CHAPTER - 7
BICTEGRAVIR, EMTRICITABINE AND TENOFOVIR ALAFENAMIDE

Bictegravir:

Bictegravir is a recently approved investigational drug that has been used in trials studying the treatment of HIV-1 and HIV-2 infection. It has been approved for HIV-1 monotherapy combined with 2 other antiretroviral in a single tablet (50 mg bictegravir, 200 mg emtricitabine, 25 mg tenofovir alafenamide).

Structure:

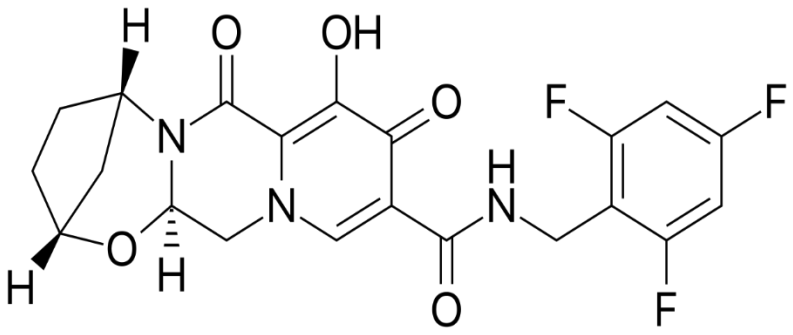

Fig. 7.1: Structure of Bictegravir

IUPAC Name	**:**	(1S,11R,13R)-5-hydroxy-3,6-dioxo-N-[(2,4,6-trifluorophenyl)methyl] -12-oxa-2,9-diazatetracyclo[11.2.1.02,11.04,9]hexadeca-4,7-diene-7-carboxamide
Molecular formula	**:**	$C_{21}H_{18}F_3N_3O_5$
Molecular Weight	**:**	449.386
Solubility	**:**	Soluble in ACN, Water, and Methanol
P^{ka}	**:**	9.81

Emtricitabine:

Emtricitabine, a drug approved by FDA for the treatment of HIV-1 and sold under the brand name Emtriva, a nucleoside reverse transcriptase inhibitor (NTRI). It is used both for adults and children with HIV infection.

Structure:

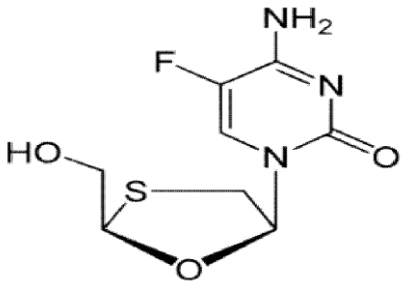

Fig. 7.2: Structure of Emtricitabine

IUPAC Name	**:**	4-amino-5-fluoro-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5- yl]-1, 2-dihydropyrimidin-2-one
Molecular formula	**:**	$C_8H_{10}FN_3O_3S$
Molecular Weight	**:**	247.248 g/mol
Solubility	**:**	Soluble in ACN, Water, and Methanol
P^{ka}	**:**	14.29

Tenofovir Alafenamide:

Tenofovir Alafenamide, sold under the brand name Vemlidy, a Nucleoside reverse transcriptase inhibitor (NTRI) and a prodrug of tenofovir. It was approved by FDA in 2016. It is used for the medical treatment of HIV infection along with Hepatitis-B, in the form of Tenofovir disoproxil fumarate (TDF). High antiviral activity and better distribution into lymphoid tissues can be seen by tenofovir alafenamide.

Structure:

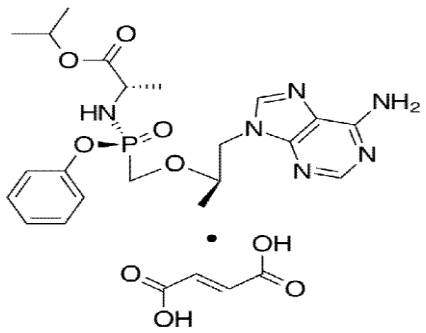

Fig. 7.3: Structure of Tenofovir alafenamide fumarate

IUPAC Name	**:**	Isopropyl (2S)-2-[[[(1R)-2-(6-aminopurin-9-yl)-1-methyl-ethoxy] methyl-phenoxy-phosphoryl] amino] propanoate
Molecular formula	**:**	$C_{21}H_{29}N_6O_5P$
Molecular Weight	**:**	476.466 g/mol
Solubility	**:**	Soluble in methanol, Acetonitrile and water
P^{ka}	**:**	11.36

MATERILAS AND METHODS

Table 7.1: List of chemicals and reference standards

S. No	Chemical/reagents	Grade make	Make
1	Orthophosphoric Acid (OPA	HPLC	HiMedia Laboratories Pvt. Ltd
2	Water	HPLC	Merk
3	Acetonitrile	HPLC	Merck
4	Triethylamine	HPLC	Merck
5	Potassium dihydrogen phosphate	AR	Thermo Fisher Scientific India Pvt.
6	Torsemide	Reference standard	Lupin labs Ltd
7	Spironolactone	Reference standard	Lupin labs Ltd

Table 7.2: List of instrument details

S. No	Instruments	Make/model
1	HPLC	Agilent Equipped with a UV-Visible detector
2	Column	Phenomenex Luna ODS (250 x 4.6 mm), 5µm
3	Pump	LC20AT
4	Detector	UV-2489
5	Analytical Balance	Shimadzu
6	pH meter	Range from 0-14 (Labindia 352)
7	Water bath Sonicator	Loba Life

PREPARATION OF SOLUTIONS:

Preparation of 0.1% Ortho phosphoric acid:

Pipette out 0.1 mL^{-1} of ortho phosphoric acid in 100 mL^{-1} of volumetric flask and make up the volume to 100 mL^{-1} with HPLC water.

PREPARATION OF STANDARD STOCK SOLUTION:

Preparation of bictegravir standard stock solution:

10 mg of bictegravir was weighed accurately and transferred into 10 mL^{-1} volumetric flask and dissolved in distilled water and then made up the remaining volume to 10 mL^{-1} with HPLC water to get the concentration of 1000 µg/ mL^{-1}.

Preparation of emtricitabine standard stock solution:

Weighed accurately 10 mg of emtricitabine and transferred into 10 mL^{-1} volumetric flask and dissolved in HPLC water and made up the volume with HPLC water to get the concentration of 1000µg/ mL^{-1}.

Preparation of tenofovir alafenamide standard stock solution:

Weighed accurately 10 mg of tenofovir alafenamide and transferred into 10 mL^{-1} volumetric flask and dissolved in HPLC water and made up with HPLC water to get the concentration of 1000 µg/ mL^{-1}.

PREPARATION OF WORKING STANDARD SOLUTION:

Preparation of bictegravir working standard solution:

Pipetted out 1 mL^{-1} of bictegravir standard stock solution into a 10 mL^{-1} volumetric flask. Diluted up to 10 mL^{-1} volume with mobile phase and mixed well. From working standard solutions, further dilutions were made up to get concentrations up to 5-160 µg/ mL^{-1}.

Preparation of emtricitabine working standard solution:

Pipetted out 1 mL^{-1} of emtricitabine standard stock solution into a 10 mL^{-1} volumetric flask. Diluted up to 10 mL^{-1} volume with mobile phase and mixed well. From working standard solutions, further dilutions were made up to 5-160µg/ mL^{-1}.

Preparation of tenofovir alafenamide standard solution:

Pipetted out 1 mL^{-1} of tenofovir alafenamide standard stock solution into a 10 mL^{-1} volumetric flask. Diluted up to 10 mL^{-1} volume with mobile phase and mixed well. From working standard solutions, further dilutions were made up to 5-160 µg/ mL^{-1}.

Preparation of sample solution:

The formulation containing bictegravir 200 mg, emtricitabine 100 mg and tenofovir alafenamide 25mg. Weighed 20 tablets and determined the average weight and crushed to fine powder. Weighed accurately tablet powder equivalent to 31.6 mg of bictegravir, 35 mg of emtricitabine and 1.5 mg of tenofovir alafenamide transferred into 10 mL^{-1} volumetric flask, and dissolve it in HPLC water and make the final volume to 10 mL^{-1} with water. From the solution 1 mL^{-1} was pipetted out into a 10 mL^{-1} volumetric flask and made up to 10 mL^{-1} with mobile phase and used for further dilutions.

METHOD DEVELOPMENT AND OPTIMIZATION

Method development involves in the evaluation and optimization of the various stages of sample preparation, chromatographic separation, qualification and quantification. Optimization of various parameters was performed in order to develop a selective and sensitive method for analysis on HPLC using UV detection.

Trial 1:

Chromatographic conditions:

Mobile phase	:	Methanol and orthophosphoric acid taken in the ratio 75:25
Flow rate	:	1.0 mL^{-1} /min
Column	:	Phenomenex, ODS 250 x 4.6 mm 5µm.
Detector wave length	:	280nm
Column temperature	:	30°C
Injection volume	:	20µg/ mL^{-1}
Run time	:	20 min
Observation	:	The peaks were not resolved

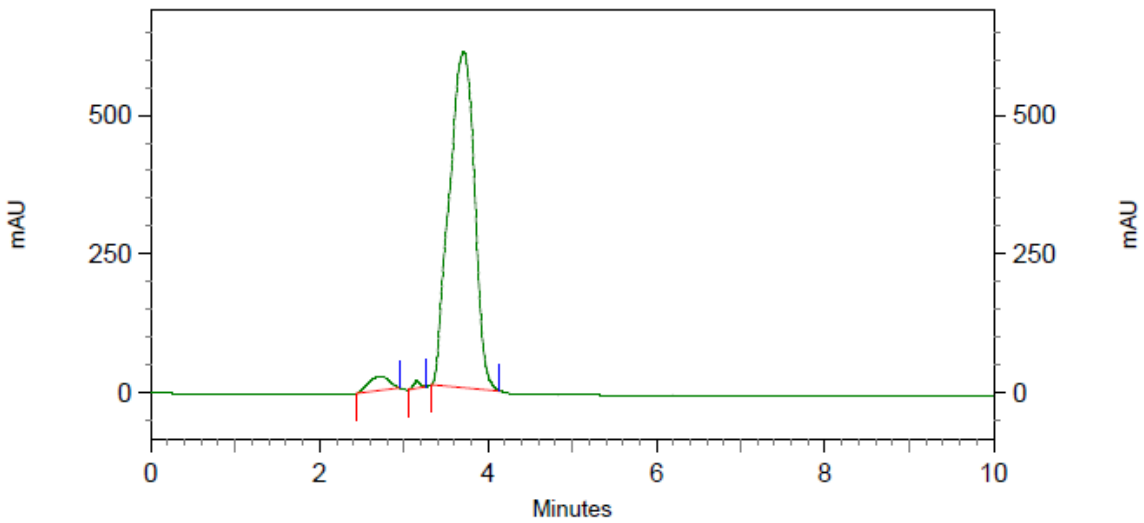

Fig. 7.4: Chromatogram for Trail-I

Trial 2:

Chromatographic conditions	**:**	
Mobile phase	**:**	Acetonitrile and ortho phosphoric acid taken in the ratio 60:40
Flow rate	**:**	0.8 mL^{-1} /min
Column	**:**	Phenomenex, ODS 150 x 4.6 mm 5µm.
Detector wave length	**:**	270 nm
Column temperature	**:**	30°C
Injection volume	**:**	20µg/ mL^{-1}
Run time	**:**	10 min
Observation	**:**	three peaks are resolved with splitting

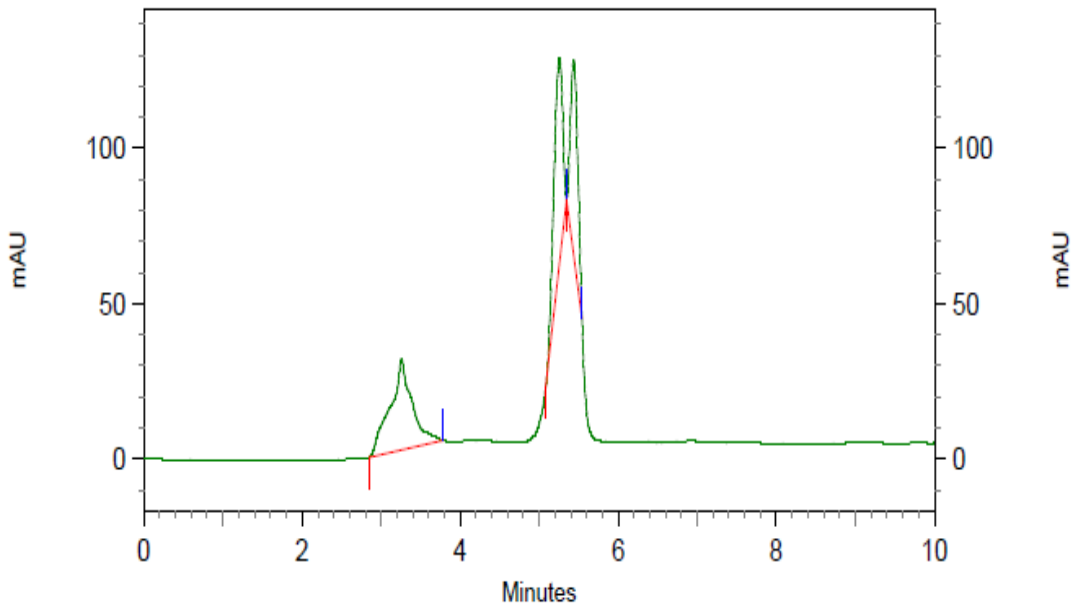

Fig. 7.5: Chromatogram for trail-II

Trial 3:

Chromatographic conditions	:	Acetonitrile and ortho phosphoric acid taken in the ratio 60:40
Mobile phase	:	Acetonitrile and ortho phosphoric acid taken in the ratio 60:40
Flow rate	:	0.8 mL^{-1} /min
Column	:	Phenomenex, ODS 150 x 4.6 mm 5µm.
Detector wave length	:	270nm
Column temperature	:	30°C
Injection volume	:	20 μL^{-1}
Run time	:	10 min
Observation	:	Peaks tailing and fronting were observed for three drugs

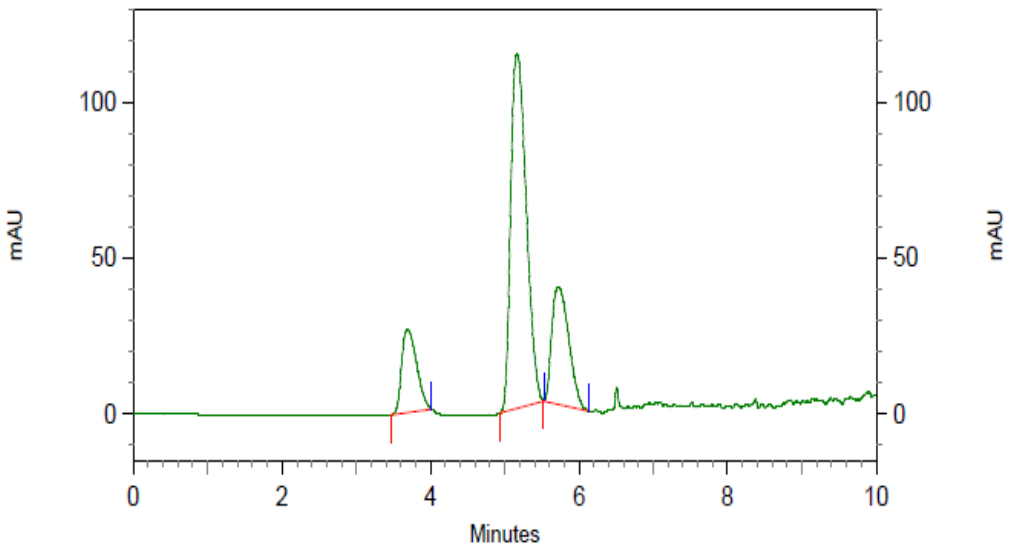

Fig. 7.6: Chromatogram for trail-III

Trial 4:

Chromatographic conditions:	:	
Mobile phase	:	Acetonitrile and ortho phosphoric acid in the ratio of 70:30
Flow rate	:	0.8 mL^{-1} /min
Column	:	Phenomenex, ODS 150 x 4.6 mm 5µm.
Detector wave length	:	270 nm
Column temperature	:	30°C
Injection volume	:	20µg/ mL^{-1}
Run time	:	10 min
Observation	:	Three peaks are resolved fronting and tailing are observed for levodopa and carbidopa

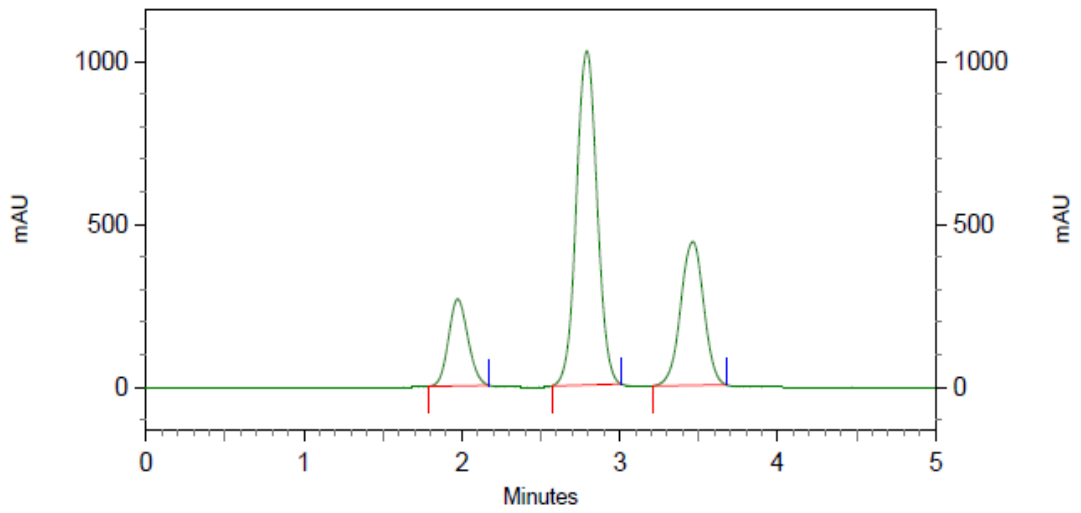

Fig. 7.7: Chromatogram for trail-IV

Choice of stationary phase

Initially the separation was tried with different columns having different dimensions like diameter and length and pore size. Finally good separation with finest peak shape was achieved with the analytical column Inertsil ODS-C_{18}; 5µm (4.6 X 250mm).

Chromatographic conditions:	**:**	
Mobile phase	**:**	Acetonitrile and 0.1% Ortho phosphoric acid in the ratio of 50:50 (v/v/)
Flow rate	**:**	1.0 mL^{-1} /min
Column	**:**	Phenomenex, ODS 150 x 4.6 mm 5µm.
Detector wave length	**:**	270 nm
Column temperature	**:**	30°C
Injection volume	**:**	20 μL^{-1}
Run time	**:**	5 min
Observation	**:**	Peaks tailing and fronting were not observed for three drugs

Selection of mobile phase

Several systematic test plans were performed to optimize the mobile phase. Different solvents like methanol, water and acetonitrile in different ratios and different pH values of the mobile phase ratios, by using different buffer solutions in order to get sharp peak and base line separation of the components and without interference of the excipients. Satisfactory peak symmetry, resolved and free from tailing was obtained in mobile phase acetonitrile: methanol: 0.1% triethylamine buffer (pH-3.0) 25:35:40 (v/v/v).

Selection of the mobile phase flow rate

Flow rates of the mobile phase were changed from 0.5-1.0 ml/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.0 mL/min flow rate was ideal for the successful elution of the analyte.

Optimized Chromatographic onditions: After series of trials, the chromatographic conditions was accomplished with following

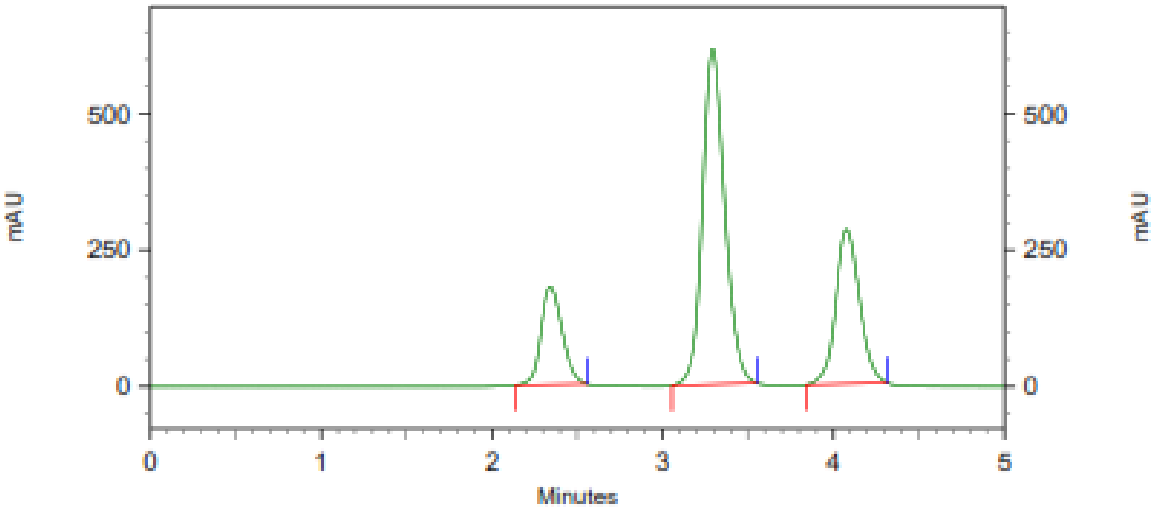

Fig. 7.8: Optimized chromatogram of Bictegravir, Emtricitabine and Tenofovir alafenamide

Discussion: Bictegravir, Emtricitabine and Tenofovir alafenamide were eluted at 2.56 min, 3.57 min and 3.503 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

METHOD VALIDATION

After the development of RP-HPLC method for the estimation of drug in a dosage form, validation of the method was performed. This section describes the procedure followed for validation of the developed method.

Specificity

Interference was not observed with the standard peaks and the chromatograms of Standard and Sample were identical with same retention time.

Acceptance Criteria:

No Interference should be observed at the retention time of standard peaks in the blank.

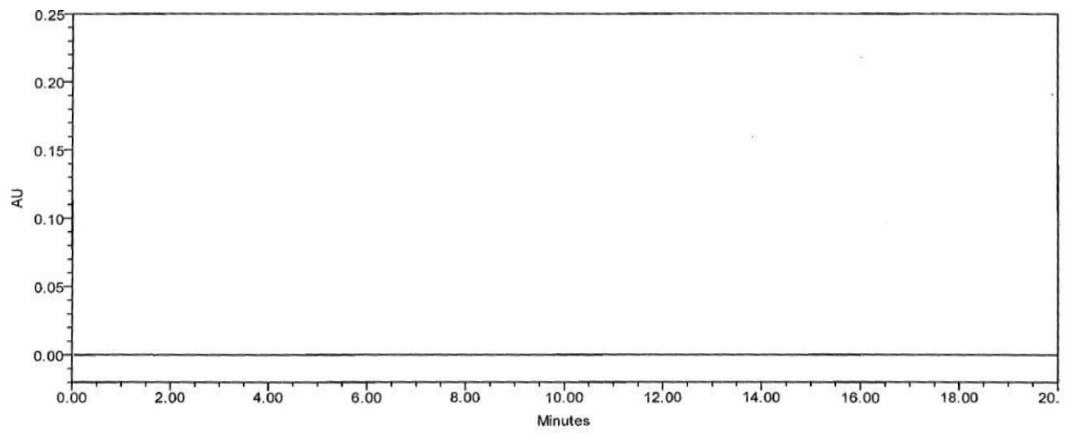

Fig. 7.9: Chromatogram of blank

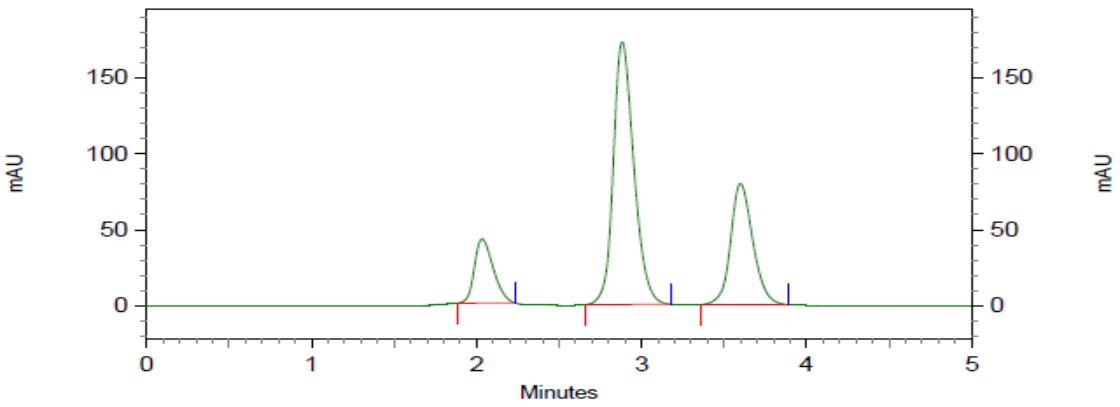

Fig. 7.10: Chromatogram of Standard

System Suitability

System suitabiliy of %Relative standard deviation of individual area response of six replicate injections for bictegravir, emtricitabine and tenofovir alafenamide was found to be 0.42 and 0.84 respectively. The %Relative standard deviation of areas of six replicate injections for bictegravir, emtricitabine and tenofovir alafenamide were found to be within limits. The tailing factor for bictegravir, emtricitabine and tenofovir alafenamide peaks was found to be 1.19 and 0.97 respectively. The tailing factor for bictegravir, emtricitabine and tenofovir alafenamide peaks was found to be within limits. The number of theoretical plates for bictegravir, emtricitabine and tenofovir alafenamide were found to be 2227 and 3036 respectively. The resolution was found to be 4.6 respectively which are well within the limits.

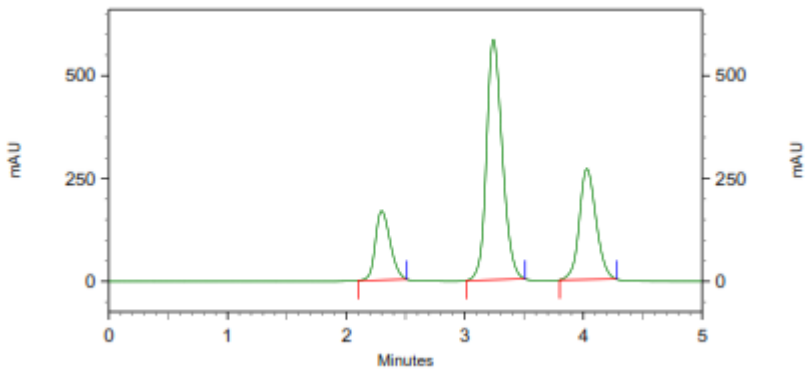

Fig. 7.11: Standard chromatogram of bictegravir, emtricitabine and tenofovir alafenamide

Table 7.3: System suitability Data

S. No	Injection Number	Peak area for Bictegravir	Peak area for Emtricitabine	Peak area for Tenofovir alafenamide	Acceptance criteria
1	01	24712691	91156709	45245759	The % RSD of peak areas of Bictegravir and Emtricitabine and Tenofovir alafenamide should not be more than 2.0
2	02	24463324	91701339	44503119	
3	03	24246704	91987990	45054196	
4	04	24549943	90158178	44649392	
5	05	24711992	91329540	45123345	
6	06	24230160	91947369	45049506	
Mean		24485802.33	91380187.5	44937552.83	
%RSD		0.875502296	0.748647825	0.650627105	

System suitability parameters		Observed value		
	Bictegravir	Emtricitabine	Tenofovir alafenamide	Acceptance criteria
The Tailing for bictegravir, emtricitabine and tenofovir alafenamide in standard solution	1.2	1.24	0.94	NMT 2.0
Theoretical plates for bictegravir, emtricitabine and tenofovir alafenamide in standard solution	2125	2338	2832	NLT 2000
Retention time (min)	2.56	3.57	4.42	NA

Precision

The precision of the Relative standard deviation of individual area of bictegravir, emtricitabine and tenofovir alafenamide were found to be within limits.

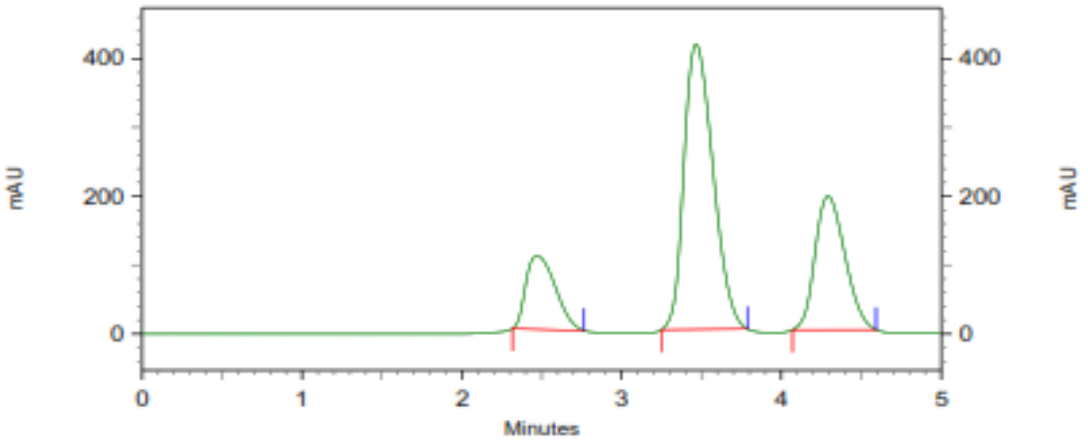

Fig. 7.12: Chromatogram of 20µg/ml

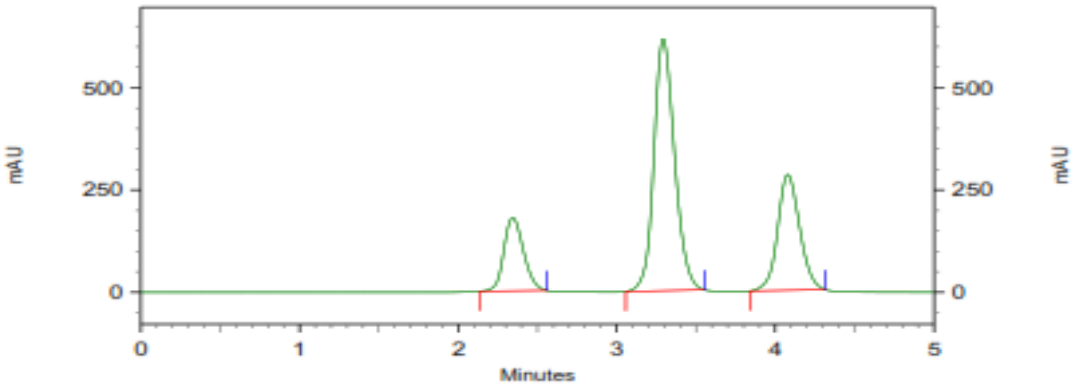

Fig. 7.13: Chromatogram of 40µg/ml

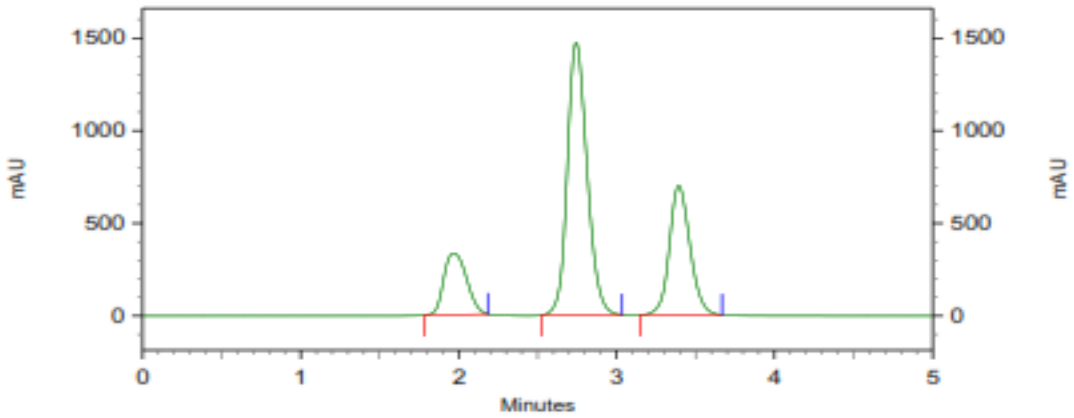

Fig. 7.14: Chromatogram of 80µg/ml

Intra-day Precision

Table 7.4: Intra-day Precision for bictegravir, emtricitabine and tenofovir alafenamide

S. No.	Injection Number	Peak area for Bictegravir	Peak area for Emtricitabine	Peak area for Tenofovir alafenamide
1	Standard 1	12721084	45439179	21306671
2	Standard 2	12489075	46463093	22042369
3	Standard 3	12556257	46757807	21895016
4	Standard 4	12412413	46606277	22066512
5	Standard 5	12617309	47179161	21478451
6	Standard 6	12899981	46894191	21620082
Mean		12616019.83	46556618	21734850.2
%RSD		1.385726869	1.28942453	1.44393721

Inter-day Precision

Table 7.5: Inter-day Precision for bictegravir, emtricitabine and tenofovir alafenamide

S. No.	Injection Number	Peak area for Bictegravir	Peak area for Emtricitabine	Peak area for Tenofovir alafenamide
1	Standard 1	12412413	46606277	22066512
2	Standard 2	11917309	45179161	21478451
3	Standard 3	11998917	46094191	21620082
4	Standard 4	12099845	46285956	21487812
5	Standard 5	12189456	45923107	21885217
6	Standard 6	12090459	45964490	21927085
Mean		12118067	46008863.67	21744193.17
%RSD		1.416538	1.037234331	1.143239276

Acceptance Criteria:

The Relative standard deviation of individual area of bictegravir, emtricitabine and tenofovir alafenamide from six standard preparations should be not more than 2.0%.

Ruggedness: The method was performed. It was found to be rugged and % of RSD (less than 2) indicating ruggedness of the method.

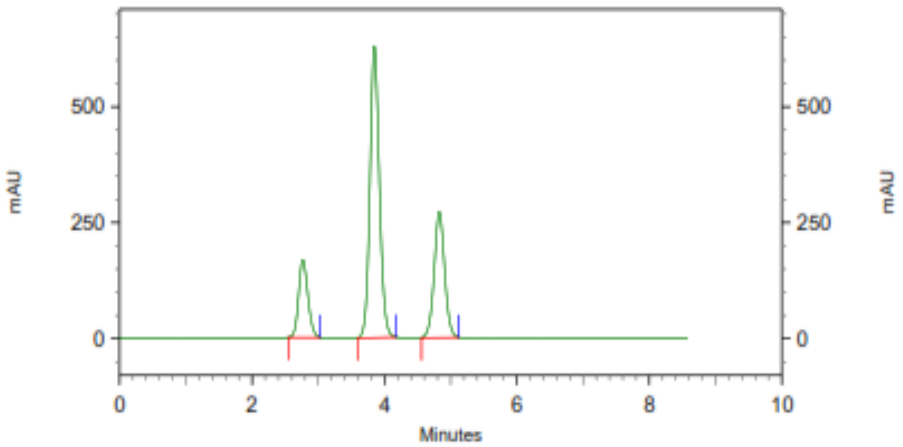

Fig. 7.15: Chromatogram of 40µg/ml

Table 7.6: Report of Ruggedness

S. No.	Concentration (µg/ mL^{-1}) of Bictegravir, Emtricitabine and Tenofovir alafenamide	Peak area of Bictegravir	Peak area of Emtricitabine	Peak area of Tenofovir alafenamide
1	40	19683049	78152534	37799115
2		19243134	79688983	38748417
3		18954123	78687933	37806560
4		19589796	76992656	37799664
5		19430985	78813195	38020838
6		19794762	77793090	37273195
		Avg: **19449308**	**Avg**: **78354732**	**Avg**: **37907965**
		Std Dev: 310406.78	**Std Dev:** 928764.10	**Std Dev:** 480810.4
		%RSD: 1.60	**%RSD:** 1.19	**%RSD:** 1.275

Linearity: For linearity, Six linear concentrations of bictegravir, emtricitabine and tenofovir alafenamide (5-160µg/ml) were injected in a triplicate manner. Average areas were mentioned above and linearity equations obtained for bictegravir, was y = 641469x, emtricitabine was y = 2E+06x and Tenofovir alafenamide was y = 1E+06x. Correlation coefficient obtained for tenofovir alafenamide 0.9992, for Emtricitabine was 0.9998 and for Tenofovir alafenamide 0.9983.

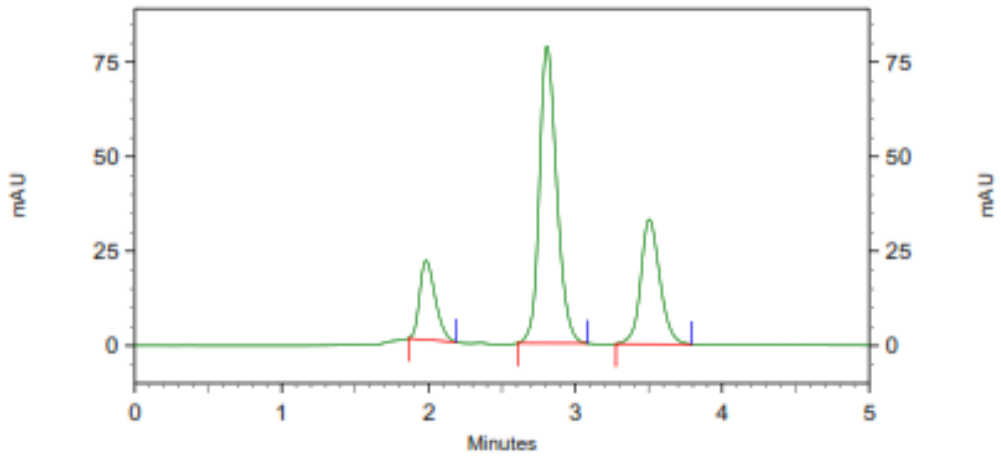

Fig. 7.16: Chromatogram of 5 µg/ml

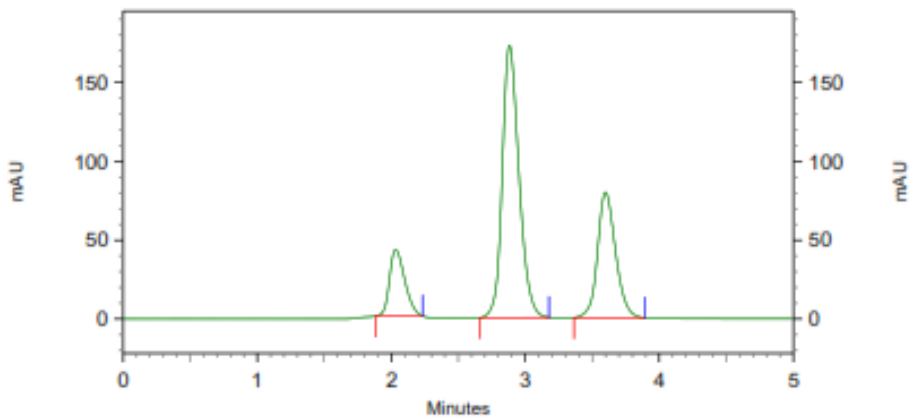

Fig. 7.17: Chromatogram of 10 µg/ml

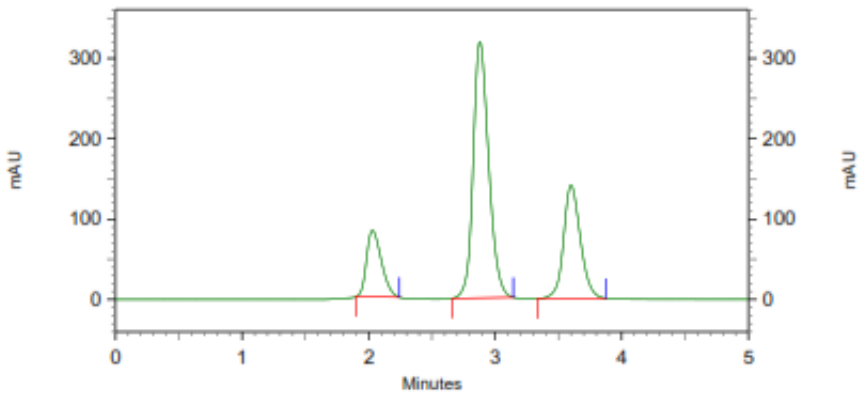

Fig. 7.18: Chromatogram of 20 µg/ml

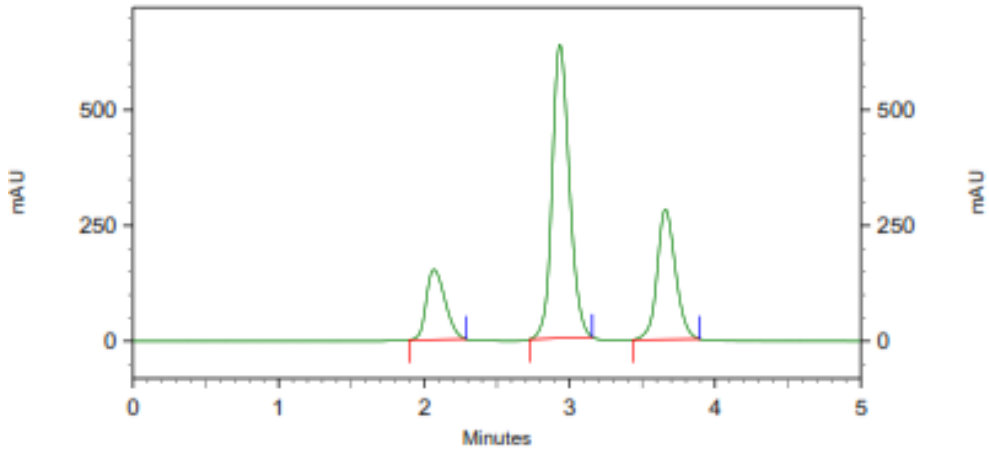

Fig. 7.19: Chromatogram of 40 µg/ml

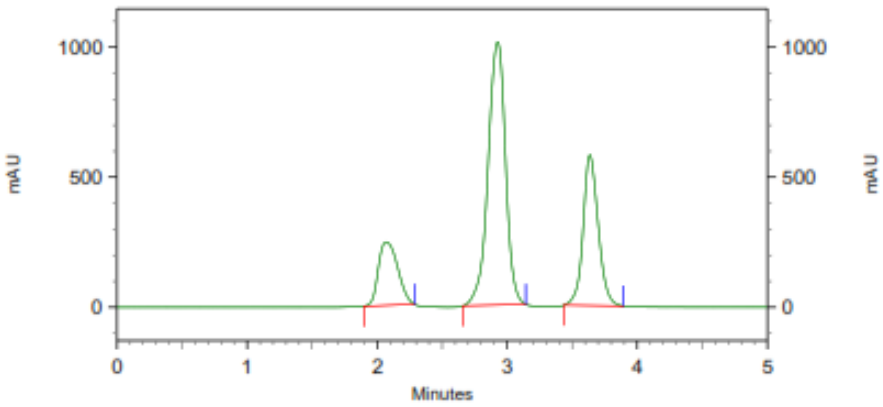

Fig. 7.20: Chromatogram of 80 µg/ml

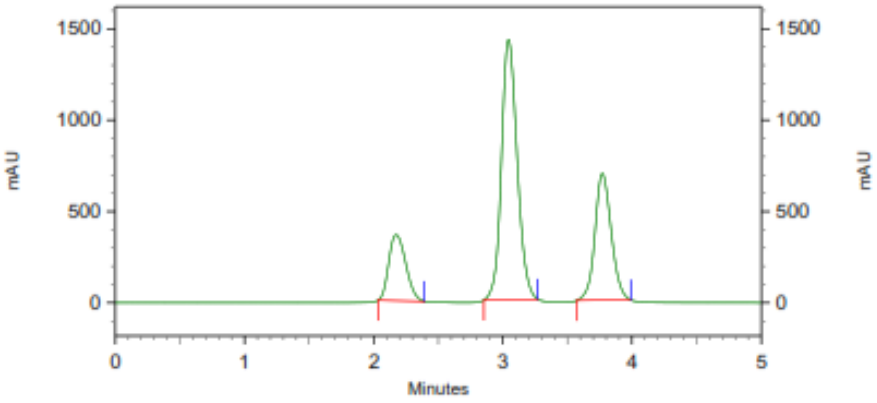

Fig. 7.21: Chromatogram of 160 µg/ml

Table 7.7: Linearity data

Standard concentration (µg/ml)	Area of Bictegravir	Standard concentration (µg/ml)	Area of Emtricitabine	Standard concentration (µg/ml)	Area of Tenofovir alafenamide
5	2603223	5	10853436	5	5002571
10	5807380	10	25098862	10	12524809
20	12333608	20	47564490	20	21927085
40	25769809	40	87897778	40	41351588
80	49232355	80	178592405	80	95516553
160	103768711	160	359415503	160	192814527
Regression $R^2 = 0.9992$		**Regression $R^2 = 0.9998$**		**Regression $R^2 = 0.9983$**	

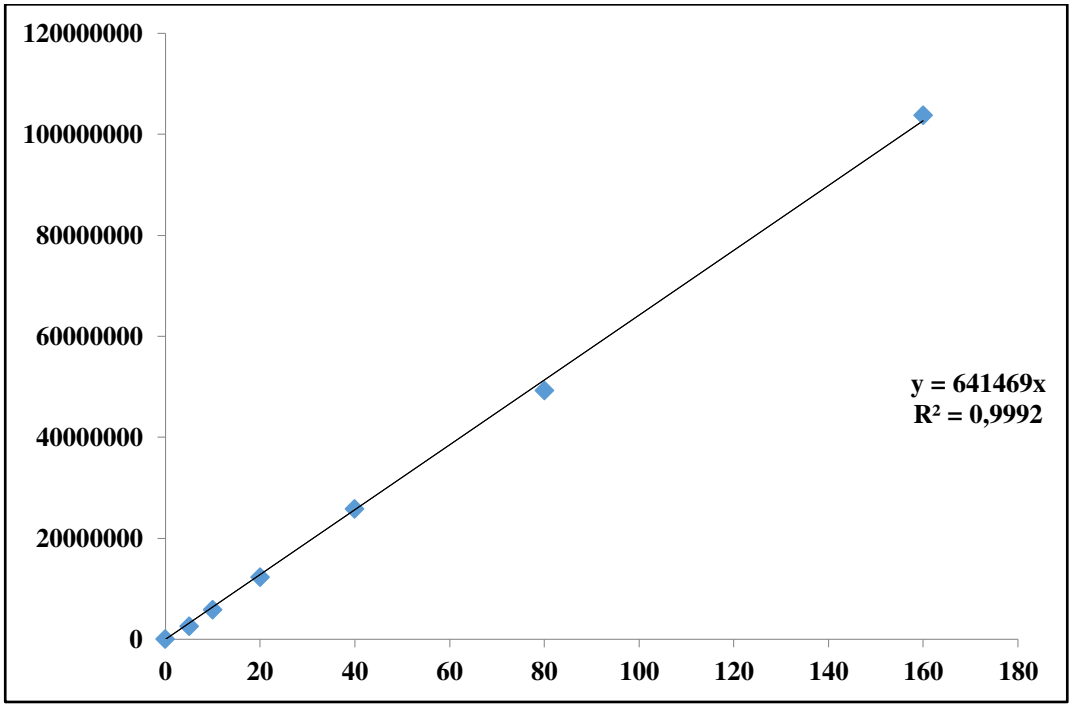

Fig. 7.22: Calibration curve for Bictegravir

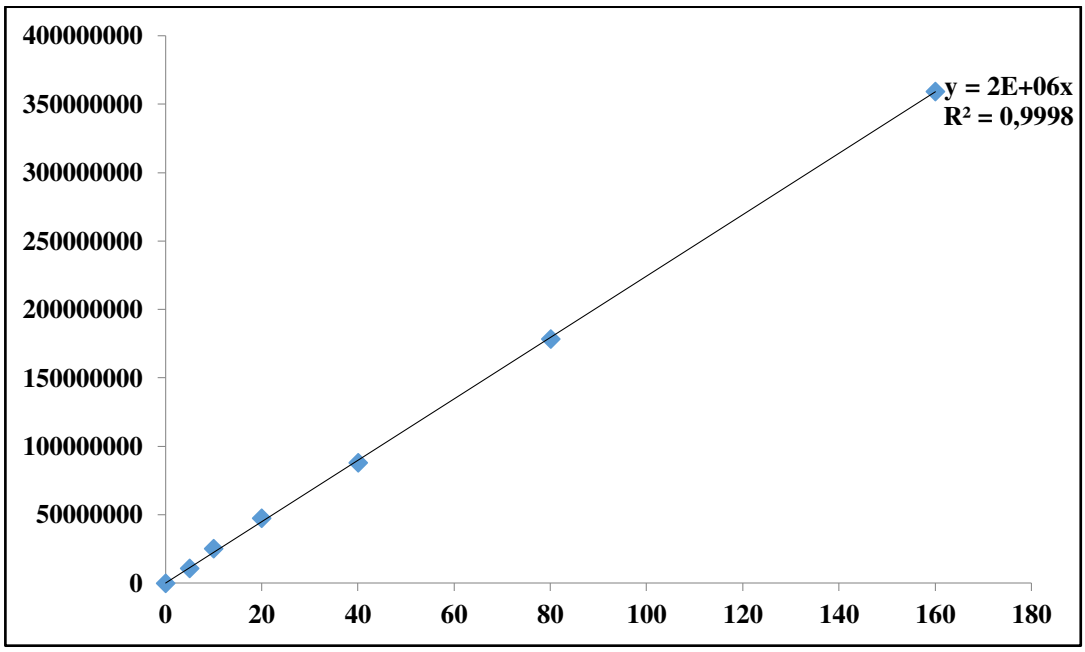

Fig. 7.23: Calibration curve for Emtricitabine

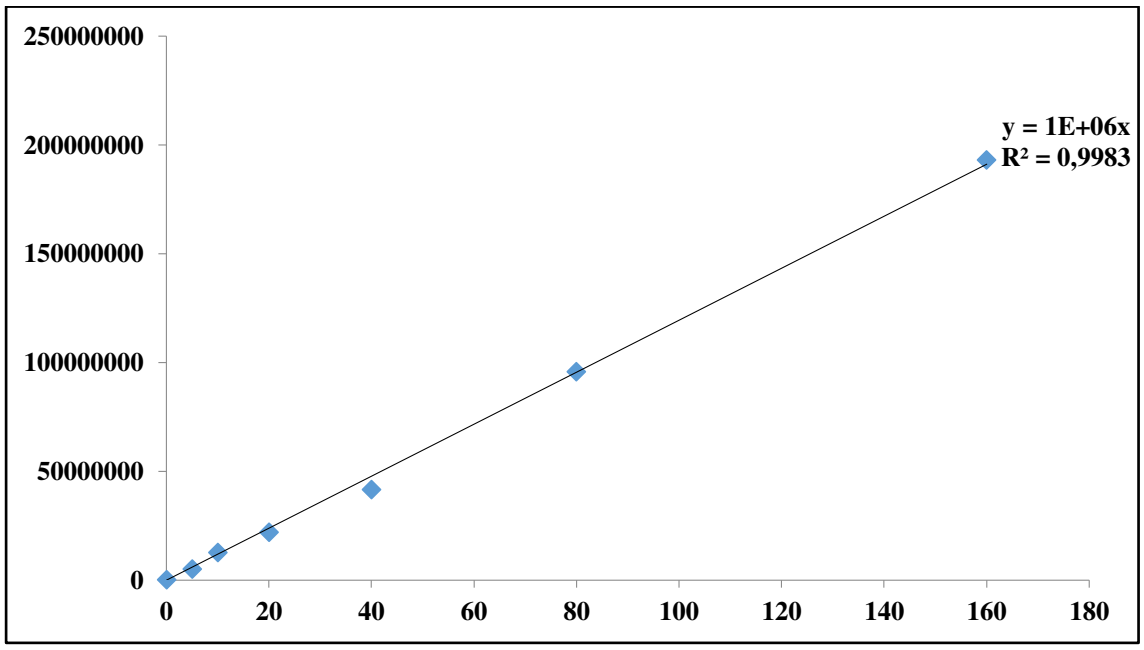

Fig. 7.24: Calibration curve for Tenofovir alafenamide

Acceptance Criteria: The Correlation coefficient should not be less than 0.997.

LOD and LOQ

LOD and LOQ of bictegravir, emtricitabine and tenofovir alafenamide were found be 0.89, 1.32, 1.03 and 2.72, 4.00, 3.13, respectively.

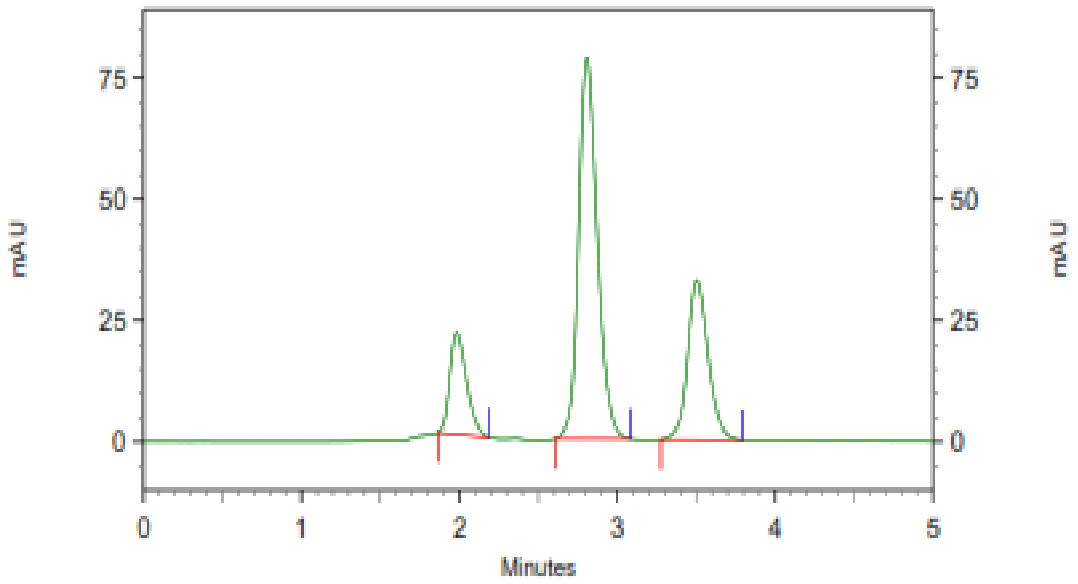

Fig. 7.25: Chromatogram of LOQ

Table 7.8: Report of LOD and LOQ

S. No.	Drugs	LOD (µg/ml)	LOQ (µg/ml)
1	Bictegravir	0.89	2.72
2	Emtricitabine	1.32	4.00
3	Tenofovir alafenamide	1.03	3.13

Accuracy:

The mean % Recovery of were found be 0.89, 1.32, 1.03 and 2.72, 4.00, 3.13, respectively, were found to be within limits at each level.

The % RSD of recovery of were found be 0.89, 1.32, 1.03 and 2.72, 4.00, 3.13, respectively, from the three sample preparations was found to be 0.40 and 1.22 at 50% level and 0.39 and 0.54 at 150% level respectively.

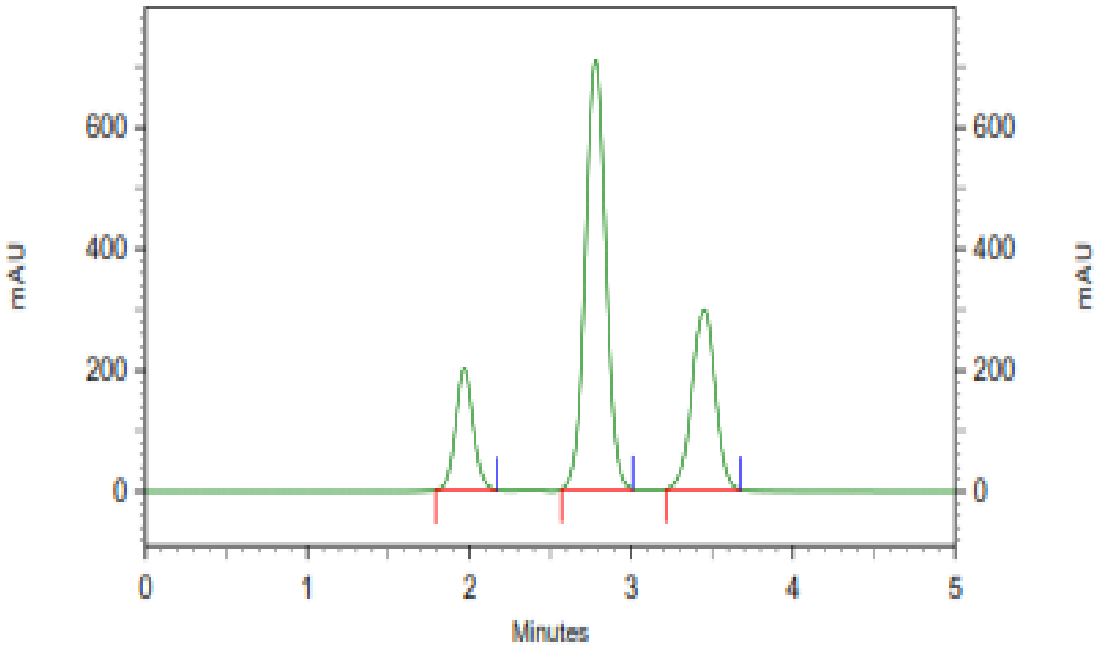

Fig. 7.26: Chromatogram of 60µg/ml

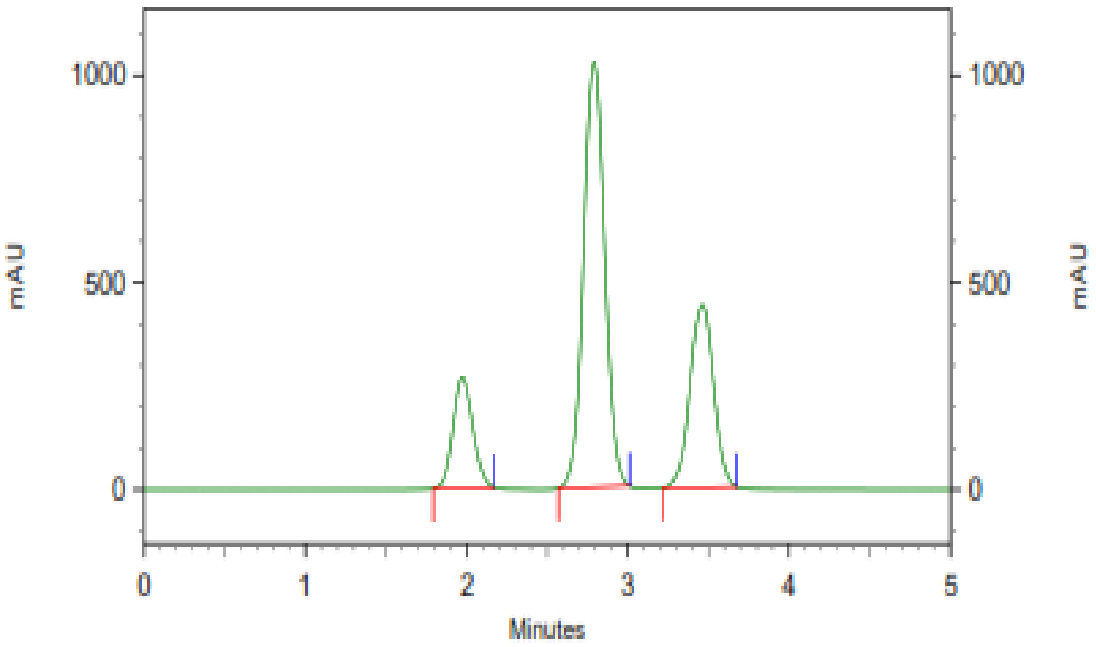

Fig. 7.27: Chromatogram of 80µg/ml

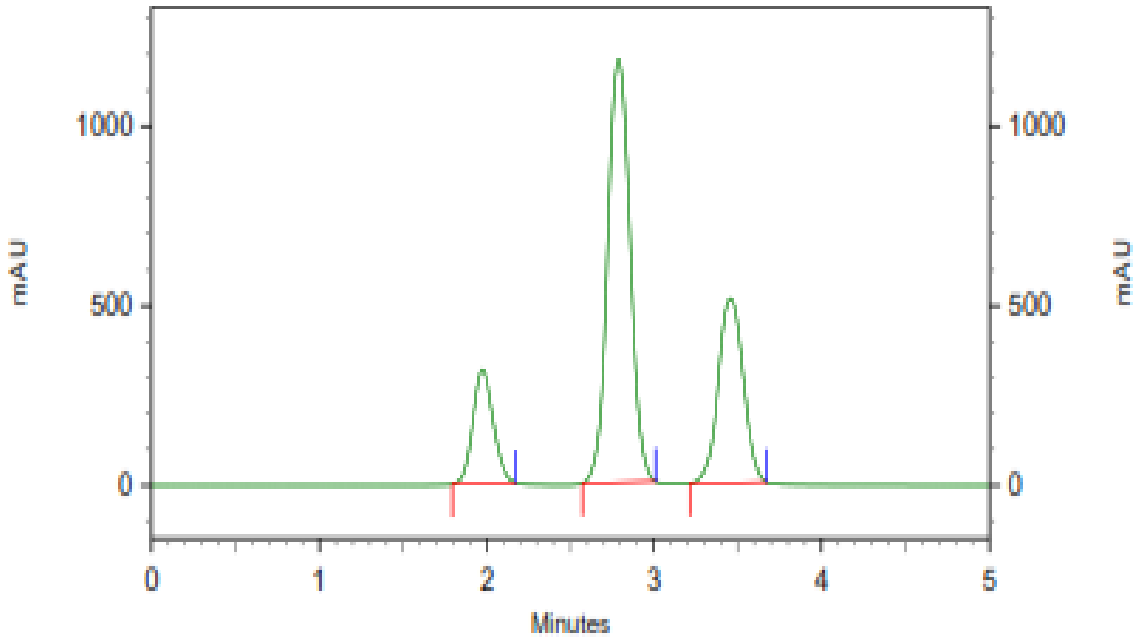

Fig. 7.28: Chromatogram of 100µg/ml

Table 7.9: Accuracy data

% Recovery	Target conc (µg/ mL^{-1})	Spiked conc (µg/ mL^{-1})	Final conc (µg/ mL^{-1})	Conc obtained in Bictegravir	% of Assay in Bictegravir	Conc obtained in Emtricitabine	% of Assay in Emtricitabine	Con obtained in Tenofovir alafenamide	% of assay in Tenofovir alafenamide
50	40	20	60	59.985	99.97	59.545	99.24	59.994	99.99
	40	20	60	59.825	99.71	59.549	99.25	59.966	99.94
	40	20	60	60.556	100.93	60.008	100.01	60.097	100.16
100	40	40	80	80.262	100.33	80.000	100.00	80.010	100.01
	40	40	80	80.058	100.07	80.459	100.57	79.308	99.13
	40	40	80	80.106	100.13	80.918	101.15	79.334	99.17
150	40	60	100	99.236	99.24	100.000	100.00	100.000	100.00
	40	60	100	99.379	99.38	104.052	104.05	100.107	100.11
	40	60	100	99.334	99.33	99.465	99.47	100.436	100.44

Acceptance Criteria:

The mean % Recovery of were found be 0.89, 1.32, 1.03 and 2.72, 4.00, 3.13, respectively, at each level should be not less than 95.0% and not more than 105.0%.

The %RSD of recovery of were found be 0.89, 1.32, 1.03 and 2.72, 4.00, 3.13, respectively, from the three sample preparations at 50% and 150% levels should not be more than 5.0%.

Robustness:

From the obtained values %RSD was found to be within the range of 0.6%-1.2% which states the method is acceptable.

Table 7.10: Report of Robustness – Bictegravir

S. No.	Parameter	Condition	System suitability results		
			% RSD	USP tailing	USP Plate Count
1	Flow rate by ± 10%	1.2 ml	0.94	0.99	2878
		1.0 ml	1.05	0.83	2695
		1.4 ml	1.10	1.01	2308
2	Column Oven temperature by ± 5°C	20°C	1.00	1.02	2603
		25°C	0.95	1.11	3256
		30°C	0.82	1.23	3968
3	Wavelength of analysis ± 5nm	275 nm	0.59	1.10	2965
		270 nm	0.66	1.14	2664
		265 nm	0.80	1.01	2723
4	Organic composition of mobile phase by ± 5%	55:55	0.65	1.23	2527
		50:50	0.78	1.14	2692
		45:55	0.85	1.12	3052

Table 7.11: Report of of Robustness – Levodopoa

S. No.	Parameter	Condition	System suitability results		
			% RSD	USP tailing	USP Plate Count
1	Flow rate by ± 10%	1.2 ml	1.05	1.21	3638
		1.0 ml	1.11	1.23	3410
		1.4 ml	1.20	1.50	2308
2	Column Oven temperature by ± 5°C	20°C	0.96	1.24	2603
		25°C	0.85	1.22	2850
		30°C	0.86	1.04	2652
3	Wavelength of analysis ± 5nm	275 nm	0.99	0.91	2921
		270 nm	0.81	0.96	3652
		265 nm	0.79	0.86	2121
4	Organic composition of mobile phase by ± 5%	55:55	0.69	0.83	2542
		50:50	0.58	0.86	2721
		45:55	0.72	0.79	2533

Table 7.12: Report of of Robustness - Carbidopoa

S. No.	Parameter	Condition	System suitability results		
			% RSD	USP tailing	USP Plate Count
1	Flow rate by ± 10%	1.2 ml	1.18	0.55	2531
		1.0 ml	1.05	0.68	2456
		1.4 ml	1.15	0.70	3210
2	Column Oven temperature by ± 5°C	20°C	1.22	1.32	2900
		25°C	1.14	1.21	2533
		30°C	1.17	1.17	2411
3	Wavelength of analysis ± 5nm	275 nm	0.56	0.86	2865
		270 nm	0.72	0.84	2456
		265 nm	0.65	0.79	2741
4	Organic composition of mobile phase by ± 5%	55:55	0.79	0.76	2648
		50:50	0.73	0.68	2315
		45:55	0.75	0.82	2145

Acceptance criteria:

% RSD should not be more than 2%. Theoretical plates should not less than 2000. Tailing factor should not more than 2.0.

ASSAY:

The commercial marketed formulation containing 200 mg of Bictegravir, and 100 mg Emtricitabine and 25 mg of Tenofovir alafenamide. The sample solution was treated same as standard solution. The solutions were injected into HPLC.

Six replicates of the samples solutions were injected for quantitative analysis .The amounts of bictegravir, emtricitabine and tenofovir alafenamide estimated were found to 99.5% and

99.98% and 99.68% respectively. A good separation and resolution of all the drugs indicates that there were no interference from the excipients commonly present In pharmaceutical formulations. This showed that the estimation of dosage form was accurate within given acceptable level of 95% to 105%.

Amount found in tablet =

$$\frac{\text{Concentration found from graph x Dilution factor}}{\text{Weight of tablet powder x 1000}} \text{ x Average weight of tablet}$$

Table 7.13: Assay results

Tablet Sample	Label Claim (mg)	Amount Present	Assay %
Bictegravir	200	199.08	99.54%
Emtricitabine	100	99.98	99.98%
Tenofovir alafenamide	25	24.62	99.68%

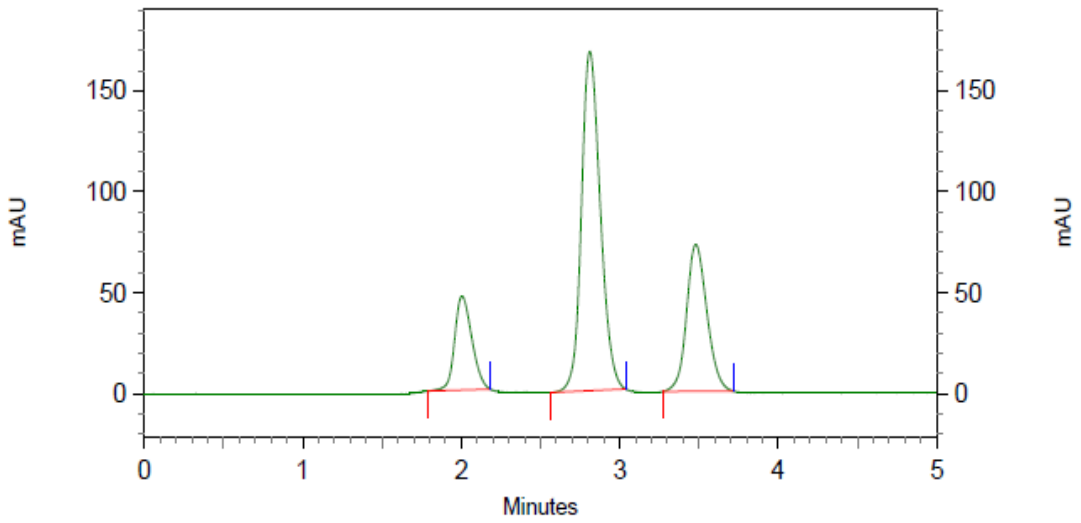

Fig. 7.29: Sample (Test) Chromatogram of Bictegravir, Emtricitabine and Tenofovir alafenamide