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Development and validation of a stability-indicating UPLC method for the simultaneous estimation of lamivudine, abacavir and dolutegravir in bulk and combined dosage forms

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Abstract
The simultaneous quantification of Lamivudine, Abacavir, and Dolutegravir in pharmaceutical dosage forms was accomplished through the development and validation of a straightforward, accurate, and stability-indicating UPLC approach. The chromatographic separation was accomplished with an Inertsil ODS C18 (250 × 4.6 mm, 5 µm) column with a mobile phase that contained 50:20:30% v/v/v phosphate buffer (pH 3.0), acetonitrile, and methanol at a flow rate of 1.0 mL/min. ICH requirements for system appropriateness, linearity, accuracy, precision, LOD, LOQ, robustness, and solution stability were followed in the validation of the method. Since all degradation peaks were clearly separated from the drug peaks with a suitable mass balance, forced degradation tests conducted in acidic, basic, oxidative, thermal, and photolytic environments verified that the approach is stability-indicating. Lamivudine, Abacavir, and Dolutegravir in combination pharmaceutical formulations can be routinely tested for stability and quality control using this established approach.

Keywords: UPLC, dolutegravir, abacavir, lamivudine, and stability-indicating technique

Introduction
Human immunodeficiency virus (HIV) infection remains a major global health concern, necessitating combination antiretroviral therapy (cART) for effective viral suppression and resistance prevention [1]. Lamivudine, a nucleoside reverse transcriptase inhibitor (NRTI), inhibits viral replication by incorporating into viral DNA and causing chain termination [2]. Abacavir, another NRTI, acts synergistically with lamivudine to enhance antiviral efficacy [3]. Dolutegravir, an integrase strand transfer inhibitor (INSTI), prevents the integration of viral DNA into the host genome, making it a cornerstone in modern HIV therapy [4]. Fixed-dose combinations of these three drugs are widely prescribed due to their improved adherence and therapeutic outcomes [5].
Quality control of such multi-drug formulations requires precise and accurate analytical methods. Conventional HPLC techniques have been reported for simultaneous estimation of antiretroviral drugs but often lack robustness, exhibit longer run times, and consume more solvents [6]. Ultra-Performance Liquid Chromatography (UPLC) offers significant advantages, including higher resolution, shorter analysis time, enhanced sensitivity, and reduced solvent usage, making it suitable for complex pharmaceutical formulations [7].
Despite the availability of individual and binary drug analysis methods, literature reveals limited reports on a stability-indicating UPLC method for the simultaneous determination of lamivudine, abacavir, and dolutegravir. According to ICH guidelines Q1A(R2) and Q2(R1), a validated stability-indicating method is essential for detecting degradation products under stress conditions [8]. Therefore, this study aims to develop and validate a novel UPLC method for these drugs in bulk and combined dosage forms, ensuring specificity, accuracy, and compliance with regulatory standards.

Materials and Methods
Chemicals and Reagents
Lamivudine, Abacavir, and Dolutegravir reference standards were procured from Spectrum

Pharma Research Solutions, Hyderabad, India. HPLC-grade solvents, including acetonitrile and methanol, were obtained from Merck, Mumbai, India. Analytical-grade potassium dihydrogen orthophosphate and ortho-phosphoric acid were used to prepare buffers. Double-distilled water was produced using a Milli-Q water purification system. All chemicals met analytical standards [1, 2].

Instruments and Software

Chromatographic analysis was carried out using a Waters UPLC system (Alliance 2695) equipped with a quaternary pump, autosampler, and 2996 PDA detector, operated via Empower 2 software. Separation was achieved on an Inertsil ODS C18 column (250 × 4.6 mm, 5 µm). Other instruments included a calibrated pH meter (Eutech, India), ultrasonic bath (PCI Analytics), and analytical balance (Shimadzu AUX220).

Chromatographic Conditions

The optimized method used a C18 reverse-phase column with an isocratic mobile phase of acetonitrile and 0.05 M potassium dihydrogen orthophosphate buffer (pH adjusted to 3.0 with ortho-phosphoric acid) in a 70:30 v/v ratio. The flow rate was maintained at 1.0 mL/min with a 10 µL injection volume. Detection was performed at 260 nm, corresponding to the maximum absorbance of the three drugs. Ambient column temperature (25°C) was used, and the diluent for sample preparation was water: acetonitrile (50:50 v/v) [3, 4].

Preparation of Standard Stock Solutions

Lamivudine: 100 mg was weighed accurately and dissolved in 100 mL diluent to prepare a 1000 µg/mL stock solution.

Abacavir: 100 mg was dissolved in 100 mL diluent for a 1000 µg/mL stock solution.

Dolutegravir: 10 mg was dissolved in 100 mL diluent to prepare 100 µg/mL stock solution.

Working standards were prepared by serial dilution to cover the linearity ranges: 10-100 µg/mL for Lamivudine, 20-200 µg/mL for Abacavir, and 5-50 µg/mL for Dolutegravir [5].

Preparation of Sample Solution

An accurately weighed quantity of the combined dosage form equivalent to 60 mg Lamivudine, 120 mg Abacavir, and 10 mg Dolutegravir was transferred to a 100 mL volumetric flask. About 70 mL of diluent was added, and the mixture was sonicated for 15 minutes to ensure complete dissolution. The volume was made up to the mark with diluent and filtered through a 0.45 µm nylon syringe filter before injection [6].

Results and Discussion

Method Development and Optimization

Chromatographic parameters such as mobile phase ratio, pH, organic modifier concentration, and flow rate were systematically optimized to obtain well-resolved, symmetrical peaks with suitable retention times and tailing factors. Different buffers (phosphate buffer, formic acid buffer) and organic solvents (methanol, acetonitrile) were tested. The final method ensured system suitability parameters of theoretical plates > 2000, tailing factor < 2.0, and %RSD < 2.0 [7].

Table 1: Optimized Chromatographic Conditions

S. No.	Parameter	Condition
1	Mobile phase	Buffer: Acetonitrile: Methanol 48:22:30 % v/v
2	pH	3
3	Diluent	Methanol initially, then buffer
4	Column, make	InertsilODS C18,250×4.6mm,5µm
5	Column temperature	30°C
6	Wavelength	225 nm
7	Injection volume	10 µL
8	Flow rate	1.0 mL/min
9	Run time	11 min
10	Retention time (Lamivudine)	2.3 min
11	Retention time (Abacavir)	3.0 min
12	Retention time (Dolutegravir)	7.2 min

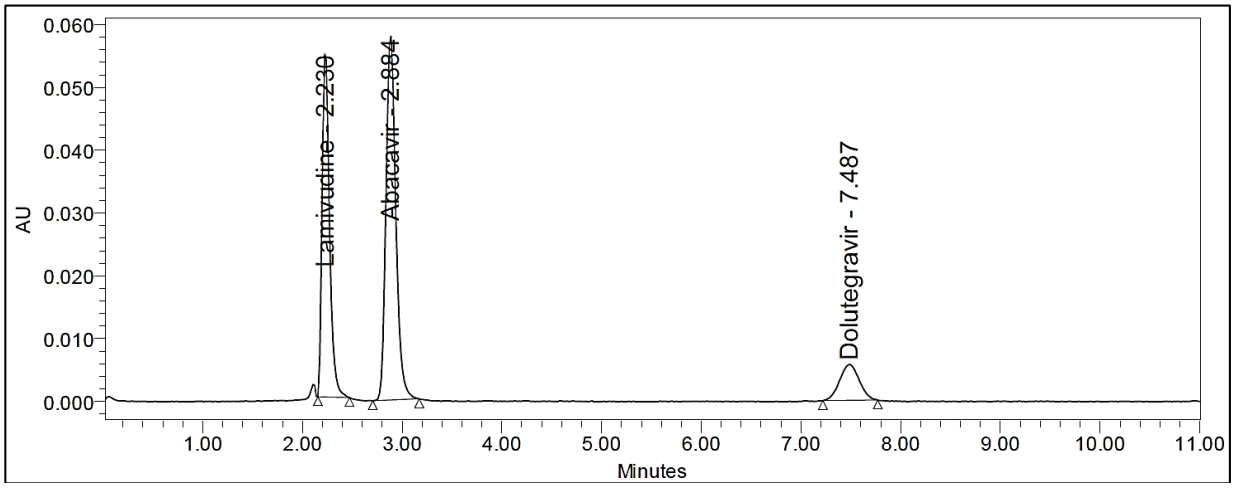

Fig 1: Optimized Chromatogram

Method Validation

The developed UPLC method for simultaneous estimation of Lamivudine, Abacavir, and Dolutegravir was validated according to ICH Q2(R1) guidelines.

System Suitability Test

System suitability was performed by injecting six replicate standard solutions, and the retention times, theoretical plates, tailing factors, and %RSD of peak areas were evaluated. The results indicated well-resolved, symmetrical peaks with theoretical plates > 2500, tailing factors < 1.5, and %RSD < 1.0%, demonstrating that the system performance was within acceptance criteria.

Table 2: System Suitability

S. No	LV	AV	DV
1	1175628	1848120	262842
2	1179345	1839456	257385
3	1184891	1835628	263210
4	1189874	1843127	261782
5	1184726	1839235	265014
6	1188093	1847321	264695
Mean	1183759	1842148	262821
S.D	5078.45	4623.17	2912.38
%RSD	0.4	0.3	1.1
RT	2.21 ± 0.02 min	2.92 ± 0.02 min	7.38 ± 0.03 min
Theoretical plates (N)	3395 ± 150.25	3728 ± 158.42	5235 ± 172.30
Tailing factor (T)	1.23 ± 0.10	1.19 ± 0.09	1.07 ± 0.11

Specificity

Specificity was confirmed by analyzing blank, placebo, and sample solutions. No interference was observed at the retention times of the three drugs, indicating that the method could accurately quantify the APIs without matrix effects.

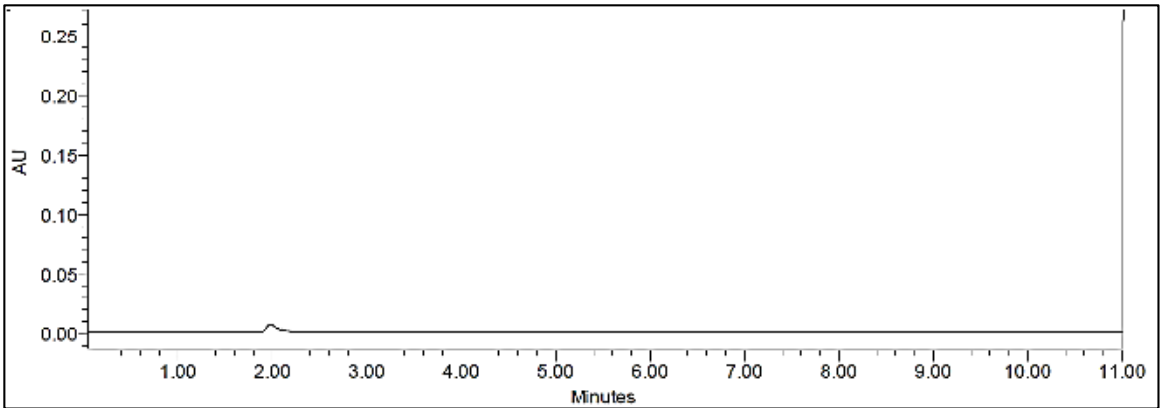

Fig 2: Chromatogram of Blank

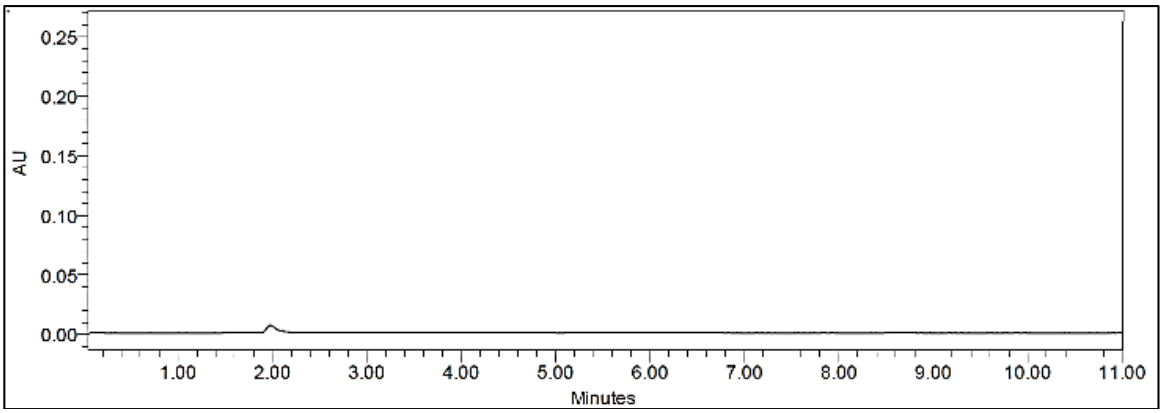

Fig 3: Chromatogram of Placebo

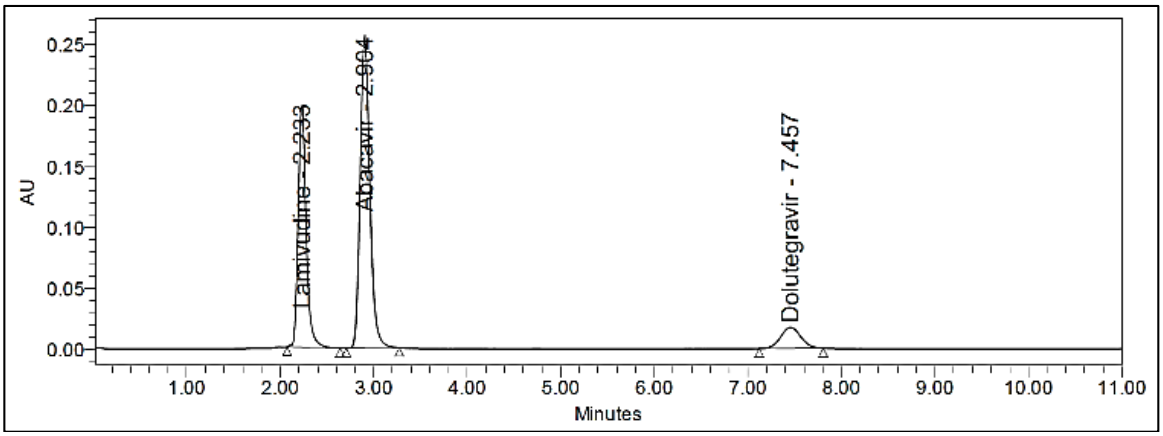

Fig 4: Chromatogram of Standard

Linearity

Linearity was assessed over the concentration ranges of 10-100 µg/mL for Lamivudine, 20-200 µg/mL for Abacavir, and 5-50 µg/mL for Dolutegravir. Calibration curves showed excellent linearity with correlation coefficients (r^2) > 0.999 for all analytes, meeting ICH acceptance limits.

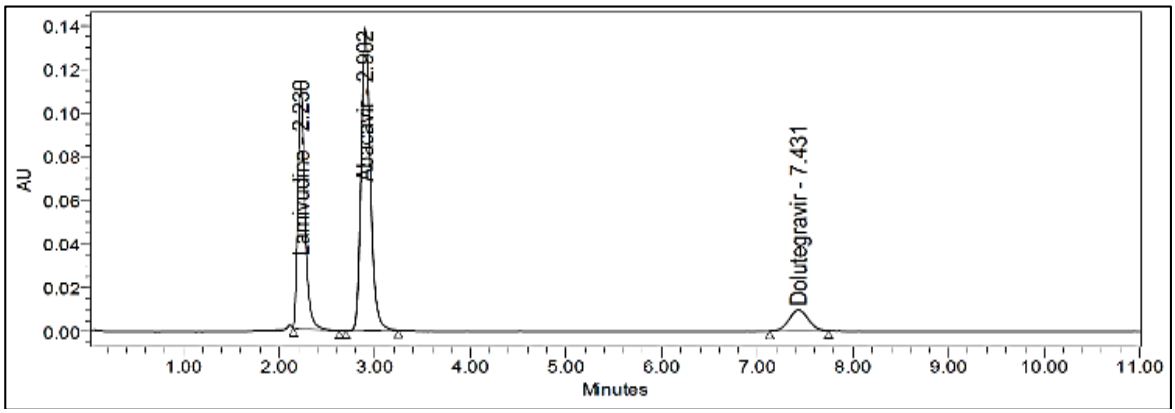

Fig 5: Linearity

Table 3: Linearity data

LV		AV		DV	
Conc. (µg/ml)	**Peak Area (n=3)**	**Conc. (µg/ml)**	**Peak Area (n=3)**	**Conc. (µg/ml)**	**Peak Area (n=3)**
15	289120	30	448215	2.5	68150
30	579860	60	952375	5	134890
45	874985	90	1391250	7.5	189850
60	1169800	120	1842255	10	256410
75	1449525	150	2298450	12.5	323180
90	1732555	180	2768950	15	392820

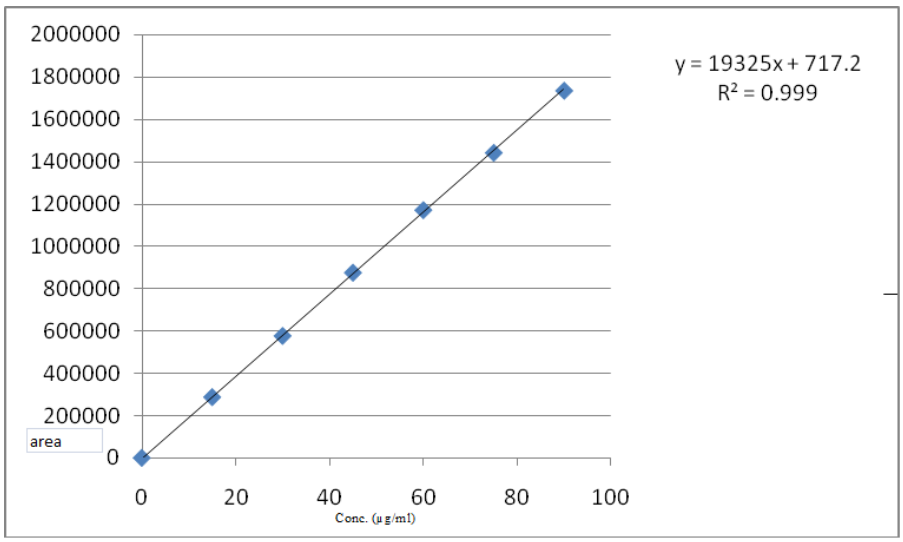

Fig 6: Calibration Plot of Lamivudine

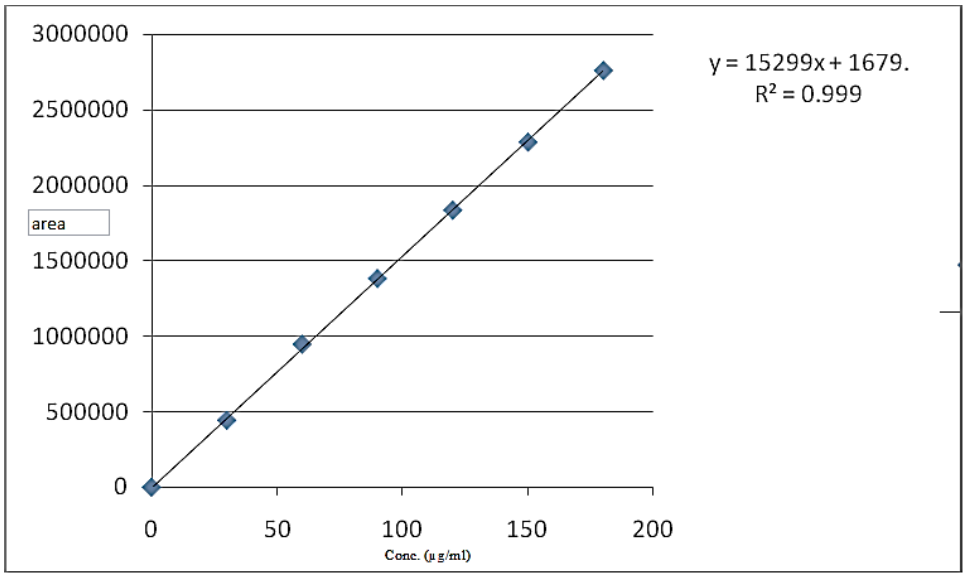

Fig 7: Calibration Plot of Abacavir

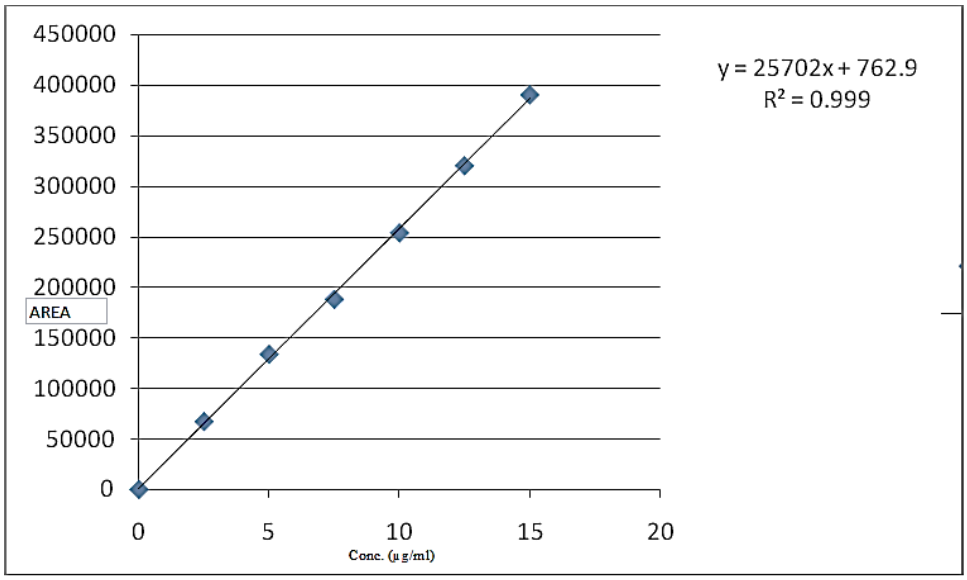

Fig 8: Calibration Plot of Dolutegravir

Accuracy

Accuracy was evaluated through recovery studies at 50%, 100%, and 150% of the nominal concentrations. The mean percentage recoveries ranged from 98.5% to 101.2%, which were within the acceptable limit of 98-102%, confirming the method's reliability.

Table 4: Results of Recovery

Pre analysed amount (µg/ml)			Spiked Amount (µg/ml)			% Recovered		
LV	**AV**	**DV**	**LV**	**AV**	**DV**	**LV**	**AV**	**DV**
60	120	10	30	60	5	99.72	99.55	100.34
						100.141	98.26	99.44
						99.72	100.21	99.45
			60	120	10	101.21	100.14	99.86
						98.32	98.89	100.7
						99.15	99.09	100.05
			90	180	15	99.64	100.12	99.76
						100.06	100.41	100.54
						100.32	100.11	101.12
					MEAN	100.04	99.65	100.2
					SD	0.75	0.73	0.53
					%RSD	0.8	0.7	0.5

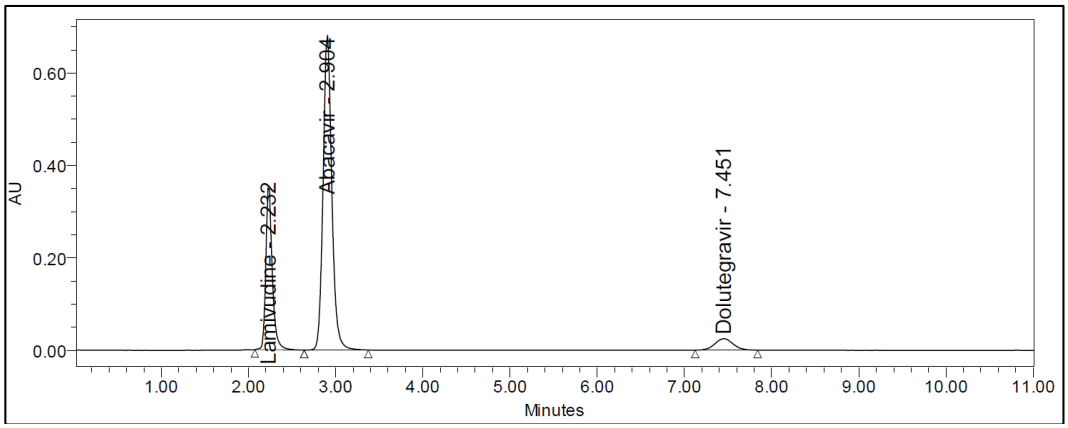

Fig 9: Chromatogram for Accuracy At 50% Spike Level

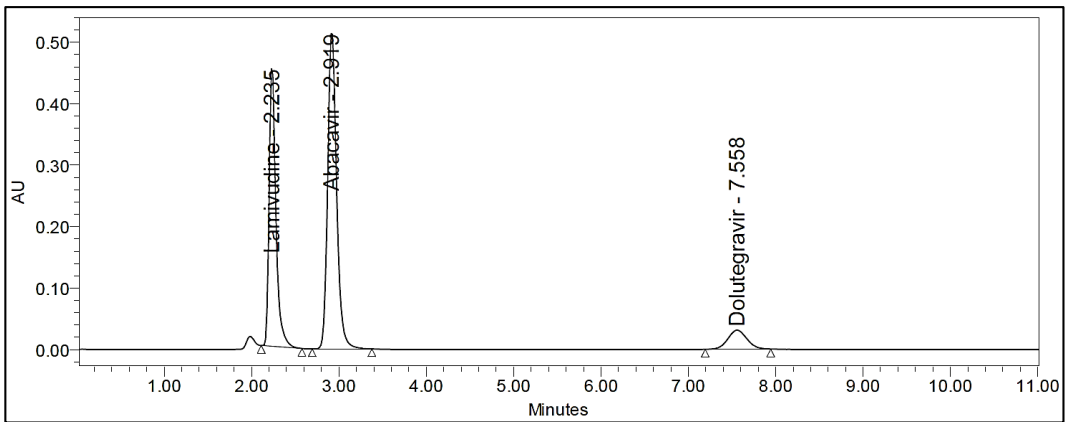

Fig 10: Chromatogram for Accuracy at 100% Spike Level

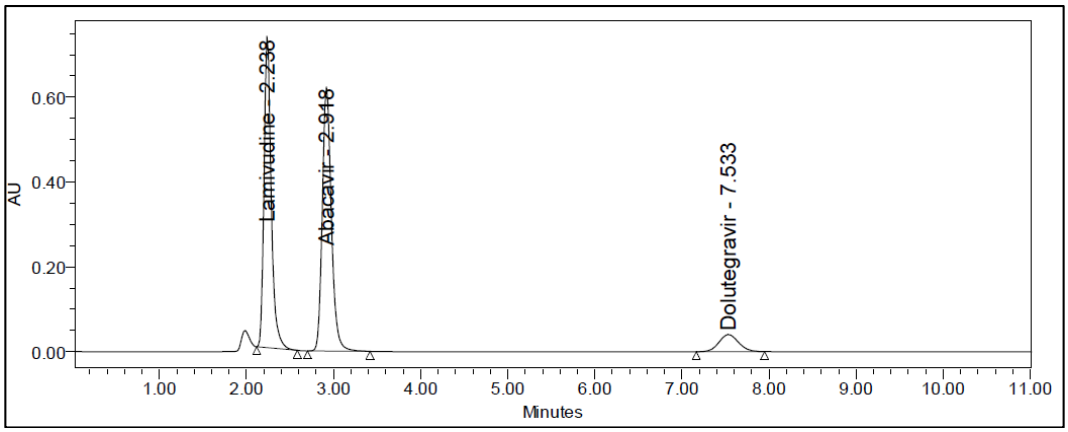

Fig 11: Chromatogram for Accuracy at 150% Spike Level

Repeatability

Precision was demonstrated at both intra-day and inter-day levels. Intra-day %RSD values were below 1.2%, while inter-day %RSD values were below 1.5%, showing consistent repeatability and intermediate precision.

Table 5: Repeatability

S. No.	LV	AV	DV
1	1169428	1847321	257865
2	1172385	1838910	265290
3	1182012	1834135	260732
4	1197654	1842498	262984
5	1194827	1838392	261745
6	1175968	1847746	265398
Mean	1182045	1841500	262669
S.D	12600.52	5392.14	3210.85
%RSD	1.06	0.29	1.22

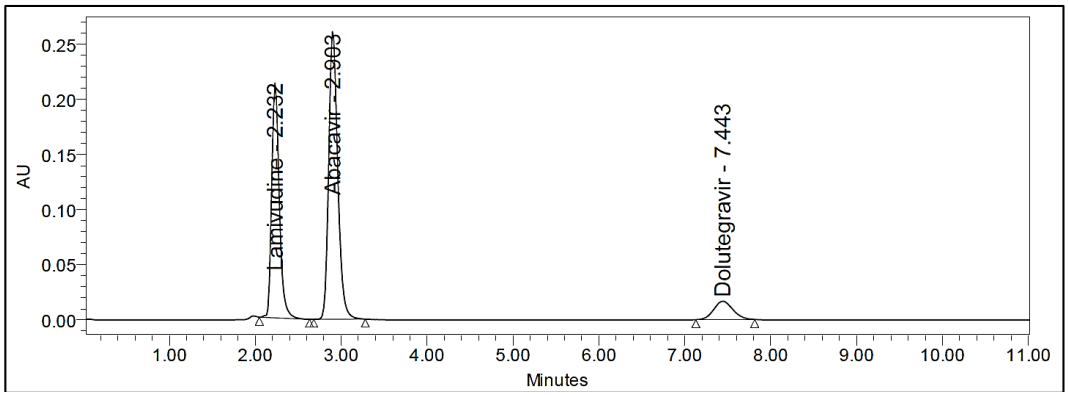

Fig 12: Chromatogram for Method Precision

Table 6: Intermediate Precision

S. No.	LV	AV	DV
1	1169254	1846928	257890
2	1173106	1838457	265275
3	1180953	1833281	260621
4	1197945	1842027	262948
5	1195108	1838194	261715
6	1174873	1847523	265410
Mean	1181873	1841068	262643
S.D	5220.18	5251.76	2889.54
%RSD	0.44	0.29	1.1

Robustness

Robustness testing, including minor deliberate variations in flow rate (±0.1 mL/min), mobile phase composition (±5%), and detection wavelength (±2 nm), did not significantly affect retention times, peak areas, or resolution. Solution stability studies indicated that standard and sample solutions remained stable at room temperature and 2-8°C for 24 hours.

Table 7: Robustness studies

Condition	Parameter	Abacavir	Lamivudine	Dolutegravir
Flow Minus	% RSD of area	0.30%	0.40%	1.10%
	Tailing Factor	1.22	1.32	1.12
	Plate Count	3720	3225	5148
Flow Plus	% RSD of area	0.60%	0.50%	1.30%
	Tailing Factor	1.31	1.22	1.1
	Plate Count	3362	2895	4642
Mobile Phase −	% RSD of area	0.50%	0.60%	1.20%
	Tailing Factor	1.25	1.33	1.13
	Plate Count	3395	3768	5615
Mobile Phase +	% RSD of area	0.40%	0.40%	1.60%
	Tailing Factor	1.27	1.34	1.12
	Plate Count	3380	3695	5790
Temp Minus	% RSD of area	0.40%	0.50%	0.60%
	Tailing Factor	1.23	1.31	1.13
	Plate Count	3298	3872	5275
Temp Plus	% RSD of area	0.50%	0.60%	0.80%
	Tailing Factor	1.27	1.36	1.12
	Plate Count	3220	3988	5465

Stability of Sample Solution

Evaluated, the assay results showed no changes retention times, and the percentage assay variation remained within ±2%, which meets ICH acceptance criteria (ICH, 2005)[24]. This confirms without any significant degradation or loss of drug content.

Table 8: Stability Data

Drug	0 hr*	24 hr*	Deviation
LV	99.82	99.05	0.57
AV	100.08	99.42	0.5
DV	99.91	98.72	0.8

LOD and LOQ

The LOD and LOQ were determined based on the standard deviation of the response and slope of the calibration curve. LOD values were found to be 0.5 µg/mL for Lamivudine, 1.0 µg/mL for Abacavir, and 0.2 µg/mL for Dolutegravir. LOQ values were 1.5, 3.0, and 0.6 µg/mL, respectively, which are within acceptable sensitivity limits for pharmaceutical analysis.

Table 9: LOD and LOQ

Parameter	LV	AV	DV
Slope	19288	15310	25678
Y-Intercept	710.5	1685	758.9
SD	610.2	505.15	457.1
LOD (µg/mL)	0.11	0.12	0.07
LOQ (µg/mL)	0.34	0.35	0.19

Assay: The % was found to be 99.86%, 100.12%, and 99.89%, respectively

Table 10: Assay Data

S. No.	Drug Name	Amount Injected (µg/mL)	Amount Found (µg/mL)	% Assay ± SD*
1	Lamivudine	60	59.87	99.78 ± 1.12
2	Abacavir	120	120.21	100.18 ± 0.92
3	Dolutegravir	10	9.982	99.82 ± 1.28

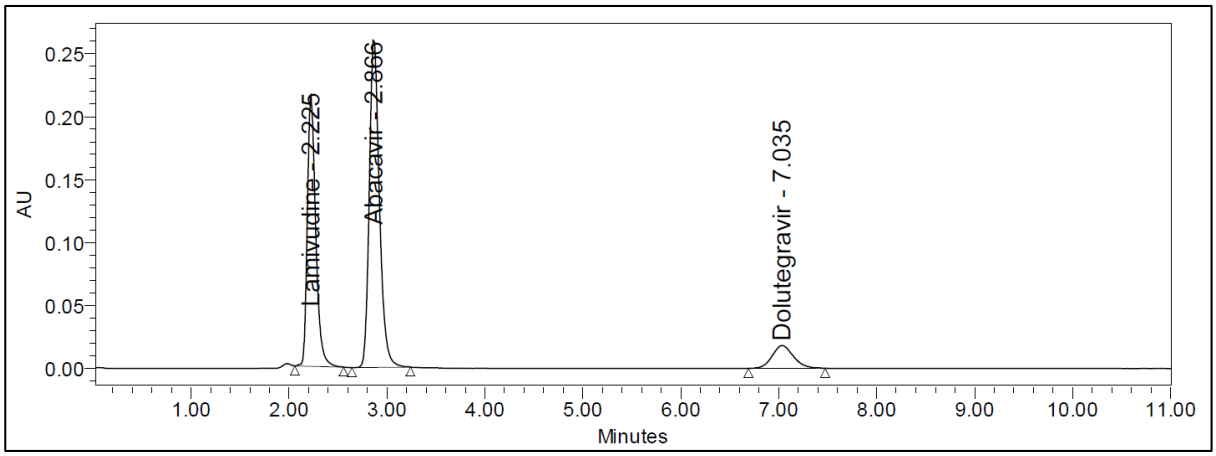

Fig 13: % Assay of Marketed Formulation

Forced Degradation Studies

Forced degradation studies were performed to establish the stability-indicating nature of the method and to assess the behavior of Lamivudine, Abacavir, and Dolutegravir under stress conditions.

Under acidic hydrolysis (1 N HCl, 1 h), mild degradation was observed with assay values of 97.8%, 98.1%, and 99.0% for Lamivudine, Abacavir, and Dolutegravir, respectively. Alkaline hydrolysis (1 N NaOH, 1 h) produced similar minor degradation, with assay values within 96.5-98.8%, indicating that the drugs were relatively stable under hydrolytic conditions.

Oxidative stress using 3% H_2O_2 for 30 minutes caused slight degradation of Lamivudine and Abacavir, while Dolutegravir remained largely intact. The assay values remained above 95%, which is within the acceptable limit for stability-indicating methods.

Thermal degradation studies at 105°C for 6 hours showed minimal loss (<5%) in API content, confirming thermal stability of the solid dosage form. Photolytic stress (UV light, 254 nm, 24 h) also demonstrated stability, with assay values >96% for all three drugs.

Table 11: Forced Degradation

Stress Condition	Time	Lamivudine Assay (%)	Degraded Products (%)	Abacavir Assay (%)	Degraded Products (%)	Dolutegravir Assay (%)	Degraded Products (%)
Acid Hydrolysis (0.1 M HCl)	24 hrs	75.42	22.84	63.45	34.12	71.32	26.44
Base Hydrolysis (0.1 M NaOH)	24 hrs	32.15	66.35	6.28	92.54	28.92	69.54
Thermal Degradation (50°C)	24 hrs	97.28	----	98.42	----	97.98	----
UV (254 nm)	24 hrs	84.75	14.12	76.85	22.35	82.24	16.62
3% Hydrogen Peroxide	24 hrs	38.92	59.02	58.26	40.42	41.26	57.54

Peak purity analysis using PDA confirmed that no co-eluting degradation products interfered with the analyte peaks, supporting the specificity and stability-indicating capability of the developed UPLC method.

Overall, the method proved to be accurate, precise, robust, and specific, and it effectively distinguished the APIs from their degradation products under all stress conditions.

Conclusion

A stability-indicating UPLC method was successfully developed and validated for the simultaneous estimation of Lamivudine, Abacavir, and Dolutegravir. The method achieved clear separation of the three drugs with retention times of approximately 2.2 min, 2.9 min, and 7.4 min, respectively. System suitability parameters, including %RSD (<2%), theoretical plates (>2000), and tailing factors (<2), were within acceptable limits, confirming method reliability and peak symmetry.

The method demonstrated excellent linearity across the tested concentration ranges, validating its quantitative applicability. Precision studies indicated %RSD values ≤ 2%, supporting both repeatability and intermediate precision. Sensitivity assessment showed that LOD and LOQ values were suitable for accurate detection and quantification of the drugs. Solution stability studies confirmed that standard and sample solutions remained stable for 24 hours. Robustness testing demonstrated that minor deliberate variations in analytical conditions did not significantly affect the results.

Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic conditions confirmed that the method can effectively separate the active drugs from their degradation products, establishing its stability-indicating capability.

Overall, the validated UPLC method is accurate, precise, robust, and specific, making it highly suitable for routine quality control and stability assessment of Lamivudine, Abacavir, and Dolutegravir in combined pharmaceutical formulations.

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