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A Validated RP-HPLC method for Simultaneous Estimation of Gemcitabine and Clarithromycin in Bulk and Pharmaceutical Dosage form

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A B S T R A C T

A simple, accurate and reproducible RP-HPLC method has been developed for simultaneous estimation of Abacavir and Lamivudine in bulk and tablet dosage form. Which are widely used as anti HIV drugs. Chromatography was carried out the column inertsil ODS C_{18} (250×4.6mm, 5µm) using a mobile phase composition of Methanol and Potassium dihydrogen Phosphate buffer p^H-4.0 (60:40) at flow rate of 1.0ml/min. The detection was made at 257nm.The retention times of Abacavir and Lamivudine are 3.0min and 3.9min respectively. The method was validated for system suitability, precision, linearity, accuracy, robustness, LOD and LOQ. The drug obeys linearity within the concentration range of 15-90µg/ml for Abacavir and 7.5-45µg/ml for Lmivudine .The proposed method was validated as per ICH guide lines and it was found to be Suitable for the routine quality control analysis of the bulk and in its tablet dosage forms.

Keywords: Abacavir, Lamivudine, RP-HPLC, Methanol, Anti retroviral agents.

A R T I C L E I N F O

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1. Introduction

In Generally chromatographic methods are (HPLC, HPTLC & GC) used because it fulfills requirement of various guidelines by effectively separating degraded product from the drug substance. Because the High-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery,

development, and production [1,2]. Abacavir and lamivudine are synthetic nucleoside revers transcriptase analogs (NRTI) used to treat HIV and AIDS. Lamivudine and Abacavir combined formulations are designed as they exhibit synergistic effect in activity against HIV-virus. Abacavir is chemically (Aba)[1R-4-(2amino -6-cyclopropyl amine) purine -9-yl]-1-cyclopent -2enyl] methanol. Lamivudine is chemically (Lam) 4 amino-1-[2R, 5S]-2-(hydroxymethyl)-1, 3-oxathiolan-5yl] pyrimidine-2-one [3,4].

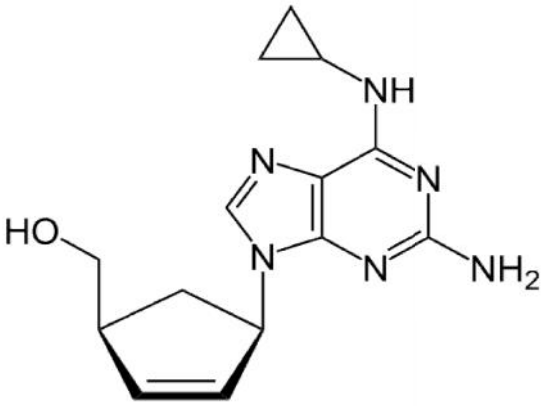

Figure 1: Structure of Abacavir

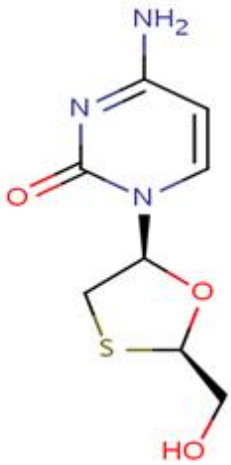

Figure 2: Structure of Lamivudine

2. Materials and Methods

Instrumentation:

Chromatographic separations were made on Inertsil ODS C_{18} (250×4.6mm, 5µm) and the injected volume was 20µL and the column was maintained at ambient temperature. The absorbance was monitored at 257nm. Chromatographic analysis was performed on Waters 2690 HPLC. Empower 2 software was used for quantitative determination at eluted peaks. The Detection was achieved by a UV detector. Dissolution of compound was enhanced by sonication on Branson Ultrasonic Bath. The PH of the solution was adjusted by using digital PH meter.

Chemicals and Reagents:

Abacavir and Lamivudine generously supplied by Hetero drugs Pvt.ltd, Hyd. Each film coated tablet contains 600mg of Abacavir and 300mg of Lamivudine. Methanol HPLC grade and Potassium dihydrogen phosphate is of analytical grade.

Preparation of Stock solution:

The solution was prepared by dissolving 15mg of accurately weighed Abacavir and 7.5 mg Lamivudine in Mobile phase, in two 50 mL volumetric flasks separately and sonicated for 20min. From the above solutions take 1 mL from each solution into a 10 mL volumetric flask and then made up to volume with mobile phase and sonicated for 10min.

Preparation of sample drug solution:

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 15 mg Abacavir and 7.5 mg Lamivudine was weighed and dissolved in the mobile phase with the aid of ultra sonication for 20 min. The content was diluted to 10 mL with mobile phase to furnish a stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 0.4 mL of the filtrate was pipette out in to a 10 ml dry clean volumetric flaskand the volume is made up to the mark with solvent.

Method validation:

The proposed method for assay was subjected to validation as per ICH guidelines to test its suitability for intended purpose.

System suitability test:

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. Accuracy can also be described as the extent to which test results generated by the method and the true value agree.

Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the

sample. Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) or by separately weighing synthetic mixtures of the test product components. ICH recommends that, for the establishment of linearity, a minimum of five concentrations normally be used.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Limit of detection:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The limit of detection (LOD) is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified.

Limit of quantification:

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The limit of quantitation (LOQ) is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products [4,5].

3. Results and Discussion

Method development*:*

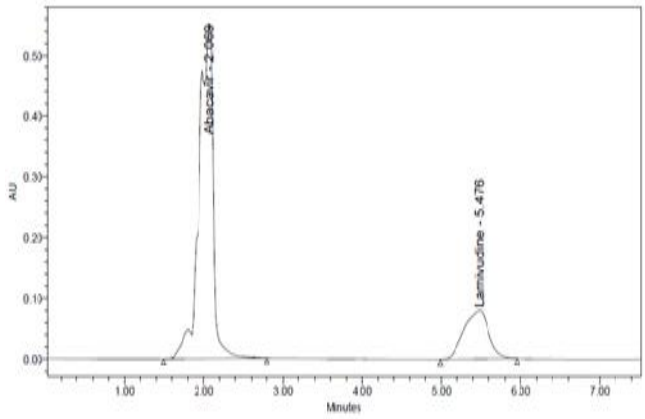

Figure 3: Chromatogram of Trial 1

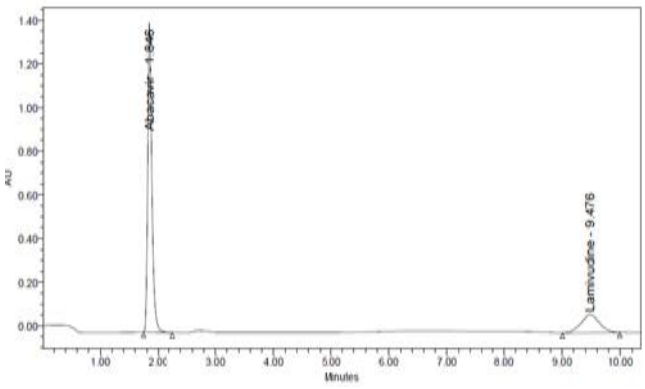

Figure 4: Chromatogram of Trial 2

Optimized method:

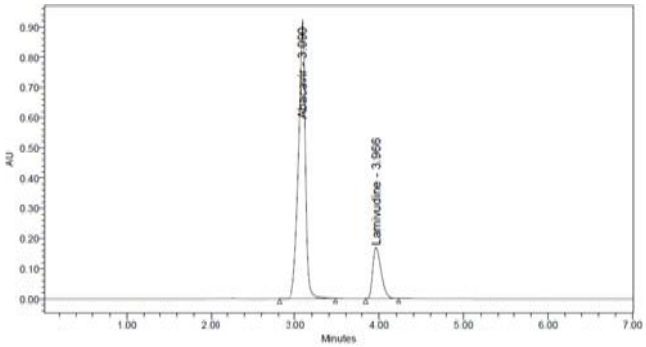

Figure 5: Chromatogram of optimized method

Validation Data

System suitability*:*

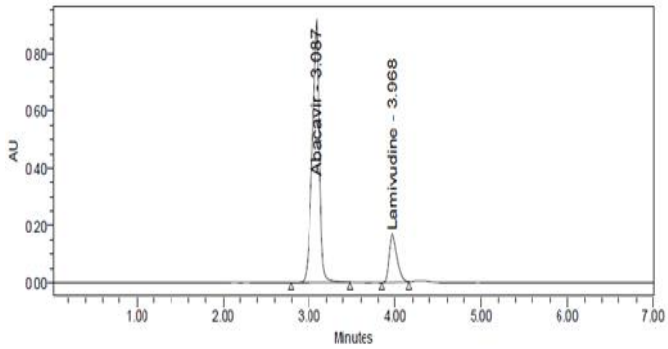

Figure 6: Chromatogrm for system suitability

Precision:

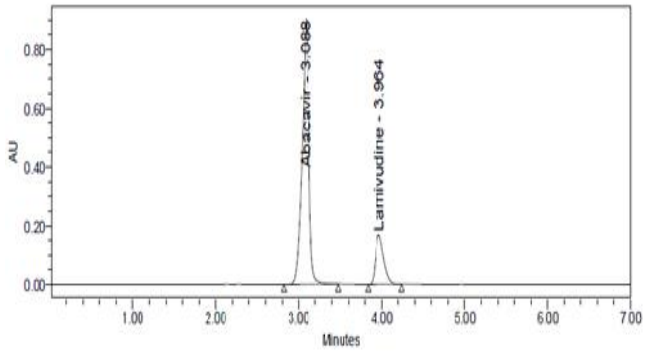

Figure 7: Intraday chromatogram of Abacavir and Lamivudine

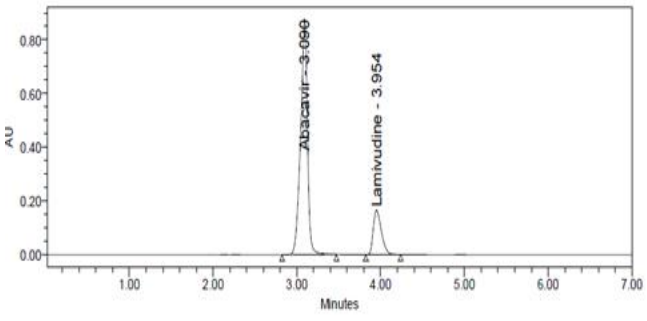

Figure 8: Interday chromatogram of Abacavir and Lamivudine

Accuracy:

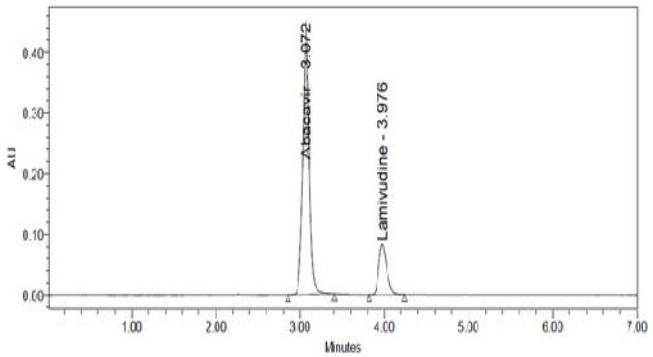

Figure 9: Chromatogram for accuracy 50%

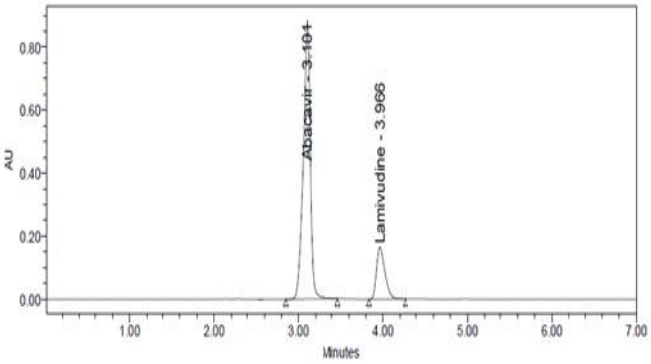

Figure 10: Chromatogram for accuracy 100%

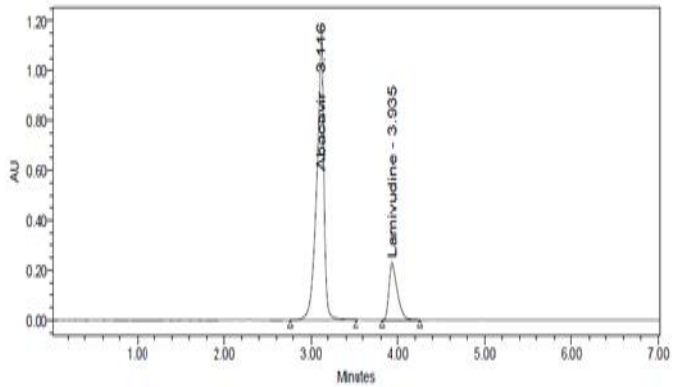

Figure 11: Chromatogram for accuracy 150%

Linearity:

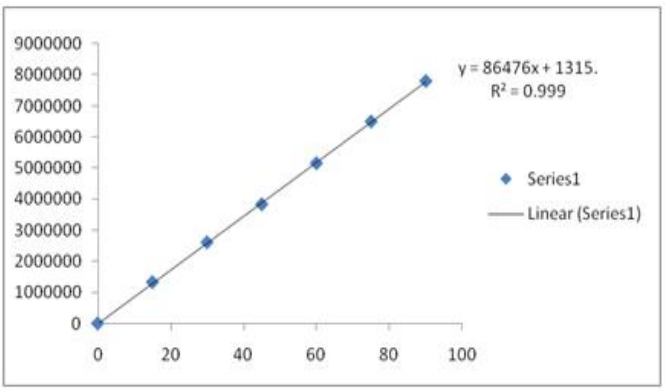

Figure 12: Linearity plot for Abacavir

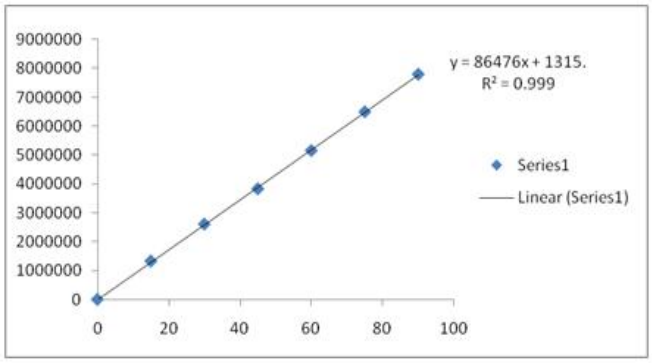

Figure 13: Linearity plot for Lamivudine

Chromatograms for linearity

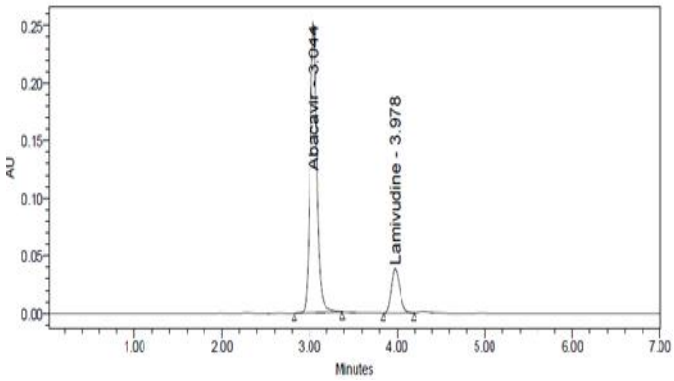

Figure 14: Chromatogram of Linearity for 25%

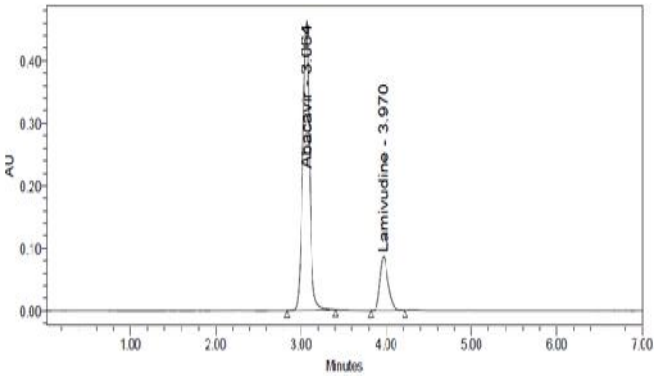

Figure 15: Chromatogram of linearity for 50%

Robustness:

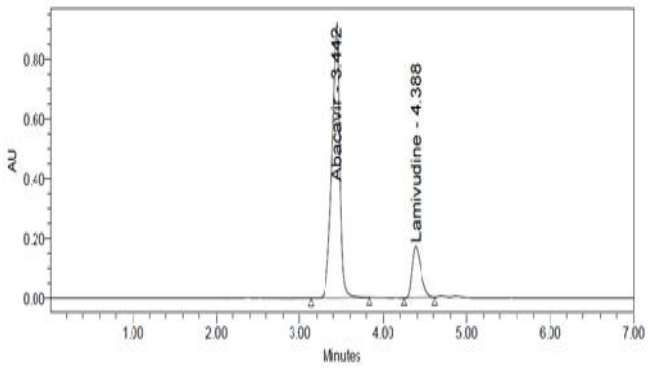

Figure 16: Chromatogram of low flow rate for Abacavir and Lamivudine

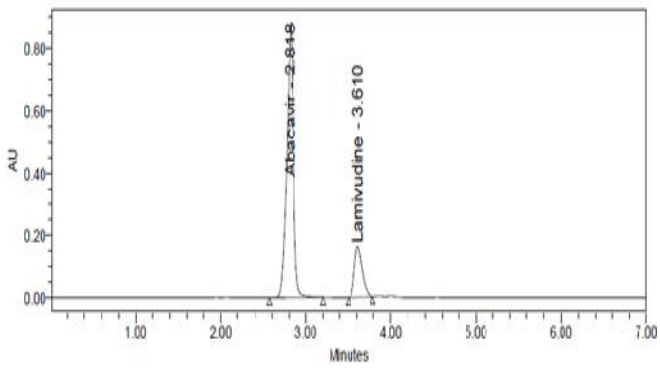

Figure 17: Chromatogram of high flow rate for Abacavir and Lamivudine

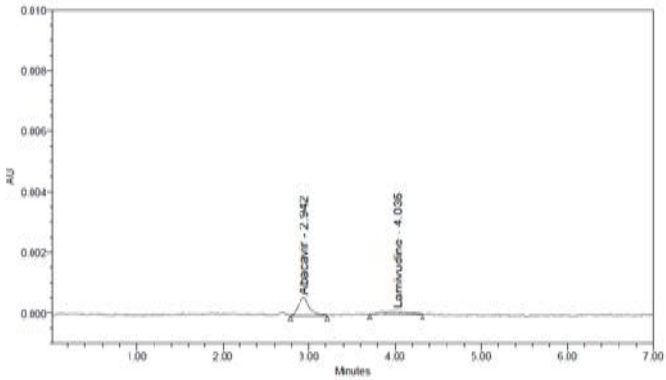

Figure 19: Chromatogram for limit of quantification for Abacavir and Lamivudine

Limit of Detection (LOD):

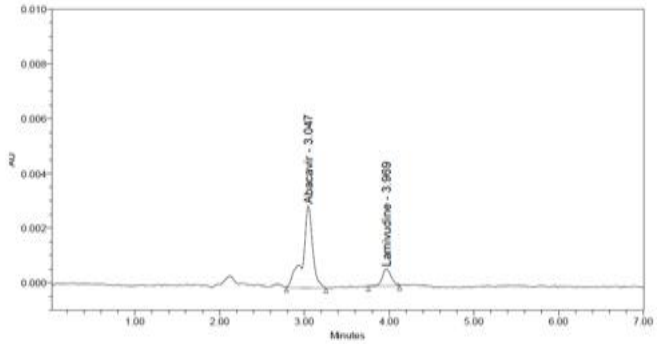

Figure 18: Chromatogram for limit of detection for Abacavir and Lamivudine

Assay:

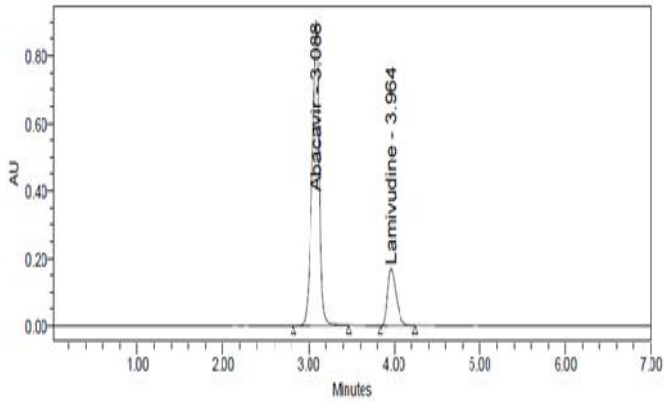

Figure 20: Chromatogram for assay

Limit of quantification (LOQ):

Table 1: System Suitability patameters for Abacavir

Injection	Retention time	Peak area	USP plate cout	USP Tailing
1	3.084	Peak Area	6104	0.87
2	3.085	5165118	6550	0.85
3	3.086	5131380	6267	0.85
4	3.087	5160426	6357	0.85
5	3.087	5174749	6608	0.85
6	3.085	5165391	6550	0.85
Mean		5131381		
SD		5154741	-------	-------
% RSD		0.362	-------	-------

Table 2: System Suitability parameters for Lamivudine

Injection	Retention time	Peak area	USP Plate count	USP Tailing
1	3.968	113257	7678	1.33
2	3.974	1136680	7832	1.33
3	3.974	1132876	7942	1.33
4	3.976	1134503	7733	1.33
5	3.978	1134502	7973	1.33
6	3.966	1134503	7733	1.33
Mean		1134272		
SD		1469.946	-------	-------
% RSD		0.12	-------	-------

Table 3: Accuracy studies for Abacavir

Concentration of spiked level	Amount added	Amount found	% Recovery	% Recovery mean	% RSD
50% Injection1	20	19.86	99.30		
50% Injection 2	20	19.98	99.90		
50% Injection 3	20	20.12	99.20	99.46	0.38
100%Injection1	40	39.54	98.85		
100%Injection2	40	39.82	99.95		
100%Injection3	40	39.96	99.90	99.76	0.18
150%Injection1	60	59.92	99.86		
150%Injection2	60	60.08	100.13		
150%Injection3	60	60.02	100.03	100.00	0.13

Table 4: Accuracy studies for Lamivudine

Concentration of spiked level	Amount added	Amount found	% Recovery	% Recovery mean	% RSD
50% Injection1	20	19.85	99.25		
50% Injection 2	20	19.96	99.80		
50% Injection 3	20	20.12	100.6	99.88	0.67
100%Injection1	40	39.74	99.35		
100%Injection2	40	40.08	100.2		
100%Injection3	40	40.24	100.6	99.81	0.39
150%Injection1	60	59.04	99.40		
150%Injection2	60	59.62	99.36		
150%Injection3	60	59.89	99.81	99.19	0.72

Table 5: Calibration data of Abacavir and Lamivudine

S.NO	Concentration Abacavir(µg/ml)	Response	Concentration Lamivudine(µg/ml)	Response
0	0	0	0	0
1	15	1329215	7.5	268657
2	30	2611655	15	564910
3	45	3836217	22.5	843844
4	60	5160593	30	1102478
5	75	6505013	37.5	1394280
6	90	7806467	45	1660779

Table 6: Robustness data of Abacavir

Flow rate (ml/min)	Retention time	Peak area	Tailing factor	USP plate count	% RSD
Low flow (0.8ml)	3.442	5838521	0.85	6443	0.3
High flow (1.2ml)	2.818	4712855	0.88	6194	0.5

Table 7: Robustness data of Lamivudine

Flow rate (ml/min)	Retention time	Peak area	Tailing factor	USP plate count	%RSD
Low flow (0.8ml)	4.388	1274497	0.85	6443	0.1
High flow (1.2ml)	3.610	1023556	1.32	7327	0.6

Table no: 8 Assay results

S.NO	Abacavi % assay	Lamivudine % asaay
1	99.27	98.57
2	100.68	98.05
3	99.16	100.90
4	100.23	101.65
5	100.47	100.47
6	99.31	100.27
AVG	99.85	99.98
STDEV	0.6842	1.3907
%RSD	0.69	1.39

4. Conclusion

Finally with the above experimental data and results the developed RP-HPLC method is suitable for determination of Abacavir and Lamivudine. Percentage of recovery shows that the proposed method is free from interferences of excipients used in the formulation. Therefore the proposed method is simple, precise, and accurate that can be effectively applied for routine analysis in quality control department of bulk drug and its tablet dosage forms.

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