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Development and Validation of Stability Indicating Method for the Simultaneous Estimation of Batcaver Sulfate, Lamivudine and Dolutegravir Sodium in Pharmaceutical Dosage forms by RP-HPLC

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Original Research Article

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Abstract: A simple, rapid, specific, stability indicating method was developed and validated for the simultaneous estimation of Abacavir sulfate, Lamivudine and Dolutegravir sodium in pharmaceutical dosage form using RP-HPLC. The chromatographic separation was done using BDS column of dimensions 250mm x 4.6mm, 5µ particle size with mobile phase consisting of potassium dihydrogen phosphate buffer and acetonitrile in the ratio 45:55%v/v run on an isocratic mode of flow rate 1.0ml/min. The column oven temperature was maintained at 30ºC. The detection was done at a wavelength of 240nm. The developed method was validated in accordance with ICH guidelines, evaluating accuracy, precision, ruggedness, robustness, LOD, LOQ, stability parameters and found to be within the limits. The method obeys Beer's law in the concentration range of 150µg/ml – 900µg/ml for Abacavir, 75µg/ml – 450µg/ml for Lamivudine and 12.5µg/ml – 75µg/ml for Dolutegravir with correlation coefficients of 0.9999, 0.9996 and 0.9999 for the three drugs respectively. Forced degradation studies were conducted by exposing the standard drug solution to the various stressed conditions such as acidic, basic, oxidative, thermal, neutral and photolytic conditions. The net degradation for the drugs was found to be within the limits.

Keywords: Abacavir sulfate, Lamivudine, Dolutegravir sodium, RP-HPLC, Stability indicating method, Method development, Validation.

INTRODUCTION

Abacavir sulfate [1, 2] (Figure 1A) is chemically designated as bis ([(1S, 4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl] cyclopent-2-en-1-yl] methanol); sulfuric acid. It is white to off-white solid, freely soluble in water and methanol. It has pKa values of 5.77 and 15.41. It acts as antiretroviral drug by inhibiting nucleoside reverse transcriptase, and hence used for the treatment of HIV/ AIDS. Lamivudine [3, 4] (Figure 1B) is chemically designated as 4-Amino-1-[(2*R*, 5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one. It is white or almost white powder, soluble in water, sparingly soluble in methanol and practically insoluble in acetone. It has a pKa value of 14.29. It acts as antiretroviral drug by inhibiting nucleoside reverse transcriptase, and hence used for the treatment of HIV / AIDS and chronic Hepatitis B at low dose. Dolutegravir sodium [5, 6] (Figure 1C) is chemically designated as (4R,12aS)-N-[(2,4-Difluorophenyl)methyl]-3,4,6,8,12,12a-hexahydro-7-hydroxy-4-methyl-6,8-ioxo-2H pyrido[1′,2′:4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide sodium salt. It is white to light yellow powder, slightly soluble in water. It has a pKa value 10.1. It acts antiretroviral drug by inhibiting HIV integrase, and hence used for the treatment of HIV / AIDS. Literature survey [7-13] reveals that there are only few methods developed for the simultaneous estimation of Abacavir, Lamivudine and Dolutegravir in pharmaceutical dosage forms. The present study aimed to develop and validate the stability indicating method for the simultaneous determination of Abacavir, Lamivudine and Dolutegravir in pharmaceutical dosage form by RP-HPLC.

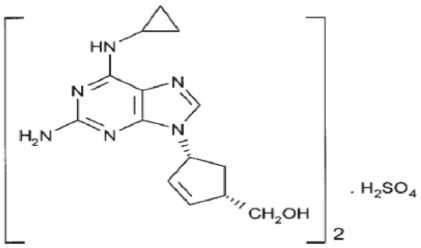

Fig-1A: Chemical structure of Abacavir sulfate

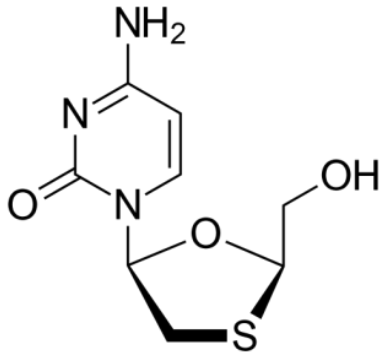

Fig-1B: Chemical structure of Lamivudine

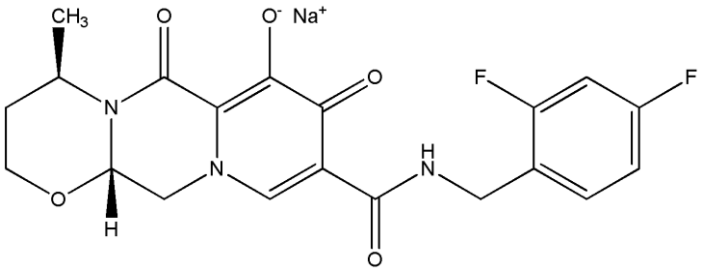

Fig-1C: Chemical structure of Dolutegravir sodium

MATERIAL AND METHODS

Chemicals and Reagents

Abacavir sulfate, Lamivudine and Dolutegravir sodium working standards were supplied by Spectrum labs, Hyderabad, India as gift samples. The tablets were purchased from local pharmacy. All the chemicals used for the development of method were of AR grade. All the solvents used for method development were of HPLC grade.

Analytical instruments and Chromatographic conditions

The separation of drugs was done using HPLC Waters 2998 model equipped with an autosampler, Photo diode array detector with empower 2 software. Column used for separation was BDS (250mm x 4.6 mm, 5µ) with mobile phase consisting of Potassium dihydrogen phosphate and acetonitrile in the ratio 45:55% v/v on isocratic mode at 1.0ml/min flow rate. The detection was done at 240nm and column oven temperature was maintained at 30ºC. The other instruments used were pH meter (EI), Digital Balance (Infra Instruments), Ultrasonic Bath (Wadegati), Hot air oven (Cisco).

Preparation of mobile phase

Transfer 1.36g of potassium dihydrogen phosphate in to a 1000mL volumetric flask; add about 100ml of milli-Q water and mix. Finally make volume up to the mark with milli-Q water. Adjust the pH to 5.8.

Mixture of above phosphate buffer and Acetonitrile in the ratio 45:55 (%v/v) respectively was used as mobile phase.

Preparation of standard and sample solutions

Dissolve 120mg of Abacavir working standard, 60mg of Lamivudine and 10mg of Dolutegravir working standard in 100ml of diluent. Dilute 1ml of the above stock solution to 10ml with diluent.

20 tablets (Triumeq) were weighed accurately and the average weight was calculated. An amount equivalent to 120mg of Abacavir was weighed and dissolved in 100ml of diluent using sonicator for 30min with intermediate shaking. The above solution was filtered using HPLC filters. 1mL of the above solution

was pipette into 10mL volumetric flask and made up with diluent.

Method Validation [14,15]

The developed method was validated as per ICH guidelines. The following parameters were validated; accuracy, precision, linearity, specificity, ruggedness, robustness and stability. Forced degradation studies [16] were also conducted by exposing the drugs solution to various conditions such as acidic, basic, peroxide, thermal, neutral and photolytic conditions.

RESULTS AND DISCUSSION

For the development of method for the simultaneous determination of Abacavir, Lamivudine and Dolutegravir, initially various mobile phase compositions and columns were tried for eluting the drugs with good peaks and parameters. Potassium dihydrogen phosphate and acetonitrile in the ratio 45:55%v/v at flow rate 1.0ml/min was selected as mobile phase. BDS (250mm x 4.6mm, 5µ) column was selected as stationary phase for separation of drugs. The column oven temperature was maintained at 30ºC. The detection wavelength was selected by scanning the drugs solution in the UV range of 400nm – 200nm and was found to be 240nm as shown in figure 2.

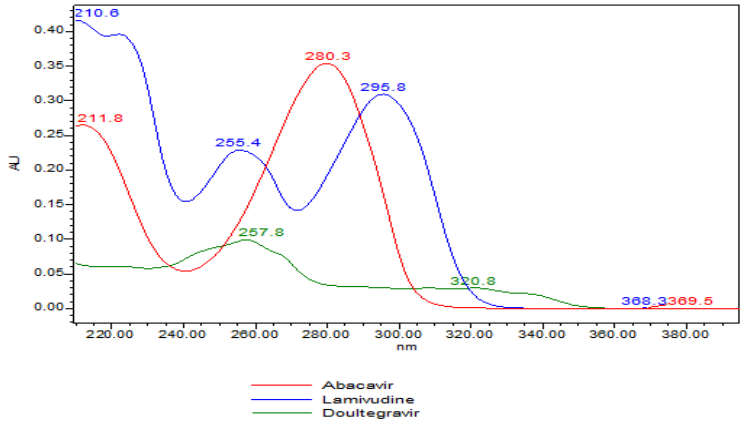

Fig-2: Overlain UV Spectrum

The standard, sample and blank solutions were prepared and injected into the chromatographic system. The system suitability parameters were noted and the chromatograms were shown in figure 3A, 3B and 3C respectively.

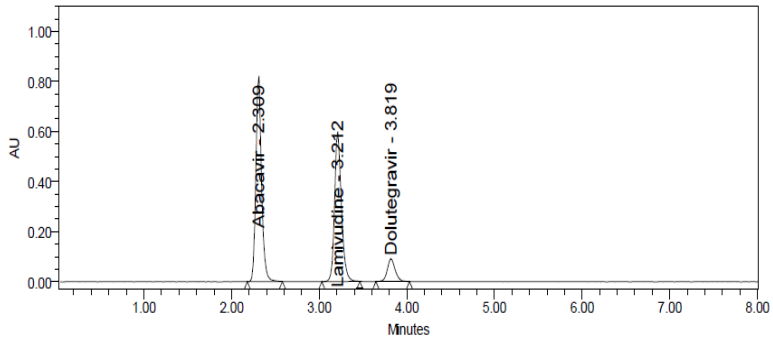

Fig-3A: Standard Chromatogram

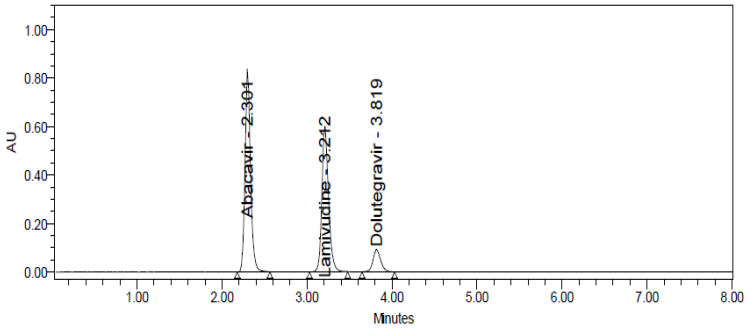

Fig-3B: Sample Chromatogram

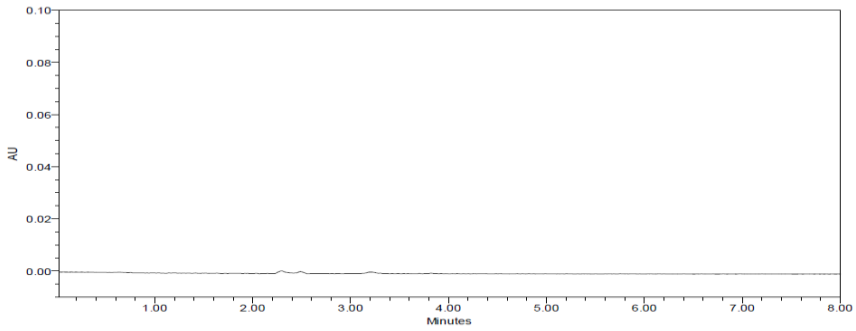

Fig-3C: Blank Chromatogram

The specificity of the method was determined by comparing with placebo and observed for any interference. No interference was observed at retention times of Abacavir, Lamivudine and Dolutegravir peaks when compared with placebo solution. The placebo chromatogram was shown in figure 4.

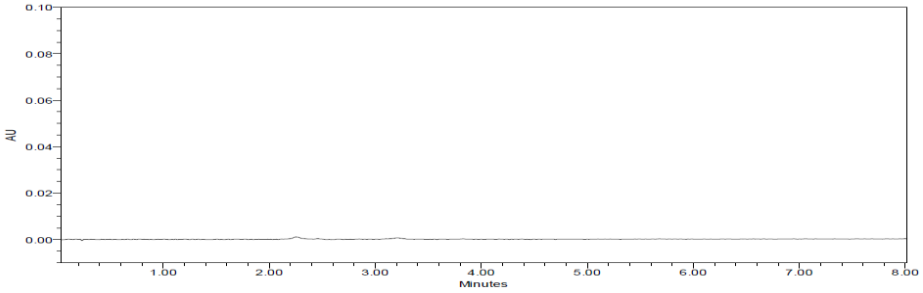

Fig-4: Placebo Chromatogram

The method obeys Beer's law in the concentration range of 150µg/ml – 900µg/ml for Abacavir, 75µg/ml – 450µg/ml for Lamivudine and 12.5µg/ml – 75µg/ml for Dolutegravir with correlation coefficient of 0.9999, 0.9996 and 0.9999 for Abacavir, Lamivudine and Dolutegravir respectively, indicates that the method is linear. The linearity plots were shown in figure 5 and results were summarized in table 1.

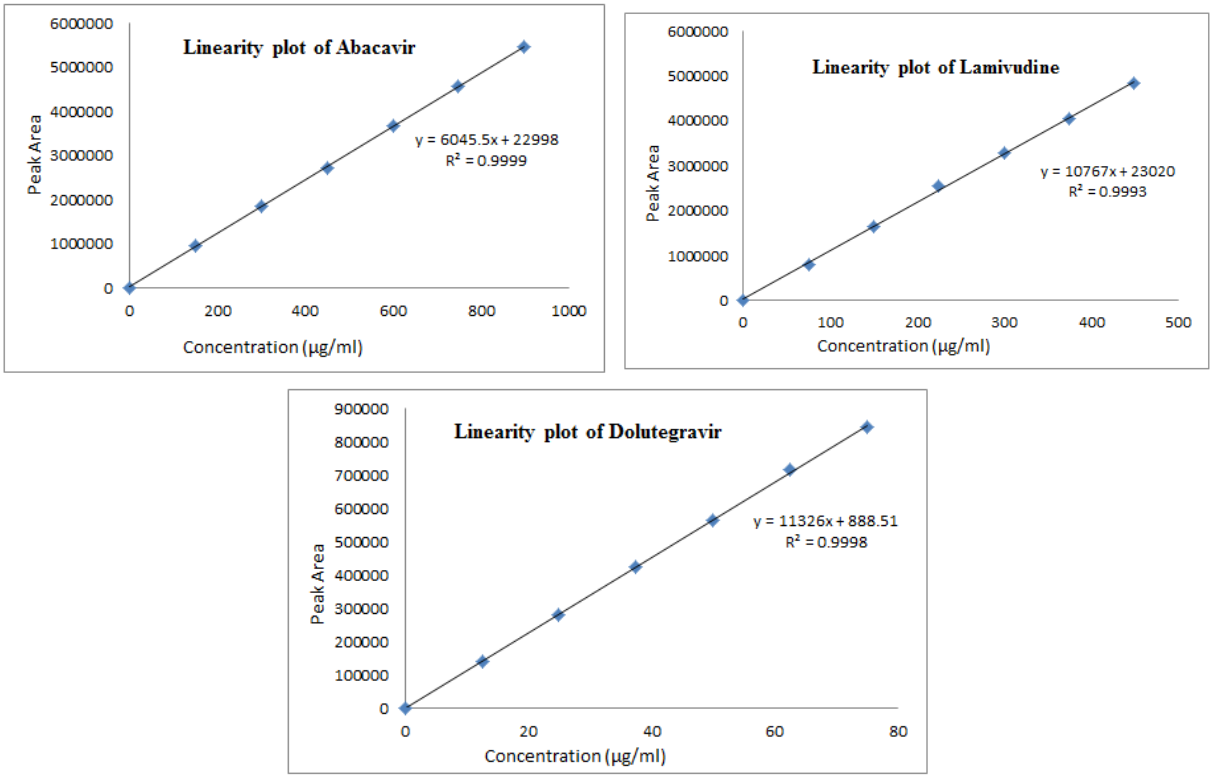

Fig-5: Linearity plot of A) Abacavir B) Lamivudine and C) Dolutegravir

Table-1: Linearity results

Parameter	Abacavir	Lamivudine	Dolutegravir
Linearity (µg/ml)	150 – 900	75 – 450	12.5 – 75.0
Regression equation, y=mx+c	y = 6045.5x + 22998	y = 10767x + 23020	y = 11326x + 888.51
Slope, m	6045.5	10767	11326
Y-intercept, c	22998	23020	888.51
Regression coefficient, r^2	0.9999	0.9993	0.9998
Correlation coefficient, r	0.9999	0.9996	0.9999

The % recovery for Abacavir, Lamivudine and Dolutegravir were found to be 99.80% - 100.21%, 99.36% - 99.79% and 99.80% - 100.10% respectively. The % RSD for Abacavir, Lamivudine and Dolutegravir were found to be 0.2, 0.5 and 0.2 respectively as the results were within the limits indicating the method to be accurate and precise. The Limit of Detection (LOD) for Abacavir, Lamivudine and Dolutegravir were found to be 1.69µg/ml, 1.23µg/ml and 0.04µg/ml respectively. The Limit of Quantitation (LOQ) for Abacavir, Lamivudine and Dolutegravir were found to be 5.11µg/ml, 3.74µg/ml and 0.11µg/ml respectively. The method was found to be rugged, robust and stable in solution for 24hours. The results are summarized in table 2.

Table-2: System Suitability and Validation Parameter Results

Parameter	Abacavir	Lamivudine	Dolutegravir
Specificity	Specific	Specific	Specific
Precision (% RSD)	0.2	0.5	0.2
Accuracy (% Recovery)	99.80%-100.21%	99.36%-99.79%	99.80%-100.10%
Linearity range (µg/ml)	150 – 900	75 – 400	12.5 – 75.0
Correlation coefficient, r	0.9999	0.9996	0.9999
Limit of Detection (µg/ml)	1.69	1.23	0.04
Limit of Quantitation (µg/ml)	5.11	3.74	0.11
Ruggedness (%RSD)	0.2	0.7	0.4
Robustness	Robust	Robust	Robust
Solution stability	Stable	Stable	Stable
USP Plate Count	6066	8154	9106
USP Tailing Factor	1.17	1.20	1.12
USP Resolution		6.8	3.9

Forced degradation studies were conducted and the net degradation was found to be within the limits indicating that the drugs are stable at various stress conditions. The results were summarized in table 3 and chromatograms were shown in figure 6.

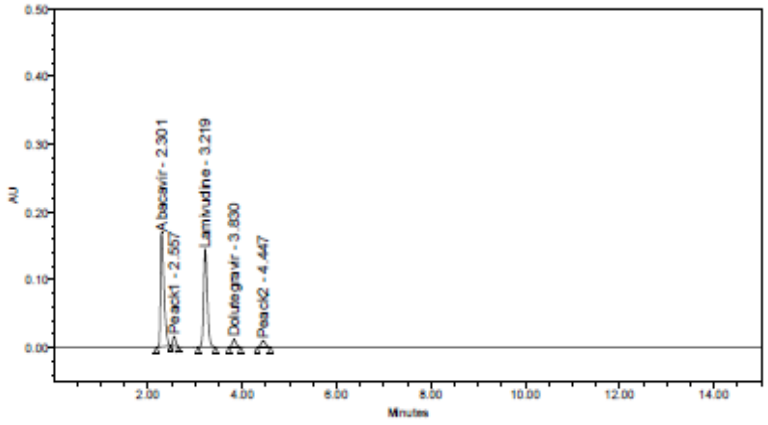

Fig-6A: Acid degradation chromatogram

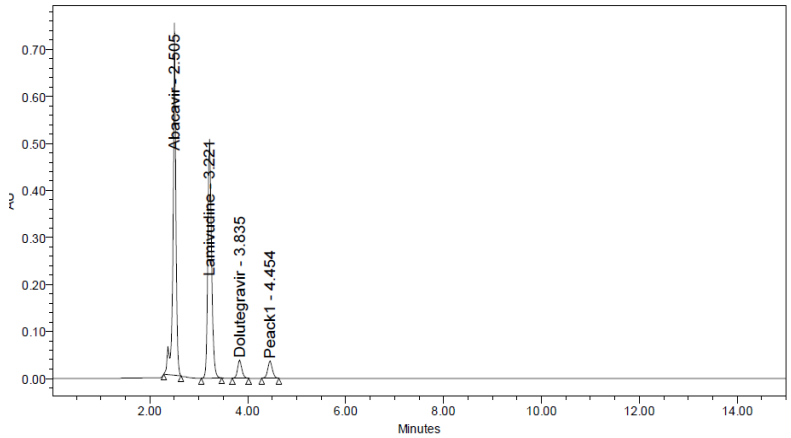

Fig-6B: Base degradation chromatogram

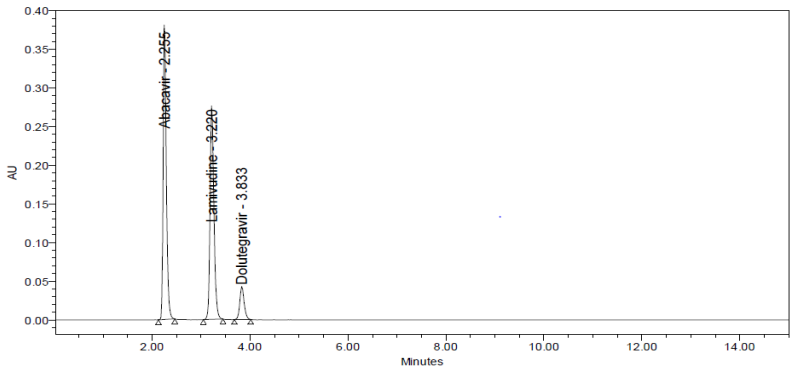

Fig-6C: Peroxide degradation chromatogram

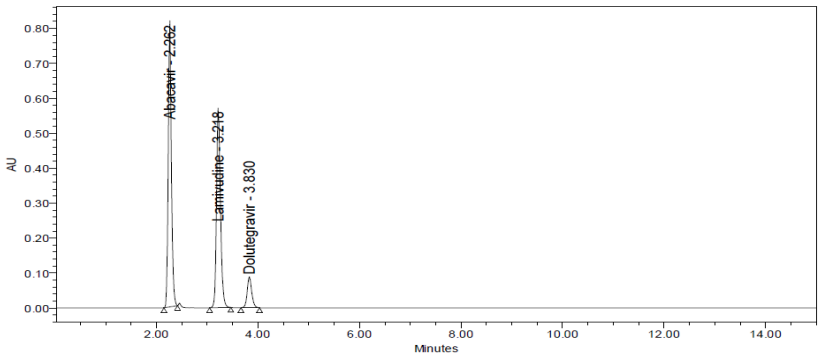

Fig-6D: Water stress study chromatogram

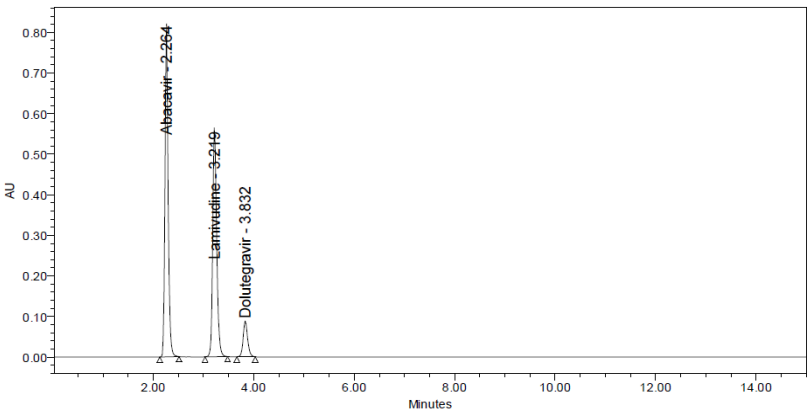

Fig-6E: Photo stability degradation chromatogram

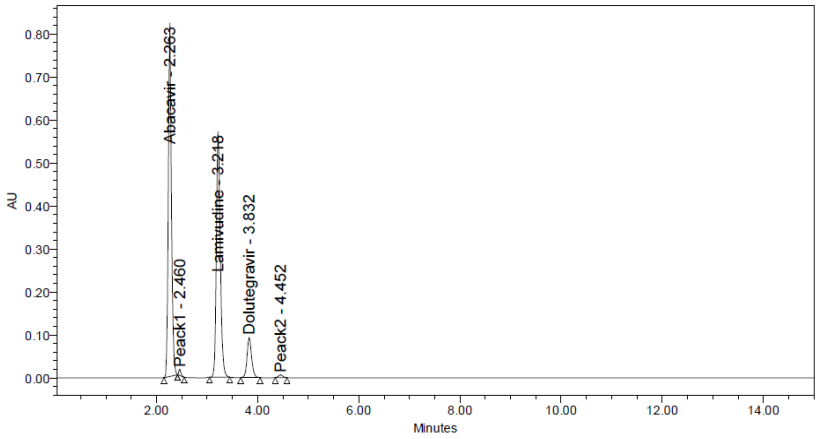

Fig-6F: Dry heat study chromatogram

Table-3: Forced degradation studies results

Drug	Parameters	Stress Condition					
		Acidic	Basic	Oxidative	Photolytic	Neutral	Dry heat
Abacavir	% Assay	95.63	97.20	98.24	99.59	99.49	99.60
	Purity Angle	0.280	0.430	0.168	0.272	0.328	0.384
	Purity Threshold	0.306	0.725	0.289	1.023	0.958	1.000
	% Degradation	4.37	2.80	1.76	0.41	0.51	0.40
Lamivudine	% Assay	95.41	97.55	98.18	98.44	99.06	98.26
	Purity Angle	0.209	0.095	0.134	0.094	0.099	0.142
	Purity Threshold	0.319	0.281	0.288	0.282	0.280	0.280
	% Degradation	4.59	2.45	1.82	1.56	0.94	1.74
Dolutegravir	% Assay	95.56	97.30	98.85	99.52	99.18	99.51
	Purity Angle	1.181	0.436	0.384	0.195	0.189	0.197
	Purity Threshold	1.589	0.668	0.625	0.410	0.404	0.404
	% Degradation	4.44	2.70	1.15	0.48	0.82	0.49
% Area of degradation Peak		6.88	3.94	-	-	-	1.10

CONCLUSION

A specific, accurate stability indicating method was developed for the simultaneous determination of Abacavir, Lamivudine and Dolutegravir in pharmaceutical dosage form using RP-HPLC. The developed method is validated as per ICH guidelines and found to be accurate, specific, precise, linear, rugged, and robust and stable in solution. Forced degradation studies confirmed that the drugs are stable at high concentrations of various stress conditions.

The proposed method is used for the simultaneous estimation of Abacavir, Lamivudine and Dolutegravir in routine and quality control analysis of pharmaceutical formulations.

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