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A Novel Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Bictegravir, Emtricitabine and Tenofovir in Pure and Fixed Dose Combination

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ABSTRACT

A novel, simple, precise, accurate stability indicating liquid chromatography method was developed for the separation and simultaneous quantification of bictegravir, emtricitabine, tenofovir in bulk drug and pharmaceutical formulations. Separation was achieved on ProntoSILHypersorb ODS C18 column using mobile phase of 0.1 M sodium perchlorate, methanol in the ratio of 65:35 (v/v), pH 4.8 at a flow rate of 1.0 mL/min and UV detection was monitored at a wavelength of 258 nm. In these conditions, well resolved peaks were observed with acceptable system suitability at a retention time of 4.6 min for bictegravir, 7.0 min for emtricitabine and 10.1 min for tenofovir. Very high correlated linearity range was found to be 5-30 µg/mL for bictegravir, 20-120 µg/mL for emtricitabine and 2.5-15 µg/mL for tenofovir. The method can separate and identify the unknown degradation compounds formed during stress degradation study.

KEYWORDS

Bictegravir, Emtricitabine, Tenofovir, HPLC method, Forced degradation.

INTRODUCTION

Bictegravir (BTV, Fig. 1) is an integrase inhibitors class drug used to treat HIV infections [1]. Bictegravir prevent multiplying HIV by blocking HIV enzyme (integrase) and thus it reduces the amount of HIV in the body [2]. No serious side effects occurred during the usage of bictegravir except common side effects like diarrhea and headache [3].

Emtricitabine (ETC, Fig. 1) is used to treat and prevent HIV infection in adults and children [4] and belongs to class of nucleoside reverse transcriptase inhibitor (NRTI). The most common side effects associated with the use of emtricitabine are diarrhea, headache, nausea and rash.

Tenofovir (TNF, Fig. 1) is a nucleotide analog reverse-transcriptase inhibitor and is recommended to use in combination with other antiretrovirals and is prescribed for the treatment of chronic hepatitis B and to prevent and treat HIV/AIDS [5]. Dizziness, nausea and diarrhea are the most common side effects associated with the uses of tenofovir.

Literature survey reveals that few analytical methods were reported for the estimation of emtricitabine and tenofovir in single dosage forms using HPLC [6] and UV visible spectro-

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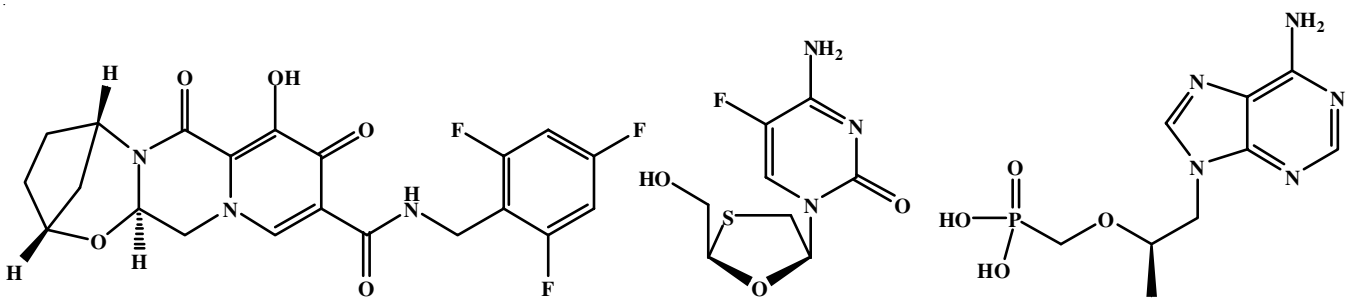

Fig. 1. Molecular structures of bictegravir, emtricitabine and tenofovir

photometer [7-13]. Few simultaneous UV [14-20], HPLC [21-25] methods in formulation analysis and few LCMS methods for bio analysis [26-28] were reported for emtricitabine and tenofovir. The other methods reported [29-43] were found to be estimation of emtricitabine and tenofovir in combination with other drugs in different sources using different analytical techniques.

The novelty of the present method is that there are no HPLC methods reported for the simultaneous separation and quantification of bictegravir, emtricitabine and tenofovir. Hence the present work aimed to develop a stability indicating liquid chromatography method for the separation and quantification of bictegravir, emtricitabine and tenofovir in bulk drug and pharmaceutical formulations.

EXPERIMENTAL

Working standard drug bictegravir was obtained from Gilead Sciences India Private Limited, New Delhi and emtricitabine, tenofovir were obtained from Cipla Limited, Kukatpally, Hyderabad, Telangana as gift samples.

Equipment: To develop a high pressure liquid chromatographic method for simultaneous estimation of bictegravir, emtricitabine and tenofovir, isocratic PEAK HPLC instrument with ProntoSILHypersorb ODS C18 column (250 mm × 4.6 mm, 5 µ) and Electronic balance-DENVER (SI234) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC-7000 UV-detector. A 20 µL rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

Standard stock solution: Standard stock solution of bictegravir, emtricitabine and tenofovir was prepared by accurately weighing about 100 mg of each drug in 100 mL volumetric flask separately and sonicated to dissolve it completely using 50 mL methanol. The final volume on the three volumetric flasks were made up to 100 mL using same solvent and then the contents were filtered through Ultipor N66 Nylon 6, 6 membrane sample filter paper. 1000 µg/mL standard stock solution concentration of bictegravir, emtricitabine and tenofovir were obtained separately. Calibration curve dilutions were prepared from the standard stock solution based on the formulation dosage of the three drugs bictegravir, emtricitabine and tenofovir. Equal volume of bictegravir, emtricitabine and tenofovir in each calibration level was mixed to get a combined calibration solutions having known concentrations of bictegravir, emtricitabine and tenofovir. Then the drugs were dissolved with 25 mL of methanol and sonicated to dissolve it completely and made up to the mark with the same solvent. The contents were filtered through Ultipor N66 Nylon 6, 6 membrane sample filter paper. Appropriate volumes of these solutions were further diluted with mobile phase to get required concentrations for construction of calibration curve.

Sample preparation: Sample solution was prepared by a composite of 20 bictegravir, emtricitabine and tenofovir combination tablets (BIKTARVY®: 50 mg of bictegravir, 200 mg of emtricitabine and 25 mg tenofovir) were grinded to a fine, uniform size powder. An amount of drug equivalent to 100 mg of emtricitabine was accurately weighed and quantitatively transferred into 100 mL volumetric flask. Approximately 30 mL mobile phase was added and the solution was sonicated for 15 min. The flask was made up to volume with mobile phase and mixed well. Then the solution is filtered through 0.45 µm nylon 6,6 membrane filter paper and then diluted with mobile phase to a concentration of 80 µg/mL of emtricitabine. Then based on the label claim of the both the drugs in the formulation, a concentration of 20 µg/mL of bictegravir and 10 µg/mL of tenofovir solution was obtained.

Method development: Systematic method development strategies were followed for separation and simultaneous quantification of bictegravir, emtricitabine and tenofovir using HPLC. Wavelength of the detector was initially selected based on the iso-absorption wavelength of bictegravir, emtricitabine and tenofovir in UV spectrophotometer and finalized based on the peak area responses of the drugs in HPLC method optimization studies. Mobile phase was selected by systematic change in the mobile phase composition and pH of the mobile phase. Different ratio of mobile phases consists of methanol, acetonitrile, phosphate, acetate, sulphate buffers and perchlorate buffers in different pH ranges of 3-7 and flow rate of 0.7-1.5 were studied. Separation was carried in different configurations of stationary phases including C8 and C18 columns. The optimized conditions that give best results in terms of system suitability and specificity were considered for further validation study.

Method validation: The method conditions that produce acceptable system suitability and specificity were considered for further validation study as per ICH guidelines. The validation includes the parameters like linearity range, accuracy, precision, ruggedness, robustness, sensitivity and forced degradation studies. The applicability of the development for the estimation of bictegravir, emtricitabine and tenofovir in pharma-

ceutical formulation was studied using marketed dosage form BIKTARVY®.

RESULTS AND DISCUSSION

Considering the solubility and the stability of bictegravir, emtricitabine and tenofovir the solvents water, basic water, acidic water, methanol, ethanol and acetonitrile were tested as diluents for the preparation of standard and sample solutions. The acetonitrile and ethanol solutions did not provide well zero crossing points for bictegravir, emtricitabine and tenofovir and these solutions were not used. After these preliminary tests, methanol was used as the diluent to develop the method and afforded satisfactory solubility and stability of bictegravir, emtricitabine and tenofovir. This solvent offers an additional advantage because it shows lower absorption in the UV region, indicating that it did not affect the analysis of bictegravir, emtricitabine and tenofovir.

The iso-absorption wavelength of bictegravir, emtricitabine and tenofovir in UV spectrophotometer was found to be 258 nm confirms that the suitable detector wavelength for the analysis of bictegravir, emtricitabine and tenofovir was found to be 258 nm. The optimized separation of bictegravir, emtricitabine and tenofovir was achieved on ProntoSILHypersorb ODS C18 column using mobile phase of 0.1 M sodium perchlorate, methanol in the ratio of 65:35 (v/v), pH 4.8 at a flow rate of 1 mL/min. In these conditions the retention time was found to be 4.6 min for bictegravir, 7 min for emtricitabine and 10.1 min for tenofovir. The blank chromatogram doesn't show any detection at the time of bictegravir, emtricitabine and tenofovir confirms that the method was found to be specific for the separation and detection of bictegravir, emtricitabine and tenofovir only. Hence the developed method was found to be specific. The standard chromatograms of bictegravir, emtricitabine and tenofovir are given in Fig. 2.

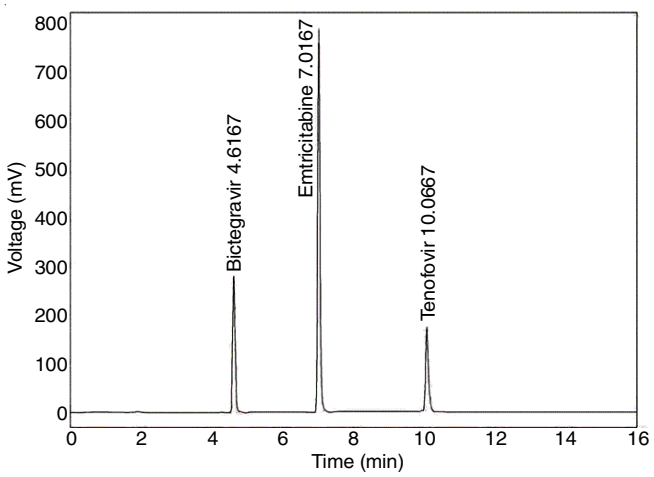

Fig. 2. Standard chromatogram of bictegravir, emtricitabine and tenofovir in the developed method

In the developed method conditions, the number of theoretical plates were found to be 4246 for bictegravir, 6327 for emtricitabine and 9678 min for tenofovir where as tail factor was found to be 0.71 for bictegravir, 1.42 for emtricitabine and 1.25 for tenofovir. A very high resolution of 6.89 was observed for emtricitabine and 9.45 for tenofovir. Hence in the method, well resolved peaks were observed with acceptable system suitable parameters.

The accurate fit six point calibration curve was observed within the concentration range of 5-30 µg/mL (y = 5462.x + 19.37; R^2 = 0.999) for bictegravir, 20-120 µg/mL (y = 2992.x + 411.8; R^2 = 0.999) for emtricitabine and 2.5-15 µg/mL (y = 5744.x + 252.0; R^2 = 0.999) for tenofovir. The correlation coefficient was found to be very high for all the three drugs in the study. Results are given in Table-1.

TABLE-1
LINEAR CALIBRATION CURVE RESULTS FOR BICTEGRAVIR, EMTRICITABINE AND TENOFOVIR

Bictegravir		Emtricitabine		Tenofovir	
Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area
5	26513.1	20	59376.2	2.5	14548.9
10	55284.5	40	120348.7	5.0	29617.1
15	82319.6	60	181624.6	7.5	43247.5
20	109376.4	80	239192.4	10.0	57391.6
25	136897.7	100	301958.3	12.5	72168.4
30	163287.9	120	357289.1	15.0	86359.1

Repeatability and reproducibility of the developed method was studied by precision studies. Standard solution at a concentration of 20, 80 and 10 respectively for bictegravir, emtricitabine and tenofovir was studied six times in the same day for intra-day precision and six times in three different days for inter-day precision and six times by two different analysts in the same day. The % RSD of six replicate experiments was studied and the % RSD was found to be 0.519 % for bictegravir, 0.810 % for emtricitabine and 0.533 % RSD for tenofovir. The % RSD in inter-day precision was found to be 0.525 % for bictegravir, 0.547 % for emtricitabine and 0.571 for tenofovir. The % RSD for ruggedness was found to be 0.506 % for bictegravir, 0.712 % for emtricitabine and 0.577 for tenofovir. The % RSD in all the experiments was found be less than 1 for bictegravir, emtricitabine and tenofovir confirms that the method was found to be precise. The results are given in Tables 2-4 respectively for bictegravir, emtricitabine and tenofovir.

The effect on separation and quantification of bictegravir, emtricitabine and tenofovir with small variation in the developed method conditions was confirmed by robustness study. Robustness was studied by change in mobile phase ratio as 0.1 M sodium perchlorate:methanol in the ratio of 70:30 (MP 1), 0.1 M sodium perchlorate:methanol in the ratio of 60:40 (MP 2). The pH of the mobile phase was adjusted to 4.9 (pH 1), 4.7 (pH 2) and the wavelength of the detector was changed

TABLE-2
INTRA-DAY PRECISION RESULTS FOR BICTEGRAVIR, EMTRICITABINE AND TENOFOVIR

S. No.	Bictegravir	Emtricitabine	Tenofovir
1	109536.2	238695.1	57401.5
2	109624.4	239416.5	57829.4
3	109568.2	237362.8	57719.6
4	109558.7	236697.3	57362.4
5	108391.1	235237.2	57064.8
6	108568.6	234469.8	57134.1
RSD (%)	0.519	0.810	0.533

TABLE-3
INTER-DAY PRECISION RESULTS FOR BICTEGRAVIR, EMTRICITABINE AND TENOFOVIR

S. No.	Bictegravir	Emtricitabine	Tenofovir
1	108736.1	236776.1	57201.1
2	108134.5	239561.4	57263.4
3	109348.8	239718.7	57294.5
4	109437.2	238961.5	57321.9
5	109567.4	237061.6	57898.3
6	108556.7	239337.9	57905.2
RSD (%)	0.525	0.547	0.571

TABLE-4
RUGGEDNESS RESULTS FOR BICTEGRAVIR, EMTRICITABINE AND TENOFOVIR

S. No.	Bictegravir	Emtricitabine	Tenofovir
1	109537.5	238607.1	57405.6
2	108834.6	239213.6	57239.4
3	109138.1	237164.2	56719.5
4	108354.9	235273.4	57581.1
5	109634.7	235197.5	57321.2
6	109816.3	236281.7	57643.7
RSD (%)	0.506	0.712	0.577

as 253 nm (WL 1) and 263 nm (WL 2). In all the changed conditions the % change of bictegravir, emtricitabine and tenofovir was studied. The % change was found to be 0.14-1.14 for bictegravir, 0.01-1.51 % for emtricitabine and 0.26-1.04 % for tenofovir (Table-5). The % change was found to be less than 2 for all the three drugs in all the conditions confirm that the method was found to be robust.

The spiked recovery studies were carried for the determination of accuracy of the developed method. 50, 100 and 150 % spiked levels was studied for the determination of accuracy of bictegravir, emtricitabine and tenofovir in the developed method. The % recovery was calculated in each spiked level and the average recovery was found to be 99.72, 99.83 and 98.44 for bictegravir, 99.23, 98.13 and 99.41 for emtricitabine and 99.57, 100.06 and 99.81 tenofovir respectively for 50, 100 and 150 % spiked levels. The % recovery was found to be within the acceptable limit of 98-102 for bictegravir, emtricitabine and tenofovir in all the spiked levels confirms that the method was found to be accurate. The precision study results for the method developed for bictegravir, emtricitabine and tenofovir are given in Table-6.

The ability of the developed method for the separation and estimation of bictegravir, emtricitabine, tenofovir and detection of degradation products formed during the stress degradation study was confirmed in stress degradation study. In this method the standard drug was exposed to 0.1 N HCl solution (acidic), 0.1 N NaOH solution (basic), 3 % hydrogen peroxide solution (peroxide), 60 °C temperature (thermal), fluorescent light (light) and ultraviolet light (UV) for 24 h. The stress exposed samples were neutralized and were analyzed in the developed method. The % assay (Table-7) was calculated by comparing the unstressed standard stock solution of the same concentration. The % degradation was found to be very high in acidic conditions for bictegravir (5.42 %), acidic (3.73 %) and UV (3.73 %) conditions for emtricitabine and peroxide for tenofovir (4.35 %).

The number of degradation products were found to be high in acidic condition. In this condition four degradation products were observed along with bictegravir, emtricitabine and tenofovir (Fig. 3). In base (Fig. 4), peroxide (Fig. 5) and UV light (Fig. 6) degradation results shows three additional peaks along with standard confirms that three additional degradation products were formed in these conditions. In fluorescent light (Fig. 7) and thermal degradation (Fig. 8) conditions, two additional degradation products were observed, which confirms that two degradation compounds were formed along with standard bictegravir, emtricitabine and tenofovir. In all the stress degradation conditions, the % degradation was found to be very less for bictegravir, emtricitabine and the degradations compounds were effectively separated by the method. Hence the developed method was found to be stable.

TABLE-5
ROBUSTNESS RESULTS FOR BICTEGRAVIR, EMTRICITABINE AND TENOFOVIR

Condition	Bictegravir		Emtricitabine		Tenofovir	
	Peak area	Assay (%)	Peak area	Assay (%)	Peak area	Assay (%)
MP 1	109534.7	100.14	239226.1	100.01	57196.1	99.65
MP 2	109386.6	100.00	239434.3	100.10	57247.5	99.74
pH 1	108961.1	99.62	235594.2	98.49	57623.8	100.40
pH 2	108665.2	99.34	236874.6	99.03	57834.2	100.77
WL 1	109637.9	100.23	237807.9	99.42	57798.4	100.70
WL 2	108139.7	98.86	239374.5	100.07	57991.3	101.04

TABLE-6
ACCURACY RESULTS FOR BICTEGRAVIR, EMTRICITABINE AND TENOFOVIR

Drug	Concentration (µg/mL)				Concentration recovered Mean ± SD	% Recovery Mean ± SD
	Level	Target	Spiked	Total		
Bictegravir	50 %	10	5	15	14.95 ± 0.01	99.72 ± 0.08
	100 %	10	10	20	19.96 ± 0.02	99.83 ± 0.14
	150 %	10	10	25	24.61 ± 0.02	98.44 ± 0.09
Emtricitabine	50 %	40	20	60	59.54 ± 0.04	99.23 ± 0.06
	100 %	40	40	80	78.51 ± 0.03	99.13 ± 0.04
	150 %	40	60	100	99.41 ± 0.03	99.41 ± 0.03
Tenofovir	50 %	5	2.5	7.5	7.46 ± 0.01	99.57 ± 0.16
	100 %	5	5.0	10.0	10.00 ± 0.01	100.06 ± 0.12
	150 %	5	7.5	12.5	12.47 ± 0.003	99.81 ± 0.02

TABLE-7
FORCED DEGRADATION STUDY RESULTS FOR BICTEGRAVIR, EMTRICITABINE AND TENOFOVIR

Condition	Bictegravir			Emtricitabine			Tenofovir		
	Area	Assay	Degraded	Area	Assay	Degraded	Area	Assay	Degraded
Acidic	103458.0	94.58	5.42	230287.5	96.27	3.73	55493.5	96.69	3.31
Base	104982.9	95.99	4.01	233289.6	97.53	2.47	55484.7	96.67	3.33
Light	106254.7	97.15	2.85	234019.5	97.84	2.16	56025.1	97.62	2.38
Peroxide	106982.5	97.82	2.18	231907.8	96.95	3.05	54896.5	95.65	4.35
Thermal	103928.5	95.01	4.99	232412.5	97.16	2.84	55019.8	95.86	4.14
UV	105241.3	96.22	3.78	230287.6	96.27	3.73	55741.6	97.13	2.87

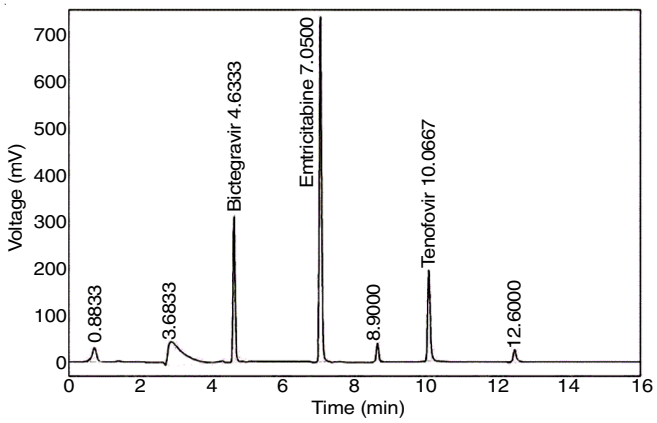

Fig. 3. Acid degradation chromatogram of bictegravir, emtricitabine and tenofovir in the developed method

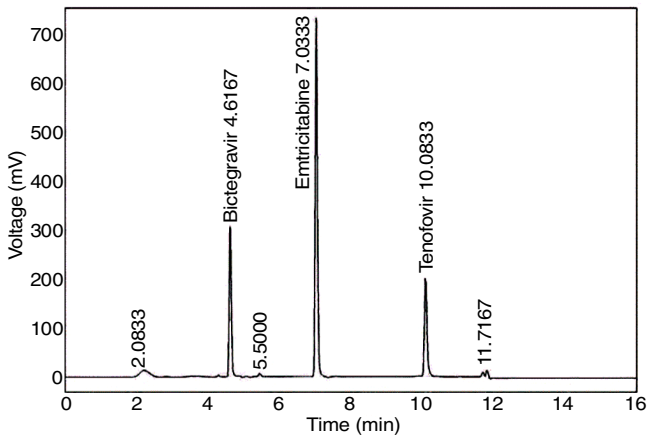

Fig. 4. Base degradation chromatogram of bictegravir, emtricitabine and tenofovir in the developed method

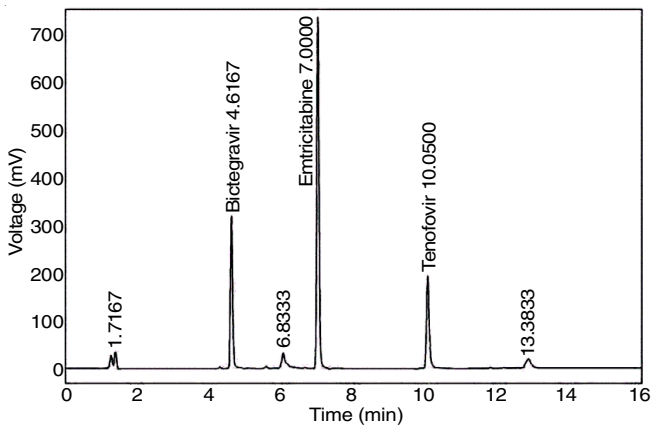

Fig. 5. Peroxide degradation chromatogram of bictegravir, emtricitabine and tenofovir in the developed method

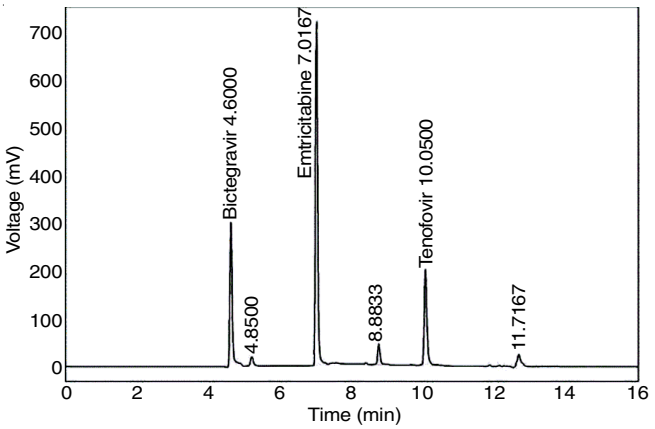

Fig. 6. UV degradation chromatogram of bictegravir, emtricitabine and tenofovir in the developed method

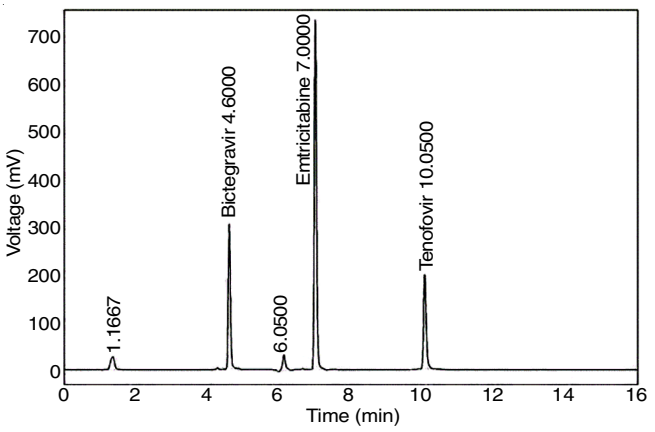

Fig. 7. Light degradation chromatogram of bictegravir, emtricitabine and tenofovir in the developed method

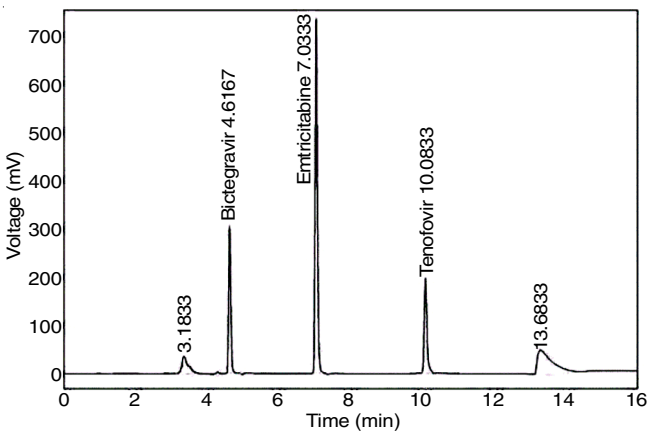

Fig. 8. Thermal degradation chromatogram of bictegravir, emtricitabine and tenofovir in the developed method

Formulation analysis was carried for the determination of applicability of the method for the analysis of bictegravir, emtricitabine and tenofovir in pharmaceutical formulations. The sample solution prepared from BIKTARVY® was analyzed in the developed method and the % assay was calculated. The % assay was found 99.60 % for bictegravir, 98.32 % for emtricitabine and 99.28 % for tenofovir in the developed method (Table-8). In the formulation chromatogram (Fig. 9) peaks corresponding to only bictegravir, emtricitabine and tenofovir were observed and no additional detections were observed. This confirms that the method was found to be suitable for the estimation of bictegravir, emtricitabine and tenofovir in formulations and formulation excipients don't interfere with the results.

TABLE-8
FORMULATION ANALYSIS RESULTS FOR
BICTEGRAVIR, EMTRICITABINE AND TENOFOVIR

Dosage (mg)	Concentration (µg/mL)		Assay (%)
	Prepared	Obtained	
Bictegravir - 50	20	19.92	99.60
Emtricitabine - 200	80	78.65	98.32
Tenofovir - 25	10	9.93	99.28

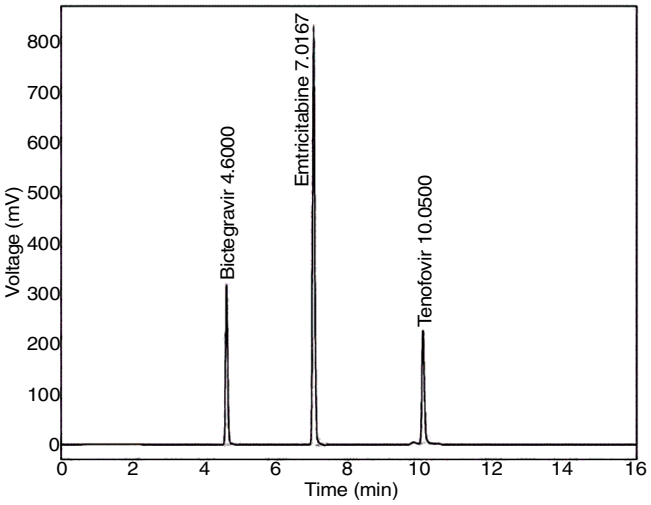

Fig. 9. Formulation chromatogram of bictegravir, emtricitabine and tenofovir in the developed method

Conclusion

A novel stability indicating liquid chromatography method was developed for the separation and simultaneous quantification of bictegravir, emtricitabine and tenofovir. The method was validated as per ICH guidelines and was found to be simple, precise, sensitive and accurate. As there is no other method reported for the simultaneous quantification of bictegravir, emtricitabine and tenofovir, the method developed here will be the first choice for the analysis of bictegravir, emtricitabine and tenofovir in bulk drug and pharmaceutical formulations.

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