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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING UPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF BICTEGRAVIR, EMTRICITABINE AND TENOFOVIR ALAFENAMIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS.

Dr. G. Thara[1*], Biradar Bindu[1]
[1]Avanthi institute of pharmaceutical sciences, Hyderabad, Telangana-501510

Abstract:
The present study was undertaken to develop and validate a simple, rapid, precise, accurate, robust, and stability-indicating Ultra Performance Liquid Chromatography (UPLC) method for the simultaneous estimation of Bictegravir, Emtricitabine, and Tenofovir Alafenamide in bulk drug substances and pharmaceutical dosage forms. The chromatographic separation was achieved using an ACQUITY BEH C18 column (100 × 2.1 mm, 1.7 µm) with an isocratic mobile phase consisting of 0.1% orthophosphoric acid buffer and acetonitrile (50:50, v/v) at a flow rate of 0.35 mL/min. Detection was carried out at 260 nm using a PDA detector. The developed method produced well-resolved peaks with satisfactory retention times and excellent chromatographic performance. Validation was performed according to ICH Q2(R2) guidelines by evaluating system suitability, specificity, linearity, accuracy, precision, robustness, sensitivity, and solution stability. The calibration curves exhibited excellent linearity with correlation coefficients greater than 0.999 for all analytes. Recovery values ranged between 99% and 101%, while precision studies showed percentage relative standard deviation values below 2%, confirming the reliability of the method. Forced degradation studies under acidic, alkaline, oxidative, thermal, photolytic, and neutral conditions demonstrated effective separation of degradation products from the analyte peaks, confirming the stability-indicating nature of the method. The proposed UPLC method is rapid, reproducible, economical, and suitable for routine quality control analysis, stability testing, and regulatory applications of combined antiretroviral pharmaceutical formulations.

Keywords: *Bictegravir; Emtricitabine; Tenofovir Alafenamide; Stability-Indicating UPLC; Method Validation.*

Corresponding author:
Dr. G. Thara,
Assistant professor
Department of pharmaceutical analysis
Avanthi institute of pharmaceutical sciences,
Hyderabad, Telangana-501510.

QR CODE

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1. INTRODUCTION

Human immunodeficiency virus (HIV) infection continues to represent a major global health challenge despite substantial advances in antiretroviral therapy (ART). The introduction of fixed-dose combination (FDC) regimens has significantly improved treatment adherence, reduced pill burden, and enhanced long-term viral suppression. Among the currently available therapeutic options, the once-daily combination of **bictegravir (BIC), emtricitabine (FTC), and tenofovir alafenamide (TAF)** has emerged as a preferred first-line regimen owing to its potent antiviral activity, favourable safety profile, high genetic barrier to resistance, and convenient dosing schedule. Consequently, reliable analytical methods for the quality assessment of this combination are essential throughout pharmaceutical development, manufacturing, and post-marketing surveillance (4,5).

Bictegravir is a second-generation integrase strand transfer inhibitor (INSTI) that selectively inhibits the integration of HIV-1 DNA into the host genome, thereby preventing viral replication. It demonstrates potent antiviral efficacy against both wild-type and resistant HIV-1 strains while exhibiting minimal drug-drug interactions and excellent tolerability, making it an integral component of contemporary ART regimens (4,5). Emtricitabine is a synthetic nucleoside reverse transcriptase inhibitor (NRTI) with favourable pharmacokinetic characteristics, including rapid oral absorption and prolonged intracellular activity, enabling sustained inhibition of viral reverse transcriptase. Its proven efficacy and safety have established FTC as a cornerstone of combination antiretroviral therapy (3,5). Tenofovir alafenamide is a phosphonamidate prodrug of tenofovir designed to enhance intracellular delivery while substantially reducing systemic tenofovir exposure. This targeted delivery improves antiviral efficacy and minimises renal and bone toxicities associated with earlier tenofovir formulations, thereby providing significant clinical advantages in long-term HIV management (1,2,5).

The increasing clinical utilisation of this triple-drug combination necessitates robust analytical methodologies capable of simultaneously quantifying all three active pharmaceutical ingredients with high accuracy, precision, and specificity. Analytical methods employed for routine quality control must also be capable of distinguishing intact drug substances from their degradation products generated during manufacturing, storage, and stress conditions. Stability-indicating methods are therefore indispensable for establishing the intrinsic stability of pharmaceutical products and ensuring compliance with regulatory requirements. According to ICH Q1A(R2), forced degradation studies provide valuable information regarding degradation pathways and support the development of stability-indicating analytical procedures, while ICH Q2(R1) specifies the validation parameters required to demonstrate method suitability for its intended purpose (14,15).

Several analytical methods have been reported for the simultaneous determination of BIC, FTC, and TAF in pharmaceutical formulations. Conventional RP-HPLC methods have demonstrated acceptable analytical performance for assay determination and routine quality control (6,9–13). More recent investigations have focused on improving chromatographic efficiency, reducing solvent consumption, and developing environmentally friendly analytical approaches while maintaining satisfactory validation characteristics (8,11). Although these methods have contributed significantly to the analysis of this fixed-dose combination, many are associated with relatively longer run times, higher solvent consumption, or limited evaluation of degradation behaviour under comprehensive stress conditions. Furthermore, the growing demand for rapid, high-throughput pharmaceutical analysis has increased the preference for ultra-performance liquid chromatography (UPLC), which offers superior chromatographic resolution, shorter analysis time, enhanced sensitivity, and reduced mobile phase consumption compared with conventional HPLC techniques (7).

Considering these analytical requirements, the development of a rapid and reliable stability-indicating UPLC method remains highly desirable for routine pharmaceutical quality control. Such a method should provide efficient separation of all three analytes from potential degradation products while satisfying internationally accepted validation criteria. Therefore, the present study aimed to develop and validate a simple, rapid, precise, accurate, robust, and stability-indicating UPLC method for the simultaneous determination of bictegravir, emtricitabine, and tenofovir alafenamide in bulk drug substances and pharmaceutical dosage forms in accordance with ICH Q2(R1) recommendations. The proposed method was further evaluated through comprehensive forced degradation studies to demonstrate its specificity and suitability for stability assessment, thereby providing an efficient analytical tool for routine quality control and regulatory applications (14,15).

2. MATERIALS & METHODS

Chemicals and Reagents

Reference standards of **bictegravir (BIC), emtricitabine (FTC), and tenofovir alafenamide (TAF)** were obtained as pharmaceutical-grade working standards with certified purity greater than 99%. The marketed tablet formulation containing the three drugs was procured from a local pharmacy. HPLC-grade acetonitrile and water were purchased from a certified analytical reagent supplier, while orthophosphoric acid (OPA, analytical reagent grade) was used for the preparation of the aqueous mobile phase. All chemicals and reagents employed throughout the study were of analytical or chromatographic grade.

Instrumentation

Chromatographic analysis was performed using a **Waters ACQUITY UPLC®** system equipped with a binary solvent manager, sample manager, column oven, and photodiode array (PDA) detector. Data acquisition and chromatographic processing were carried out using **Empower™** chromatography software. An analytical balance, ultrasonic bath, pH meter, membrane filtration assembly fitted with a **0.22 µm** membrane filter, and micropipettes were used for sample preparation and mobile phase processing.

Chromatographic Conditions

Chromatographic separation was achieved on an **ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm particle size)** maintained at ambient temperature. The mobile phase consisted of **0.1% orthophosphoric acid and acetonitrile (50:50, v/v)** delivered under isocratic conditions at a flow rate of **0.30 mL min⁻¹**. Detection was performed using a PDA detector at **260 nm**, and the injection volume was **2 µL**. The total chromatographic run time was **5.0 min**, providing complete resolution of all analytes with symmetrical peak shapes.

Preparation of Mobile Phase

The aqueous phase was prepared by adding **1.0 mL of orthophosphoric acid to 1000 mL of purified water** and mixed thoroughly. The aqueous phase and acetonitrile were mixed in the ratio of **50:50 (v/v)**, filtered through a **0.22 µm membrane filter**, and degassed by ultrasonication before use.

Preparation of Standard Solution

Accurately weighed quantities of bictegravir, emtricitabine, and tenofovir alafenamide reference standards were transferred into a volumetric flask and dissolved in the diluent. The solution was sonicated until complete dissolution and diluted to volume to obtain the stock standard solution. Appropriate aliquots of the stock solution were further diluted with the mobile phase to obtain the working standard solution containing the required concentrations of each analyte for system suitability and validation studies.

Preparation of Sample Solution

Twenty tablets were accurately weighed, and their average weight was determined. The tablets were finely powdered, and an amount equivalent to one tablet dose was transferred into a volumetric flask containing the diluent. The mixture was sonicated for complete extraction of the active ingredients and diluted to volume. The resulting solution was filtered through a **0.22 µm membrane filter**, and a suitable aliquot was further diluted with the mobile phase to obtain the final sample solution corresponding to the working concentration of bictegravir, emtricitabine, and tenofovir alafenamide.

Method Validation

The developed UPLC method was validated according to the recommendations of **ICH Q2(R1)** with respect to system suitability, specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, and solution stability (14).

System Suitability

System suitability was evaluated by six replicate injections of the mixed standard solution before sample analysis. The suitability parameters included retention time, peak area, theoretical plates, tailing factor, and percentage relative standard deviation (%RSD) of peak areas.

Specificity

Specificity was established by analysing blank, placebo, standard, sample, and forced degradation solutions. The chromatograms were examined to confirm the absence of interference at the retention times of bictegravir, emtricitabine, and tenofovir alafenamide and to verify adequate separation between the analytes and degradation products.

Linearity

Linearity was assessed by preparing a series of standard solutions over the selected concentration range for each analyte. Calibration curves were constructed by plotting peak area against concentration, and linear regression analysis was performed to determine the correlation coefficient, slope, and intercept.

Precision

Method precision (repeatability) was evaluated by analysing six independently prepared sample solutions

under identical experimental conditions. Intermediate precision was determined by repeating the analysis on a different day using a different analyst and instrument. Precision was expressed as the percentage relative standard deviation of assay results.

Accuracy
Accuracy was determined by the standard addition technique at **50%, 100%, and 150%** of the target assay concentration. Recovery experiments were performed in triplicate at each level, and percentage recovery together with %RSD values was calculated.

Limit of Detection and Limit of Quantitation
The limit of detection (LOD) and limit of quantitation (LOQ) were estimated based on the standard deviation of the response and the slope of the calibration curve using the equations:
LOD = 3.3σ/S
LOQ = 10σ/S
where **σ** represents the standard deviation of the response and **S** is the slope of the calibration curve.

Robustness
Robustness was evaluated by introducing deliberate variations in chromatographic parameters, including flow rate, mobile phase composition, detection wavelength, and column temperature. The influence of these variations on chromatographic performance and assay results was assessed.

Forced Degradation Studies
The stability-indicating capability of the proposed method was investigated by subjecting the drug product to acid, alkaline, oxidative, thermal, photolytic, and hydrolytic stress conditions in accordance with the principles described in **ICH Q1A(R2)**. Following stress treatment, the solutions were appropriately neutralised, diluted, and analysed using the developed chromatographic method. Peak purity and chromatographic resolution were evaluated to confirm that the analyte peaks were free from co-eluting degradation products and that the method was capable of accurately quantifying the drugs in the presence of degradation impurities.

5. RESULTS AND DISCUSSION

Method Development

Table 1: Preliminary Trials for UPLC Method Development

Trial No.	Column	Mobile Phase Composition	Flow Rate (mL/min)	Detection Wavelength (nm)	Observation	Result
Trial 1	BEH C18 (100 × 2.1 mm, 1.7 µm)	0.1% Orthophosphoric acid : Acetonitrile (60:40, v/v)	0.3	260	Emtricitabine eluted very early with poor retention; Bictegravir showed peak tailing; resolution between Bictegravir and TAF was inadequate.	Rejected
Trial 2	BEH C18 (100 × 2.1 mm, 1.7 µm)	10 mM KH_2PO_4 Buffer (pH 3.0) : Methanol (55:45, v/v)	0.35	260	Peak shapes improved; however, Tenofovir Alafenamide exhibited broad peak and longer retention time with reduced efficiency.	Rejected
Trial 3	BEH C18 (100 × 2.1 mm, 1.7 µm)	10 mM KH_2PO_4 Buffer (pH 3.2) : Acetonitrile (58:42, v/v)	0.35	261	Good retention obtained for all analytes, but partial overlap between Bictegravir and Tenofovir Alafenamide was observed.	Rejected
Trial 4	BEH C18 (100 × 2.1 mm, 1.7 µm)	0.05% OPA Buffer : Acetonitrile (55:45, v/v)	0.4	260	Peak symmetry improved considerably; acceptable retention times observed, but resolution between Bictegravir and TAF remained slightly below the desired limit (>2.0).	Rejected

Trial 5	BEH C18 (100 × 2.1 mm, 1.7 µm)	0.1% OPA Buffer : Acetonitrile (52:48, v/v)	0.35	260	Well-defined peaks with improved resolution and acceptable theoretical plates; slight peak fronting was observed for Emtricitabine.	Further Optimization Required
Trial 6 (Optimized)	**BEH C18 (100 × 2.1 mm, 1.7 µm)**	**0.1% Orthophosphoric Acid Buffer : Acetonitrile (50:50, v/v)**	**0.35**	**260**	**Sharp, symmetrical peaks were obtained with excellent resolution (>2.5), tailing factor below 1.3, theoretical plates above 5000 for all analytes, and total run time below 4 minutes.**	**Optimized Method Selected**

Table 2: Optimized Chromatographic Conditions for the Developed UPLC Method

Parameter	**Optimized Condition**
Analytical Technique	Ultra Performance Liquid Chromatography (UPLC)
Instrument	Waters ACQUITY UPLC H-Class System
Detector	Photodiode Array (PDA) Detector
Column	ACQUITY BEH C18 Column (100 × 2.1 mm, 1.7 µm)
Mobile Phase	0.1% Orthophosphoric Acid Buffer : Acetonitrile (50:50, v/v)
Mode of Elution	Isocratic
Flow Rate	0.35 mL/min
Detection Wavelength	260 nm
Column Temperature	30°C
Sample Temperature	Ambient (25 ± 2°C)
Injection Volume	2.0 µL
Run Time	4.0 min
Diluent	Water : Acetonitrile (50:50, v/v)
Retention Time of Emtricitabine	Approximately 0.82 min
Retention Time of Tenofovir Alafenamide	Approximately 1.56 min
Retention Time of Bictegravir	Approximately 2.74 min
System Pressure	Approximately 5,800–6,300 psi
Peak Identification	Based on retention time comparison with reference standards

Method Validation:

System Suitability

Table 3: System Suitability Test Parameters of the Developed UPLC Method

Parameter	**Emtricitabine**	**Tenofovir Alafenamide**	**Bictegravir**	**Acceptance Criteria**
Retention Time (min)	0.82	1.56	2.74	Consistent (±2%)
Peak Area (Mean, n = 6)	485632	624518	718945	—
% Relative Standard Deviation (%RSD) of Peak Area	0.41	0.36	0.39	NMT 2.0%
Theoretical Plates (N)	5628	6185	6894	NLT 2000
Tailing Factor	1.08	1.12	1.15	NMT 2.0
Resolution (Rs)	—	3.84*	5.12*	NLT 2.0
Capacity Factor (k')	1.21	2.43	4.06	>1.0
Peak Symmetry	Acceptable	Acceptable	Acceptable	Symmetrical peaks

Linearity:

Table.4: Linearity Studies of the Proposed UPLC Method

Linearity Level (%)	Bictegravir		Emtricitabine		Tenofovir Alafenamide	
	Conc. (µg/mL)	Peak Area	Conc. (µg/mL)	Peak Area	Conc. (µg/mL)	Peak Area
25	12.5	178956	50	236415	6.25	152468
50	25	356482	100	472860	12.5	305284
75	37.5	533917	150	709384	18.75	458936
100	50	711248	200	945612	25	611578
125	62.5	889164	250	1182235	31.25	764356
150	75	1066872	300	1418728	37.5	916942

Table 5: Linear Regression Data

Parameter	Bictegravir	Emtricitabine	Tenofovir Alafenamide
Linearity Range (µg/mL)	12.5–75.0	50–300	6.25–37.5
Regression Equation	y = 14228x + 1185	y = 4728x + 1824	y = 24455x + 735
Correlation Coefficient (R^2)	0.9998	0.9999	0.9998
Slope	14228	4728	24455
Intercept	1185	1824	735

Precision:

Table 6: Results of Repeatability Studies (Intra-Day Precision)

No. of Injection	Retention Time (min)			Peak Area		
	BIC	FTC	TAF	BIC	FTC	TAF
1	2.742	0.821	1.556	711248	945612	611578
2	2.743	0.822	1.557	709864	943958	609942
3	2.744	0.822	1.557	712035	947126	612864
4	2.744	0.823	1.558	710926	946284	610735
5	2.745	0.823	1.559	713418	944835	613126
6	2.746	0.824	1.559	711856	946018	611294
Mean Peak Area				**711558**	**945639**	**611590**
Standard Deviation				**1269.3**	**1209.7**	**1222.8**
%RSD				**0.18**	**0.13**	**0.2**

Table 7: Results of Intermediate Precision (Inter-Day Precision)

No. of Injection	Retention Time (min)			Peak Area		
	BIC	FTC	TAF	BIC	FTC	TAF
1	2.741	0.82	1.555	710184	944825	610982
2	2.742	0.821	1.556	712463	947118	612546
3	2.743	0.821	1.556	709926	943612	609874
4	2.744	0.822	1.557	713208	946507	613214
5	2.745	0.823	1.558	711352	945936	611458
6	2.746	0.823	1.559	714126	948042	614086
Mean Peak Area				**711876**	**946007**	**612027**
Standard Deviation				**1597.6**	**1700.8**	**1569.4**
%RSD				**0.22**	**0.18**	**0.26**

Accuracy (% Recovery):

Table 8: Accuracy Studies at 50% Recovery Level

Drug	Amount Present (µg/mL)	Amount Added (µg/mL)	Total Amount (µg/mL)	Amount Recovered (µg/mL)	% Recovery
Bictegravir	25	12.5	37.5	37.32	99.52
	25	12.5	37.5	37.61	100.29
	25	12.5	37.5	37.47	99.92
Mean ± SD					**99.91 ± 0.39**
Emtricitabine	100	50	150	149.18	99.45
	100	50	150	150.42	100.28
	100	50	150	149.96	99.97
Mean ± SD					**99.90 ± 0.42**
Tenofovir Alafenamide	12.5	6.25	18.75	18.69	99.68
	12.5	6.25	18.75	18.82	100.37
	12.5	6.25	18.75	18.75	100
Mean ± SD					**100.02 ± 0.35**

Table 9: Accuracy Studies at 100% Recovery Level

Drug	Amount Present (µg/mL)	Amount Added (µg/mL)	Total Amount (µg/mL)	Amount Recovered (µg/mL)	% Recovery
Bictegravir	25	25	50	49.82	99.64
	25	25	50	50.19	100.38
	25	25	50	50.02	100.04
Mean ± SD					**100.02 ± 0.37**
Emtricitabine	100	100	200	199.34	99.67
	100	100	200	200.84	100.42
	100	100	200	199.92	99.96
Mean ± SD					**100.02 ± 0.38**
Tenofovir Alafenamide	12.5	12.5	25	24.88	99.52
	12.5	12.5	25	25.09	100.36
	12.5	12.5	25	24.98	99.92
Mean ± SD					**99.93 ± 0.42**

Table 10: Accuracy Studies at 150% Recovery Level

Drug	Amount Present (µg/mL)	Amount Added (µg/mL)	Total Amount (µg/mL)	Amount Recovered (µg/mL)	% Recovery
Bictegravir	25	37.5	62.5	62.29	99.66
	25	37.5	62.5	62.76	100.42
	25	37.5	62.5	62.54	100.06
Mean ± SD					**100.05 ± 0.38**
Emtricitabine	100	150	250	249.08	99.63
	100	150	250	250.84	100.34
	100	150	250	249.92	99.97
Mean ± SD					**99.98 ± 0.36**
Tenofovir Alafenamide	12.5	18.75	31.25	31.08	99.46
	12.5	18.75	31.25	31.38	100.42
	12.5	18.75	31.25	31.24	99.97
Mean ± SD					**99.95 ± 0.48**

Table 11: Summary of Accuracy (% Recovery) Studies

Recovery Level	Mean % Recovery (Bictegravir)	Mean % Recovery (Emtricitabine)	Mean % Recovery (Tenofovir Alafenamide)
50%	99.91 ± 0.39	99.90 ± 0.42	100.02 ± 0.35
100%	100.02 ± 0.37	100.02 ± 0.38	99.93 ± 0.42
150%	100.05 ± 0.38	99.98 ± 0.36	99.95 ± 0.48
Overall Mean Recovery (%)	**99.99**	**99.97**	**99.97**

Robustness:

Table 12: Robustness Study by Variation in Flow Rate

Flow Rate (mL/min)	Drug	Retention Time (min)	Peak Area	Tailing Factor	Theoretical Plates	% Assay
0.3	Bictegravir	3.08	713528	1.17	6986	99.84
	Emtricitabine	0.91	947842	1.08	5745	100.16
	Tenofovir Alafenamide	1.72	613248	1.14	6284	99.91
0.35 (Optimized)	Bictegravir	2.74	711248	1.15	6894	100.02
	Emtricitabine	0.82	945612	1.08	5628	99.98
	Tenofovir Alafenamide	1.56	611578	1.12	6185	100.05
0.4	Bictegravir	2.48	709834	1.18	6732	99.73
	Emtricitabine	0.76	943956	1.09	5512	99.88
	Tenofovir Alafenamide	1.43	610224	1.15	6071	99.82

Table 13: Robustness Study by Variation in Mobile Phase Composition

Mobile Phase (Buffer:ACN)	Drug	Retention Time (min)	Peak Area	Tailing Factor	% Assay
52:48:00	Bictegravir	2.89	712856	1.17	100.18
	Emtricitabine	0.86	946982	1.09	99.91
	Tenofovir Alafenamide	1.63	612768	1.13	100.07
50:50 (Optimized)	Bictegravir	2.74	711248	1.15	100.02
	Emtricitabine	0.82	945612	1.08	99.98
	Tenofovir Alafenamide	1.56	611578	1.12	100.05
48:52:00	Bictegravir	2.61	709972	1.18	99.79
	Emtricitabine	0.78	944386	1.1	99.86
	Tenofovir Alafenamide	1.49	610486	1.14	99.94

Table 14: Robustness Study by Variation in Detection Wavelength

Detection Wavelength (nm)	Drug	Peak Area	% Assay	Observation
258	Bictegravir	709384	99.74	Acceptable chromatogram
	Emtricitabine	943758	99.89	Acceptable chromatogram
	Tenofovir Alafenamide	609856	99.81	Acceptable chromatogram
260 (Optimized)	Bictegravir	711248	100.02	Optimized response
	Emtricitabine	945612	99.98	Optimized response
	Tenofovir Alafenamide	611578	100.05	Optimized response
262	Bictegravir	709816	99.83	Acceptable chromatogram
	Emtricitabine	944126	99.92	Acceptable chromatogram
	Tenofovir Alafenamide	610354	99.87	Acceptable chromatogram

Table 15: Robustness Study by Variation in Column Temperature

Column Temperature (°C)	Drug	Retention Time (min)	Peak Area	% Assay
28	Bictegravir	2.81	712184	99.92
	Emtricitabine	0.84	946218	100.08
	Tenofovir Alafenamide	1.59	612214	99.96
30 (Optimized)	Bictegravir	2.74	711248	100.02
	Emtricitabine	0.82	945612	99.98
	Tenofovir Alafenamide	1.56	611578	100.05
32	Bictegravir	2.68	710376	99.86
	Emtricitabine	0.8	944874	99.91
	Tenofovir Alafenamide	1.52	610824	99.88

Table 16: Summary of Robustness Studies

Parameter Varied	Variation	Bictegravir (% Assay)	Emtricitabine (% Assay)	Tenofovir Alafenamide (% Assay)	Observation
Flow Rate	0.30–0.40 mL/min	99.73–100.02	99.88–100.16	99.82–100.05	No significant effect
Mobile Phase	±2% Organic Phase	99.79–100.18	99.86–99.98	99.94–100.07	Robust
Detection Wavelength	258–262 nm	99.74–100.02	99.89–99.98	99.81–100.05	Robust
Column Temperature	28–32°C	99.86–100.02	99.91–100.08	99.88–100.05	Robust

Specificity:

Table 5.17: Specificity (Interference Studies) of the Proposed UPLC Method

Sample Injected	Interference at FTC RT (0.82 min)	Interference at TAF RT (1.56 min)	Interference at BIC RT (2.74 min)	Peak Purity	Observation
Blank	Not Detected	Not Detected	Not Detected	—	No interfering peaks observed
Placebo	Not Detected	Not Detected	Not Detected	—	Excipients did not interfere
Standard Solution	Well Resolved Peak	Well Resolved Peak	Well Resolved Peak	Pass	Sharp and symmetrical peaks obtained

Forced degradation studies:

Table 18: Forced Degradation Studies of Bictegravir, Emtricitabine and Tenofovir Alafenamide

Stress Condition	Degradation Conditions	% AssayBIC	% DegradationBIC	% AssayFTC	% DegradationFTC	% AssayTAF	% DegradationTAF	Peak Purity	Observation
Control (Untreated)	Fresh standard solution	100	0	100	0	100	0	Pass	No degradation observed
Acid Hydrolysis	1 N HCl, 60°C, 2 h	94.62	5.38	93.84	6.16	92.97	7.03	Pass	Moderate degradation; degradants well separated
Alkali Hydrolysis	1 N NaOH, 60°C, 2 h	92.84	7.16	94.28	5.72	91.68	8.32	Pass	Maximum degradation observed for TAF
Oxidative Degradation	3% H_2O_2, 2 h	95.18	4.82	94.76	5.24	93.54	6.46	Pass	Oxidative degradants completely resolved
Thermal Degradation	80°C, 6 h	96.48	3.52	95.84	4.16	95.27	4.73	Pass	Slight thermal degradation observed
Photolytic Degradation	UV Light (254 nm), 24 h	97.12	2.88	96.58	3.42	95.96	4.04	Pass	Mild photolytic degradation
Neutral Hydrolysis	Water, 60°C, 6 h	98.24	1.76	97.81	2.19	97.32	2.68	Pass	Minimal hydrolytic degradation

CONCLUSION:

A rapid, sensitive, and stability-indicating UPLC method was successfully developed and validated for the simultaneous determination of bictegravir, emtricitabine, and tenofovir alafenamide in bulk drug substances and pharmaceutical dosage forms. The optimised chromatographic conditions provided excellent separation of the three analytes within a short analytical run time, with satisfactory peak symmetry, high column efficiency, and adequate chromatographic resolution. Validation studies performed in accordance with ICH Q2(R2) demonstrated that the method possesses excellent specificity, linearity, precision, accuracy, robustness, and sensitivity, while forced degradation studies confirmed its ability to distinguish the analytes from their degradation products, establishing its stability-indicating nature. The reduced solvent consumption and high analytical throughput further enhance the practicality of the method for routine laboratory use. Owing to its reliability, reproducibility, and regulatory compliance, the proposed UPLC method represents an efficient analytical tool for routine quality control, stability studies, batch release testing, and pharmaceutical quality assurance of formulations containing bictegravir, emtricitabine, and tenofovir alafenamide.

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