RESEARCH ARTICLE

Revolutionizing HIV Treatment: Pioneering Ultra Performance Liquid Chromatography for Emtricitabine, Dolutegravir, and Tenofovir Tablet Validation

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ABSTRACT

An uncomplicated and precise method has come into existence for the simultaneous quantification of emtricitabine, dolutegravir, and tenofovir in solid dosage forms. The chromatographic analysis utilized a Hibar100 column (50×2.1 mm, 2 µm) with a mobile phase of 0.1% OPA and acetonitrile in a 60:40 v/v ratio at a constant flow rate of 1.0 mL/min, maintaining a temperature of 30°C. The perfected wavelength at 260.0 nm revealed retention times of 1.951, 1.180, and 1.584 minutes for dolutegravir, emtricitabine, and tenofovir, respectively.

Keywords: Emtricitabine, Dolutegravir, Tenofovir, UPLC.

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Conflict of interest: None

INTRODUCTION

Ultra-performance liquid chromatography (UPLC) revolutionizes analytical separation science by advancing speed, sensitivity, and resolution with fine particles and higher flow rates. This article explores UPLC's potential in pharmaceutical analysis, aiming to accelerate analysis while maintaining quality compared to conventional high-performance liquid chromatography (HPLC) methods. Emtricitabine, an NRTI, is pivotal in human immunodeficiency virus (HIV) treatment, inhibiting reverse transcriptase and preventing HIV-1 with emtricitabine alafenamide. Tenofovir, an antiviral derived from adenosine monophosphate, is available as tenofovir disoproxil and tenofovir alafenamide since 2008, improving oral bioavailability. Dolutegravir, marketed as Tivicay, treats HIV-1 in mature persons and adolescents ≥12 years weighing ≥40 kg, with 52.6 mg of dolutegravir sodium equivalent to 50 mg of dolutegravir free acid. FDA approved dolutegravir on August 12, 2013.[1-16] The structures of emtricitabine, tenofovir, and dolutegravir are shown in Figures 1, 2, and 3, respectively (Figures 1-3).

MATERIALS AND METHODS

Chemicals

Acetonitrile, HPLC Water, $N(CH_2CH_3)$, KH_2PO_4, and H_3PO_4 were obtained from Merck India Ltd, Mumbai, India. The APIs of emtricitabine, tenofovir, and dolutegravir standards were obtained from Hetero Labs, Hyderabad.

The Instrumentation

The Waters Acquity System features binary pumps, a TUV detector, and an autosampler seamlessly integrated with Empower 2 Software. [17-20]

Method Optimization

After thorough experimentation with various mobile phase compositions, 0.1% OPA: Acetonitrile in a 60:40 v/v ratio emerged as the most effective choice for optimal separation and analytical performance. Utilizing a UV spectrum wavelength of 260 nm, the developed UPLC method enabled robust absorbance of both drugs, facilitating their accurate quantification. This optimized method was smoothly applied for the simultaneous evaluation of the combined drugs in vitro, demonstrating its efficacy for precise and efficient analysis.[21]

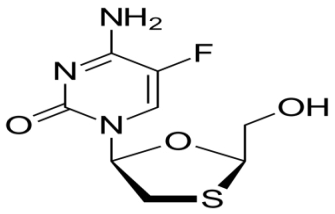

Figure 1: Structure of emtricitabine

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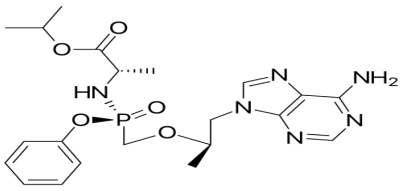

Figure 2: Structure of tenofovir

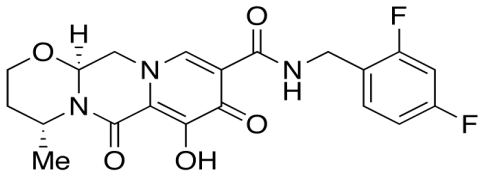

Figure 3: Structure of dolutegravir

Validation Procedure

The analytical method underwent rigorous authentication according to ICH guidelines, ensuring reliability and quality. It covered specifications such as system suitability, precision, accuracy, linearity, robustness, limit of detection (LoD), limit of quantitation (LoQ), forced degradation, and stability, meeting industry standards for pharmaceutical analysis.[22-30]

Validation

System suitability specifications

System suitability was evaluated by taking the necessary steps with standard solutions of tenofovir, emtricitabine, and dolutegravir. Six consecutive injections were made, and key specifications like peak tailing, resolution, and USP plate count were calculated. The %RSD for the area of these injections was required to be ≤2% to ensure system stability and consistency for accurate quantification of the compounds.

Degradation studies

Degradation studies are crucial for assessing pharmaceutical stability and behavior under various conditions. These include forced degradation, thermal, photostability, hydrolysis, oxidation, and pH stability tests. The aim is to identify degradation pathways and ensure drug safety and efficacy. Analytical techniques like HPLC, MS, and spectroscopy monitor chemical changes, detect degradation products, and evaluate stability under stress conditions. These studies inform drug formulation, storage conditions, and regulatory compliance to maintain product quality over time.[31,32]

RESULTS AND DISCUSSION

Optimized Method

The analysis utilized a mobile phase consisting of 0.1% H_3PO_3 and CH_3CN in a 60:40 v/v ratio, with a flux of 1-mL/min. A Hibar 100 x 2.1 mm column with 2 µm particles was employed. Detection occurred at an observation of 260.0 nm, with the column temperature held at 30°C. Each injection volume was 1-mL, and the total run time for the analysis was 4 minutes. The diluent used was a mixture of water and acetonitrile in 50:50 v/v ratio (Figure 4).

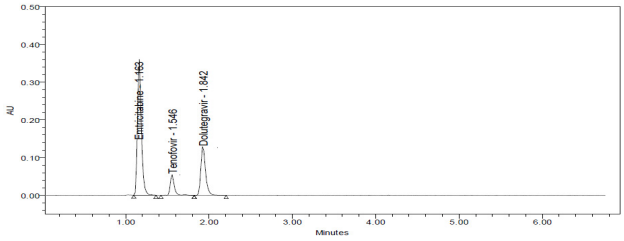

Figure 4: Optimized chromatogram

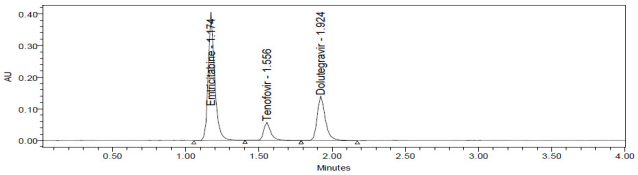

Figure 5: Chromatogram for system suitability

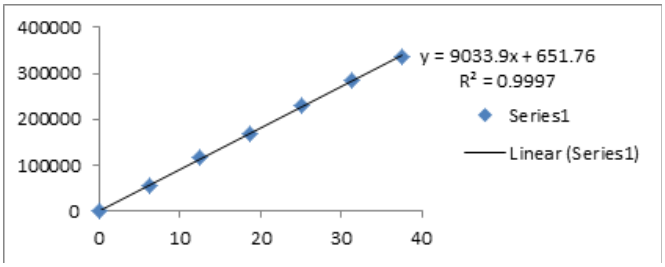

Figure 6: Calibration curve of tenofovir

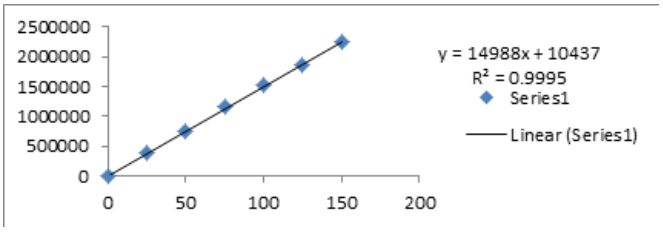

Figure 7: Calibration curve of emtricitabine

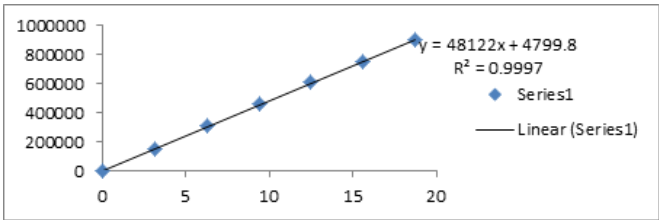

Figure 8: Calibration curve of dolutegravir

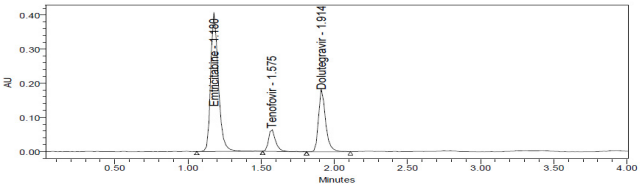

Figure 9: Linearity 100% chromatogram

Table 1: System relevancy results

S. No.	*Emtricitabine*			*Tenofovir*				*Dolutegravir*			
Inj	*Run time (min)*	*TP*	*Tailing*	*Run time (min)*	*TP*	*Tailing*	*RS*	*Run time (min)*	*TP*	*Tailing*	*RS*
1	1.174	2776	1.24	1.541	5151	1.27	4.1	1.87	5991	1.32	3.5
2	1.176	2886	1.25	1.55	5185	1.28	4	1.878	6099	1.32	3.4
3	1.18	2919	1.26	1.555	5058	1.28	4	1.907	5975	1.34	3.6
4	1.181	2948	1.25	1.556	5102	1.28	4.1	1.908	6145	1.31	3.5
5	1.186	2854	1.25	1.564	5079	1.27	4	1.924	6032	1.32	3.5
6	1.199	2851	1.25	1.584	5181	1.28	4.1	1.951	6022	1.33	3.6

Table 2: Linearity results

S. No.	*Tenofovir*		*Emtricitabine*		*Dolutegravir*	
	Conc (µg/mL)	*Peak area*	*Conc (µg/mL)*	*Peak area*	*Conc (µg/mL)*	*Peak area*
1	6.25	56334	25	378226	3.125	152278
2	12.5	115591	50	755585	6.25	312586
3	18.75	167812	75	1164216	9.375	462895
4	25	228769	100	1531036	12.5	607432
5	31.25	284851	125	1865225	15.625	748804
6	37.5	336908	150	2247311	18.75	907578

Table 3: System precision results

S. No	*Area of tenofovir*	*Area of emtricitabine*	*Area of dolutegravir*
1	227299	1519830	610546
2	225249	1515637	595582
3	227736	1492055	604272
4	225123	1519364	605553
5	222602	1497910	612308
6	224164	1491701	604130
Mean	225362	1506083	605399
S.D	1924.1	13616.7	5890.7
%RSD	0.9	0.9	1

Table 4: Repeatability results

S. No	*Area of tenofovir*	*Area of emtricitabine*	*Area of dolutegravir*
1	224464	1494424	598949
2	226184	1492275	607672
3	225436	1512857	614475
4	225043	1509815	608881
5	223335	1515580	606506
6	224763	1491540	597201
Mean	224871	1502749	605614
S.D	960.1	11148	6471.8
%RSD	0.4	0.7	1.1

Table 5: Intermediate precision results

S. No	*Area of tenofovir*	*Area of emtricitabine*	*Area of dolutegravir*
1	224995	1486682	486933
2	224953	1493336	478806
3	225056	1498890	490580
4	222620	1497820	479690
5	225815	1481720	483068
6	222583	1503116	503971
Mean	224337	1493594	487175
S.D	1381.2	8067.3	9345.5
%RSD	0.6	0.5	1.9

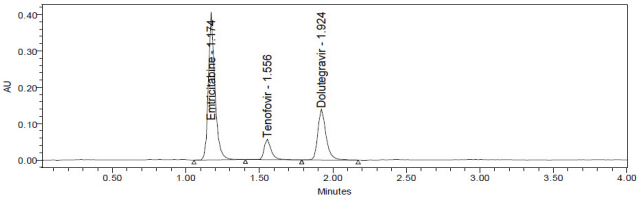

Figure 10: System precision chromatogram

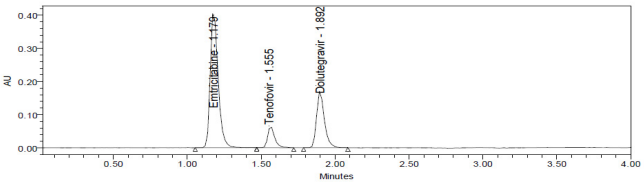

Figure 12: Intermediate precision chromatogram

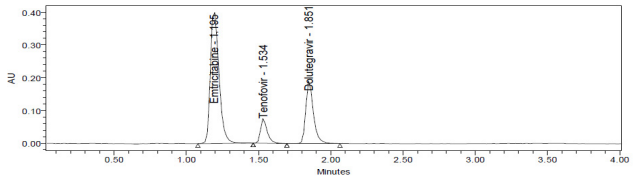

Figure 11: Repeatability chromatogram

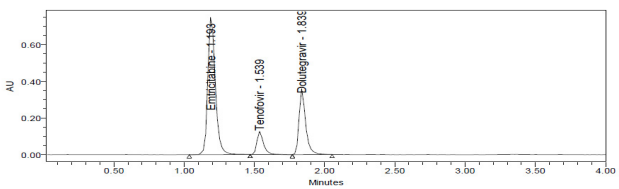

Figure 13: Accuracy 100% chromatogram

Table 6: Accuracy results

	Tenofovir				*Emtricitabine*				*Dolutegravir*			
Level (%)	*Amount Spiked*	*Amount recovered*	*% Recovery*	*Mean %Recovery*	*Amount Spiked*	*Amount recovered*	*% Recovery*	*Mean % Recovery*	*Amount Spiked*	*Amount recovered*	*% Recovery*	*Mean % Recovery*
	(µg/mL)	*(µg/mL)*			*(µg/mL)*	*(µg/mL)*			*(µg/mL)*	*(µg/mL)*		
	12.5	25	101.16		50	100	98.42		25	25	101.16	
50	12.5	25	98.45		50	100	98.96		25	25	98.45	
	12.5	25	99.59		50	100	99.71		25	25	99.59	
	25	25	100.1		100	100	98.51		50	25	100.1	
100	25	25	99.58	99.64	100	100	101.21	99.48	50	25	99.58	99.64
	25	25	98.85		100	100	100.71		50	25	98.85	
	37.5	25	98.98		150	100	98.34		75	25	98.98	
150	37.5	25	99.86		150	100	99.11		75	25	99.86	
	37.5	25	100.2		150	100	100.33		75	25	100.2	

Table 7: Sensitivity results

Sample	*LoD (µg/mL)*	*LoQ(µg/mL)*
Tenofovir	0.06	0.18
Emtricitabine	0.47	1.44
Dolutegravir	0.11	0.34

Table 8: Robustness results

S. No.	*Condition*	*%RSD dolutegravir*	*%RSD emtricitabine*	*%RSD tenofovir*
1	F.R (-) 0.9 mL/min	0.4	0.8	0.8
2	F.R (+) 1.1 mL/min	1.3	0.40	0.6
3	M.P (-) 65 W:35M	1.5	0.20	1.4
4	M.P (+) 55 W:45M	0.2	1.00	1.1
5	(-) 25°C	0.2	0.9	0.5
6	(+) 35°C	1	0.7	1.4

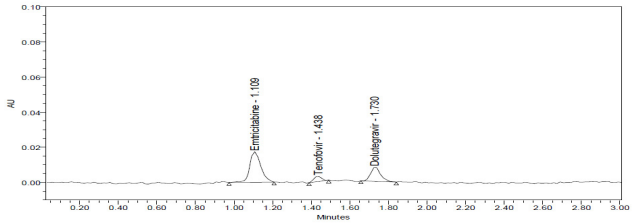

Figure 14: LoD chromatogram for standard

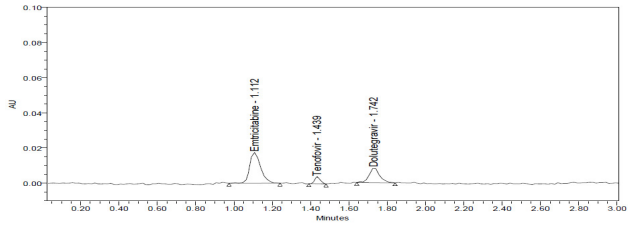

Figure 15: LoQ chromatogram for standard

Table 9: Assay results

S. No	*Tenofovir*			*Emtricitabine*			*Dolutegravir*		
	Stnd Area	*Sample area*	*%Assay*	*Stnd Area*	*Sample area*	*%Assay*	*Stnd Area*	*Sample area*	*%Assay*
1	227299	224464	99.40	1519830	1494424	99.03	1681191	1693761	100.31
2	225249	226184	100.16	1515637	1492275	98.89	1693923	1698036	100.56
3	227736	225436	99.83	1492055	1512857	100.25	1699326	1709571	101.25
4	225123	225043	99.66	1519364	1509815	100.05	1687072	1689419	100.05
5	222602	223335	98.90	1497910	1515580	100.43	1679077	1707150	101.10
6	224164	224763	99.53	1491701	1491540	98.84	1680435	1675492	99.23
Avg	225362	224871	99.58	1506083	1502749	99.58	1686837	1695572	100.42
Stdev	1924.1	960.1	0.43	13616.7	11148.0	0.739	8238.0	12493.5	0.740
%RSD	0.9	0.4	0.4	0.9	0.7	0.7	0.5	0.7	0.7

Table 10: Degradation results

S. No.	Degradation condition	Tenofovir		Emtricitabine		Dolutegravir	
		%Undegraded	%Degraded	%Undegraded	%Degraded	%Undegraded	%Degraded
1	Acid	95.98	4.02	96.21	3.79	96.06	3.94
2	Alkali	96.73	3.27	96.42	3.58	96.26	3.74
3	Oxidation	95.16	4.84	95.62	4.38	95.79	4.21
4	Thermal	97.64	2.36	97.87	2.13	97.81	2.19
5	UV	98.40	1.60	98.65	1.35	98.27	1.73
6	Water	99.34	0.66	99.59	0.41	99.39	0.61

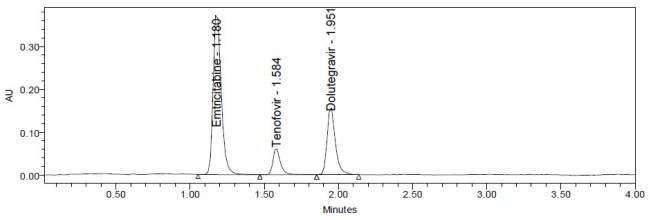

Figure 16: Chromatogram for working standard solution

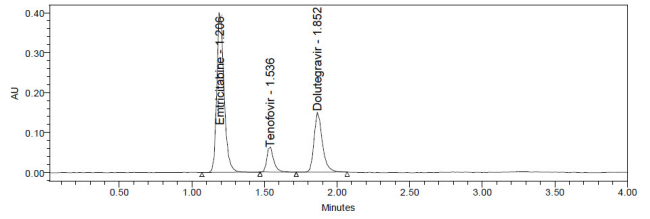

Figure 17: Chromatogram for working sample solution

Method Validation

System suitability

System suitability specifications were determined and met the required standards, ensuring reliable performance of the chromatographic system (Figure 5, Table 1).

Linearity

The calibration curves for tenofovir, emtricitabine, and dolutegravir showed good linearity with high correlation coefficients, indicating accurate quantification over the tested concentration ranges (Figures 6-9, Table 2).

Precision

The method showed excellent precision with low %RSD values for both repeatability and intermediate precision tests, confirming the method's reliability (Figures 10-12, Tables 3-5).

Accuracy

Recovery studies demonstrated the method's accuracy, with average % recoveries close to 100% for all three drugs (Table 6, Figure 13).

Detection and quantification limits

The LoD and LoQ values were low, allowing for the sensitive detection and quantification of the analytes (Figures 14-15, Table 7).

Robustness

The method proved to be robust, with small changes in chromatographic conditions not significantly affecting the results (Table 8).

Assay of formulation

The %assay values for tenofovir, emtricitabine, and dolutegravir were close to 100%, demonstrating the method's applicability for routine quality control of tablet formulations (Figures 16 and 17, Table 9).

Degradation studies

Forced degradation studies indicated the method's capability to detect degradation products, confirming its stability-indicating nature (Table 10).

CONCLUSION

A precise method has come into existence for the simultaneous estimation of dolutegravir, emtricitabine, and tenofovir in tablet form. Retention times were 1.951, 1.180, and 1.584 minutes, respectively, with %RSD for system precision at 1.0, 0.9, and 0.9%. Method precision %RSD values were 1.1, 0.7, and 0.4%. %Recovery rates were 100.04, 99.48, and 99.58%. LoD/LoQ values (ppm) were emtricitabine: 0.47/1.44, dolutegravir: 0.11/0.34, tenofovir: 0.06/0.18. Regression equations were tenofovir: y = 9033x + 651.7, emtricitabine: y = 14988x + 10437, dolutegravir: y = 33277x + 12509. Reduced retention times indicate a simple and cost-effective method suitable for routine standard control in industries.

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