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Research Article

VALIDATED RP-HPLC FOR SIMULTANEOUS ESTIMATION OF ABACAVIR AND LAMIVUDINE IN TABLET DOSAGE FORM

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ABSTRACT

A simple and sensitive reverse phase HPLC method has been developed for simultaneous analysis of Abacavir and Lamivudine in combined dosage form. The method utilizes sample preparation followed by separation on an Inertsil ODS column 150mm length, 4.6mm inner diameter, with 5µm particle size. Analytes were monitored by UV detection at 254nm using an isocratic mode with mixed phosphate buffer: Acetonitrile in the ratio 70:30 as mobile phase. The flow rate was set at 1.0ml/min and effluent was monitored at 254nm.The method was validated for system suitability, linearity, precision, accuracy, robustness and stability of standard solution. The drug obeys linearity within the concentration range of 20-120µg/ml for Abacavir and 10-60µg/ml for Lamivudine,the percentage recovery values of pure drug were in between 99.0 to 99.9 respectively. The retention time was 2.620min and 4.307min for Abacavir and Lamivudine respectively.

Keywords: Lamivudine, Abacavir, High performance liquid chromatography, Acetonitrile

INTRODUCTION

The combinations of two or more drugs in the pharmaceutical dosage forms are very much useful in multiple therapies. Market survey revealed that, day-by-day new drugs and their combination with another drugs are being introduced in market as they have more patient compliance than a single drug[1].Abacavir chemicaly called {(1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]cyclopent-2-en-1-yl}methanol and lamivudine is 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one,both has Anti retroviral (Nucleoside reverse transcriptase inhibitors) activity[2,3], used to treat the human immune deficiency virus that causes the AIDS,especially the lamivudine also used to treat the severe acute HBV hepatitis [4,5]. Lamivudine is practically insoluble in acetone,very soluble in water and methanol[6] .Literature survey reveals the few analytical methods were evoked for the simultaneous estimations of abacavir and lamivudine by modern analytical instruments like,LC-MS-MS[7], HPTLC [8] and UV[9]. So attempt has been made to develop a simple, accurate, precise method for simultaneous estimation of abacavir and lamivudine in tablet dosage forms by using RP-HPLC method.

MATERIALS AND METHODS

The HPLC system used was Shimadzu Separation ModuleLC-20AT Prominence Liquid Chromatography with UV Detector. The chromatogram was recorded at and peaks quantified by means of PC based Spinchrom software.

Solvents used

Acetronitrile and water for buffer HPLC grade, Potassium dihydrogen ortho phosphate, Di-Potassium hydrogen ortho phosphate used as a solvent in the study and obtained from MERCK Company, Mumbai.

Prepration of standard stock solution

Accurately weighed and transfered 100mg of Abacavir and 50mg lamivudine working sample into a 100ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10mins and then made up to volume with mobile phase.

Sample Preparation

Accurately weighed and transfered 100mg of Abacavir and 50mg lamivudine working sample into a 100ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10mins and then made up to volume with mobile phase.

Chromatographic condition

The chromatographic separation was performed at ambient temperature on a reverse phase Inertsil ODS (150×4.6mm) 5µ column. Mobile phase was made up of acetonitrile: phosphate buffer in a ratio 70:30. The mobile phase was filtered, degassed before use. The flow rate was adjusted to 1.0ml/min; the detector wavelength was set at 254nm. The injector volume of the standard and sample was 20µl.

Method Validation

The method was validated as per international conference on harmonization (ICH) guidelines [10, 11]

Linearity

Linearity of the method was determined by mean of calibration graph using an increasing amount of each analyst. Linearity was evaluated by visual inspection of a calibration graph. At least three concentration levels were tested in agreement to ICH. The slope, intercept was reported as required by ICH. LOD and LOQ were estimated from the standard deviation of the response and the slope of the calibration curve. The standard deviation can be determined either from the standard deviation of multiple blank samples or from the standard deviation of the intercepts of the regression lines done in the range of the detection limit.

Assay

Calculate the percentage purity of Abacavir and Lamivudine present in the tablet using the formula:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where,

AT = average area counts of sample preparation.

As= average area counts of standard preparation.

WS= Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = Label Claim

Accuracy

The accuracy of the method was measured by recovery studies and ascertained by standard addition method. A known amount of pure drug at three different levels i.e. 80 %, 100 %, and 120 % was added to pre-analyzed sample solutions and total concentration was determined using the proposed method.

Precision

Precision was investigated at three levels, intra-day, inter-day, and reproducibility. The intra- and inter- day variability were assessed by using standard drug solution at three different concentration. Intra-day precision was carried out by analyzing the drug solutions within same day. The inter-day precision was measured using standard solution over three consecutive days. Reproducibility of the method was determined by performing same analytical procedure at different laboratories using same experimental design.

System Suitability Tests (SST)

Once a method or system has been validated the task becomes one of routinely checking the Suitability of the system to perform within the validated limits. The simplest form of an HPLC system suitability test involves a comparison of the chromatogram trace with a standard trace. This allows a comparison of the peak shape, peak width, and baseline resolution. Alternatively these parameters can be calculated experimentally to provide a quantitative system suitability test report such as number of theoretical plates (efficiency), Capacity factor, Separation (relative retention), Resolution, Tailing factor. These are measured on a peak or peaks of known retention time and peak width.

Specificity

Specificity was studied in order to assess unequivocally an analyst in the presence of components that may be expected to be present. Specificity was confirmed by obtaining positive results (by comparison with a known reference material) from samples containing the analyst, coupled with negative results from samples which do not contain the analyst. The parameters like retention time (Rt), resolution (RS) capacity factor, tailing factor were calculated.

Robustness

The robustness of the method was investigated under a variety of conditions including changes of pH of the eluent, flow rate and of buffer composition. The obtained results were compared with that of standard results.

Ruggedness

Ruggedness is a measurement of reproducibility of test results under the variation in condition normally expected from laboratory to laboratory and from analyst to analyst. Degree of representative of test results is then determined as a function of the assay variable. By analysis of aliquots from homogenous lots in different laboratories, by different analyst, using operational and environmental conditions that may differs but is still within the specified parameter of the assay variable.

RESULT AND DISCUSSION

Method Development

Different columns containing octyl and octadecylsilane stationary phases were tried for the separation and resolution. It was found the Inertsil ODS column offered more advantages. Individual drug solution was injected into the column and elution pattern of all the drugs and resolution parameters were studied. In addition to this, UV spectra of individual drugs were recorded at the wavelength from 200-400nm and the response for optimization was compared. The choice of wavelength 254nm was considered satisfactory, permitting the detection of both drugs with adequate sensitivity.

Method Validation

Linearity

Linear curves for the two drugs were obtained throughout the concentration ranges studied. Regression analysis was done on the peak areas of the two drugs (y) Vs concentration (x). The linear ranges of abacavir and lamivudine are 20-120µg/ml, 10-60µg/ml respectively showed in (Table no: 1) (Fig 1a &1 b).

Table 1: Linearity studies of Abacavir and Lamivudine by RP-HPLC

S. No	Conc. Taken in µg/ml Abacavir	Peak area of Abacavir	Conc. taken in µg/ml Lamivudine	Peak area of Lamivudine
1	20	356.698	10	1189032
2	40	686.406	20	1497165
3	60	976.18	30	1851004
4	80	1288.844	40	2186380
5	100	1632.858	50	2548658
6	120	2012.779	60	

Correlation coefficient 0.998 0.999

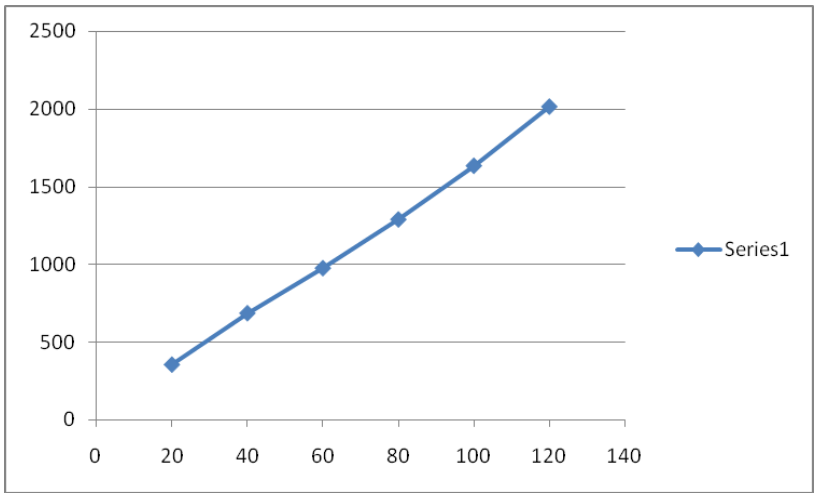

Fig. 1a: Linearity graph of Abacavir

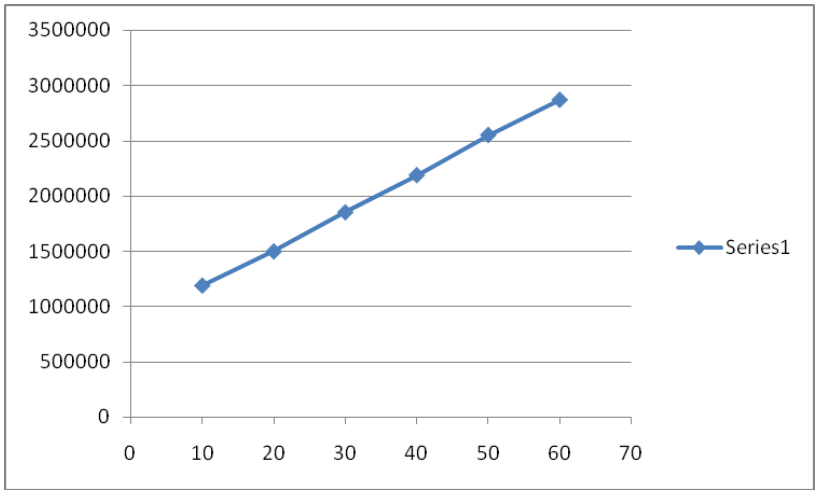

Fig. Ib: Linearity graph of Lamivudine

Assay

Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and percentage purity was calculated. Recovery studies were also carried out. The Chromatograms were shown in (Fig 2, 3,). The results were discussed in (Table 2 and 3).

Table 2: Assay of Abacavir and Lamivudine by RP-HPLC

S. No	Abacavir				Lamivudine			
	Standard Rt	Sample Rt	Standard area	Sample area	Standard Rt	Sample Rt	Standard area	Sample area
01	2.507	2.497	1695.983	1701.579	4.157	4.143	386.693	405.474
02	2.507	2.497	1700.93	1705.882	4.160	4.157	402.523	405.799
03	2.507	2.497	1691.960	1703.730	4.160	4.157	397.146	405.567
MEAN			1696.293	1703.731	4.159	4.155	395.454	405.613
STDEV			4.490033	3.04268			6.572385	0.22981
%RSD			0.264697	0.178589			1.6619858	0.056654

Table 3: Assay of Abacavir and Lamivudine by RP-HPLC

S. No	Abacavir		Lamivudine	
01	Spl. Area	1703.731	Spl. Area	405.613
02	Std.Area	1696.293	Std.Area	395.454
03	Std. Wt	49.8mg	Std. Wt	24.3mg
04	Spl.Wt	85.2mg	Spl.Wt	85.2mg
05	LC	600mg	LC	300mg
06	Avg.Wt	1023.2mg	Avg.Wt	1023.3mg
07	Std.Purity	99.85%	Std.Purity	99.86%
08	Assay %	99.96	Assay %	99.65

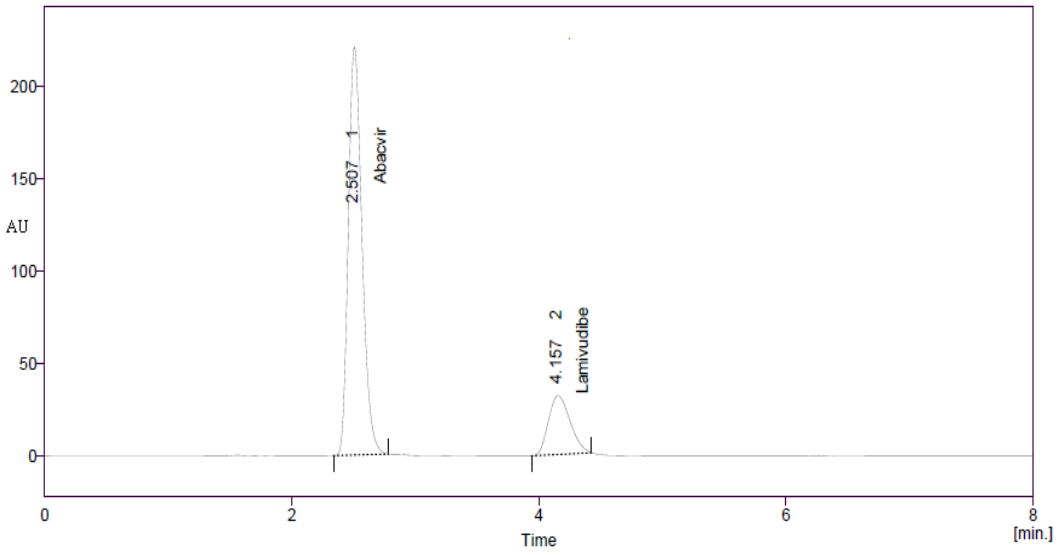

Fig. 2: Chromatogram of standard (100µg/ml)

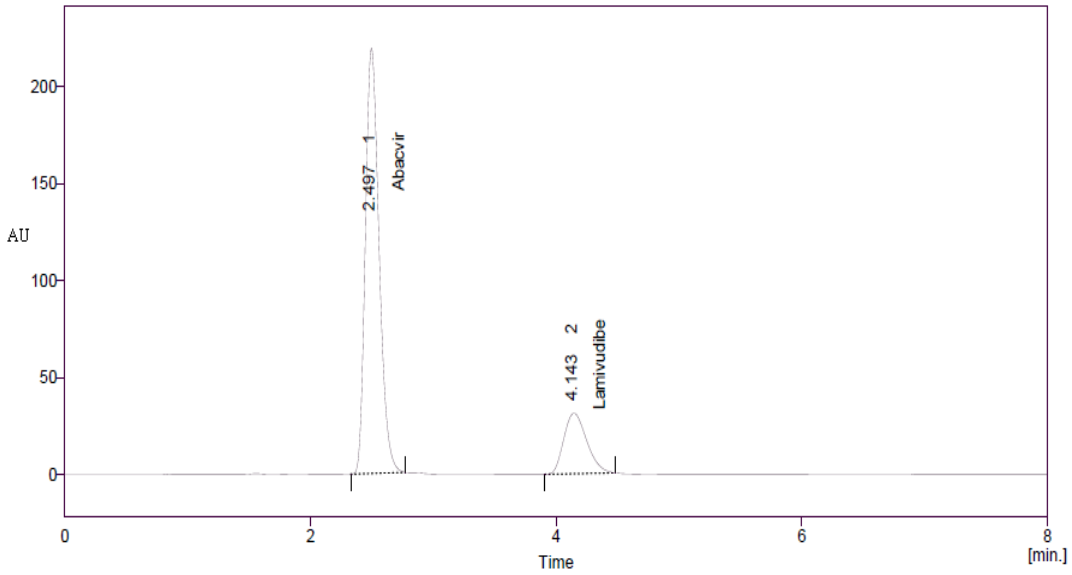

Fig. 3: Chromatogram of sample (100µg/ml)

Accuracy

Inject the standard solution, Accuracy 90µg/ml, 110µg/ml, 130µg/ml solutions. Calculate the amount found and amount added for Abacavir and Lamivudine and calculate the individual recovery and mean recovery values. The results were discussed in the (Table 4, 5)

Precision

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The results were discussed in the Table 6 (Method and precision of Abacavir) and Table 7 (Method System precision of Lamivudine).

System suitability

A Standard solution of Abacavir and Lamivudine working standard was prepared as per procedure and was injected six times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.. The results were discussed in (Table 8).

Specificity

Stress degradation studies by treating with alkali and acid

Abacavir and Lamivudine was treated with Heat, 0.1M NaOH and 0.1M HCl. Solutions of sample was prepared as per test procedure and injected into the HPLC system.Chromatogram of degradants should not show any peak at the retention time of analyte peak.

There is no interference due to degradants at the retention time of analyte. The chromatograms were shown in (Fig 4 and 5).

Table 4: Accuracy of Abacavir

S. No	Concentration in µg/ml	Percentage Recovery	Mean percentage recovery	Standard deviation	Relative standard deviation
1	40	99.40119	99.40119	2.074304	0.580016
2	40	99.40119			
3	40	99.40119			
4	50	99.20424	99.20424	2.051591	0.470294
5	50	99.20424			
6	50	99.20424			
7	60	99.6764	99.6764	5.89134	1.137313

Table 5: Accuracy of Lamivudine

S. No	Concentration in µg/ml	Percentage Recovery	Mean percentage recovery	Standard deviation	Relative standard deviation
1	80	99.13438	99.13438	6.972176	0.463415
2	80	99.13438			
3	80	99.13438			
4	100	99.94599	99.94599	2.995057	0.161553
5	100	99.94599			
6	100	99.94599			
7	120	99.25805	99.25805	5.740154	2.638048
8	120	99.25805			
9	120	99.25805			

Table 6: Method and System Precision of Abacavir

Injection number(100µg/ml)	Retention time of Abacavir	Area	% Assay
1	2.487	1704.777	99.41
2	2.49	1678.033	100.99
3	2.493	1687.676	100.41
4	2.497	1674.856	101.18
5	2.493	1681.65	100.77
Avg	2.492	1685.398	100.55
SD	0.003742	11.83384	0.699
%RSD	0.150147	0.702139	0.682

Table 7: Method and System Precision of Lamivudine

Injection number(100µg/ml)	Retention time of Lamivudine	Area	% Assay
1	4.107	398.07	98.92
2	4.107	396.023	99.43
3	4.11	396.935	99.20
4	4.117	399.94	98.94
5	4.12	396.091	99.41
Avg	4.1122	397.4118	99.18
SD	0.005975	1.6375	0.245
%RSD	0.145298	0.412	0.241

Table 8: System suitability of Abacavir and Lamivudine

Parameters	Data of Abacavir	Data of Lamivudine
No. of Theoretical plates	2456	2586
Tailing factor	1.393	1.432
Retention Time	2.527	4.177

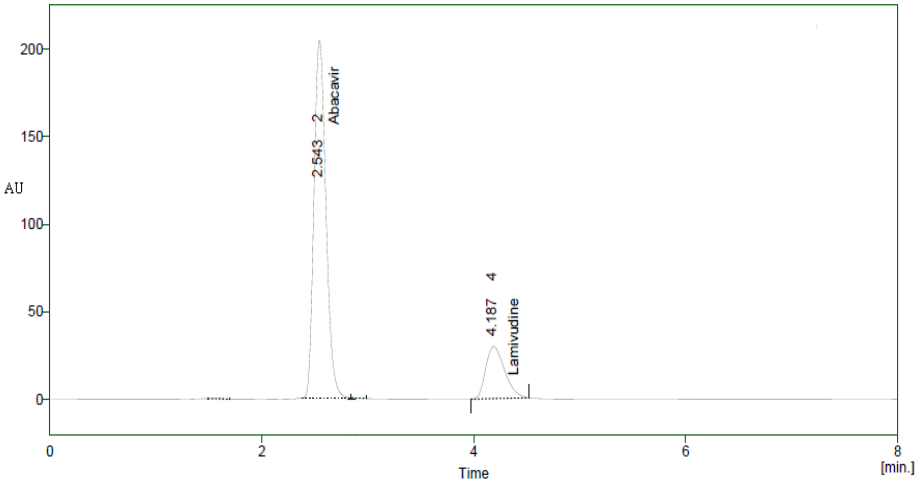

Fig. 4: Chromatogram of Specificity (0.1 M Hcl)

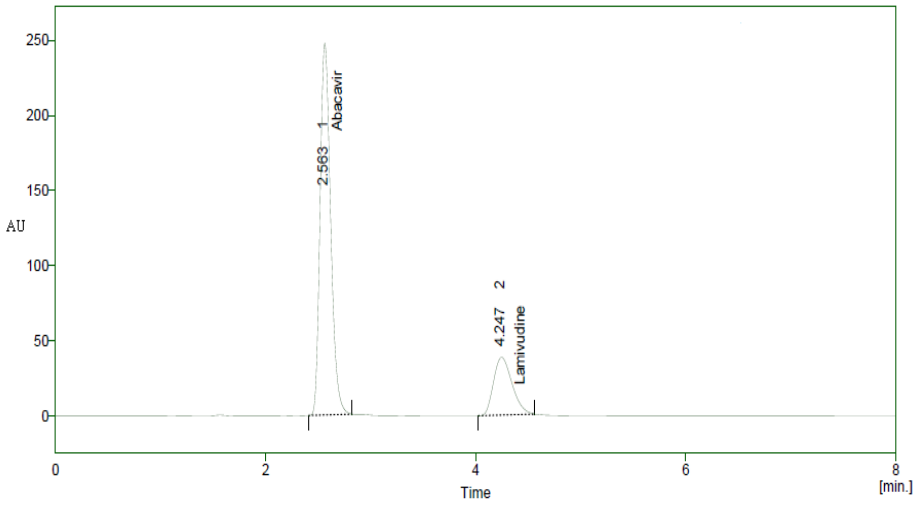

Fig. 5: Chromatogram of Specificity (0.1 M NaoH)

Robustness

(a) Effect of variation of Flow rate

A study was conducted to determine the effect of variation in flow rate by injecting 0.9and 1.1ml/min. Standard solution was prepared and injected into the HPLC system. The retention time values were measured and are given in the (Table 9).

(b) Effect of variation of wavelength

A study was conducted to determine the effect of variation in wavelength. Standard solution was prepared and injected into the HPLC system at 252nm and 256nm. The effect of variation in wavelength was evaluated. The results were discussed in (Table 9)

Table 9: Robustness of Abacavir and Lamivudine

Proposed variations(Abacavir)		Asymmetry factor	Acceptance criteria
Variation in Flow Rate	0.9ml	1.387	In between 0.5 and 2.0
	1.1ml	1.407	
Variation in Wavelength	252nm	1.429	
	256nm	1.464	

Proposed variations(Lamivudine)		Asymmetry factor	Acceptance criteria
Variation in Flow Rate	0.9ml	1.500	In between 0.5 and 2.0
	1.1ml	1.442	
Variation in Wavelength	252nm	1.535	
	256nm	1.500	

Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It is checked that the results are reproducible under differences in conditions, analysts and instruments. Hence the proposed method was found to be rugged.

The Chromatograms were shown in (Fig 6 and 7). The results were discussed in (Table 10).

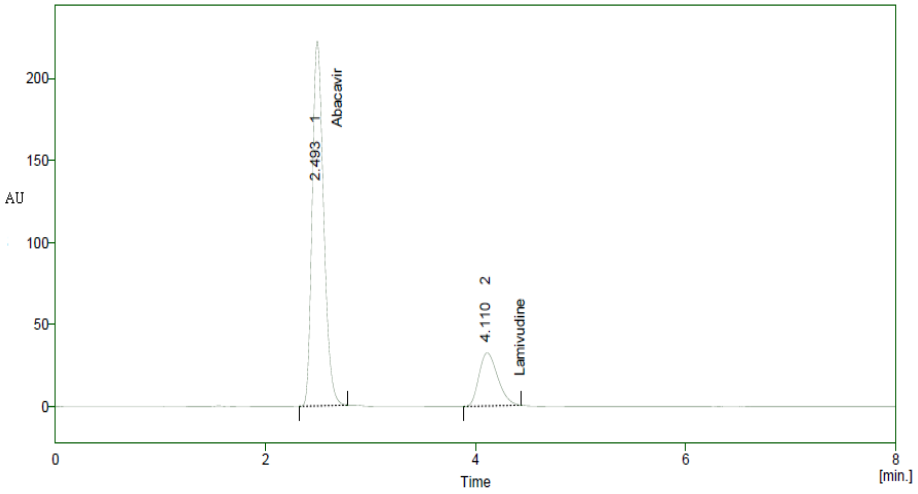

Fig. 6: Chromatogram of Ruggedness Analyst-1

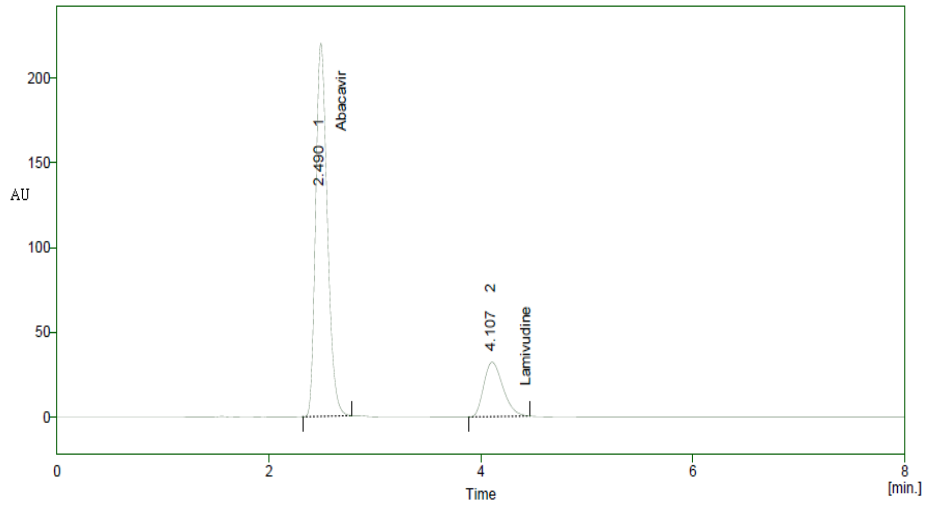

Fig. 7: Chromatogram of Ruggedness Analyst-2

Table 10: Ruggedness of Abacavir and Lamivudine

	R_t of Abacavir	R_t of Lamivudine	Areas of R_t of Abacavir	Areas of Lamivudine
Analyst1(100mcg)	2.493	4.110	1687.676	401.082
Analyst2(100mcg)	2.490	4.107	1681.709	404.594
Mean	2.491	4.108	1684.69	402.838
SD	0.0021	0.0021	4.219	2.65
%RSD	0.150	0.103	0.25	0.65

CONCLUSION

The proposed HPLC method provides as a fast, accurate, rugged assay with stability indicating potential for these two drugs in tablet or in solution. In conclusion, the developed method is strongly recommended for the assay of two drugs in the locally available pharmaceutical dosage form i.e. tablet.

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