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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHOD FOR THE SIMULTANEOUS ESTIMATION OF LAMIVUDINE, STAVUDINE AND EFAVIRENZ IN PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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ABSTRACT
A stability indicating method was developed for the simultaneous estimation of Lamivudine, Stavudine and Efavirenz in pharmaceutical dosage form by reverse phase high performance liquid chromatography (RP-HPLC) and validated. The chromatographic separation was performed using the Kromasil C_{18} (250mm × 4.6mm, 5µ) column run in an isocratic mode with a flow rate of 1mL/min at ambient temperature. The mobile phase consists of 0.1% O-Phosphoric acid, Acetonitrile and Methanol in the ratio 70:20:10 (v/v/v/) and detected at the wave length 226nm. The retention times for Lamivudine, Stavudine and Efavirenz were found to be 2.39min, 3.06min and 3.53min respectively. The drugs obeyed Beer's law in the concentration range of 18.75µg/mL – 112.50µg/mL, 5µg/mL – 30µg/mL and 75µg/mL – 450µg/mL respectively. The method was validated as per ICH guidelines for accuracy, precision, specificity, ruggedness, robustness and stability. The standard solution was subjected to stress conditions such as acidic, basic, oxidative, neutral, photolytic and thermal conditions. The degradation was found to be within the limits.

KEYWORDS: Lamivudine, Stavudine, Efavirenz, Stability indicating, Method development, Validation, RP-HPLC.

INTRODUCTION
Lamivudine, (LAM)[1] (Fig.1A), 4-Amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-diydropyrimidin-2-one, is a white powder, soluble in water, sparingly soluble in methanol with pKa 14.29. It is used as antiretroviral drug in the treatment of HIV and AIDS. Stavudine (STA)[2] (Fig.1B), 1-[(2*R*,5*S*)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl]-5-methyl-1,2,3,4-tetrahydropyrimidine-2,4-dione, is a white crystalline powder, soluble in water, sparingly soluble in ethanol with pKa 9.95. It is used as antiretroviral drug in the treatment of HIV and AIDS. Efavirenz (EFA)[3] (Fig.1C), (4S)-6-Chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2-one, is a white to slightly pink powder, soluble in methanol with pKa 12.52. It acts as antiretroviral agent and used in the treatment of HIV infection and AIDS. According to the literature survey[4,8], very few methods were developed for the simultaneous estimation of Lamivudine, Stavudine and Efavirenz in pharmaceutical dosage forms. The present study aimed to develop and validate the stability indicating method for the simultaneous estimation of Lamivudine, Stavudine and Efavirenz in pharmaceutical dosage form using RP-HPLC.

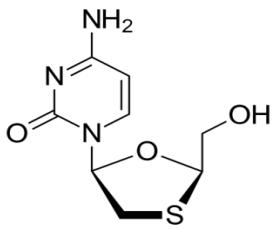

Fig.1A: Chemical Structure of Lamivudine.

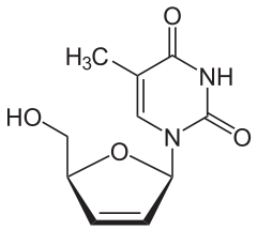

Fig. 1B: Chemical Structure of Stavudine.

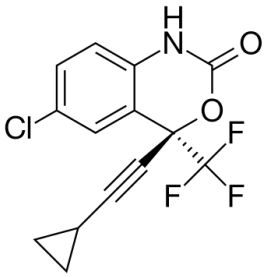

Fig.1C: Chemical Structure of Efavirenz.

MATERIALS AND METHOD

Lamivudine, Stavudine and Efavirenz working standards were supplied by Hetero Drugs Pvt. Ltd., Hyderabad, India as gift samples. The tablets were purchased from local pharmacy. All the chemicals used in the method were of AR grade. All the solvents used were of HPLC grade.

The HPLC analysis was performed using Waters 2998 model equipped with an autosampler, Photo Diode Array detector and done on empower software. Column used was Kromasil C18 (250mm × 4.6mm, 5µ).

Preparation of Buffer (0.1% OPA): Dilute 1ml of Ortho Phosphoric acid (OPA) with 1000mL of water in 1000mL volumetric flask.

Preparation of Mobile Phase: Mixture of Buffer, Acetonitrile, Methanol in the ratio 70:20:10 (%v/v/v) respectively was used as mobile phase.

Preparation of Diluent: Mixture of water and acetonitrile in the ratio 50:50 (%v/v) respectively.

Preparation of Standard solution

Prepare standard solution using Lamivudine, Stavudine and Efavirenz working standards of concentrations 75µg/mL, 20µg/mL and 300µg/mL respectively with diluent.

The standard solution was injected into the HPLC system and chromatogram was recorded (Fig.2A).

Preparation of Sample solution

The average weight of 20 tablets (Emduo E 40) was calculated. Then the tablets were crushed and fine powder was collected. An amount equivalent to 7.5mg of Lamivudine, 2mg of Stavudine and 30mg of Efavirenz was weighed and sample solution was prepared of concentrations 75µg/mL, 20µg/mL and 300µg/mL respectively with diluent.

The sample solution was injected into the HPLC and chromatogram was recorded (Fig.2B). A blank solution was also injected and chromatogram was recorded (Fig.2C).

Method validation[9-11]

The standard solution was injected into the HPLC system six times and system suitability parameters were noted in the table 1.

The specificity study was conducted using placebo solution. The placebo interference with the peaks of drugs is to be noted (Fig.3).

Precision (%RSD) was determined by injecting the six samples of solution. To determine the accuracy of the test method, samples were prepared by spiking drug materials with the equivalent amount of placebo at 50%, 100% and 150% of the target concentration. The average % recoveries were determined.

Linearity was determined by preparing the series of standard solutions and injecting into the HPLC system. A graph is plotted to concentration versus peak area, results and graphs were summarized in table 2 and figures 4A, 4B and 4C. LOD and LOQ were determined using the formula mentioned in ICH guidelines, based on calibration curves.

Ruggedness (%RSD) was determined by analyzing the samples on different days. Robustness was determined by varying the optimum conditions such as ±5% of organic phase, ±0.2mL/min flow rate and ±5ºC column oven temperature with respect to test method.

The stability of drugs in solution was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of drug solution for 24h under laboratory conditions.

Forced degradation studies[12] were conducted by exposing the standard solution to the stress conditions like acidic (hydrochloric acid), basic (sodium hydroxide), oxidative (hydrogen peroxide), neutral (water), photolytic (UV light) and thermal (heat) conditions. The chromatograms were recorded (Fig.5) and results were summarized in table 3.

RESULTS

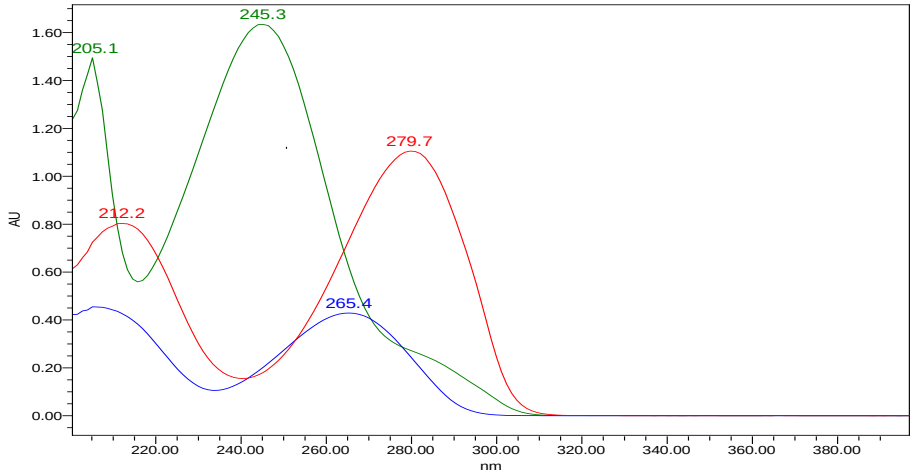

Fig. 6: Overlay UV Spectrum of LAM, STA and EFA.

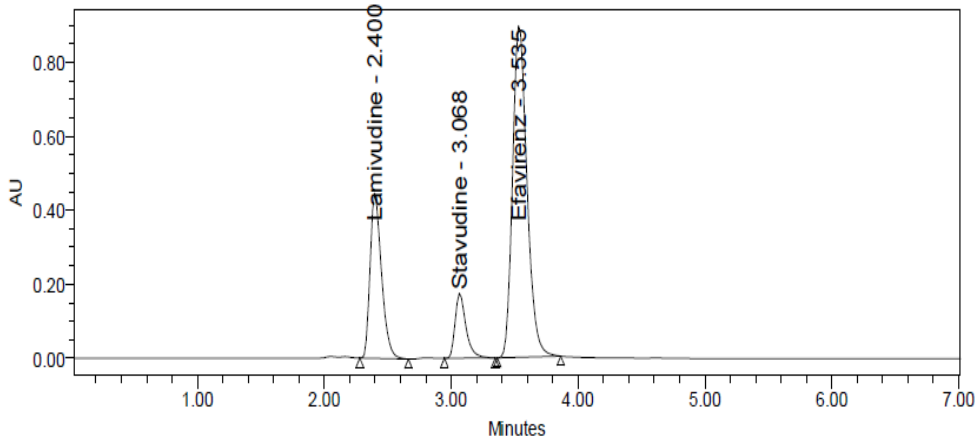

Fig. 2A: Standard chromatogram.

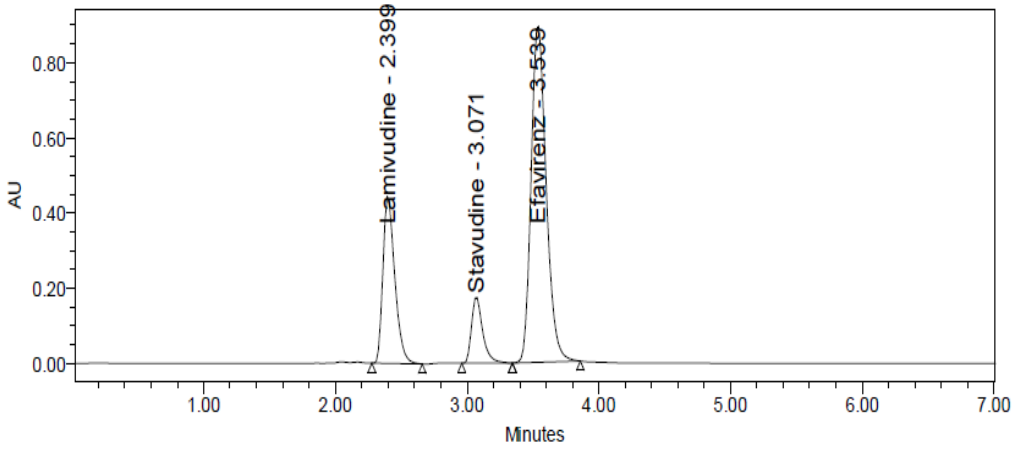

Fig. 2B: Sample chromatogram.

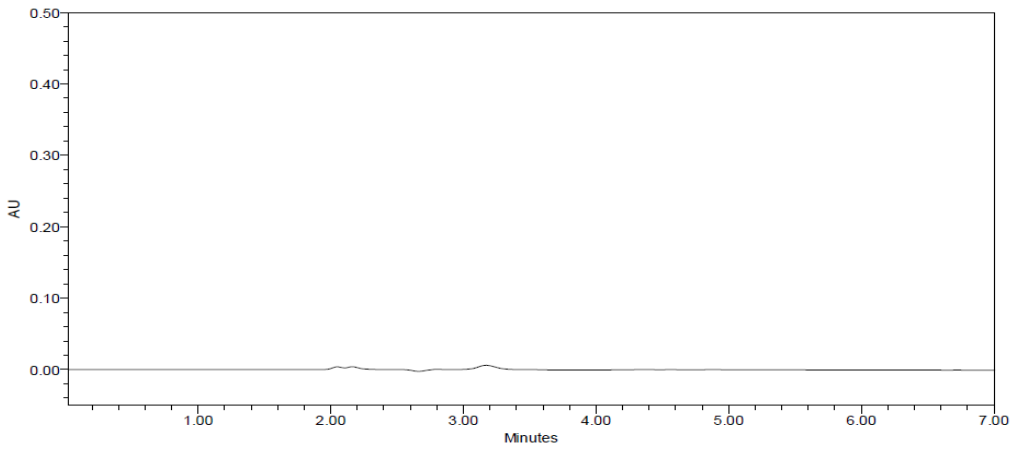

Fig. 2C: Blank chromatogram.

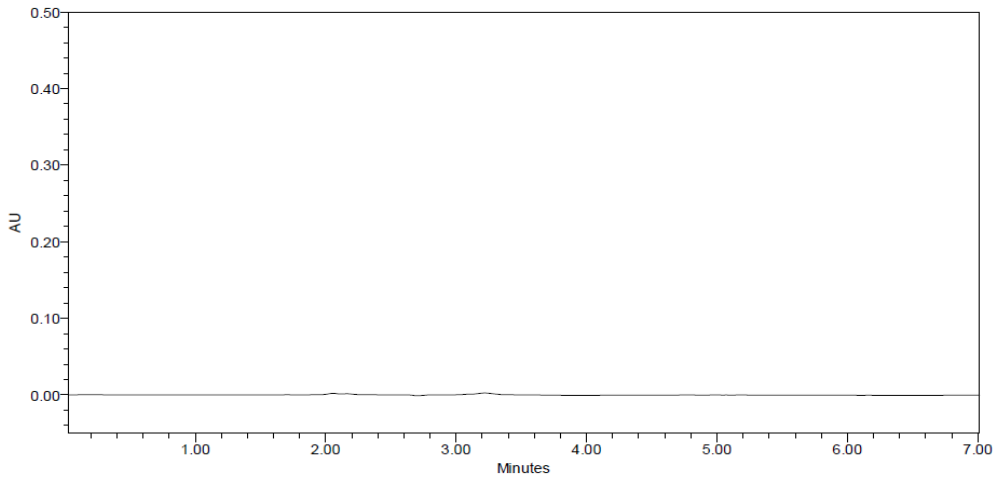

Fig. 3: Placebo chromatogram.

Table 1: System suitability and validation parameter results.

Parameter	Lamivudine	Stavudine	Efavirenz
Specificity	Specific	Specific	Specific
Precision (%RSD)	0.7	0.7	1.1
Accuracy (% Recovery)	99.90%-100.55%	99.90%-100.30%	99.76%-99.90%
Linearity range (µg/ml)	18.75 -112.50	5 -30	75 - 450
Correlation coefficient, r	0.9995	0.9995	0.9996
Limit of Detection (µg/ml)	0.09	0.11	0.28
Limit of Quantitation (µg/ml)	0.29	0.33	0.86
Ruggedness (%RSD)	0.3	0.4	0.1
Robustness	Robust	Robust	Robust
Stability	Stable	Stable	Stable
USP Plate Count	3237	6739	4637
USP Tailing factor	1.35	1.41	1.22
USP Resolution		4.1	2.6

Table 2: Linearity results.

Parameter (Unit)	Lamivudine	Stavudine	Efavirenz
Linearity range (µg/mL)	18.75-112.50	5-30	75-450
Regression equation, y=mx	y=38265x+70813	y=52791x+30072	y=23772x+172096
Slope, m	38265	52791	23772
Regression coefficient, r^2	0.9991	0.9991	0.9992
Correlation coefficient, r	0.9995	0.9995	0.9996

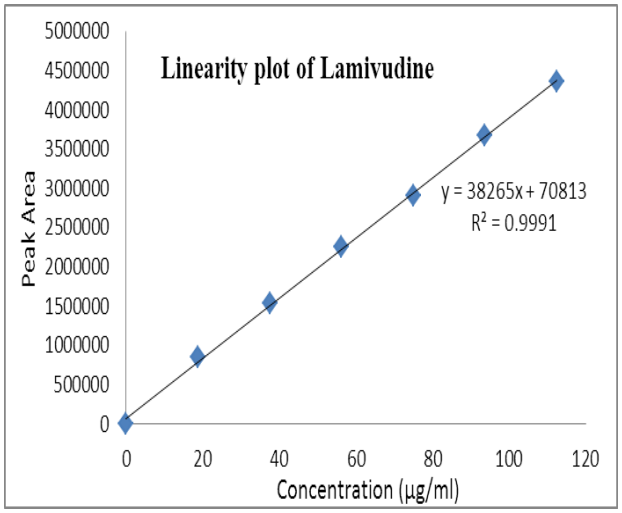

Fig. 4A: Linearity plot of Lamivudine.

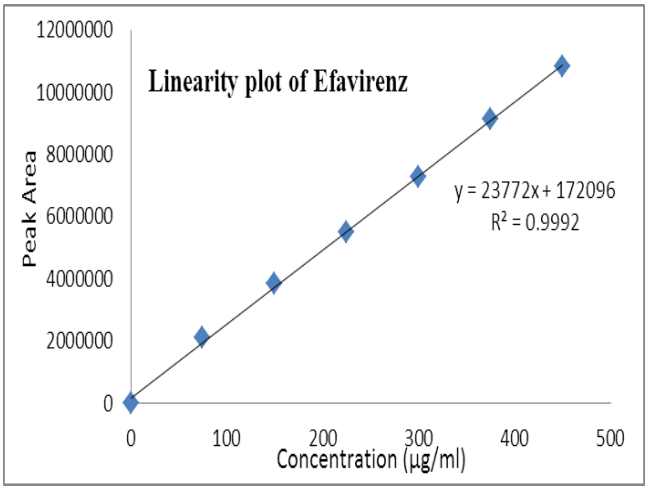

Fig. 4C: Linearity plot of Efavirenz.

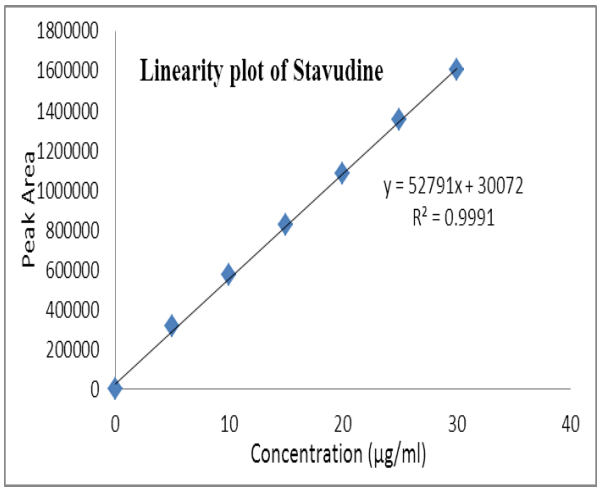

Fig. 4B: Linearity plot of Stavudine.

Table 3: Forced degradation studies results.

Drug	Parameters	Stress Condition					
		Acidic	Basic	Oxidative	Photolytic	Neutral	Dry heat
Lamivudine	% Assay	96.81	97.11	97.66	99.31	99.69	99.08
	Purity Angle	0.217	0.268	0.121	0.213	0.186	0.184
	Purity Threshold	0.308	0.300	0.288	0.278	0.276	0.273
Stavudine	% Assay	95.97	96.15	96.30	99.21	99.25	98.89
	Purity Angle	0.100	0.163	0.108	0.134	0.216	0.177
	Purity Threshold	0.249	0.246	0.249	0.255	0.248	0.243
Efavirenz	% Assay	95.92	97.90	98.79	99.33	99.78	99.04
	Purity Angle	0.189	0.171	0.201	0.210	0.236	0.135
	Purity Threshold	0.285	0.290	0.308	0.324	0.321	0.314
% Area of degradation Peak		0.300	0.190	-	-	-	-

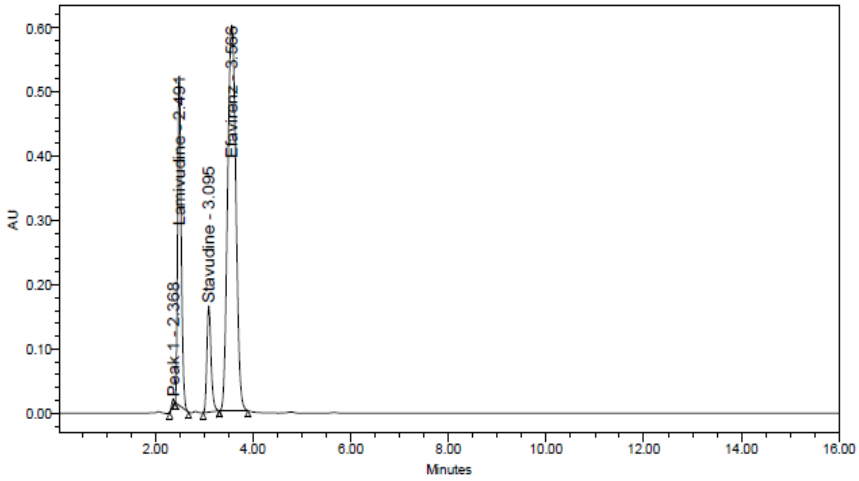

Fig. 5A: Acid Degradation study chromatogram.

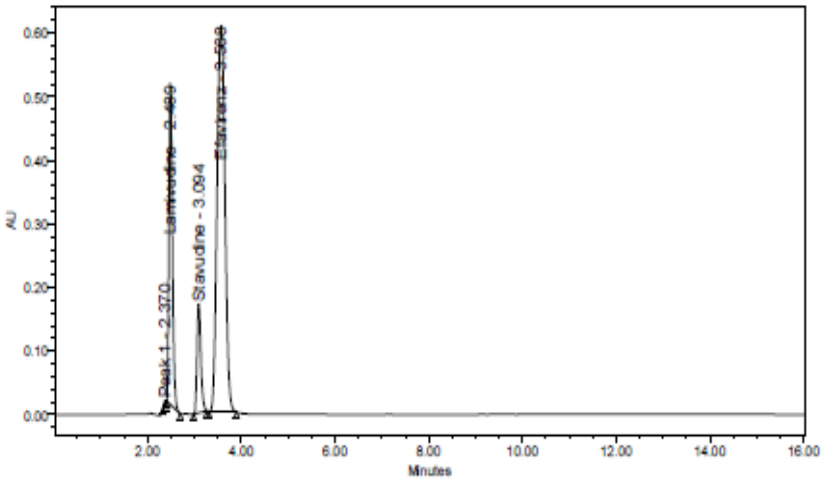

Fig. 5B: Base Degradation study chromatogram.

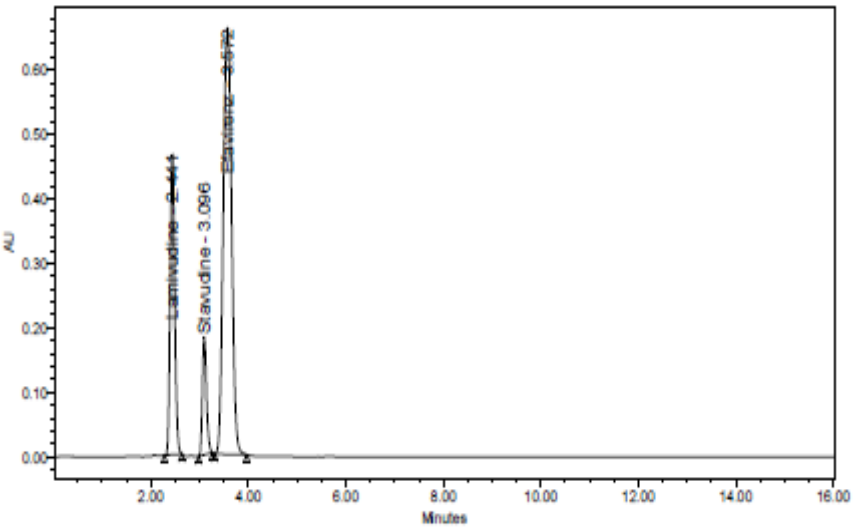

Fig. 5C: Oxidative Degradation study chromatogram.

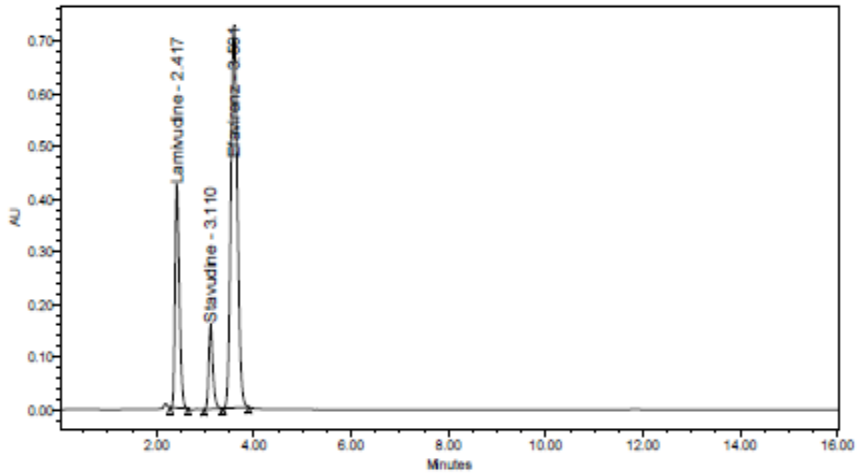

Fig. 5D: Neutral Degradation study chromatogram.

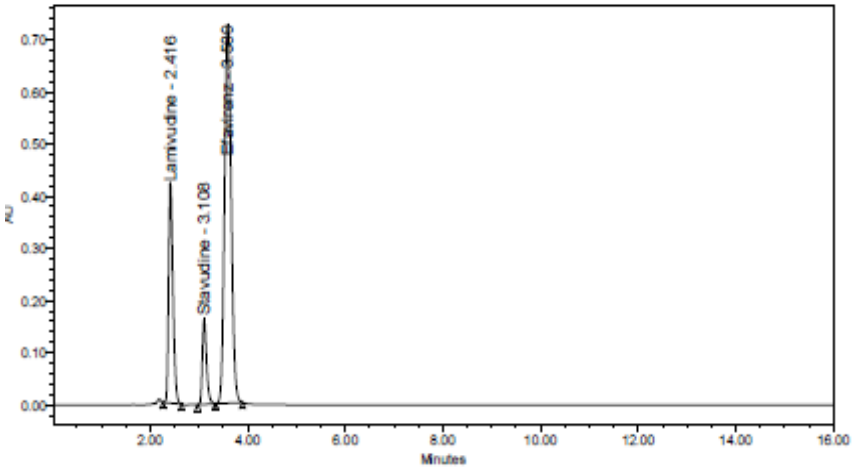

Fig. 5E: Photolytic Degradation study chromatogram.

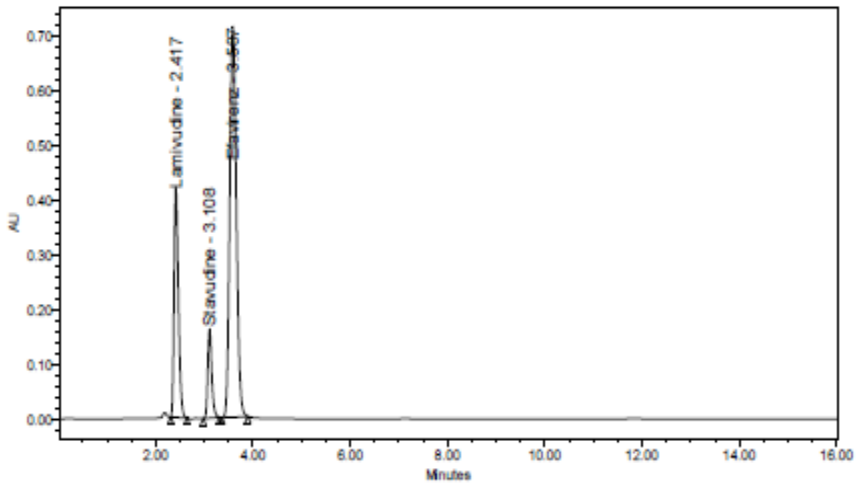

Fig. 5F: Thermal Degradation study chromatogram.

DISCUSSION

At the starting, various mobile phase ratios were tried to separate the drugs. Based on their peak parameters, run time and resolution, optimized conditions were determined.

The standard solution of 10µg/mL was prepared and scanned in the range of 200-400nm. 226nm was selected as detection wavelength based on the overlay UV spectrum (Figure 6). The chromatographic separation was performed using Kromasil C18, 250mm×4.6mm, 5µcolumn. The mobile phase consists of 0.1% OPA,

acetonitrile and methanol in the ratio 70:20:10(%v/v/v) run in an isocratic mode and flow rate 1mL/min. The retention times of Lamivudine, Stavudine and Efavirenz were found to be 2.39min, 3.06min and 3.53min respectively.

A linear response was observed in the concentration range of 18.75µg/mL – 112.50µg/mL for Lamivudine, 5µg/mL – 30µg/mL for Stavudine and 75µg/mL – 450µg/mL for Efavirenz, with correlation coefficient of 0.999.

The %RSD for Lamivudine, Stavudine and Efavirenz were found to be 0.7, 0.7 and 1.1 respectively. The % recoveries were found to be 99.90% - 100.55% for Lamivudine, 99.90% - 100.30% for Stavudine and 99.76% - 99.90% for Efavirenz.

The results of ruggedness, robustness and stability confirmed that the developed method is rugged, robust and stable up to 24h.

The forced degradation studies confirmed that the drugs were stable under stress conditions such as acidic, basic, oxidative, neutral, photolytic and thermal conditions. The net degradation was found to be within the limits. The peak purity angle is less than the peak purity threshold.

CONCLUSION

A stability indicating RP-HPLC method was developed for the simultaneous estimation of Lamivudine, Stavudine and Efavirenz in bulk drug and pharmaceutical dosage form. The method was validated according to ICH guidelines. The method was found to accurate, precise, specific, stable, rugged and robust. From the degradation studies, it is concluded that the drugs were stable in stress conditions.

The proposed method is used for the simultaneous estimation of Lamivudine, Stavudine and Efavirenz in routine and quality control analysis of tablet formulations.

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