IJPSR (2020), Volume 11, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 January 2020; received in revised form, 08 February 2020; accepted, 24 February 2020; published 01 March 2020

STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF EMITRICITABINE, TENOFOVIR, COBICISTAT AND ELVITEGRAVIR

P. D. Chaithanya Sudha *, P. Sohail and Ushashree Avulapati

Department of Pharmaceutical Analysis, Krupanidhi College of Pharmacy Bengaluru - 560035, Karnataka, India.

Keywords:

Emtricitabine (EMT), Tenofovir (TNF), Cobicistat (COB), Elvitegravir (ELV), RP-HPLC, Degradation studies, and validation

Correspondence to Author:
Dr. P. D. Chaithanya Sudha,

Associate Professor,
Department of Pharmaceutical Analysis, Krupanidhi College of Pharmacy Bengaluru - 560035, Karnataka India.

E-mail: chaithumpharm@gmail.com

ABSTRACT: A stability-indicating RP-HPLC method for the simultaneous determination of emtricitabine (EMT), tenofovir (TNF), cobicistat (COB) and elvitegravir (ELV) in solid dosage forms. The waters 2695, High-Performance Liquid Chromatographic system with column kromasil C_{18}, 250 × 4.6 mm, 5 µ. The detector used is PDA detector at 288 nm. The mobile phase used in this method is pH-3.5 phosphate buffer and acetonitrile in the ratio of 60:40% V/V. Flow rate used for this proposed method is 1.0 ml/min. The retention times observed are 2.304 min, 2.691 min, 3.185 min and 4.537 min for emtricitabine, tenofovir, cobicistat, and elvitegravir respectively. The linearity calculated was found to be within the range. The % recoveries for EMT, TNF, COB and ELV were within the acceptance criteria. These drugs were found to be stable at forced degradation studies and results are within the limits. The proposed method can be used for the quality control of the combination in the pharmaceutical dosage forms.

INTRODUCTION: Emtricitabine is NRTI and is an analog of cytidine which inhibits the reverse transcriptase enzyme that copies the HIV RNA into a new viral DNA. By interrupting this process the drug lowers the amount of the HIV or viral load in the patient's body and increases the number of immune system cells. Emtricitabine is used in prevention and treatment of HIV infection. It is a nucleoside reverse transcriptase inhibitor. It is used in a combination with tenofovir and efavirenz and is approved by USFDA.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(3).1452-66

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(3).1452-66

Tenofovir alafenamide is NRTI used to treat chronic hepatitis B and to prevent HIV. It causes premature termination of DNA transcription and prevents viral replication. This drug shows high anti-HIV activity in the male genital tract. Tenofovir inhibits the activity of the HIV-1 reverse transcriptase by competing with the natural substrate, deoxyadenosine 5' triphosphate and by DNA chain termination.

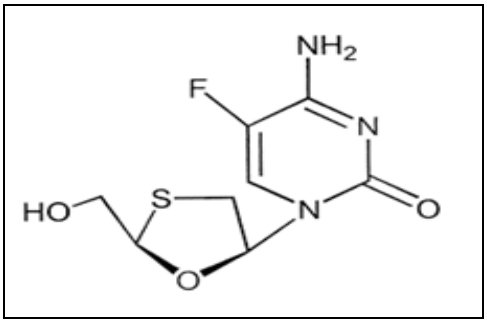

FIG. 1: CHEMICAL STRUCTURE OF EMITRICITABINE

IUPAC Name: 4-amino-5-flouro-1-(2R, 5S)-2-(hydroxymeythyl)-1, 3-oxathiolan-5-yl-1,2-dihydro - pyrimidine-2-one.

Molecular Formula: $C_8H_{10}N_3O_3SF$.

Molecular Weight: 247.248 g/mol.

Category: Anti-viral agent.

Tenofovir disproxal is used in the prevention of HIV infection and to treat chronic hepatitis-B. It is used alone and in combination with tenofovir and/or efavirenz and/or emtricitabine. It is a nucleoside reverse transcriptase inhibitor.

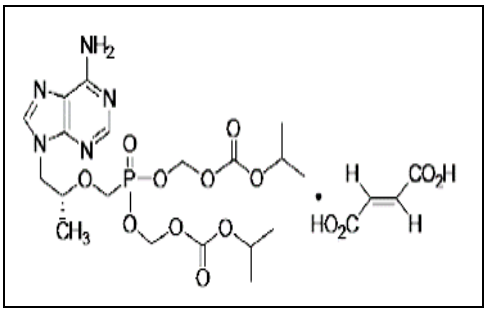

FIG. 2: CHEMICAL STRUCTURE OF TENOFOVIR ALAFENAMIDE

IUPAC Name: Bis-(isopropoxycarbonyl) oxy-methyl (2R)-1-(6-amino-9H-purine-9-yl)-2- pro-panyl oxy-methyl) phosphonate.

Molecular Formula: $C_9H_{14}N_5O_4P$.

Molecular Weight: 287.213 g/mol.

Category: Anti-viral agent.

Cobicistati is used for the treatment of HIV. It is a pharmacokinetic enhancer that inhibits the cytochrome P450 forms. In combination with the other retroviral agents increases systemic exposure.

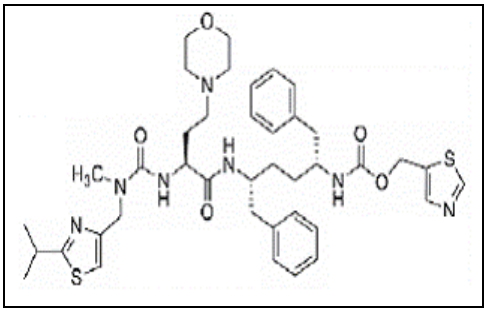

FIG. 3: CHEMICAL STRUCTURE OF COBICISTAT

IUPAC Name: (1, 3-thiazol-5-yl) methyl, N-(2R, 5R)-5-(2S)-2-methyl (2-propan-2-yl)-1, 3-thiazol-4 -yl methyl carbamoyl amino-4-(morpholin-4-yl) butanamido-1, 6-diphenylhexan-2-yl carbamate.

Molecular Formula: $C_{40}H_{53}N_7O_5S_2$.

Molecular Weight: 776.03 g/mol.

Elvitegravir is an integrase strand inhibitor used to treat HIV infection. This is mainly used with other antiretroviral agents.

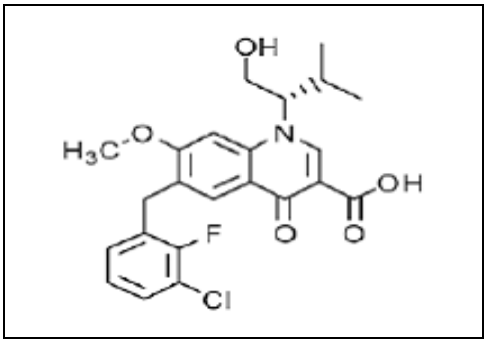

FIG. 4: CHEMICAL STRUCTURE OF ELVITEGRAVIR

IUPAC Name: 6-(3-chloro-2-fluorophenyl) methyl -1-(2S)-1-hydroxy-3-methyl butan-2-yl-7- methoxy -4-oxo-1, 4-dihydroquinoline-3-carboxylic acid.

Molecular Formula: $C_{23}H_{23}ClFNO_5$.

Molecular Weight: 447.883 g/mol.

Genovya is the combination of the above four drugs which indicates a complete regimen for the treatment of HIV-1 infection in adults and pediatric patients.

MATERIALS AND METHODS:

Standard Preparation: Accurately weighed and transferred 50 mg of emtricitabine, 2.5 mg of tenofovir alafenamide, 37.5 mg of cobicistat and 37.5 mg of elvitegravir working standards into a 25 ml clean dry volumetric flasks, add 10 ml of diluent, sonicated for 10 min and makeup to the final volume with diluents. 1000 µg/ml of emtricitabine, 50 µg/ml of tenofovir alafenamide 750 µg/ml of cobicistat and 750 µg/ml of elvitegravir). 1 ml of the above stock solution was transferred to a 10ml volumetric flask and made up with diluent. 100 µg/ml of emtricitabine, 5 µg/ml of tenofovir alafenamide 75 µg/ml of cobicistat and 75 µg/ml of elvitegravir).

Sample Preparation: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 60 mL of diluent added and sonicated for 25 min, further the volume made

up with diluents and filtered. 2000 µg/ml of emtricitabine, 100 µg/ml of tenofovir alafenamide, 1500 µg/ml of cobicistat and 1500 µg/ml of elvitegravir). 0.5 ml of filtered sample stock solution was transferred to a 10 ml volumetric flask and made up with diluent. 100 µg/ml of emtricitabine, 5 µg/ml of tenofovir alafenamide, 75 µg/ml of cobicistat and 75 µg/ml of elvitegravir).

0.01N KH_2PO_4 Buffer Preparation: Accurately weighed 1.36 gm of potassium dihydrogen orthophosphate in a 1000 ml of volumetric flask add about 900 ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 3.5 with dil. Ortho-phosphoric acid solution.

Mobile Phase: The mobile phase is prepared by mixing Buffer and ACN in the ratio 60:40% V/V.

Diluent Preparation: 0.01N KH_2PO_4: Acetonitrile (50:50).

Instrumentation and Chromatographic Conditions: The waters 2695, High-Performance Liquid Chromatographic system is used for the method development. The following chromatographic conditions are used.

Chromatographic Conditions:
Flow rate: 1.0 ml/min
Column: Kromasil C_{18}, 250 × 4.6 mm, 5µ.
Detector wavelength: 288.0 nm
Column temperature: 30 ºC
Injection volume: 10.00 µL
Run time: 8.0 min
Diluent: 0.01N KH_2PO_4: Acetonitrile (50:50)

Method Development: To optimize chromatographic conditions, the effect of chromatographic variables such as different columns, mobile phase composition, and flow rates were studied at constant conditions such as appropriate wavelength 288 nm, injection volume of 10 µl and run time of about 8 min throughout the trials to achieve the best possible separation and resolution. The conditions which produce the best resolution tailing factor, USP plate count were selected for the estimation. The resulting chromatograms were recorded and chromatographic parameters such as tailing factor, USP plate count and resolution were calculated.

Forced Degradation Studies: A stress study was conducted to demonstrate the effective separation of degradations from the main analyte peaks of the sample when exposed to the following stress conditions. All the stressed samples were suitable diluted to required concentration with diluent and injected twice into the HPLC system by using optimized chromatographic conditions and the chromatograms were recorded and evaluated for peak purity. The % of degradation of emtricitabine, tenofovir, cobicistat, and elvitegravir were calculated.

TABLE 1: STRESS CONDITIONS FOR THE PROPOSED RP-HPLC METHOD

Type of degradation	Stress condition
Control	Un degraded
Acid degradation	Refluxed with 2N HCl at 60 °C for 30 min
Alkali degradation	Refluxed with 2N NaOH at 60 °C for 30 min
Peroxide degradation	Refluxed with 20% of H_2O_2 on a heating mantle at 60 °C for 30 min
Photolytic (UV) degradation	Exposed to UV light at 241nm for about 3 days
Thermal degradation	Heated in an oven at 105 °C for 6 h
Neutral degradation	Reflux on water bath 60 °C for 6 h

Method Validation:

System Suitability Studies: The system stability test was carried out by injecting five replicate injections of 10 µL of the standard solutions of emtricitabine, tenofovir, cobicistat, and elvitegravir into the chromatographic system by using optimized chromatographic conditions. The system suitability parameters were evaluated for tailing factor, % relative standard deviation for retention time and peak areas, resolutions and theoretical plates were determined.

Linearity of Detector Response: The linearity of an analytical method was established by preparing a series of linearity solutions (25-150% level) by diluting aliquots of 0.25, 0.5, 0.75, 1.0 and 1.25 ml were taken from stock solution of concentration 100 µg/ml of emtricitabine, 5 µg/ml of tenofovir, 75 µg/ml of cobicistat and 75 µg/ml of elvitegravir and then diluted up to mark with diluent. Such that the final concentrations were in the range 25-150 µg/ml emtricitabine, 1.25-7.5 µg/ml tenofovir, 18.75-112.5 µg/ml cobicistat and 18.75-112.5 µg/ml elvitegravir. The volume of 10 µl of each sample was injected five times for each

concentration level in triplicate into the chromatographic system and the chromatographs were recorded. The calibration curve was constructed by plotting the peak area versus drug concentration. A linear relationship between peak area vs. concentration was observed in the range of study (concentration in µg/ml on X-axis and peak area response on Y-axis) from this calculate the correlation coefficient, slope and intercept.

Precision:

System Precision repeatability / Intra-Day Precision: The system precision study was demonstrated by injecting 10 µl solution of standard preparations six times into the chromate-graphic system and chromatograms were recorded. Calculated peak areas for emtricitabine, tenofovir, cobicistat, and elvitegravir results were expressed as % RSD.

Method Precision: The method precision of the test method was conducted by 10 µl solution of sample preparations six times into the chromatographic system and chromatograms were recorded. Calculated peak areas for emtricitabine, tenofovir, cobicistat, and elvitegravir results were expressed as % RSD.

Intermediate/Inter-Day Precision Ruggedness: The intermediate precision of the method was carried out by injecting 10 µl solution of standard preparations six times into the chromatographic system on different days and chromatograms were recorded. Calculated peak areas for emtricitabine, tenofovir, cobicistat, and elvitegravir and results were expressed as % RSD.

Accuracy Recovery: The accuracy of the method was studied by % recovery across its range by making 3 different concentrations at 50%, 100% and 150% Levels using standard addition method where sample preparations were spiked with known amount of standard and then each concentration was injected triplicate into the chromatographic system and chromatograms were recorded. The % recoveries obtained from each Level for emtricitabine, tenofovir, cobicistat, and elvitegravir calculated.

Robustness: The robustness of the proposed method was determined by deliberately varying the chromatographic conditions such as mobile phase compositions, flow rate, wavelength, and column temperature.

The standard solutions prepared as per the test method were injected triplicate into the chromatograph at variable conditions such as flow rate at ± 0.1 ml/min, mobile organic phase composition by ± 10%, wavelength by ± 5 nm and column temperature by ± 5 °C. System suitability parameters were evaluated from the obtained chromatograms.

Specificity Interference Studies: Specificity of the method for the interference of the blank and the placebo was conducted by injecting blank, placebo, standard and sample solutions in triplicate as per test method. The specificity of the proposed RP-HPLC method also assessed by comparing the chromatograms obtained from blank, placebo, standard and sample solutions.

RESULTS:

System Suitability Studies: System Suitability chromatography obtained is shown in **Fig. 5** and the results of the proposed method are presented in **Table 2**.

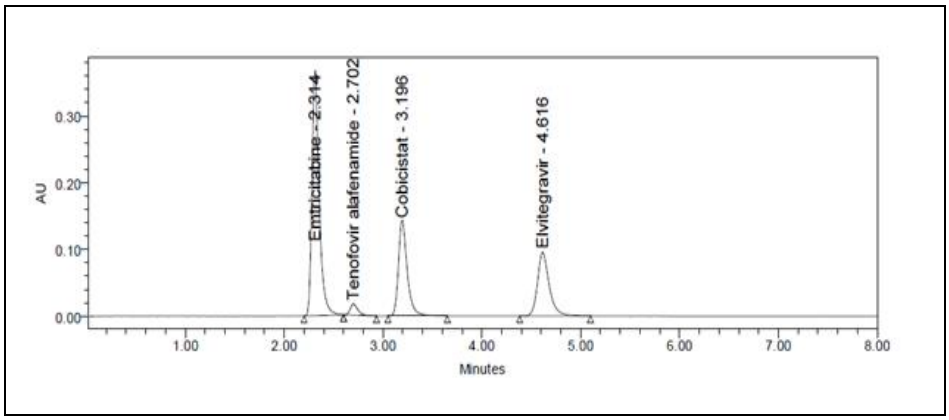

FIG. 5: CHROMATOGRAM OF SYSTEM SUITABILITY SOLUTION

TABLE 2: SYSTEM SUITABILITY TEST PARAMETERS

S. no.	Name of the drug	Rt (min)	USP resolution	USP tailing	USP plate count	% RSD
1.	Emitricitabine	2.304	-	1.38	5156	1.3
2.	Tenofovir	2.691	2.8	1.38	6246	2.0
3.	Cobicistat	3.185	3.3	1.33	6758	1.1
4.	Elvitegravir	4.537	7.7	1.32	12575	0.9

Linearity of Detector Response: The detector response was found to be linear in the concentration range of 25-150 µg/ml of emtricitabine, 1.25-7.5 µg/ml of tenofovir, 18.75-112.5 µg/ml of cobicistat and 18.75-112.5 µg/ml of elvitegravir respectively as shown in the **Fig. 6**, **7** and peak areas are measured. The calibration curves of emtricitabine, tenofovir, cobicistat, and elvitegravir are shown in **Fig. 12-15** respectively. The linearity studies and regression characteristics of the proposed method are presented in **Tables 3** and **4**.

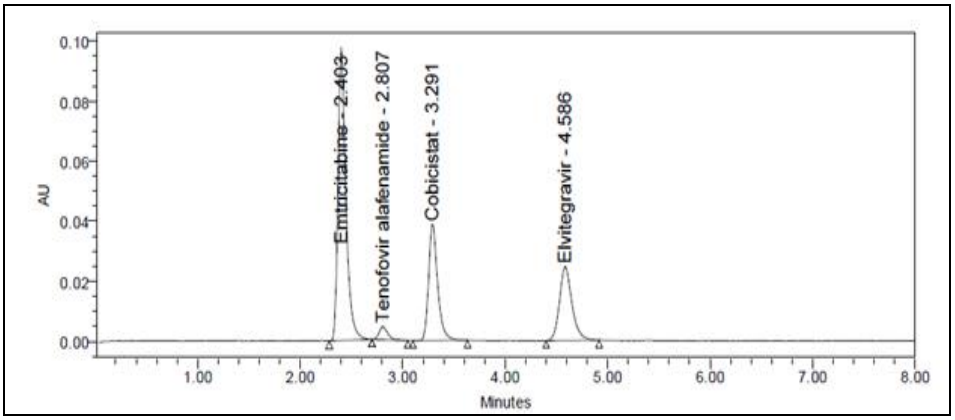

FIG. 6: HPLC CHROMATOGRAM OF 25% LEVEL OF LINEARITY SOLUTION

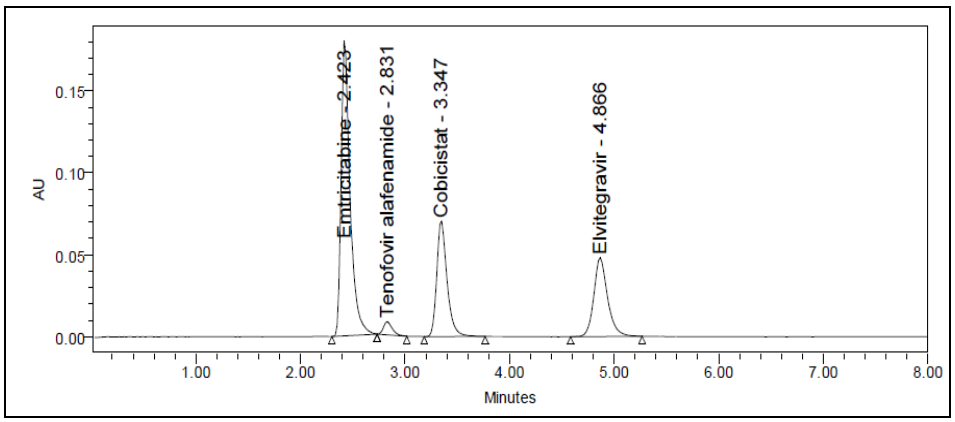

FIG. 7: HPLC CHROMATOGRAM OF 50% LEVEL OF LINEARITY SOLUTION

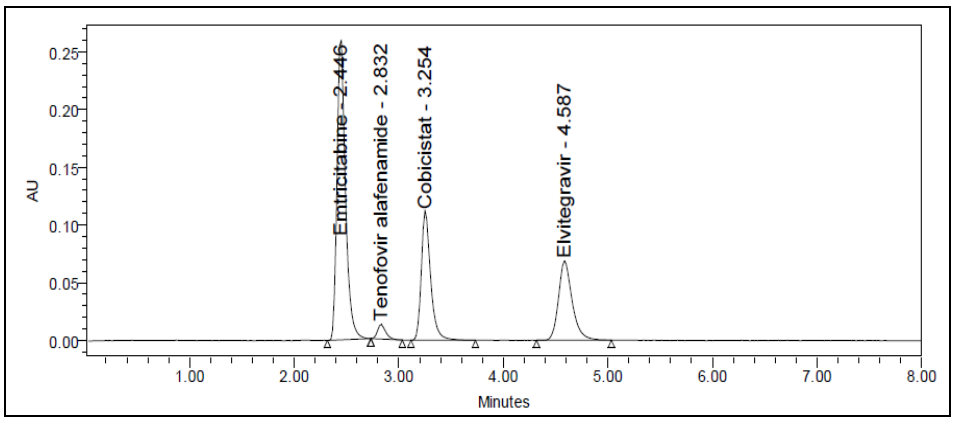

FIG. 8: HPLC CHROMATOGRAM OF 75% LEVEL OF LINEARITY SOLUTION

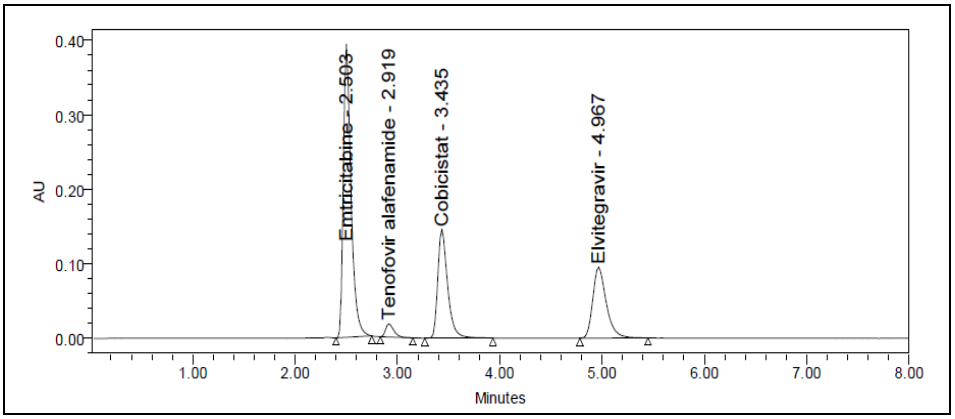

FIG. 9: HPLC CHROMATOGRAM OF 100% LEVEL OF LINEARITY SOLUTION

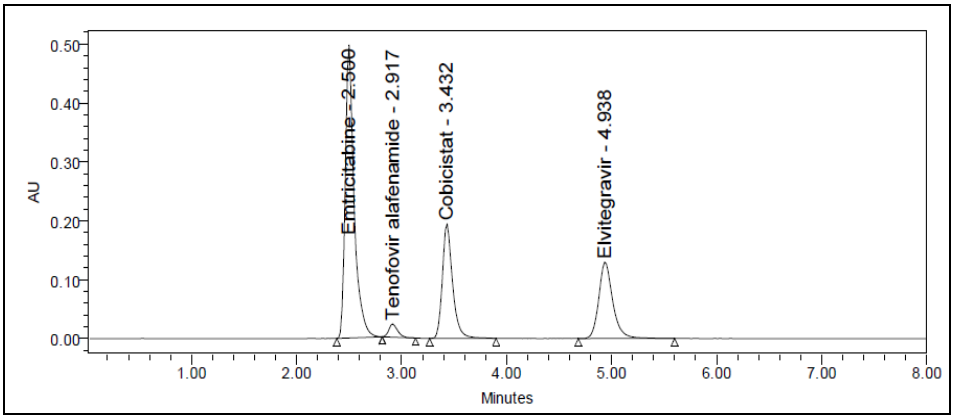

FIG. 10: HPLC CHROMATOGRAM OF 125% LEVEL OF LINEARITY SOLUTION

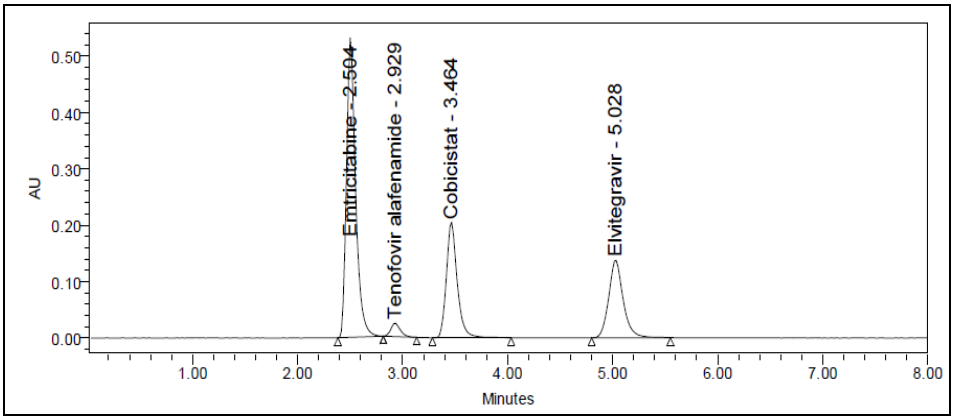

FIG. 11: HPLC CHROMATOGRAM OF 150% LEVEL OF LINEARITY SOLUTION

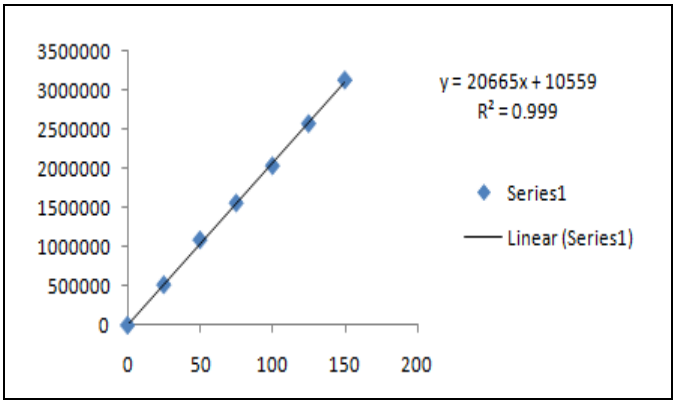

FIG. 12: STANDARD CALIBRATION GRAPH OF TENOFOVIR

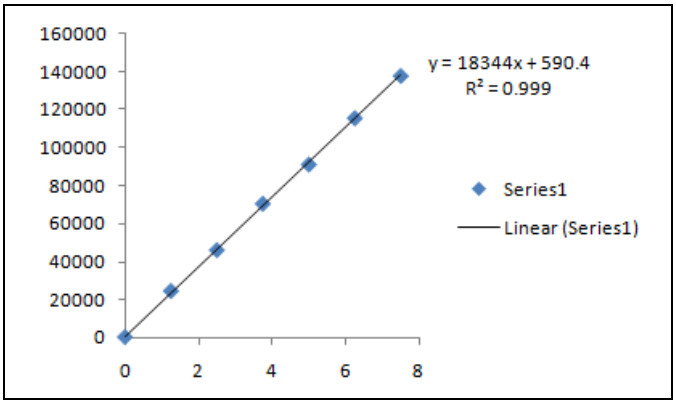

FIG. 13: STANDARD CALIBRATION GRAPH OF EMITRICITABINE

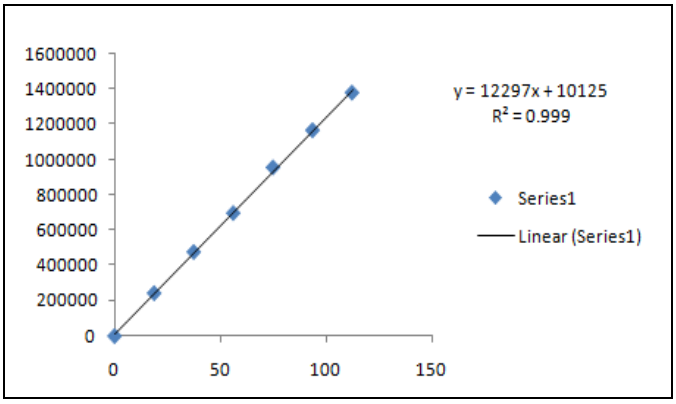

FIG. 14: STANDARD CALIBRATION GRAPH OF COBICISTAT

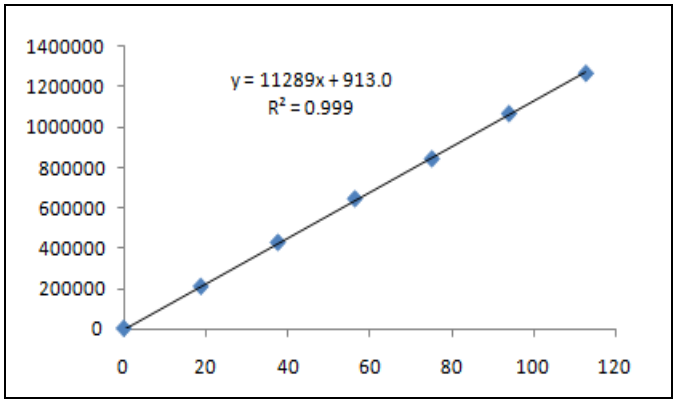

FIG. 15: STANDARD CALIBRATION GRAPH OF ELVITEGRAVIR

TABLE 3: LINEARITY STUDIES OF THE PROPOSED METHOD

% Level of concentration	Emtricitabine		Tenofovir		Cobicistat		Elvitegravir	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
25	25	514598	1.25	24368	18.75	246435	18.75	208167
50	50	1077868	2.5	46768	37.5	471870	37.5	427021
75	75	1551017	3.75	69142	56.25	693238	56.25	643175
100	100	1998668	5.0	91067	75	952015	75	841543
125	125	2582170	6.25	115163	93.75	1169913	93.75	1065752
150	150	3116961	7.5	138383	112.5	1399209	112.5	1265598

TABLE 4: REGRESSION CHARACTERISTICS OF THE PROPOSED METHOD

Parameter	Results			
	Emitricitabine	Tenofovir	Cobicistat	Elvitegravir
Linearity range (µg/ml)	25-150 µg/ml	1.25-7.5µg/ml	18.75-112.5µg/ml	18.75-112.5µg/ml
Regression equation (y=mx+b)	y = 20665x + 10559	y = 18344x + 590.4	y = 12297x + 10125	y = 11289x + 913.0
Slope(m)	20665	18344	12297	11289
Intercept(b)	10559	590.4	10125	913.0
Correlation coeefficient(r^2)	0.999	0.999	0.999	0.999

Precision:

System Precision Repeatability / Intra-Day precision: The % RSD of the peak areas for emtricitabine, tenofovir, cobicistat, and elvitegravir shown in **Table 5**. Standard chromatograms of repeatability studies are shown in **Fig. 16-21**.

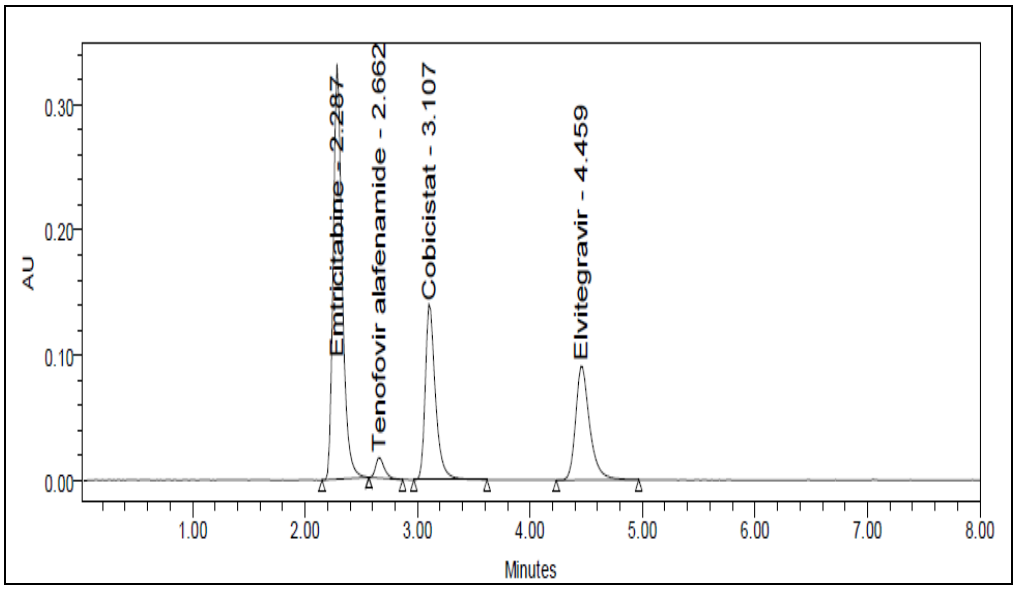

FIG. 16: HPLC CHROMATOGRAM OF SYSTEM PRECISION STUDIES 1

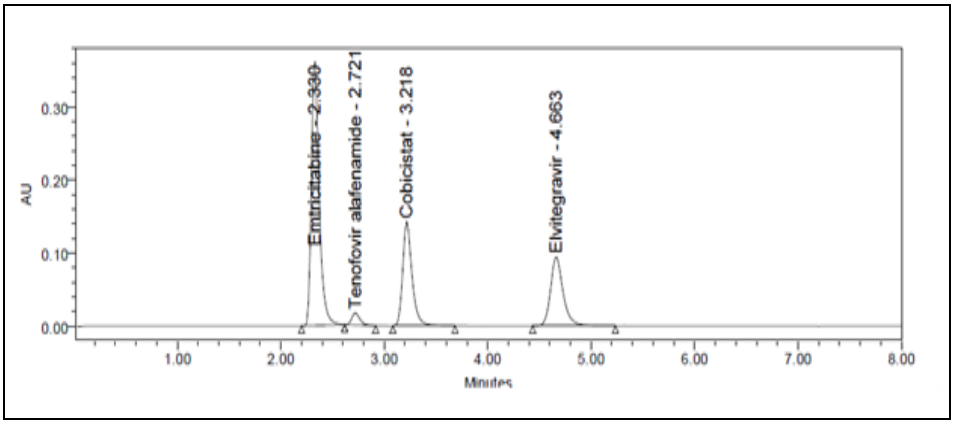

FIG. 17: HPLC CHROMATOGRAM OF SYSTEM PRECISION STUDIES 2

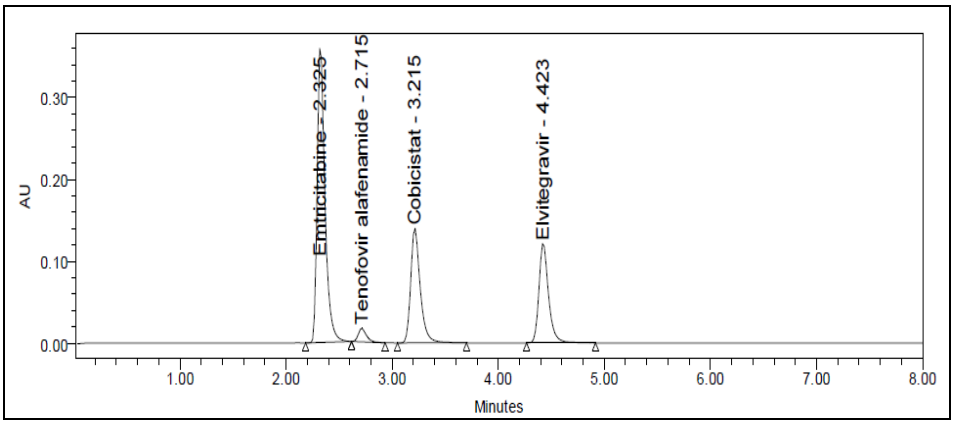

FIG. 18: HPLC CHROMATOGRAM OF SYSTEM PRECISION STUDIES 3

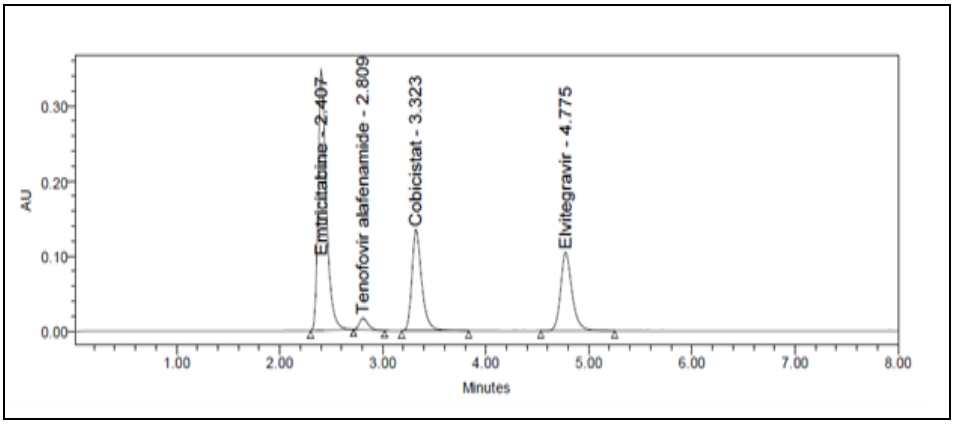

FIG. 19: HPLC CHROMATOGRAM OF SYSTEM PRECISION STUDIES 4

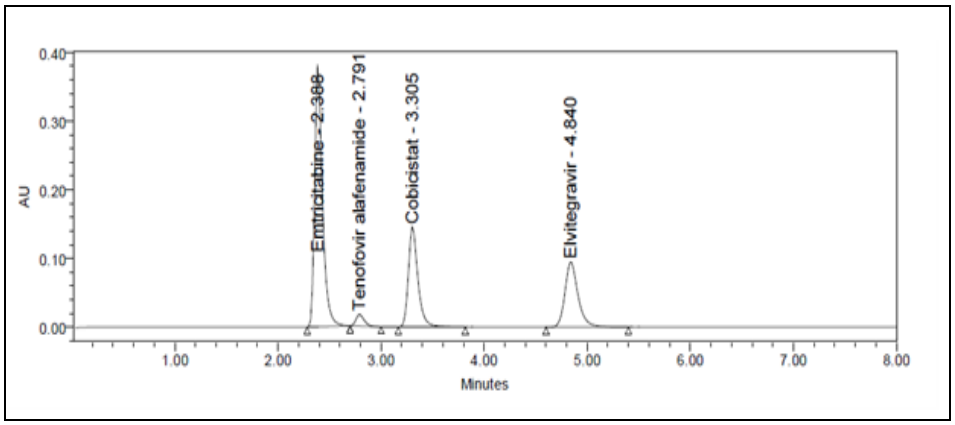

FIG. 20: HPLC CHROMATOGRAM OF SYSTEM PRECISION STUDIES 5

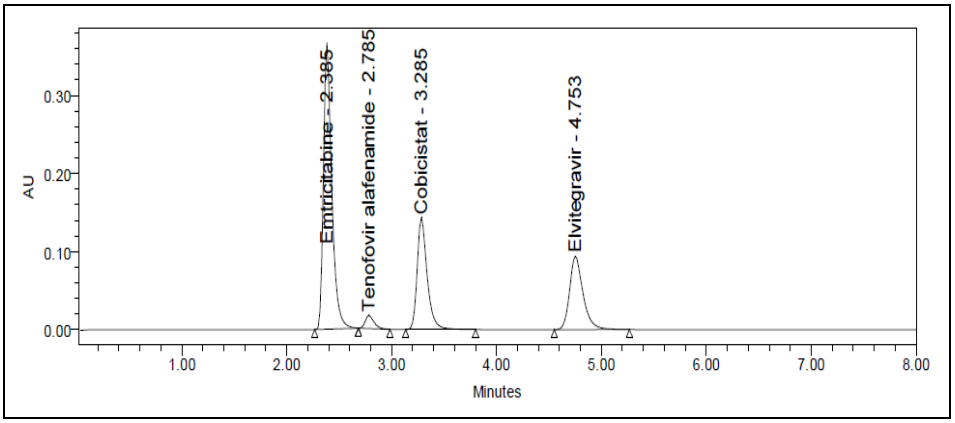

FIG. 21: HPLC CHROMATOGRAM OF SYSTEM PRECISION STUDIES 6

TABLE 5: RESULTS OF REPEATABILITY STUDIES (INTRA-DAY PRECISION)

No. of injection	Retention time (min)				Peak area			
	EMT	TNF	COB	ELV	EMT	TNF	COB	ELV
1	2.287	2.662	3.107	4.423	1833297	87475	883089	757179
2	2.325	2.715	3.215	4.459	1820568	87444	864564	788004
3	2.330	2.721	3.218	4.663	1882812	91410	888445	799509
4	2.385	2.785	3.285	4.753	1992994	95578	944683	835471
5	2.388	2.791	3.305	4.775	1979370	93709	937566	805488
6	2.407	2.809	3.323	4.840	1914090	90199	90888	835461
Statistical Parameters		Mean			1903855	90969	903206	803519
		SD			72264.9	3291.7	31692	29830.4
		% RSD			3.8	3.6	3.5	3.7

Method Precision: The % RSD of the peak areas for emtricitabine, tenofovir, cobicistat, and elvitegravir are shown in **Table 6**.

Intermediate/Intra-Day Precision: The % RSD was determined for retention time, and peak areas of emtricitabine, tenofovir, cobicistat, and elvitegravir are shown in **Tables 7-11**.

Accuracy Recovery: The % recoveries obtained from each concentration level for emtricitabine, tenofovir, cobicistat, and elvitegravir.

TABLE 6: RESULTS OF METHOD PRECISION STUDIES

No. of injection	Retention time (min)				Peak area			
	EMT	TNF	COB	ELV	EMT	TNF	COB	ELV
1	2.287	2.662	3.107	4.423	1968297	89475	933089	857179
2	2.325	2.715	3.215	4.459	1970568	90444	944564	848004
3	2.330	2.721	3.218	4.663	1982812	91410	942445	849509
4	2.385	2.785	3.285	4.753	1992994	91578	944683	835471
5	2.388	2.791	3.305	4.775	1979370	90709	937566	855488
6	2.407	2.809	3.323	4.840	1964090	90199	940888	835461
Statistical Parameters		Mean			1976355	90636	940539	846852
		SD			10737.7	783.3	4504.9	9475.2
		% RSD			0.5	0.9	0.5	1.1

TABLE 7: RESULTS OF INTERMEDIATE (INTER-DAY) PRECISION STUDIES

No. of injection	Peak area (Day-1)				Peak area (Day-2)			
	EMT	TNF	COB	ELV	EMT	TNF	COB	ELV
1	1960533	89594	936844	856509	1926467	87650	928279	844740
2	1950784	89977	953210	846871	1908601	87748	922753	846514
3	1982415	90597	953357	844519	1910325	87534	928278	846823
4	1963488	91763	946496	852880	1924571	87545	923079	847203
5	1978143	90091	943552	847710	1900990	87859	926960	849026
6	1988930	90530	933070	848263	1928989	87361	929002	846177
Mean	1972752	90425	944422	849459	1916657	87616	926392	846747
SD	14676.5	752.8	8347.6	4404.5	11501.3	175.7	2773.8	1401.1
% RSD	0.7	0.8	0.9	0.5	0.6	0.2	0.3	0.2

TABLE 8: DATA OF ACCURACY STUDIES OF EMITRICITABINE

S. no.	% Level of test conc.	Peak area	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % recovery	% RSD*
1	50	3111878	50	50.07593	100.15	100.3	1.21
2	50	3121032	50	50.5189	101.04		
3	50	3099965	50	49.49944	99.00		
4	100	4124728	100	99.08875	99.09	100.29	1.32
5	100	4179111	100	101.7204	101.72		
6	100	4145252	100	100.0819	100.08		
7	150	5209450	150	151.5795	101.05	99.8	1.58
8	150	5116316	150	147.0727	98.05		
9	150	5188611	150	150.5711	100.38		

*mean of three determinations

TABLE 9: DATA OF ACCURACY STUDIES OF TENOFOVIR

S. no.	% Level of test conc.	Peak Area	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % recovery	% RSD*
1	50	138859	2.5	2.537538	101.50	100.96	0.79
2	50	138188	2.5	2.500959	100.04		
3	50	138786	2.5	2.533559	101.34		
4	100	183761	5	4.985314	99.71	98.78	0.81
5	100	182466	5	4.914719	98.29		
6	100	182522	5	4.917771	98.36		
7	150	229899	7.5	7.500469	100.01	99.6	0.49
8	150	228603	7.5	7.429819	99.06		
9	150	229519	7.5	7.479754	99.73		

*mean of three determinations

TABLE 10: DATA OF ACCURACY STUDIES OF COBICISTAT

S. no.	% Level of test conc.	Peak area	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % recovery	% RSD*
1	50	1388890	37.5	37.12206	98.99	99.4	0.6
2	50	1393978	37.5	37.53582	100.10		
3	50	1389625	37.5	37.18183	99.15		
4	100	1860409	75	75.46629	100.62	100.05	0.56
5	100	1850057	75	74.62446	99.50		
6	100	1855031	75	75.02895	100.04		
7	150	2329249	112.5	113.5927	100.97	100.38	0.51
8	150	2317524	112.5	112.639	100.12		
9	150	2316544	112.5	112.559	100.05		

*mean of three determinations

TABLE 11: DATA OF ACCURACY STUDIES OF ELVITEGRAVIR

S. no.	% Level of test conc.	Peak area	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % recovery	% RSD*
1	50	1282457	37.5	38.05751	101.49	100.39	1.25
2	50	1272023	37.5	37.13578	99.03		
3	50	1278988	37.5	37.75106	100.67		
4	100	1694168	75	74.42774	99.24	99.66	1.05
5	100	1707912	75	75.64187	100.86		
6	100	1691255	75	74.17041	98.89		
7	150	2125611	112.5	112.5411	100.04	99.27	0.80
8	150	2105428	112.5	110.758	98.45		
9	150	2116440	112.5	111.731	99.32		

*mean of three determinations

Robustness: System suitability parameters from the obtained chromatograms were evaluated and are reported in **Tables 12-15**.

Specificity Interference Studies: The chromate-grams obtained from the blank, placebo, standard

and sample solutions were recorded and results of specificity studies are reported in **Table 16**.

Chromatograms of blank and placebo solutions showed no peaks at the retention times of emtricitabine, tenofovir, cobicistat and elvitegravir and the retention times of the analyses in standard and sample solutions were found to be same. So, the proposed RP-HPLC method was said to be specific and free from interference due to the recipient's presence in the tablets.

Forced Degradation Studies: The chromatograms of the stressed samples were evaluated for peak purity as shown in **Fig. 22-27**. These stress degradation results of Emtricitabine, Tenofovir, Cobicistat, and Elvitegravir reported in **Tables 16-20**.

TABLE 12: DATA OF ROBUSTNESS STUDIES OF EMITRICITABINE

Parameter	Optimized condition	Used condition	Peak area	Retention time	Plate count	Tailing factor
Flow rate	1 ml/min	0.9 ml/min	1963042	2.497	2061	1.1
(± 0.1 ml/min)		1 ml/min	2021928	2.535	2637	1.21
		1.1 ml/min	2067649	2.660	2895	1.20
Column temp.	30 °C	25 °C	1870361	2.370	3476	1.29
(± 5 °C)		30 °C	1833840	2.371	3308	1.20
		35 °C	1898130	2.380	3379	1.28
Mobile phase	40:60	35:65	2270513	2.749	4565	1.29
composition (5%		40:60	2279887	2.772	4993	1.32
v/v)		45:55	2255925	2.780	4774	1.32

TABLE 13: DATA OF ROBUSTNESS STUDIES OF TENOFOVIR

Parameter	Optimized condition	Used condition	Peak area	Retention time	Plate count	Tailing factor
Flow rate	1ml/min	0.9 ml/min	93714	2.916	4071	1.18
(± 0.1 ml/min)		1 ml/min	96947	2.981	2996	1.17
		1.1 ml/min	95948	3.115	3133	1.16
Column temp.	30 °C	25 °C	85629	2.736	4778	1.22
(± 5 °C)		30 °C	86195	2.755	4547	1.29
		35 °C	86265	2.781	4228	1.23
Mobile phase	40:60	35:65	107146	3.210	5585	1.27
Composition		40:60	107474	3.237	5715	1.26
(5 % v/v)		45:55	106671	3.244	5751	1.26

TABLE 14: DATA OF ROBUSTNESS STUDIES OF COBICISTAT

Parameter	Optimized condition	Used condition	Peak area	Retention time	Plate count	Tailing factor
Flow rate	1ml/min	0.9 ml/min	931537	3.496	3882	1.14
(± 0.1 ml/min)		1 ml/min	940615	3.615	3074	1.09
		1.1 ml/min	954117	3.761	3339	1.11
Column temp.	30 °C	25 °C	881834	3.121	5320	1.22
(± 5 °C)		30 °C	881330	3.188	5150	1.22
		35 °C	898973	3.294	4578	1.20
Mobile phase	40:60	35:65	1056369	3.785	6333	1.22
Composition		40:60	1061847	3.819	6511	1.22
(5 % v/v)		45:55	1051130	3.828	6319	1.23

TABLE 15: DATA OF ROBUSTNESS STUDIES OF ELVITEGRAVIR

Parameter	Optimized condition	Used condition	Peak area	Retention Time	Plate Count	Tailing factor
Flow rate	1 ml/min	0.9 ml/min	823543	5.316	5972	1.09
(± 0.1 ml/min)		1 ml/min	847519	5.664	5144	1.02
		1.1 ml/min	834522	5.789	3744	1.02
Column temp.	30 °C	25 °C	810784	4.361	4402	1.15
(± 5 °C)		30 °C	800394	4.496	4836	1.13
		35 °C	811160	4.808	5234	1.14
Mobile phase	40:60	35:65	997723	5.460	6592	1.11
Composition		40:60	988100	5.477	7013	1.14
(5% v/v)		45:55	940151	5.478	7637	1.14

TABLE 16: RESULTS OF SPECIFICITY STUDIES

S. no.	Solution	EMT		TNF		COB		ELV	
		Rt (min)	Peak area	Rt (min)	Peak area	Rt (min)	Peak area	Rt (min)	Peak area
1	Blank	-	-	-	-	-	-	-	-
2	Standard	2.304	1860533	2.691	88594	3.185	876844	4.537	786509
3	Placebo	-	-	-	-	-	-	-	-
4	Sample	2.497	1963042	2.916	93714	3.496	931537	5.316	823543

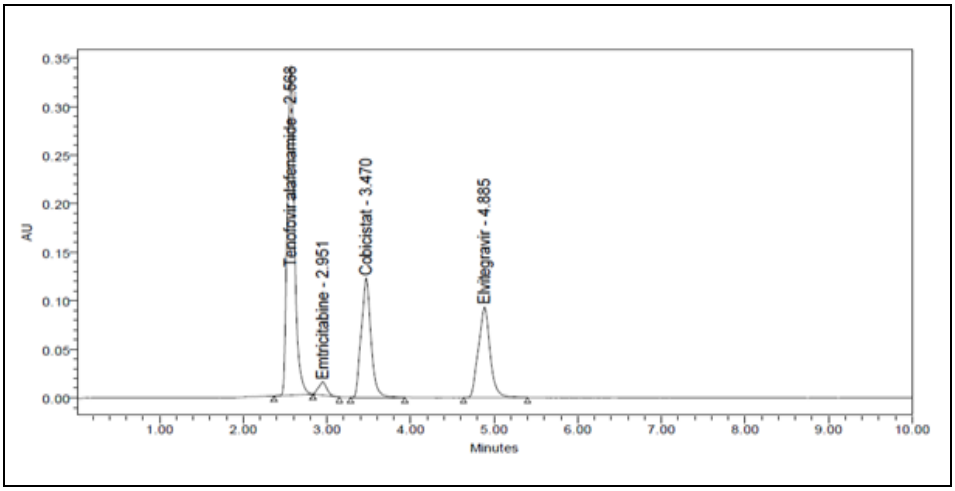

FIG. 22: HPLC CHROMATOGRAM OF ACID DEGRADED SAMPLE

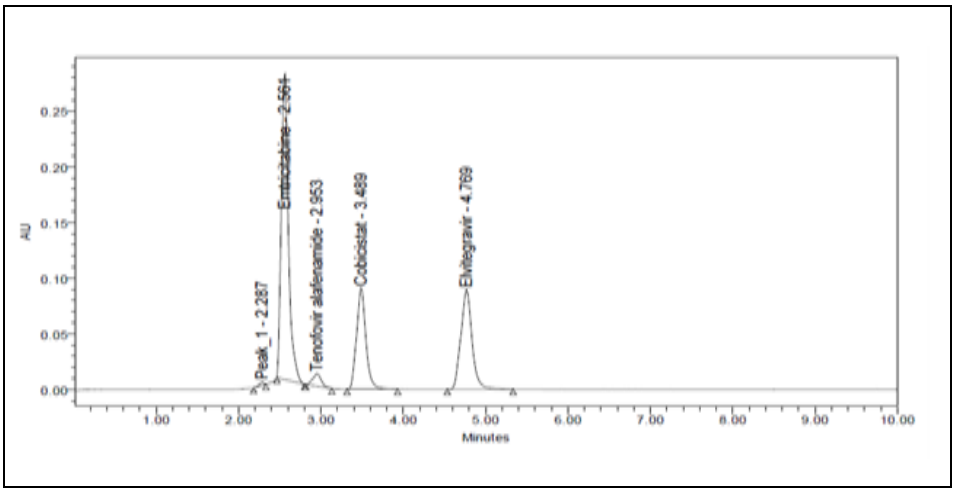

FIG. 23: HPLC CHROMATOGRAM OF ALKALI DEGRADED SAMPLE

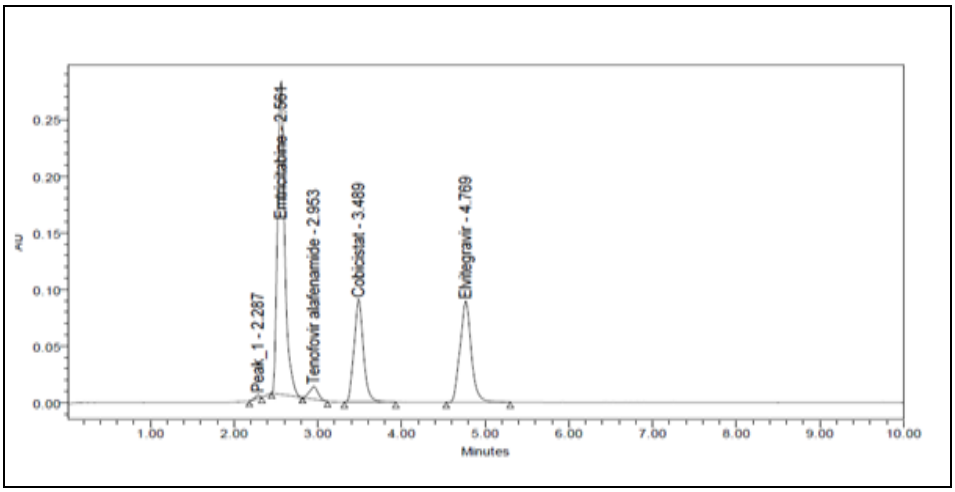

FIG. 24: HPLC CHROMATOGRAM OF PEROXIDE DEGRADED SAMPL

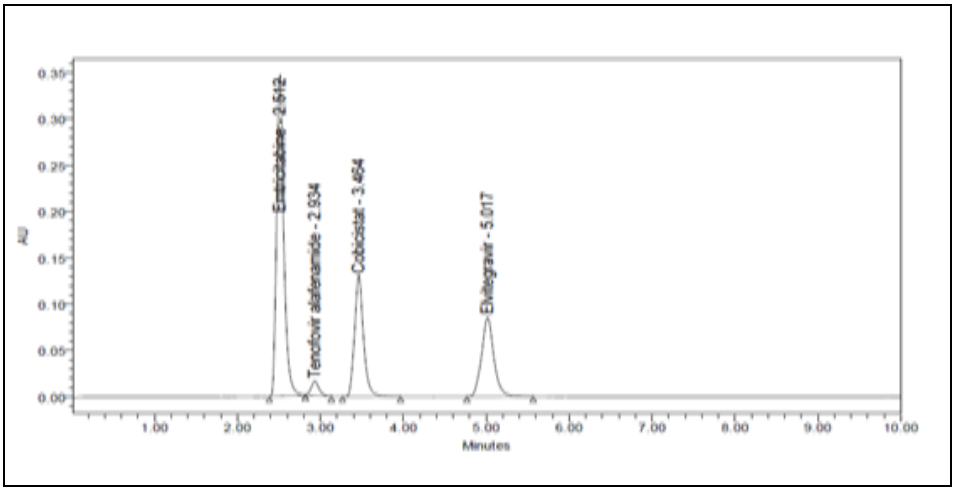

FIG. 25: HPLC CHROMATOGRAM OF THERMAL DEGRADED SAMPLE

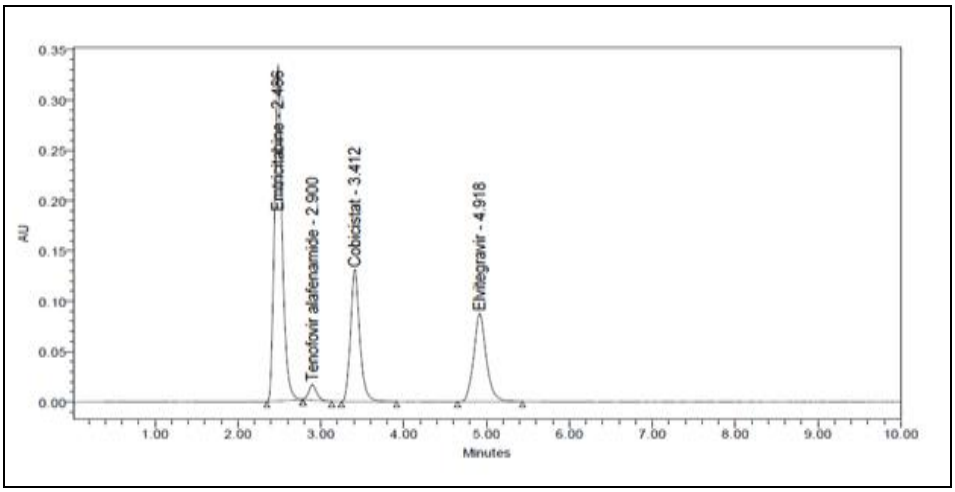

FIG. 26: HPLC CHROMATOGRAM OF UV DEGRADED SAMPLE

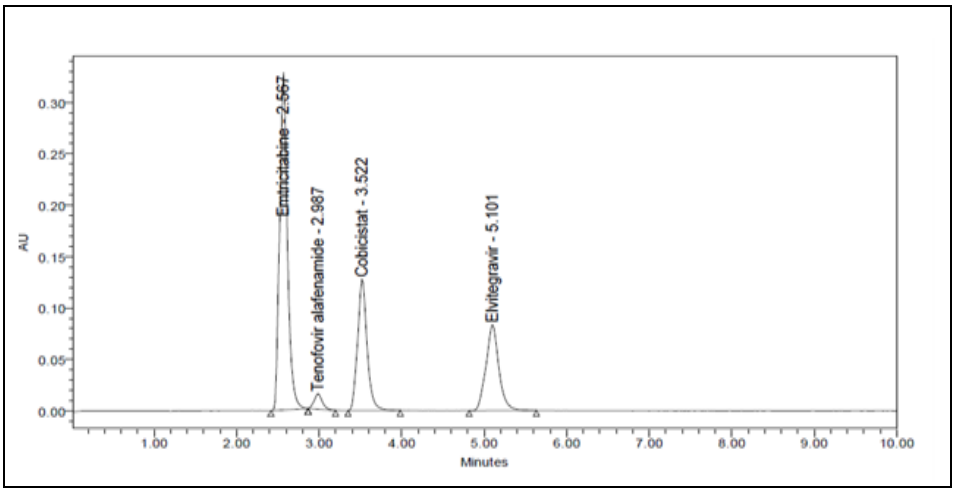

FIG. 27: HPLC CHROMATOGRAM OF NEUTRAL DEGRADED SAMPLE

TABLE 17: FORCED DEGRADATION STUDIES OF EMITRICITABINE

S. no.	Forced degradation condition	% Degradation	Peak purity
1	Acid stress	3.56	passes
2	Alkali stress	6.70	passes
3	Oxidation stress	5.50	passes
4	Thermal stress	2.40	passes
5	Photolytic Stress	1.51	Passes
6	Neutral stress	0.50	Passes

TABLE 18: FORCED DEGRADATION STUDIES OF TENOFOVIR

S. no.	Forced degradation condition	% Degradation	Peak purity
1	Acid stress	4.12	Passes
2	Alkali stress	7.42	Passes
3	Oxidation stress	8.23	Passes
4	Thermal stress	3.00	Passes
5	Photolytic Stress	2.77	Passes
6	Neutral stress	0.80	Passes

TABLE 19: FORCED DEGRADATION STUDIES OF COBICISTAT

S. no.	Forced degradation condition	% Degradation	Peak purity
1	Acid stress	4.61	passes
2	Alkali stress	4.61	passes
3	Oxidation stress	4.61	passes
4	Thermal stress	4.57	passes
5	Photolytic Stress	2.43	Passes
6	Neutral stress	0.40	Passes

TABLE 20: FORCED DEGRADATION STUDIES OF ELVITEGRAVIR

S. no.	Forced degradation condition	% Degradation	Peak purity
1	Acid stress	5.61	Passes
2	Alkali stress	5.41	Passes
3	Oxidation stress	5.54	Passes
4	Thermal stress	4.40	Passes
5	Photolytic Stress	4.73	Passes
6	Neutral stress	1.90	Passes

DISCUSSION:

System Suitability Studies: The % RSD for the peak areas of responses and retention times of five replicates injections of standard solutions of emtricitabine, tenofovir, cobicistat, and elvitegravir were found to be less than 2%.

The theoretical plates were more than 2000 for all the combinations. The tailing factor was found to be less than 2.0 and resolution between adjacent peaks was found to be more than 5.0.

Linearity of Detector Response: The detector response was found to be linear in the concentration range of 25-150 µg/ml of emtricitabine, 1.25-7.5 µg/ml of tenofovir, 18.75-112.5 µg/ml of cobicistat and 18.75-112.5 µg/ml of elvitegravir. The correlation coefficient values were found to be within the limits.

Precision:

System Precision Repeatability / Intra-Day Precision and Method Precision: The % RSD was determined for retention time and peak areas of emtricitabine, tenofovir, cobicistat and elvitegravir obtained from 6 replicates injections in the precision studies are consistent as evidenced by the values of % RSD below 2%. Hence, it can be calculated that the proposed RP-HPLC method was précised.

Accuracy Recovery: The mean % recovery from spiked samples was found to be in the range of 99.8-100.3% emtricitabine, 98.78-100.96% tenofovir, 99.4-100.38% cobicistat and 99.27-100.39% elvitegravir respectively, which were within the acceptance limit. The excellent mean recoveries and standard deviation suggested that the good accuracy of the proposed RP-HPLC method.

System Suitability: It was found that the system suitability parameters were within the limits at all variable conditions. From the results obtained, it can be concluded that the proposed RP-HPLC method is robust towards small variations.

Forced Degradation Studies: In all forced degradation samples, it was found that the peak purity of all 4 drugs passed peak purity and also found that the degradant peaks were well separated from the main analyte peaks. No significant degradant was observed under Thermal, Photolytic and neutral degradation studies emtricitabine, tenofovir, cobicistat, and elvitegravir were degraded below 5% without any major degradants.

Limit of Detection (LOD) and Quantification (LOQ): The LOD values were found to be 1.03 µg/ml, 0.03 µg/ml, 1.07 µg/ml and 0.44 µg/ml for emtricitabine, tenofovir, cobicistat, and elvitegravir respectively and LOQ values to found to be 3.13 µg/ml, 0.09 µg/ml, 3.23 µg/ml and 1.34 µg/ml for emtricitabine, tenofovir, cobicistat and elvitegravir respectively. These low LOD and LOQ values indicate that the proposed RP-HPLC method is sensitive.

CONCLUSION: The results of this investigation reveal that by applying the proposed RP-HPLC method, the retention times of emtricitabine, tenofovir, cobicistat and elvitegravir were found to be 2.304, 2.691, 3.185 and 4.137 min respectively. Quantitative linearity was obeyed in the concentration range of 25-150 µg/ml, 1.25-7.5 µg/ml, 18.75-112.5 µg/ml and 18.75-112.5 µg/ml with correlation coefficient value of 0.999. The %

RSD values obtained from the precision studies were also found to be less than 2, which indicates the precise method. The high % recoveries indicate that the proposed method was highly accurate. The low values of LOD and LOQ indicate the high sensitivity of the proposed method. The absence of interfering peaks observed in the chromatogram of blank and placebo interference studies indicates specific methods and degradants formed during stress degradation studies were also well separated and not interfere with estimation of the drugs by the proposed stability-indicating RP-HPLC method. From this study, it is concluded that the proposed stability indicating RP-HPLC method was found to be simple, accurate, precise, rapid and useful for the routine analysis of emtricitabine, tenofovir, cobicistat, and elvitegravir in bulk and pharmaceutical dosage forms. The obtained results were satisfactory as per ICH guidelines.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Pranitha D, Vanitha C, Francis P, Alagar Raja M, Vishnu Vardan P, Surendar M and David B: Simultaneous estimation of emtricitabine, tenofovir disoproxil fumarate and rilpivirine in bulk form by RP-HPLC method. J Pharm Res 2012; 5(8): 4600-02.
2. Anandakumar K, Kannan K and Vetrichelvan T: Development and validation of emtricitabine and tenofovir disoproxil fumarate in pure and fixed dose combination by UV spectroscopy. Digest Journal of Nanomaterial and Biostructure 2011; 6: 1085-90.
3. Narendra D, Satyanarayana T and Ganga RB: HPLC method development and validation for simultaneous estimation of tenofovir and emtricitabine in combined pharmaceutical dosage form. International J of Research in Pharmaand Biomedical Sciences 2012; 3: 361-67.
4. Palavan C, Ramprasad LA, Srinivasu P and Sheshagirirao JVLN: A new RP-HPLC method development for the simultaneous estimation of emtricitabine, efavirenz and tenofovir in tablet dosage form. American Journal of Pharma Tech Research 2013; 3: 547-54.
5. Ravendrababu VV, Pankaj KS and Singvi I: A new gradient liquid chromatographic method for simultaneous estimation of tenofovir disoproxil fumarate, cobicistat, emtricitabine and elvitegravir in bulk and tablet dosage form. Asian Journal of Chemistry 2014; 26: 6233-37.
6. Joshi M, Nikalje AP, Shahed M and Dehghan M: HPTLC method for the simultaneous estimation of emtricitabine and tenofovir in tablet dosage form. Indian Journal of Pharmaceutical Sciences 2009; 71: 95-97.
7. Manish G, Avinash K, Ravi Y and Tiwari P: Development and validation of UPLC method for emtricitabine, tenofovir and efavirenz in pharmaceutical preparation; Analytical Chemistry. An Indian Journal 2010; 9: 247-51.
8. Matt MK, Pilli NR and Rao JVNL: A validated liquid chromatography and tandem mass spectrometric method for simultaneous quantification of tenofovir, emtricitabine and efavirenz in human plasma and its pharmacokinetic application. Acta Chromatographica 2015; 1: 27-39.
9. Raju N and Begum S: Simultaneous RP-HPLC method for the estimation of the emtricitabine, tenofovir disoproxil fumerate and efavirenz in tablet dosage forms. Res J Pharm Tech 2008; 1: 522-5.
10. Delahunty T, Bushman L, Robbins B and Fletcher CV: The simultaneous assay of tenofovir and emtricitabine in plasma using LC/MS/MS and isotopically labeled internal standards. J Chromatogr B Analyt Technol Biomed Life Sci 2009; 877: 1907-14.
11. Mangoankar K and Desai A: Simultaneous estimation of emtricitabine, tenofovir disoproxil fumarate and efavirenz from tablets by reverse phase high-performance liquid chromatography method. Indian Drug 2008; 45(3): 188-92.
12. Joshi M, Nikalje AP, Shahed M and Dehghan M: HPTLC method for the simultaneous estimation of emtricitabine and tenofovir in tablet dosage form. Indian Journal of Pharmaceutical Sciences 2009; 71(1): 95-97.
13. Kaul N, Agrawal H, Paradkar AR and Mahadik KR: International conference on harmonization guidance in practice: stress degradation studies on lamivudine and development of a validated specific stability-indicating HPTLC assay method. Chromatographia 2004; 60(3-4): 213-21.
14. Jullien V, Tréluyer JM, Pons G and Rey E: Determination of tenofovir in human plasma by high-performance liquid chromatography with spectrofluorimetric detection. Journal of Chromatography B 2003; 785(2): 377-81.
15. Rezk NL, Crutchley RD and Kashuba ADM: Simultaneous quantization of emtricitabine and tenofovir in human plasma using high-performance liquid chromatography after solid-phase extraction. Journal of Chromatography B 2005; 822(1-2): 201-08.
16. T. Delahunty, L. Bushman, and C. V. Fletcher, Sensitive assay for determining plasma tenofovir concentrations by LC/MS/MS, Journal of Chromatography B, vol. 830, no. 1, pp.6–12, 2006.
17. Barkil ME, Gagnieu MC and Guitton J: Relevance of a combined UV and single mass spectrometry detection for the determination of tenofovir in human plasma by HPLC intherapeutic drug monitoring. Journal of Chromatography B 2007; 854(1-2): 192-97.

How to cite this article:
Sudha PDC, Sohail P and Avulapati U: Stability indicating RP-HPLC method for the simultaneous deter-mination of emitricitabine, tenofovir, cobicistat and elvitegravir. Int J Pharm Sci & Res 2020; 11(3): 1452-66. doi: 10.13040/IJPSR.0975-8232.11(3).1452-66.