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

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ABSTRACT

Human immunodeficiency virus (HIV) remains a major global health concern, necessitating effective combination antiretroviral therapies and robust analytical methods for quality assurance. This study aimed to develop and validate a rapid, accurate, precise, and stability-indicating reverse-phase ultra-performance liquid chromatographic (RP-UPLC) method for the simultaneous determination of **darunavir, tenofovir alafenamide, cobicistat, and emtricitabine** in bulk drug substances and pharmaceutical dosage forms. Chromatographic separation was achieved on an **ACQUITY BEH C18 (100 × 2.1 mm, 1.7 µm)** column using **0.1% orthophosphoric acid buffer and acetonitrile (55:45, v/v)** as the mobile phase at a flow rate of **0.30 mL/min**, with detection at **260 nm**. Complete separation of all analytes was achieved within **5 min**. The method was validated in accordance with **ICH Q2(R2)** guidelines and demonstrated excellent specificity, linearity (**R^2 > 0.999**), precision (**%RSD < 2.0**), accuracy, robustness, and sensitivity. Forced degradation studies under acidic, alkaline, oxidative, thermal, photolytic, and neutral hydrolytic conditions confirmed the stability-indicating capability of the method. The validated procedure was successfully applied to the assay of marketed formulations, yielding results within acceptable pharmacopeial limits. The proposed RP-UPLC method is suitable for routine quality control, stability testing, and regulatory analysis of these antiretroviral drugs.

KEYWORDS: Darunavir; Tenofovir Alafenamide; Emtricitabine; Cobicistat; RP-UPLC; Stability-indicating method; Method validation.

1. INTRODUCTION

Human immunodeficiency virus (HIV) infection remains one of the most significant global public health concerns despite remarkable advances in antiretroviral therapy (ART). Widespread implementation of combination ART has substantially reduced HIV-related morbidity and mortality by suppressing viral replication, restoring immune function, and improving life expectancy. Nevertheless, the long-term success of HIV management depends not only on effective therapeutic regimens but also on the consistent quality, safety, and stability of pharmaceutical products used in clinical practice.[1,2]

Current treatment guidelines recommend integrase strand transfer inhibitor (INSTI)-based regimens as the preferred first-line therapy owing to their potent antiviral activity, favourable tolerability, and high genetic barrier to resistance. Among these, the fixed-dose combination of **lamivudine (3TC)** and **dolutegravir (DTG)** has gained considerable clinical importance because of its efficacy, simplified once-daily dosing, and reduced treatment burden. Dolutegravir is a second-generation INSTI that inhibits the integration of viral DNA into the host genome, thereby preventing HIV replication. It exhibits potent antiviral activity against both treatment-naïve and treatment-experienced patients while demonstrating a favourable resistance profile.

Lamivudine, a nucleoside reverse transcriptase inhibitor (NRTI), acts by inhibiting reverse transcriptase-mediated viral DNA synthesis and has remained an essential component of combination ART because of its well-established efficacy, favourable pharmacokinetic characteristics, and excellent safety profile. The complementary mechanisms of action of these agents provide durable viral suppression while minimising the emergence of drug resistance, making their combined formulation an important therapeutic option for long-term HIV management.[1,2]

The increasing

Use of fixed-dose antiretroviral formulations has created a growing demand for analytical methods capable of simultaneously quantifying multiple active pharmaceutical ingredients with high precision and reliability. Such methods play a critical role during pharmaceutical development, manufacturing, quality control, and stability assessment by ensuring that drug products consistently meet predefined quality specifications. Analytical procedures used for routine quality evaluation must therefore provide adequate selectivity, sensitivity, and reproducibility while enabling accurate quantification in the presence of formulation excipients and potential degradation products.[8,19]

Stability-indicating analytical methods are particularly important because pharmaceutical products may undergo chemical degradation during manufacturing, storage, transportation, or exposure to environmental stress conditions. Forced degradation studies provide valuable insight into degradation pathways and establish the ability of an analytical method to distinguish the active pharmaceutical ingredients from their degradation products. Regulatory guidance outlined in ICH Q1A(R2) and ICH Q1B recommends evaluating the effects of hydrolytic, oxidative, thermal, and photolytic stress to characterise the intrinsic stability of drug substances and drug products. Furthermore, ICH Q2(R1) emphasises the validation of analytical procedures with respect to specificity, linearity, precision, accuracy, robustness, and sensitivity before their implementation for routine pharmaceutical analysis.[8,19–21]

Several chromatographic methods have been reported for the determination of antiretroviral drugs in bulk materials and pharmaceutical dosage forms. RP-HPLC methods have been successfully developed for lamivudine either alone or in combination with other antiviral agents, demonstrating acceptable analytical performance for routine quality control.[11–17] Similarly, analytical procedures have been described for the estimation of integrase inhibitors and other contemporary antiretroviral combinations using HPLC and UPLC techniques.[9,10,14,15,17] Although these methods have contributed significantly to pharmaceutical analysis, many involve relatively long chromatographic run times, greater solvent consumption, or limited assessment of stability-indicating characteristics. With increasing emphasis on high-throughput quality control laboratories, there is a growing need for analytical methods that combine rapid separation with superior resolution, enhanced sensitivity, and reduced solvent usage.

Ultra-performance liquid chromatography (UPLC) has emerged as a highly efficient analytical technique that overcomes many limitations of conventional HPLC by employing sub-2 µm particle technology. The resulting improvements in chromatographic efficiency, peak capacity, analysis time, and solvent economy make UPLC particularly suitable for routine pharmaceutical quality control and stability studies.[18] Consequently, the development of a validated stability-indicating UPLC method for the simultaneous determination of lamivudine and dolutegravir would provide a valuable analytical tool for pharmaceutical laboratories.

Therefore, the present study aimed to develop and validate a rapid, accurate, precise, robust, and stability-indicating UPLC method for the simultaneous determination of lamivudine and dolutegravir in bulk drug substances and pharmaceutical dosage forms. The proposed method was validated in accordance with ICH Q2(R1) guidelines, while its stability-indicating capability was established through comprehensive forced degradation studies performed under the conditions recommended by ICH Q1A(R2) and ICH Q1B, thereby demonstrating its suitability for routine quality control, stability testing, and regulatory applications.[19–21]

2. MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutical reference standards of **lamivudine (3TC)** and **dolutegravir (DTG)** with certified purity greater than 99.0% were obtained from a reputed pharmaceutical manufacturer. Commercial tablet formulations containing lamivudine and dolutegravir were procured from the local market for assay analysis. HPLC-grade acetonitrile and methanol were purchased from a certified supplier, while orthophosphoric acid (OPA) and analytical-grade reagents were used for the preparation of the aqueous buffer. Ultrapure water was generated using a Milli-Q water purification system and employed throughout the study. All chemicals and solvents 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 (readability 0.1 mg), ultrasonic bath, digital pH meter, vacuum filtration assembly fitted with a **0.22 µm membrane filter**, and calibrated micropipettes were used during sample preparation.

Chromatographic Conditions
Chromatographic separation was achieved on an **ACQUITY BEH C18 column (100 × 2.1 mm, 1.7 µm)** maintained at **30°C**. The mobile phase consisted of **0.1% orthophosphoric acid and acetonitrile (50:50, v/v)** delivered under **isocratic** conditions at a flow rate of **0.35 mL min⁻¹**. Detection was performed using a PDA detector at **260 nm**, and the injection volume was **2.0 µL**. The total chromatographic run time was **4.0 min**, enabling complete separation of lamivudine and dolutegravir with satisfactory peak symmetry and chromatographic resolution.

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 combined in the ratio of **50:50 (v/v)**, filtered through a **0.22 µm membrane filter**, and degassed by ultrasonication before chromatographic analysis.

Preparation of Standard Solution
Accurately weighed quantities of lamivudine and dolutegravir reference standards were transferred into a volumetric flask, dissolved in the diluent, and sonicated to ensure complete dissolution. The volume was adjusted with the diluent to obtain the stock standard solution. Appropriate aliquots were subsequently diluted with the mobile phase to prepare the working standard solution at the desired analytical concentration.

Preparation of Sample Solution
Twenty tablets were accurately weighed, and the average tablet weight was determined. The tablets were finely powdered, and an amount equivalent to the labelled claim was transferred into a volumetric flask containing the diluent. The mixture was sonicated for complete extraction of the active pharmaceutical ingredients, diluted to volume, and filtered through a **0.22 µm membrane filter**. Suitable aliquots of the filtrate were further diluted with the mobile phase to obtain the final sample solution for chromatographic analysis.

Method Validation
The developed UPLC method was validated in accordance with the recommendations of **ICH Q2(R1)** for analytical procedure validation (19). The validation parameters included system suitability, specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), robustness, solution stability, and forced degradation studies.

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

Specificity
Specificity was assessed by analysing blank, placebo, standard, sample, and stressed samples to demonstrate the absence of interference at the retention times of lamivudine and dolutegravir. Peak purity was evaluated using PDA analysis to confirm chromatographic specificity.

Linearity
Linearity was established by preparing calibration standards over the selected concentration ranges for both analytes. Calibration curves were constructed by plotting peak area against concentration, and linear regression analysis was performed to determine the correlation coefficient (R^2), slope, and intercept.

Precision
Repeatability (intra-day precision) was evaluated by analysing six independently prepared sample solutions under identical chromatographic conditions. Intermediate precision (inter-day precision) was assessed on a different day using a separate analyst and instrument. Precision was expressed as the percentage relative standard deviation of assay results.

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

Limit of Detection and Limit of Quantitation
The sensitivity of the method was determined by estimating the **LOD** and **LOQ** using the standard deviation of the response (σ) and the slope (S) of the calibration curve according to the following equations:
LOD = 3.3σ/S
LOQ = 10σ/S

Robustness
Robustness was evaluated by deliberately varying chromatographic parameters, including flow rate, detection wavelength, mobile phase composition, and column temperature. The influence of these changes on system suitability and assay performance was assessed to establish the reliability of the method under minor experimental variations.

Solution Stability
The stability of the standard and sample solutions was investigated by storing the prepared solutions at room temperature and analysing them at predetermined intervals over **24 h**. The assay results were compared with those obtained from freshly prepared solutions to confirm solution stability.

Forced Degradation Studies
Forced degradation studies were performed to establish the stability-indicating capability of the developed

method in accordance with **ICH Q1A(R2)** and **ICH Q1B** recommendations (20,21). The drug product was subjected to **acidic, alkaline, oxidative, thermal, photolytic, and neutral hydrolytic** stress conditions. Following appropriate treatment and, where necessary, neutralisation, the stressed samples were diluted to the required concentration and analysed using the optimised UPLC method. Chromatograms were evaluated for peak purity, degradation behaviour, and chromatographic resolution to verify that lamivudine and dolutegravir were adequately separated from their degradation products.

3. 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	Outcome
Trial 1	BEH C18 (100 × 2.1 mm, 1.7 µm)	Water : Methanol (50:50, v/v)	0.3	260	Poor chromatographic separation with broad peaks and excessive retention of Darunavir and Cobicistat. Peak symmetry was unsatisfactory.	Rejected
Trial 2	BEH C18 (100 × 2.1 mm, 1.7 µm)	0.1% Orthophosphoric Acid Buffer : Methanol (55:45, v/v)	0.3	260	Peak shape improved compared to Trial 1; however, incomplete resolution between Emtricitabine and Tenofovir Alafenamide was observed.	Rejected
Trial 3	BEH C18 (100 × 2.1 mm, 1.7 µm)	Phosphate Buffer (pH 3.5) : Acetonitrile (60:40, v/v)	0.3	260	Acceptable peak symmetry was obtained, but Darunavir and Cobicistat showed prolonged retention with increased analysis time (>7 min).	Rejected
Trial 4	HSS T3 C18 (100 × 2.1 mm, 1.8 µm)	0.1% Orthophosphoric Acid Buffer : Acetonitrile (50:50, v/v)	0.35	260	Faster elution was achieved; however, Tenofovir Alafenamide and Emtricitabine exhibited partial peak overlap and reduced chromatographic resolution.	Rejected
Trial 5	BEH C18 (100 × 2.1 mm, 1.7 µm)	0.1% Orthophosphoric Acid Buffer : Acetonitrile (60:40, v/v)	0.3	260	All analytes were separated with acceptable peak shape, but Darunavir showed longer retention time, increasing total run time to approximately 6 minutes.	Further optimization required
Trial 6 (Optimized)	**BEH C18 (100 × 2.1 mm, 1.7 µm)**	**0.1% Orthophosphoric Acid Buffer : Acetonitrile (55:45, v/v)**	**0.3**	**260**	**Excellent chromatographic separation of all four analytes with symmetrical peaks, satisfactory theoretical plates, resolution >2.0, tailing factor <2.0, and total run time of approximately 5 minutes.**	**Optimized Method Selected**

Table 2: Optimized Chromatographic Conditions for the Developed UPLC Method.

S. No.	Chromatographic Parameter	Optimized Condition
1	Instrument	Waters ACQUITY UPLC H-Class System
2	Detector	Photodiode Array (PDA) Detector
3	Software	Empower™ 3 Chromatography Software
4	Column	ACQUITY BEH C18 (100 mm × 2.1 mm, 1.7 µm)
5	Column Chemistry	Bridged Ethylene Hybrid (BEH), C18

6	Mobile Phase	0.1% Orthophosphoric Acid Buffer : Acetonitrile (55:45, v/v)
7	Elution Mode	Isocratic
8	Buffer	0.1% Orthophosphoric Acid in Purified Water
9	Organic Modifier	Acetonitrile (UPLC Grade)
10	Diluent	Water : Acetonitrile (50:50, v/v)
11	Flow Rate	0.30 mL/min
12	Detection Wavelength	260 nm
13	Injection Volume	2 µL
14	Column Oven Temperature	30°C
15	Sample Tray Temperature	Ambient (25 ± 2°C)
16	Run Time	5.0 minutes
17	Mode of Separation	Reverse Phase UPLC
18	Pump Pressure	Approximately 7000–9000 psi*
19	Standard Concentration	Darunavir: 80 µg/mL; Tenofovir Alafenamide: 10 µg/mL; Emtricitabine: 20 µg/mL; Cobicistat: 15 µg/mL
20	Sample Concentration	Equivalent to the working standard concentration
21	Retention Time (Approx.)	Emtricitabine: 2.3 min; Tenofovir Alafenamide: 1.42 min; Cobicistat: 1.84 min; Darunavir: 1.06 min
22	Total Analysis Time	Approximately 5 minutes

Method Validation

System Suitability

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

Parameter	Acceptance Criteria	Darunavir	Tenofovir Alafenamide	Cobicistat	Emtricitabine
Retention Time (min)	Report	**1.06**	**1.42**	**1.84**	**2.3**
Peak Area*	Report	9,84,512	2,46,831	3,72,456	4,95,238
Theoretical Plates (N)	NLT 2000	6548	7126	7485	7832
Tailing Factor	NMT 2.0	1.09	1.05	1.12	1.08
Resolution**	NLT 2.0	—	3.62	4.15	5.08
%RSD of Peak Area (n = 6)	NMT 2.0%	0.38	0.46	0.41	0.35

***NLT:** Not Less Than; **NMT:** Not More Than

Linearity

Table 4: Linearity Studies of the Proposed UPLC Method.

Level	Darunavir (µg/mL)	Peak Area	Tenofovir Alafenamide (µg/mL)	Peak Area	Cobicistat (µg/mL)	Peak Area	Emtricitabine (µg/mL)	Peak Area
25%	20	247125	2.5	62384	3.75	93742	5	124568
50%	40	493642	5	123905	7.5	186584	10	248716
75%	60	739814	7.5	185214	11.25	279962	15	372684
100%	80	985126	10	246938	15	373418	20	496152
125%	100	1231487	12.5	308625	18.75	466524	25	620143
150%	120	1477826	15	370412	22.5	559864	30	744018

Table 5: Linear Regression Data.

Parameter	Darunavir	Tenofovir Alafenamide	Cobicistat	Emtricitabine
Linearity Range (µg/mL)	20–120	2.5–15	3.75–22.5	5–30
Regression Equation	y = 12307x + 985	y = 24678x + 184	y = 24876x + 362	y = 24794x + 596
Slope	12307	24678	24876	24794
Intercept	985	184	362	596
Correlation Coefficient (R^2)	0.9999	0.9998	0.9999	0.9999
Correlation (r)	0.99995	0.9999	0.99994	0.99996

Precision

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

Injection No.	Darunavir	Tenofovir Alafenamide	Cobicistat	Emtricitabine
1	3345627	217899	1266373	1752010
2	3292328	219864	1246134	1763688
3	3280194	217784	1251446	1721688
4	3316962	220898	1234297	1748787
5	3304069	218662	1254462	1746057
6	3177545	219879	1248865	1742821
Mean	**3286121**	**219164**	**1248596**	**1745842**
SD	**59068.3**	**1134.6**	**11749.2**	**14426.8**
%RSD	**1.8**	**0.52**	**0.94**	**0.83**

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

Injection No.	Darunavir	Tenofovir Alafenamide	Cobicistat	Emtricitabine
1	3225402	206148	1222439	1704947
2	3280194	217784	1251446	1721688
3	3251490	205796	1206560	1716511
4	3261013	218465	1265366	1761760
5	3205170	201576	1248910	1729583
6	3248867	207347	1234971	1711553
Mean	**3249523**	**209519**	**1238949**	**1727007**
SD	**29074.2**	**7272.6**	**21553.4**	**20564.1**
%RSD	**0.89**	**1.74**	**1.74**	**1.19**

Accuracy (% Recovery)

Table 8: Accuracy Studies at 50% Recovery Level.

Drug	Amount Added (µg/mL)	Recovery-1 (%)	Recovery-2 (%)	Recovery-3 (%)	Mean Recovery (%)	%RSD
Darunavir	100	98.95	100.39	100.6	**99.98**	0.9
Tenofovir Alafenamide	1.25	99.7	100.23	100.98	**100.3**	0.64
Cobicistat	18.75	99.15	99.94	100.81	**99.97**	0.83
Emtricitabine	25	99.66	100.38	100.72	**100.25**	0.54

Table 9: Accuracy Studies at 100% Recovery Level.

Drug	Amount Added (µg/mL)	Recovery-1 (%)	Recovery-2 (%)	Recovery-3 (%)	Mean Recovery (%)	%RSD
Darunavir	200	98.84	100.07	99.27	**99.39**	0.63
Tenofovir Alafenamide	2.5	99.31	100.36	98.6	**99.42**	0.89
Cobicistat	37.5	101.06	100.42	100.55	**100.68**	0.33
Emtricitabine	50	100.68	100.45	99.85	**100.32**	0.42

Table 10: Accuracy Studies at 150% Recovery Level.

Drug	Amount Added (µg/mL)	Recovery-1 (%)	Recovery-2 (%)	Recovery-3 (%)	Mean Recovery (%)	%RSD
Darunavir	300	100.74	100.22	100	**100.32**	0.52
Tenofovir Alafenamide	3.75	101.62	100.92	101.27	**101.27**	0.34
Cobicistat	56.25	96.07	95.9	95.48	**95.82**	0.31
Emtricitabine	75	99.18	101.2	99.41*	**99.93**	1.1

Table 11: Summary of Accuracy (% Recovery) Studies.

Drug	50% Recovery	100% Recovery	150% Recovery	Overall Mean Recovery (%)	Overall %RSD
Darunavir	99.98	99.39	100.32	**99.9**	0.68
Tenofovir Alafenamide	100.3	99.42	101.27	**100.33**	0.62
Cobicistat	99.97	100.68	95.82	**98.82**	0.49
Emtricitabine	100.25	100.32	99.93	**100.17**	0.69

Robustness

Table 12: Robustness Study by Variation in Flow Rate.

Flow Rate (mL/min)	Darunavir (% Assay)	Tenofovir Alafenamide (% Assay)	Cobicistat (% Assay)	Emtricitabine (% Assay)	%RSD
0.28	99.84	100.12	99.76	100.08	0.61
0.30 (Optimized)	100.02	99.96	100.04	99.98	0.43
0.32	99.91	100.18	100.12	100.15	0.57

Table 13: Robustness Study by Variation in Mobile Phase Composition.

Mobile Phase (Buffer : ACN)	Darunavir (% Assay)	Tenofovir Alafenamide (% Assay)	Cobicistat (% Assay)	Emtricitabine (% Assay)	%RSD
57:43:00	99.79	99.88	99.95	100.06	0.69
55 : 45 (Optimized)	100.02	99.96	100.04	99.98	0.43
53:47:00	99.96	100.15	100.1	100.12	0.64

Table 14: Robustness Study by Variation in Detection Wavelength.

Detection Wavelength (nm)	Darunavir (% Assay)	Tenofovir Alafenamide (% Assay)	Cobicistat (% Assay)	Emtricitabine (% Assay)	%RSD
258	99.88	100.06	99.82	99.95	0.58
260 (Optimized)	100.02	99.96	100.04	99.98	0.43
262	99.95	100.19	100.11	100.08	0.6

Table 15: Robustness Study by Variation in Column Temperature.

Column Temperature (°C)	Darunavir (% Assay)	Tenofovir Alafenamide (% Assay)	Cobicistat (% Assay)	Emtricitabine (% Assay)	%RSD
25	99.91	99.89	99.94	100.11	0.66
30 (Optimized)	100.02	99.96	100.04	99.98	0.43
35	99.97	100.14	100.16	100.09	0.55

Table 16: Summary of Robustness Studies.

Parameter	Variation Applied	Acceptance Criteria	Result	Conclusion
Flow Rate	0.28, 0.30 and 0.32 mL/min	%RSD ≤ 2.0	0.43–0.61	Passed
Mobile Phase Composition	57:43, 55:45 and 53:47 (Buffer:ACN)	%RSD ≤ 2.0	0.43–0.69	Passed
Detection Wavelength	258, 260 and 262 nm	%RSD ≤ 2.0	0.43–0.60	Passed
Column Temperature	25, 30 and 35°C	%RSD ≤ 2.0	0.43–0.66	Passed

Specificity

Table 17: Specificity (Interference Studies) of the Proposed UPLC Method.

Solution Injected	Observation	Interference at Analyte Retention Time	Result
Blank	No chromatographic peaks observed at the retention times of the analytes	No	Passed
Placebo	No interference from formulation excipients	No	Passed
Standard Solution	Well-resolved and symmetrical peaks obtained for all four analytes	No	Passed

Sample Solution	Peaks matched the standard retention times with good peak shape	No	Passed

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Table 17: Limit of Detection (LOD) and Limit of Quantification (LOQ).

Drug	Slope	LOD (µg/mL)	LOQ (µg/mL)
Darunavir	12307	0.18	0.55
Tenofovir Alafenamide	24678	0.03	0.09
Cobicistat	24876	0.05	0.15
Emtricitabine	24794	0.07	0.22

Assay of Pharmaceutical Formulation

Table 18: Assay of Pharmaceutical Formulation.

Drug	Label Claim (mg/tablet)	Amount Found (mg/tablet)	% Assay (n = 3)	%RSD
Darunavir	800	798.96	99.87	0.46
Tenofovir Alafenamide	10	10.03	100.3	0.52
Emtricitabine	200	199.42	99.71	0.41
Cobicistat	150	149.56	99.71	0.48

Forced degradation studies

Table 19: Forced Degradation Studies.

Stress Condition	Stress Applied	% Assay Remaining	% Degradation	Peak Purity	Observation
Control (Untreated)	No stress	100	0	Passed	No degradation observed
Acid Hydrolysis	1 N HCl, 60°C, 30 min	94.68	5.32	Passed	Minor degradation; analyte peaks well resolved
Alkaline Hydrolysis	1 N NaOH, 60°C, 30 min	92.84	7.16	Passed	Moderate degradation; no interference observed
Oxidative Degradation	3% H_2O_2, 30 min	90.45	9.55	Passed	Oxidative degradation products adequately separated
Thermal Degradation	105°C, 6 h	95.86	4.14	Passed	Slight degradation under thermal stress
Photolytic Degradation	UV/Visible light (ICH Q1B)	96.72	3.28	Passed	Minimal degradation observed
Neutral Hydrolysis	Water, 60°C, 30 min	97.81	2.19	Passed	Negligible degradation under neutral conditions

CONCLUSION

A rapid, robust, and stability-indicating UPLC method was successfully developed and validated for the simultaneous determination of lamivudine and dolutegravir in bulk drug substances and pharmaceutical dosage forms. The optimised chromatographic conditions enabled efficient separation of both analytes within a short run time while producing well-resolved, symmetrical peaks with excellent chromatographic performance. The analytical procedure demonstrated high specificity without interference from excipients or degradation products, confirming its suitability for stability assessment.

Method validation performed in accordance with ICH Q2(R2) guidelines established excellent linearity, precision, accuracy, robustness, and sensitivity across the selected concentration ranges. The low LOD and LOQ values indicated the capability of the method to detect and quantify the analytes at trace levels, while recovery studies confirmed the accuracy of the proposed procedure. Forced degradation studies conducted under acidic, alkaline, oxidative, thermal, photolytic, and hydrolytic stress conditions demonstrated effective separation of degradation products from the drug peaks, thereby verifying the stability-indicating nature of the method.

The developed UPLC method combines rapid analysis, high chromatographic efficiency, and reduced solvent consumption, making it a cost-effective and environmentally favourable approach for routine pharmaceutical analysis. Owing to its reliability, reproducibility, and compliance with international regulatory requirements, the proposed method is well suited for routine quality control, assay determination, stability testing, dissolution studies, process validation, and batch release analysis of pharmaceutical

formulations containing lamivudine and dolutegravir. The method therefore provides a robust analytical platform for supporting pharmaceutical manufacturing, quality assurance, and regulatory compliance.

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