wish to extend my sincere thanks to my beloved guide Mrs. V. Chaithanya, Associate Professor, Department of Pharmaceutical Analysis, Sahasra Institute of Pharmaceutical Sciences, Warangal for her encouragement which made me finish this work.

ABBREVIATIONS

API's	Active Pharmaceutical Ingredients
As	Asymmetry Factor
DD Water	Double Distilled Water
F	Flow Rate
GC	Gas Chromatography
GLC	Gas-Liquid Chromatography
GSC	Gas-Solid Chromatography
h	Peak Height
HPLC	High-Performance Liquid Chromatography
ICH	International Conference On Harmonization
IP	Indian Pharmacopoeia
IUPAC	International Union of Pure and Applied Chemistry
k	Retention Factor
nm	Nanometer

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