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Development and Validation of Stability Indicating Method for the Simultaneous Quantification of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine Hydrochloride in Pharmaceutical Dosage Forms by RP-HPLC

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Original Research Article

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Abstract: A stability indicating method was developed and validated for the simultaneous quantification of Emtricitabine, Tenofovir and Rilpivirine in pharmaceutical dosage form by reverse phase high performance liquid chromatography (RP-HPLC). The chromatographic separation was performed using the Kromasil C18 (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 0.01N Potassium dihydrogen phosphate and Acetonitrile in the ratio 65:35 (v/v) and detected at the wave length 279nm. The retention times for Emtricitabine, Tenofovir and Rilpivirine were found to be 4.35min, 2.05min and 3.61min respectively. The drugs obeyed Beer's law in the concentration range of 50µg/mL to 300µg/mL for Emtricitabine, 75µg/mL to 450µg/ml for Tenofovir and 6.25µg/mL to 37.5µg/mL for Rilpivirine 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 net degradation was found to be within the limits.
Keywords: Development and Validation, Emtricitabine, Rilpivirine hydrochloride, RP-HPLC Stability indicating method, Tenofovir Disoproxil Fumarate.

INTRODUCTION

Emtricitabine (EMT) [1, 2] (Figure-1A), 4-amino-5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one, is a white to off-white powder, soluble in water and methanol and practically insoluble in methylene chloride with pKa value of 2.65.

It is used as antiretroviral drug in the treatment of HIV and AIDS. Tenofovir Disoproxil Fumarate (TDF) [3,4] (Figure-1B), [3, 4] [[(2R)-1-(6-aminopurin-9-yl)propan-2-yl] oxymethyl-(propan-2-yloxycarbonyloxymethoxy)phosphoryl] oxymethyl propan-2-ylcarbonate;(E)-but-2-enedioic acid, is a white to off-white crystalline powder, soluble in methanol and dimethyl formamide, sparingly soluble in water with pKa 3.75. It is used as antiretroviral drug in the treatment of HIV and AIDS. Rilpivirine hydrochloride (RPH) [5, 6] (Figure 1C), 4-{[4-({4-[(*E*)-2-cyanovinyl]-2, 6-dimethylphenyl} amino) pyrimidin-2-yl] amino} benzonitrile monohy drochloride, is a white powder, soluble in Soluble in N,N-dimethylformamide and N,N-dimethylacetamide with pKa value of 5.6. It acts as antiretroviral agent and used in the treatment of HIV infection and AIDS. According to the literature survey [7-11], very few methods were developed for the simultaneous estimation of Emtricitabine, Tenofovir and Rilpivirine in pharmaceutical dosage forms. The present study aimed to develop and validate the stability indicating method for the simultaneous estimation of Emtricitabine, Tenofovir and Rilpivirine in pharmaceutical dosage form using RP-HPLC.

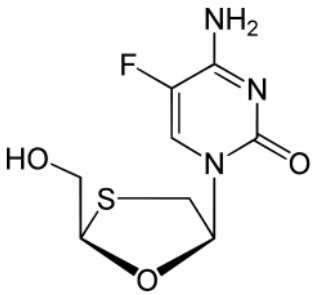

Fig-1A: Chemical Structure of Emtricitabine

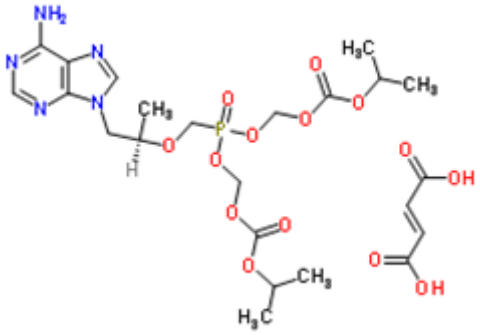

Fig-1B: Chemical Structure of Tenofovir Disoproxil Fumarate

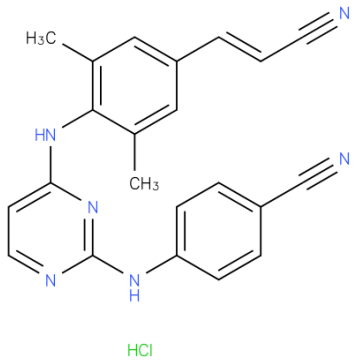

Fig-1C: Chemical Structure of Rilpivirine Hydrochloride

MATERIALS AND METHODS

Emtricitabine, Tenofovir and Rilpivirine 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 Mobile Phase: Mixture of 0.01N Potassium dihydrogen phosphate Buffer and Acetonitrile in the ratio 65:35 (v/v) respectively was used as mobile phase.

Preparation of Standard solution: (200µg/mL Emtricitabine, 300µg/mL Tenofovir Disoproxil Fumarate and 25µg/mL Rilpivirine hydrochloride) 20mg of Emtricitabine, 30mg of Tenofovir Disoproxil Fumarate and 2.5mg of Rilpivirine hydrochloride working standards were accurately weighed and transferred into a 10mL volumetric flask. 7mL of diluent was added, sonicated to dissolve and make up to final volume with diluent. From the above stock solution, 1mL was pipetted into a 10mL volumetric flask and the volume was made up to mark with diluent. The standard solution was injected into the HPLC system and chromatogram was recorded (Figure-2A).

Preparation of Sample solution

20 tablets (Complera) were weighed accurately and the average weight was calculated. Then the tablets were crushed and fine powder was collected. An amount equivalent to 20mg of Emtricitabine, 30mg of Tenofovir Disoproxil Fumarate and 2.5mg of

Rilpivirine was weighed and transferred into 10mL volumetric flask. 7mL of diluent was added and sonicated for 30min with intermediate shaking. Volume was made up with diluent. The above solution was filtered using HPLC filters. 1mL of the above solution was pipette into 10mL volumetric flask and made up with diluent.

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

Method validation [12]

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 (Figure-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-1 and Figure 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 [13] 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 (Figure 5) and results were summarized in Table-2.

RESULTS AND 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. 279nm was selected as detection wavelength based on the overlay UV spectrum. The chromatographic separation was performed using Kromasil C18, 250mm × 4.6mm, 5µ column. KH_2PO4: acetonitrile (65:35) run in isocratic mode and flow rate 1.0ml/min was selected. Retention time for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine were found to be 4.35min, 2.05min and 3.61min respectively.

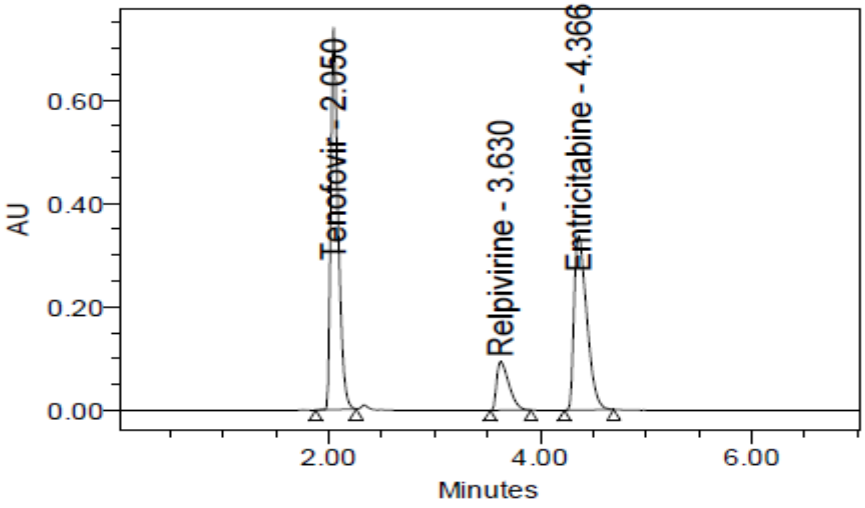

Fig-2A: Standard chromatogram

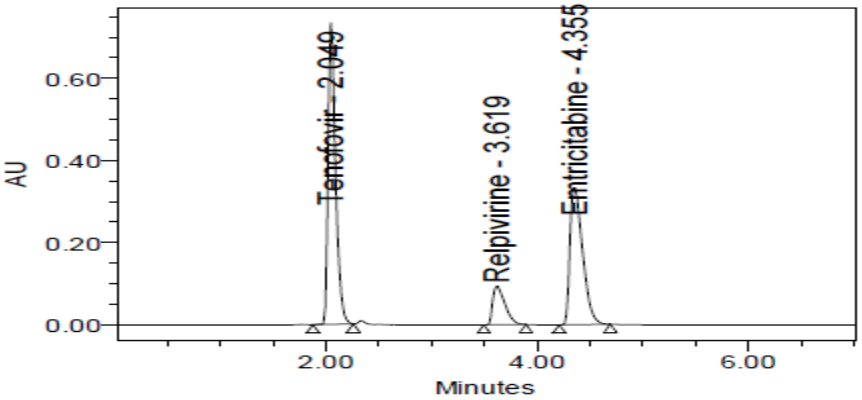

Fig-2B: Sample chromatogram

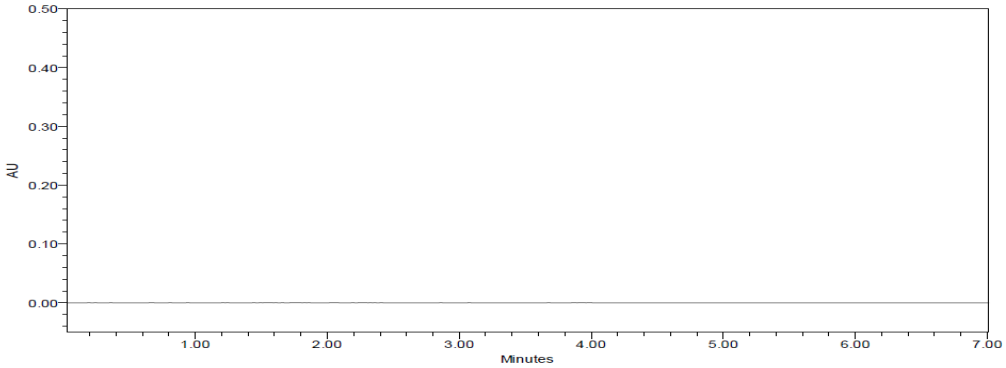

Fig-2C: Blank chromatogram

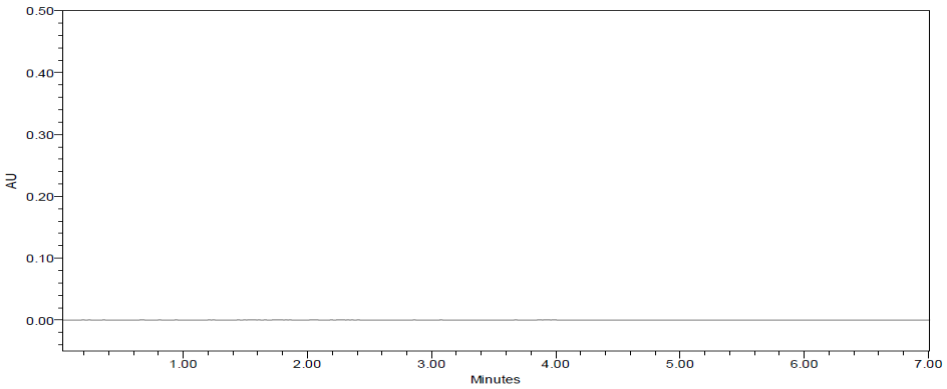

Fig-3: Placebo chromatogram

Table-1: System Suitability and Validation Parameter Results

Parameter	**EMT**	**TDF**	**RPH**
Specificity	Specific	Specific	Specific
Precision (% RSD)	0.5	0.3	0.6
Accuracy (% Recovery)	99.22%-99.83%	99.42%-99.91%	99.85%-100.09%
Linearity range (µg/ml)	50 – 300	75 – 400	6.25 – 37.5
Correlation coefficient, r	0.9997	0.9997	0.9995
Limit of Detection (µg/ml)	1.14	0.97	0.01
Limit of Quantitation (µg/ml)	3.45	2.94	0.03
Ruggedness (%RSD)	0.2	0.2	0.2
Robustness	Robust	Robust	Robust
Stability	Stable	Stable	Stable
USP Plate Count	6160	3943	4503
USP Tailing Factor	1.61	1.45	1.74
USP Resolution	3.3		9.0

A linear response was observed in the concentration range of 50µg/mL – 300µg/mL for Emtricitabine, 75µg/mL – 450µg/mL for Tenofovir and 6.25µg/mL – 37.5µg/mL for Rilpivirine with correlation coefficient of 0.999.

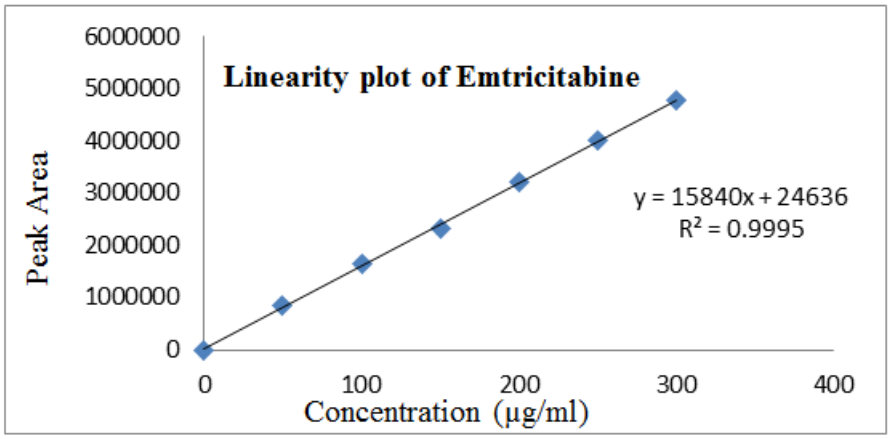

Fig-4A: Linearity plot of EMT

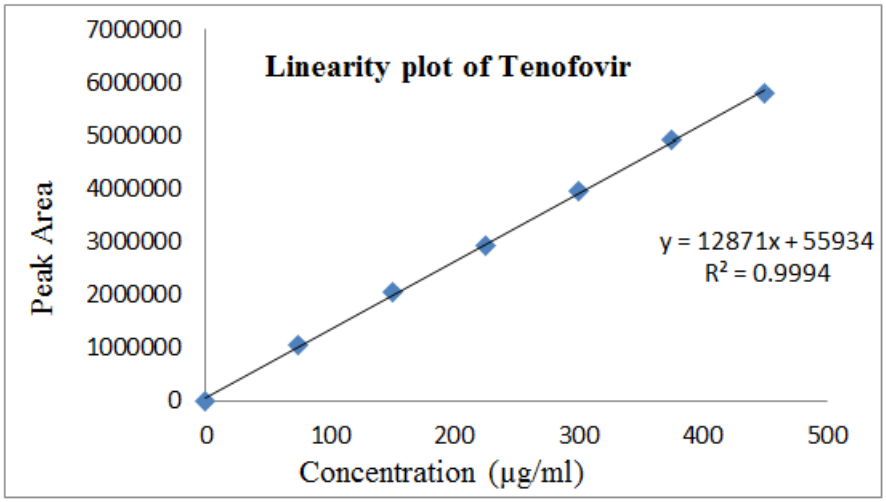

Fig-4B: Linearity plot of TDF

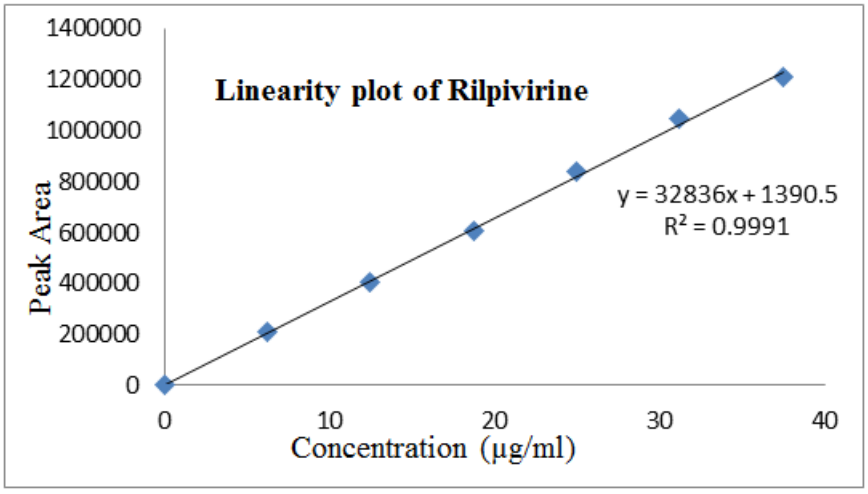

Fig-4C: Linearity plot of RPH

The %RSD for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine were found to be 0.5, 0.3 and 0.6 respectively. The % recoveries were found to be 99.22% - 99.83% for Emtricitabine,

99.42%-99.91% for Tenofovir Disoproxil Fumarate and 99.59%-100.09% for Rilpivirine.

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.

Table-2: Forced degradation studies results

Drug	Parameters	Stress Condition					
		Acidic	Basic	Oxidative	Photolytic	Neutral	Dry heat
EMT	% Assay	96.09	97.45	98.62	99.82	99.90	98.84
	Purity angle	0.063	0.065	0.065	0.070	0.075	0.074
	Purity threshold	0.268	0.270	0.270	0.276	0.279	0.276
	% Degradation	3.91	2.55	1.38	0.18	0.10	1.16
TDF	% Assay	96.17	96.53	97.83	99.19	99.71	98.99
	Purity angle	0.073	1.073	1.165	2.429	0.269	1.281
	Purity threshold	0.270	1.270	1.273	2.658	1.024	2.124
	% Degradation	3.83	3.47	2.17	0.81	0.29	1.01
RPH	% Assay	96.28	97.06	97.56	99.34	99.60	99.16
	Purity angle	0.120	0.273	0.373	0.079	0.078	0.072
	Purity threshold	0.279	0.280	0.578	0.284	0.285	0.283
	% Degradation	3.72	2.94	2.44	0.66	0.40	0.84

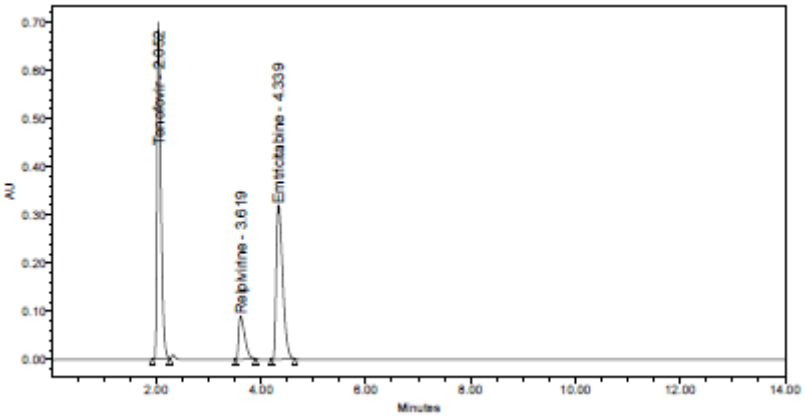

Fig-5A: Acid Degradation study chromatogram

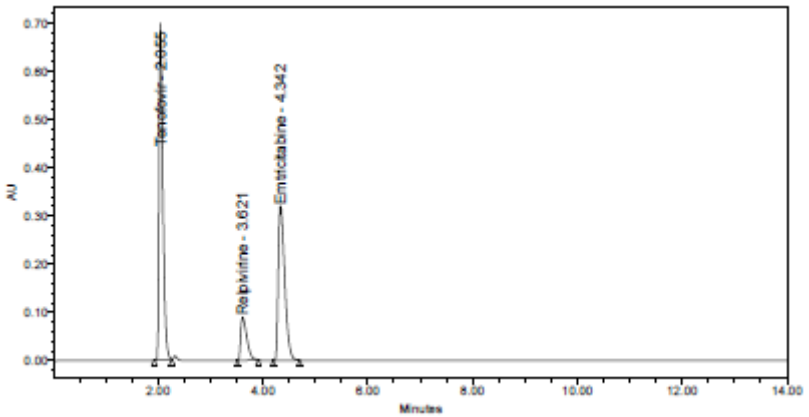

Fig-5B: Base Degradation study chromatogram

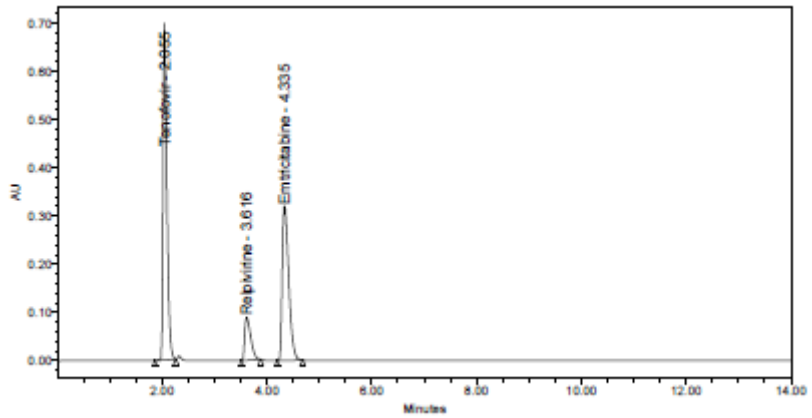

Fig-5C: Peroxide Degradation study chromatogram

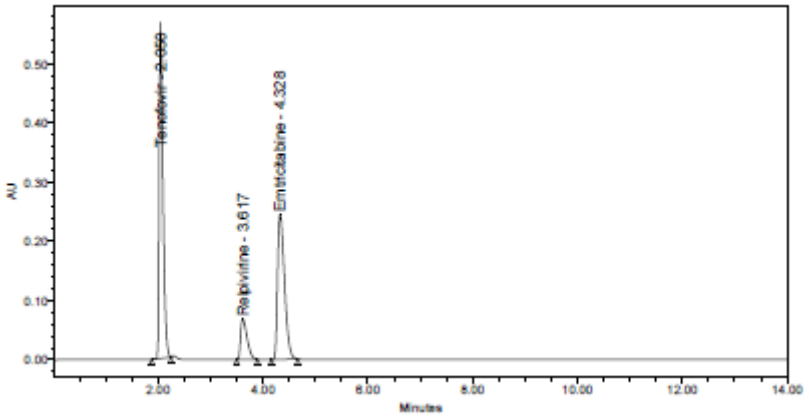

Fig-5D: Neutral Degradation study chromatogram

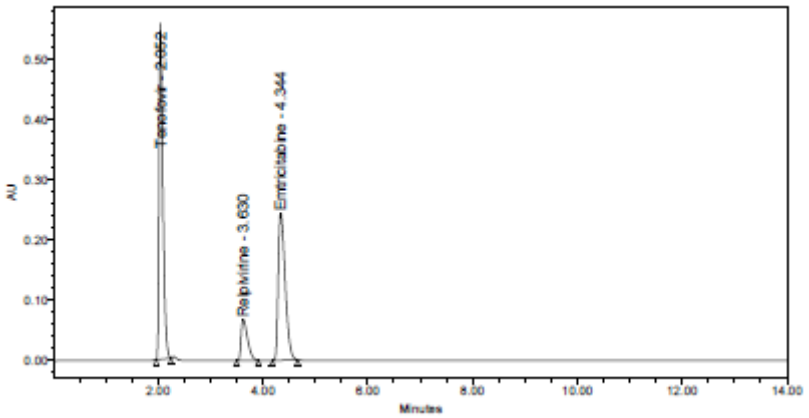

Fig-5E: Photolytic Degradation study chromatogram

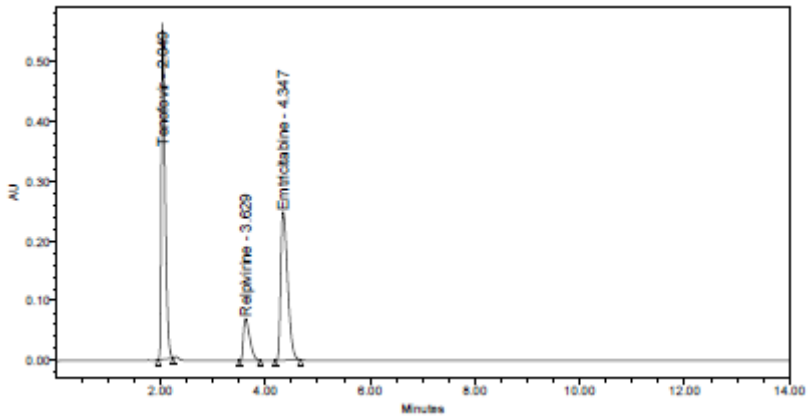

Fig-5F: Thermal Degradation study chromatogram

CONCLUSION

A stability indicating RP-HPLC method was developed for the simultaneous estimation of Emtricitabine, Tenofovir and Rilpivirine 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 Emtricitabine, Tenofovir and Rilpivirine in routine and quality control analysis of tablet formulations.

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