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Analytical method development and validation of emtricitabine, bictegravir, tenofovir alafenamide by RP-HPLC

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ABSTRACT

A simple and precise method was developed for estimating Emtricitabine, Bictegravir and Tenofovir Alafenamide. The method was found to be specific and precise. The separation was attained on Symmetry C18 (150 × 4.5mm, 5µm)column with Water : Acetonitrile : Methanol (15:35:50) % v/v as mobile phase at a flow rate of 1.0 ml/min and linearity was achieved in the concentration range of 50% to 150% of standard concentration with correlation coefficient 0.99. The percent recovery from the assay was found to be 100.80%for Emtricitabine, 100.34% for Bictegravirand 100.64% for Tenofovir Alafenamide. Specificity and precision studies for Emtricitabine, Bictegravir and Tenofovir Alafenamide were within the acceptable range. From the stability studies, the percentage variation was less than 10.0% which is the desired criteria. Therefore, this method can be adopted to estimate Emtricitabine, Bictegravir and Tenofovir Alafenamidein other pharmaceutical formulations.

Keywords: Emtricitabine, Bictegravir, Tenofovir Alafenamide, HPLC, Method development, Validation.

INRODUCTION

Emtricitabine is a nucleoside analogue and reverse transcriptase inhibitor used in combination with other agents for treatment and prevention of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS). Emtricitabine does not appear to be a significant cause of drug induced liver injury, but may cause flares of disease in patients with underlying chronic hepatitis B virus (HBV) infection.[1-7]Emtricitabine is a white to off-white powder with a solubility of approximately 112 mg/mL in water at 25 °C. The log P for emtricitabine is -0.43 and the pKa is 2.65. EMTRIVA is available as capsules or as an oral solution. Emtricitabine is a cytidine analog which, when phosphorylated to emtricitabine 5'-triphosphate, competes with deoxycytidine 5'-triphosphate for HIV-1 reverse transcriptase. As HIV-1 reverse transcriptase incorporates emtricitabine into forming DNA strands, new nucleotides are unable to be incorporated, leading to viral DNA chain termination. Inhibition of reverse transcriptase prevents transcription of viral RNA into DNA, therefore the virus is unable to incorporate its DNA into host DNA and replicate using host cell machinery. This reduces viral load.

Bictegravir is a human immunodeficiency virus (HIV) integrase strand transfer inhibitor, the fourth in this class of agents that target the viral integrase. Bictegravir is used only in combination with other antiretroviral agents in the treatment of HIV infection and it has had limited use. Bictegravir is associated with a low rate of serum aminotransferase elevations during therapy, but has not been linked to instances of acute, clinically apparent liver injury.[7-10]Bictegravir sodium is an off-white to yellow solid with a solubility of 0.1 mg per mL in water at 20 °C.This single dose medication inhibits the strand transfer of viral DNA into the human genome, preventing HIV-1 virus replication and propagation. In vitro, bictegravir has shown powerful antiviral activity against HIV-2 and various subtypes of HIV-1. It has shown synergistic effects when combined with other ARVs, including tenofovir alafenamide (TAF),

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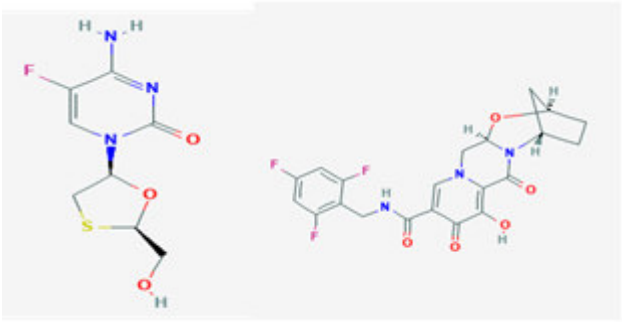
emtricitabine (FTC), and darunavir (DRV). Bictegravir: integrase strand transfer inhibitor; INSTI), an HIV-1 encoded enzyme necessary for viral replication. Inhibition of the integrase enzyme prevents the integration of HIV-1 into host DNA, blocking the conversion of the HIV-1 provirus and progression of the virus.

Tenofovir alafenamide is a prodrug of tenofovir. It was developed by Gilead Sciences based on the protide technology of Chris McGuigan for use in the treatment of HIV/AIDS and chronic hepatitis B, and is applied in the form of tenofovir alafenamide fumarate (TAF). Closely related to the commonly used reverse-transcriptase inhibitor tenofovir disoproxil fumarate (TDF), TAF has greater antiviral activity and better distribution into lymphoid tissues than that agent.[11-21]Tenofovir alafenamide fumarate is a white to off-white or tan powder with a solubility of 4.7 mg per mL in water at 20 °C.Tenofovir alafenamide presents 91% lower plasma concentration with an intracellular presence of about 20-fold higher when compared to tenofovir disoproxil. This is due to its prolonged systemic exposure and its higher intracellular accumulation of the active metabolite tenofovir diphosphate. Tenofovir alafenamide accumulates more in peripheral blood mononuclear cells compared to red blood cells. Once activated, tenofovir acts with different mechanisms including the inhibition of viral polymerase, causing chain termination and the inhibition of viral synthesis.

From the literature survey, it was revealed that few UV spectrophotometric method was developed but were not economical. Moreover, RP-HPLC[22, 23] and LC-MS[24] and derivative methods were also developed which estimates Emtricitabine, Bictegravir and Tenofovir Alafenamide either individually or in combination. In the present research work, a new method was developed to estimate Emtricitabine, Bictegravir and Tenofovir Alafenamide simultaneously and validated as per ICH guidelines.[25]



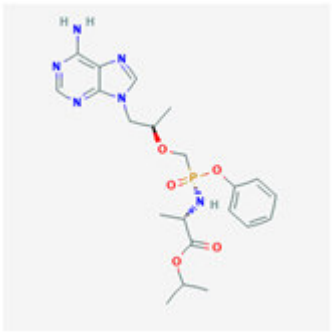


Figure 1: Structure of Emtricitabine, Bictegravir **Figure 2: Structure of Tenofovir Alafenamide**

MATERIALS AND METHODS

Gift samples of emtricitabine, bictegravir and tenofovir alafenamide were received from startech lab, Hyderabad. KH2PO4 was purchased from Final chemicals where as water, methanol for HPLC and ortho phosphoric acid were purchased from Merck.

Instrumentation: Waters HPLC was used for the separation of emtricitabine, bictegravir and tenofovir alafenamide. UV/VIS spectrophotometer (LABINDIA UV 12.500^{+}) was used for detection. Instruments such as; pH meter used was of Adwa — AD 10100 and weighing machine was of Afcoset ER-1000A.

Method development

Mobile phase preparation

Accurately measured 500 ml (50%) of methanol, 350 ml (35%) of Acetonitrile and 150 ml (15%) of water were mixed and degassed in an ultrasonic sonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration. The Mobile phase was used as the diluent.

Preparation of standard solution

Accurately weighed 50mg of Emtricitabine, 12.5mg of Bictegravir and 6.25mg of Tenofovir alafenamide was taken in a 25 ml volumetric flask, and made up to the mark with mobile phase. Take 1ml of above solution and dilute to 10ml with diluents in order to get a concentration of 200µg/ml of Emtricitabine, 50µg/ml of Bictegravir and 25µg/ml of Tenofovir alafenamide respectively.

Preparation of sample solution

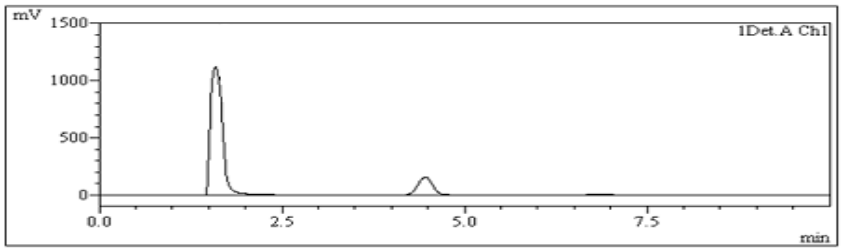
Accurately weighed 992.2 mg of tablet powder (sample) was taken in a 100ml volumetric flask, and made up to the mark with mobile phase. Take 1ml of above solution and dilute to 10ml with diluents in order to get a concentration of 200µg/ml of Emtricitabine, 50µg/ml of Bictegravir and 25µg/ml of Tenofovir alafenamide respectively.

Procedure: Mixture of with Water, Acetonitrile and Methanol in the ratio 15:35:50 % v/v was used as mobile phase which was injected into the system for 30 minutes prior to injecting the prepared solutions of standard as well as sample. Detection of the drug was achieved at the wavelength of 258nm at 30°C. After several trials, method was optimized followed by validation of the method considering various validation parameters.

RESULTS AND DISCUSSION

Method development was achieved using Symmetry C18

(150 × 4.5mm, 5µm). Mobile phase was mixture of Water, Acetonitrile and Methanol in the ratio 15:35:50 % v/v. Flow rate (1ml/min) and injection volume (20µl) was set. The peaks obtained had good resolution with the retention time 1.590, 4.462 and 6.870 for emtricitabine, bictegravir and tenofovir alafenamide respectively. Chromatogram of optimized trial is shown in figure 3.



Peak#	Ret.Time	Area	Area %	Name	Theoretical Plate#	Tailing Factor	Resoluti
1	1.590	12753966	84.285	Emtricitabine	515.581	1.454	0.000
2	4.462	2181715	14.418	Bictegravir	2311.091	1.037	8.818
3	6.870	196231	1.297	Tenofovir.A	2893.325	0.986	5.460

Figure 3: Chromatogram of optimized trial

System suitability

All the parameters were evaluated by performing system suitability studies. The recorded responses for suitability studies are depicted in table 1.

Table 1: Results of system suitability parameters

Peak#	Ret.Time	Area	Area %	Name	Theoretical Plate#	Tailing Factor	Resolution
1	1.590	12753966	84.285	Emtricitabine	515.581	1.454	0.000
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Method validation

Validation of the method was evaluated for various parameters which include linearity, specificity, robustness and stability. The method was also evaluated for specificity of the method and was found to be specific as there were no interactions found. Linearity obtained was shown to have good correlation as shown in table 2.

Linearity

The linearity range was observed over the range of 50% to 150% of standard concentration. The respective absorbance values are depicted in table 2. The linearity graph plotted is presented in figure 4and 5 for emtricitabine, bictegravir and tenofovir alafenamide respectively

.Table 2: linearity results

Emtricitabine		Tenofovir.A		Bictegravir	
50	6691572	**50**	102726	**50**	1109266
75	11420619	75	148827	75	1704468
100	13619535.5	100	206761	100	2112631
125	17127996.5	125	253209	125	2758655
150	19883695	150	298846	150	3258995
Cc	0.99	Cc	0.99	cc	0.99

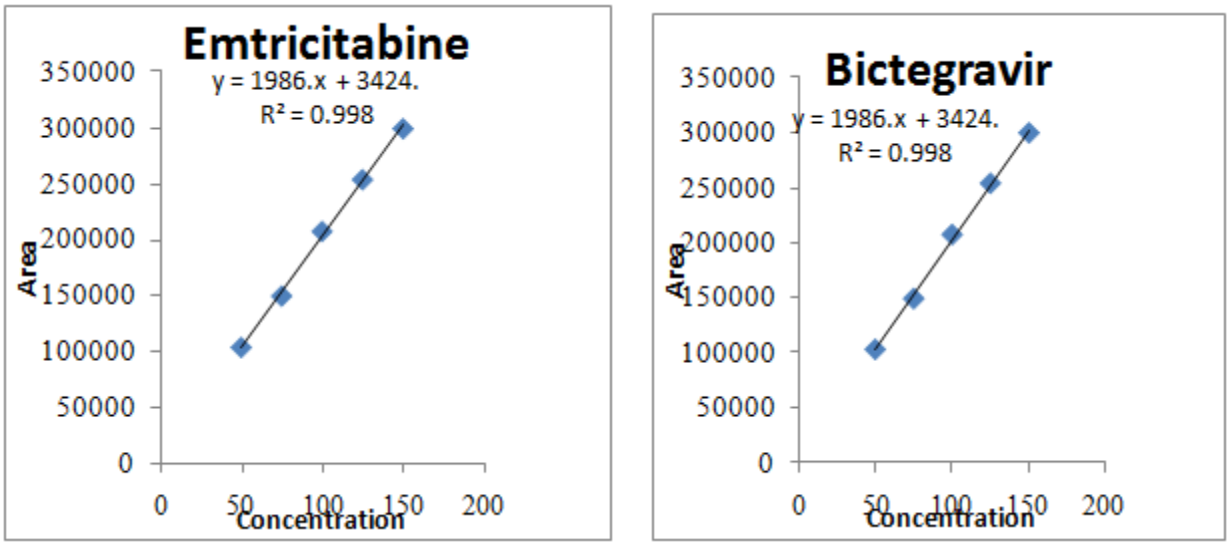

Figure 4: Linearity graph for Emtricitabine, Bictegravir

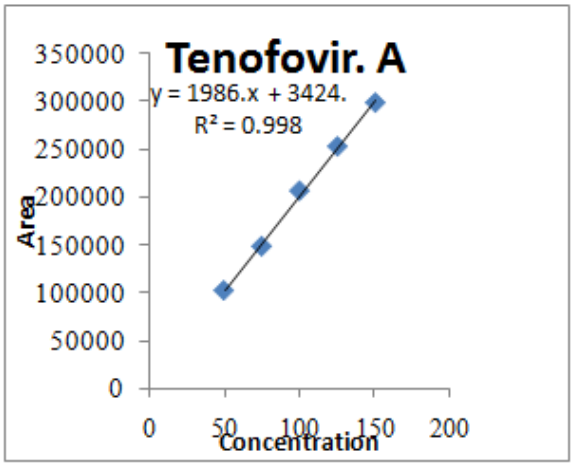

Figure 5: Linearity graph for Tenofovir Alafenamide

Accuracy

Percent recovery of sample solutions at different concentrations (50%, 100%, and 150%) was calculated. The Percent recovery of emtricitabine, bictegravir and tenofovir alafenamide are depicted in table 3,4 and 5 respectively.

Table 3: Accuracy (recovery) data for Emtricitabine

%Concentration	Area	Added amount (mg)	Amount Found (mg)	PercentRecovery	Mean Recovery
50%	6691572	25.0	25.52	101.18%	100.80%
100%	13619535.5	50.0	49.57	99.14%	
150%	19883695	75.0	76.55	102.06%	

Table 4: Accuracy (recovery) data for Bictegravir

%Concentration	Area	Added amount (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1109265.5	6.25	6.52	101.61%	100.34%
100%	2112631	12.5	12.13	99.13%	
150%	3258995	18.75	18.55	100.28%	

Table 5: Accuracy (recovery) data for Tenofovir Alafenamide

%Concentration	Area	Added amount (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	102726	3.125	3.52	101.78%	100.64%
100%	206761	6.25	5.89	99.24%	
150%	298846	9.375	9.85	101.06%	

Precision

Precision of the method was performed for both sample solutions as described under experimental work. The same method was performed on the other day for intermediate precision. The results are depicted in the table 6, 7 and 8.

Table 6: Results of precision

Method precision	STD-1	STD-2	STD-3	STD-4	STD-5	AVG	SD	% RSD
Emtricitabine	12853951	12888636	12748325	12728596	12905502	12825002	81457	0.64
Bictegravir	2232494	2219575	2209455	2216416	2231519	2221892	9939	0.45
Tenofovir.A	199620	198435	197951	196127	196521	197730.8	1427	0.72

Table 7: Results of intermediate precision – day 1

IP – 1	STD-1	STD-2	STD-3	STD-4	STD-5	AVG	SD	%RSD
Emtricitabine	12830504	12824621	12891914	12894176	12702525	12828748	77819	0.61
Bictegravir	2227557	2217498	2226908	2230217	2229167	2226269	5074	0.23
Tenofovir.A	197329	197629	197002	194949	199620	197305.8	1666	0.84

Table 8: results of intermediate precision – Day 2

Day-II-Analyst-II	STD-1	STD-2	STD-3	STD-4	STD-5	AVG	SD	%RSD
Emtricitabine	12833840	12628653	12806978	12787145	12789023	12769128	80737	0.63
Bictegravir	2223837	2196959	2199127	2223764	2209412	2210620	12919	0.58
Tenofovir.A	190053	184537	184972	188346	185418	186665	2411	1.29

Robustness

The standard and samples of the drugs were injected by changing the conditions of chromatography. There was no change observed in the parameters like tailing factor, resolution, plate count and asymmetric factor. Chromatograms for variation in flow rate are presented in figure 6 and 7 where as chromatograms for variation in composition are presented in figure 8 and 9. Their respective results are depicted in table 9-14.

Variation in flow

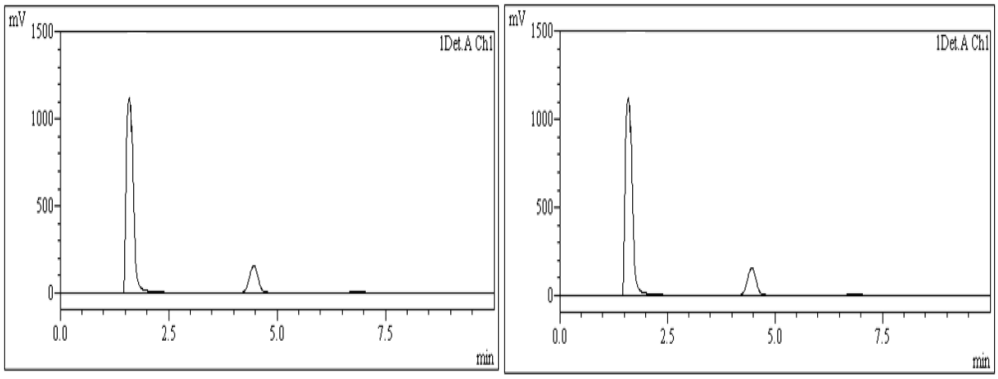

Figure 6: Chromatogram showing less flow Figure 7: Chromatogram showing more flow

Variation of mobile phase organic composition

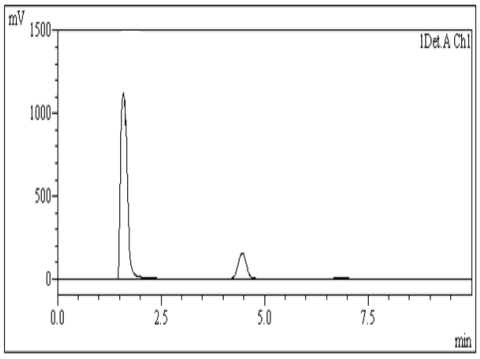

Figure 8: less organic composition

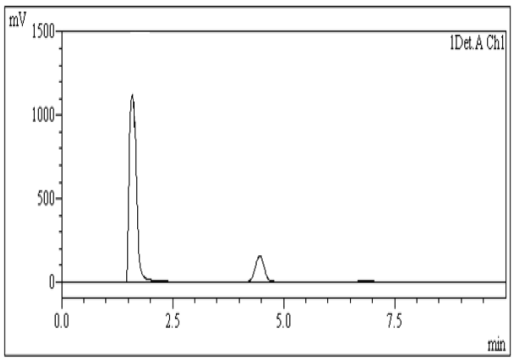

Figure 9: more organic composition

Table 9: Results for variation in flow for Emtricitabine

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	SP Tailing
1	0.9	514.586	1.453
2	1.0	515.581	1.454
3	1.1	515.987	1.454

Table 10: Results for variation in flow for Bictegravir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	SP Tailing
1	0.9	2321.286	1.037
2	1.0	2311.091	1.037
3	1.1	2341.447	1.047

Table 11: Results for variation in flow for Tenofovir Alafenamide

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	SP Tailing
1	0.9	2884.258	0.985
2	1.0	2893.325	0.986
3	1.1	2897.459	0.994

* Actual flow (1.0ml/min) was considered from Assay standard.

Table 12: Results for variation in mobile phase composition for Emtricitabine

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	515.181	1.454
2	*Actual	515.581	1.454
3	10% more	515.784	1.454

Table 13: Results for variation in mobile phase composition for Bictegravir

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2310.958	1.037
2	*Actual	2311.091	1.037
3	10% more	2311.172	1.047

Table 14: Results for variation in mobile phase composition for Tenofovir Alafenamide

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2891.325	0.976
2	*Actual	2893.325	0.986
3	10% more	2897.325	0.986

***** Results for actual Mobile phase composition have been consideredfrom Accuracy standard.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Emtricitabine, Bictegravir and Tenofovir Alafenamide was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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