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ORIGINAL ARTICLE

Analytical Method Development and Validation for The Simultaneous Estimation of Dolutegravir and Lamivudine by RP-HPLC in Bulk and Tablet Dosage Form

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ABSTRACT
A simple, precise, accurate and sensitive high performance liquid chromatographic method was developed and validated for simultaneous assessment of the Dolutegravir and Lamivudine in their tablet dosage form. The Phenomenex ODS-3 column (250 mm X 4.6 mm, 5µm) was used to establish the technique. The mobile phase used was Acetonitrile: 0.1% OPA in Water (40:60% v/v) at a flow rate of 1 mL/min with isocratic elution. The chromatographic method was injected with 20 µL of material. Using a UV detector, the two eluted chemicals were found with a wavelength of 269 nm. At 40 ℃, the column temperature was kept constant. Dolutegravir and lamivudine were shown to have retention durations of 9.8 minutes and 1.8 minutes, correspondingly. For dolutegravir and lamivudine, the linearity range was 2.5 to 7.5 µg/mL and 15 to 45 µg/mL, one-to-one, with correlation coefficients of 0.9998 and 0.9999. Dolutegravir and Lamivudine were revealed to have reclamation percentages of 99.15% and 99.67%, respectively. LOD and LOQ values for dolutegravir and lamivudine were determined to be 0.08 µg and 0.37 µg, in that order.
Keywords: *Dolutegravir, Lamivudine, RP-HPLC, Simultaneous, Validation, Method development*

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INTRODUCTION

An HIV-1 integrase inhibitor called dolutegravir prevents the viral genome from integrating into the host cell by blocking the strand transfer process. Dolutegravir [Fig. 1] is administered in conjunction with many other modern medications, including Lamivudine. Chemically Dolutegravir is (3S, 7R)-N-[(2, 4-difluorophe-nyl) methyl]-11-hydroxy-7-methyl-9, 12-dioxo-4-oxa-1, 8-diazatricyclotetradeca-10, 13-diene-13-carboxamide [1]. Lamivudine [Fig. 2] has the potential to be a nucleoside reverse transcriptase inhibitor (NRTI) and exhibits activity against hepatitis B and HIV-type I (Human Immunodeficiency Virus Type I). Lamivudine, chemically 4-amino-1-[(2R, 5S)-2-(hydroxyl methyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one. Reverse transcriptase lamivudine has been shown to be effective against the hepatitis B, HIV-1, and HIV-2 viruses [2]. The combination of Dolutegravir and Lamivudine is FDA approved manufactured by GSK Healthcare, and marketed as Dovato. Dolutegravir 50 mg and Lamivudine 300 mg are the fixed dose components of the Dovato tablet. [3]

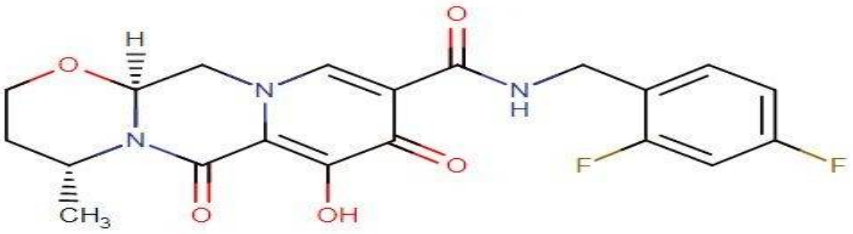


Fig. 1 Structure of Dolutegravir

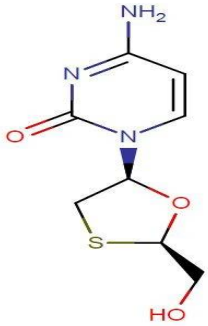


Fig. 2 Structure of Lamivudine

In the pharmaceutical sector, an efficient analytical method is required for the analysis of a medicine either on its own or in combination with other medicines. For the quantitative determination of single and multiple component combinations, a number of analytical approaches were applied. Various analytical methods including UV [4-6], HPLC [7-15], HPTLC [16, 17] and LC / MS / MS [18-22] have been described in the literature to assess Dolutegravir, Lamivudine, and certain antiviral medications by individually or in combination with other medications. There is no formal method for concurrent evaluation, according to the literature study. In both bulk and tablet dosage forms, dolutegravir and lamivudine were examined using RP-HPLC. This research is therefore being done in order to advancement and validate a new, simple method for the simultaneous assessment of dolutegravir and lamivudine in pharmaceutical dosage form and bulk. The proposed innovative method can discrete both active analytes present and validation performed according to ICH (Q1 specification) guidelines [23].

MATERIAL AND METHODS

Materials:

Dolutegravir and Lamivudine purchased from Simson Pharma Limited, Mumbai, India. The Dovato tablet purchased from the pharmacy. The formulation contains 300 mg of lamivudine and 50 mg of dolutegravir which was produced by GSK Healthcare. Thermo Fisher Scientific's Qualigens provided HPLC-grade acetonitrile, ortho-phosphoric acid (OPA), methanol, and TFAA, while Moreshwar Enterprises supplied HPLC-grade water.

Instrumentation

Chromatographic separation was carried out using the HPLC-Agilent (Model-1260 Infinity II) system, which has a UV detector, an integrated auto sampler, and a column oven. The Open lab Ezchrom workstation software was used. Phenomenex ODS-3, 250 mm X 4.6 mm, 5 µm, column was used. A Bio Technics India ultra sonicator was utilized to enrich the drug's solubility. The solution's pH was adjusted using a Labman pH meter. The samples were weighed with an Aczet balance.

Sample Preparation

Preparation of Mobile phase:

Mobile phase solution composed of HPLC- grade Acetonitrile and 0.1% OPA in Water (40: 60 % v/v).

Preparation of Diluent:

DMSO used as diluent for preparing stock solution as both drugs are soluble in DMSO.

Method Development

For the chromatographic separation, dolutegravir and lamivudine were analyzed in a Phenomenex ODS-3 column (250 mm X 4.6 mm, 5 µm). The separation were carried out with mobile phase having Acetonitrile (HPLC grade) and 0.1% OPA in water (40:60% v/v). With a UV detection wavelength of 269 nm, the solution was pumped at ambient temperature and filtered through a 0.45 µ PVDF filter at a flow rate of

1.0 ml/min. The amount injected was 20µl. With a resolution of 35.45, the retention times for dolutegravir and lamivudine were determined to be 1.8 and 9.8 minutes, respectively, during the 14-minute run time.

Standard stock solution of Dolutegravir:

Weigh accurately 21.05 mg of Dolutegravir sodium (equal to 20 mg of Dolutegravir) and transfer it to a 50 ml volumetric flask. Add 5 ml of Dimethy sulfoxide (DMSO), sonicate to dissolve it completely, and make up the volume with methanol (400 µg/mL of Dolutegravir).

Standard stock solution of Lamivudine

Weigh accurately 20 mg of Lamivudine and transfer to 50 mL volumetric flask. Add 5 mL of Dimethy sulfoxide (DMSO), sonicate to dissolve it completely, and make up the volume with methanol (400 µg/mL of Lamivudine).

Sample solution:

Weigh and calculate the average weight of 20 tablet. Using a mortar and pestle, crush the same twenty tablets, then evenly distribute the contents using butter paper. Weigh the powder material (277.60 mg) that is equivalent to 25 mg of dolutegravir and 150 mg of lamivudine. Place it into a 100 mL volumetric flask, add 10 ml of DMSO, and sonicate it for 15 minutes with intermittently shaking, and make up the volume with methanol. Use a 0.45µ syringe filter to filter the solution, discarding 3–5 ml of the filtrate. Then dilute the 0.5 ml filtrate to 25 ml with the mobile phase (5 µg/mL of Dolutegravir and 30 µg/mL of Lamivudine).

RESULTS AND DISCUSSION

As mandated by ICH Q2 (R1), the recommended method was confirmed.

System suitability test:

To verify the system's reproducibility, re-injections of drug solution at 5µg/ml Dolutegravir and 30µg/ml Lamivudine concentrations were used to check the system suitability test parameters. Study focused on system suitability parameters such as resolution of repeated injections, numeral of hypothetical plates (N), tailing factor, and retention time. The outcomes displayed in Table 1.

Table 1: System suitability test

Parameters	Dolutegravir	Lamivudine
Retention time	1.8	9.8
Number of Theoretical Plate	13402	4519
Tailing factor	1.22	1.24
Resolution	35.45	NA

Filter study:

Filtration was performed with a centrifuged (unfiltered) sample that was run through 0.45 µ nylon and 0.45 µ PVDF filters, with 5 ml of solution being discarded. (Sample tablet used). PVDF and Nylon filters are both suitable for use as they meet the requirements for filter studies.

Table 2: Filter study results

Sample	Area		% Absolute difference	
	Dolutegravir	Lamivudine	Dolutegravir	Lamivudine
Unfiltred	4538925	23615890	NA	NA
0.45 µ PVDF filter	4519614	23530142	0.43	0.36
0.45 µ Nylon filter	4527981	23551780	0.24	0.27

Solution Stability:

Table 3: Solution Stability results of Dolutegravir

Time point	Dolutegravir Sample solution		Dolutegravir Standard solution	
	Area	% Absolute difference	Area	% Absolute difference
Initial	4533292	NA	4541924	NA
12 Hours	4518601	0.32	4528810	0.29
24 Hours	4499024	0.76	4510395	0.69

Table 4: Solution Stability results of Lamivudine

Time point	Lamivudine Sample solution		Lamivudine Standard solution	
	Area	% Absolute difference	Area	% Absolute difference
Initial	23518172	NA	23587658	NA
12 Hours	23418250	0.42	23500174	0.37
24 Hours	23360278	0.67	23456580	0.56

Specificity:

Blank & Placebo did not intervene when lamivudine and dolutegravir were being kept. It has been established that lamivudine and dolutegravir are within acceptable bounds. Peak purity of dolutegravir and lamivudine, for both the standard (0.994 and 0.996) and sample (0.990 and 0.992), were within limits. The sample solution's retention time was the same as the standard solutions. As a result, the developed chromatographic technique met the requirements for specificity. The chromatograms displayed in Figures 3 to 6.

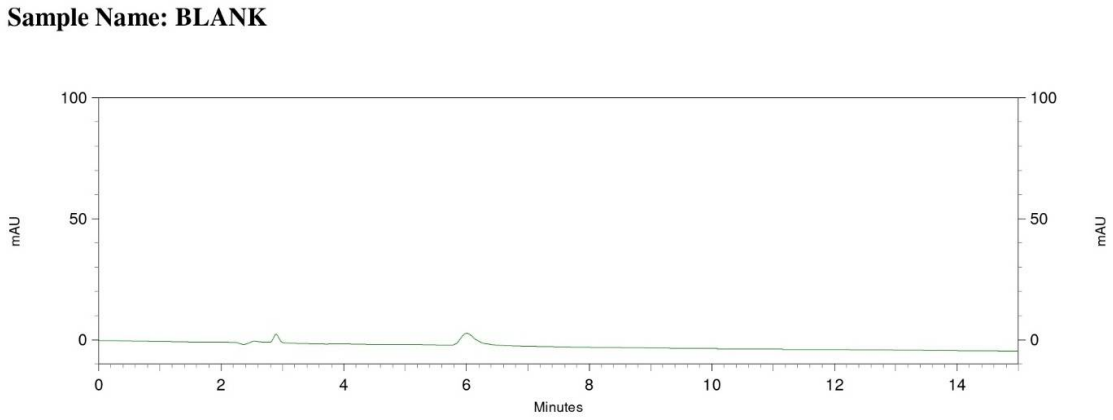


Fig. 3 Chromatogram of Blank

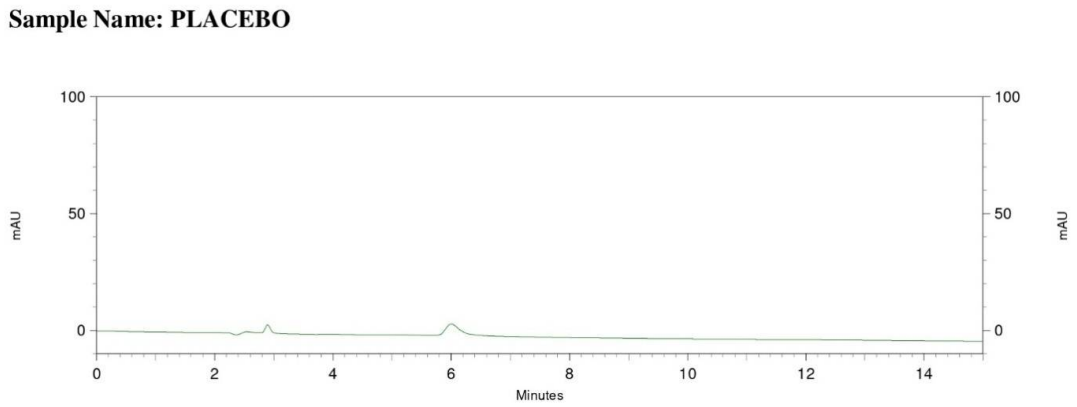
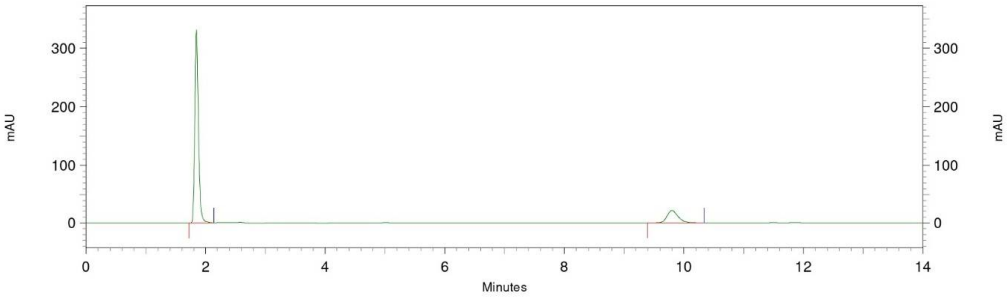


Fig. 4 Chromatogram of Placebo

Sample Name: STANDARD SOLUTION



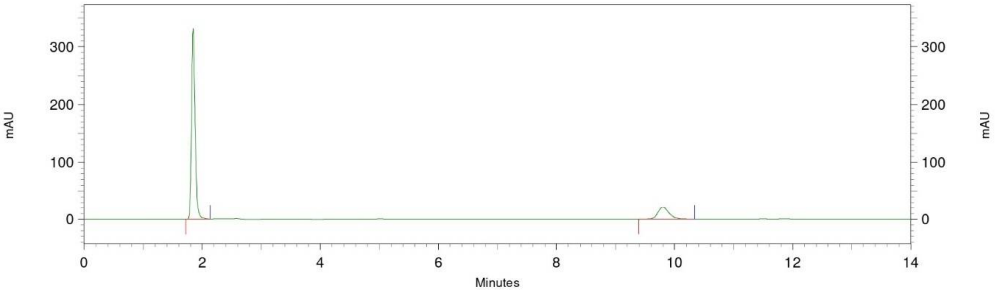
VWD: Signal A, 269 nm

Results

Name	Retention Time	Peak Purity
Lamivudine	1.86	0.996
Dolutegravir	9.81	0.994

Fig. 5 Chromatogram of Standard Solution

Sample Name: SAMPLE SOLUTION



VWD: Signal A, 269 nm

Results

Name	Retention Time	Peak Purity
Lamivudine	1.87	0.992
Dolutegravir	9.83	0.990

Fig. 6 Chromatogram of Sample Solution

Linearity:

Peak area vs. Dolutegravir and Lamivudine concentration was plotted to create a calibration graph. A set of standards of Dolutegravir and Lamivudine were prepared across a range of 2.5-7.5µg/ml and 15-45µg/ml, respectively, from the stock solution. Table 5 presented the findings.

Table 5: Linearity of Lamivudine and Dolutegravir

% level	Dolutegravir		Lamivudine	
	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
50%	2.50	2247868	15.0	11883108
75%	3.75	3431118	22.5	17727562
100%	5.00	4551325	30.0	23565225
125%	6.25	5689812	37.5	29314682
150%	7.50	6783727	45.0	35423824

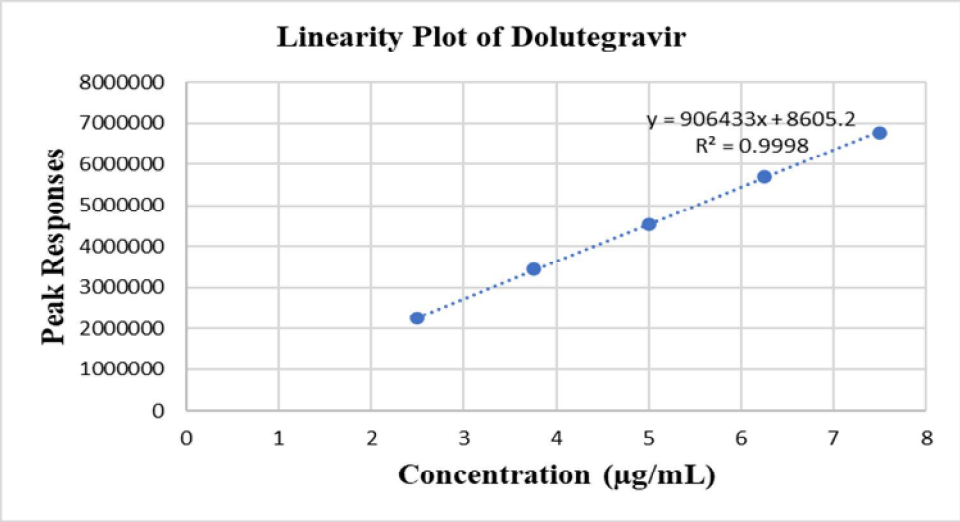


Fig. 7 Linearity of Dolutegravir

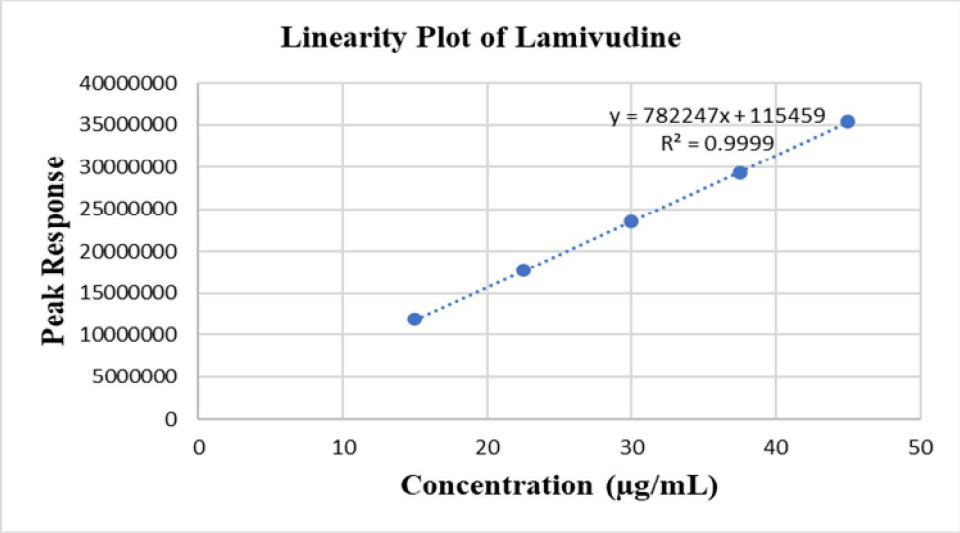


Fig. 8 Linearity of Lamivudine

Accuracy:

By calculating the recovery of dolutegravir and lamivudine added to the preset sample solution at 50%, 100%, and 150% and injected into the HPLC system, the accuracy of this process was resolute. Table 6 displays the percentage recovery of Lamivudine and Dolutegravir at each level that was determined.

Table 6: Results of accuracy study

% of target concentration	Dolutegravir (% recovery)	Lamivudine (% recovery)
50%	98.52	99.14
100%	99.48	99.59
150%	99.45	100.27
Mean % recovery (n = 3)	99.15	99.67
%RSD	0.764	0.960

Precision:

System Precision Peak Area (SPPA) and Method Precision Peak Area (MPPA)

Precision is the degree to which data values for multiple measurements made under identical analytical conditions are similar to one another. Six duplicates (n=6) of the produced solutions were injected in duplicate under the identical conditions to determine the precision of the test procedure. Consideration

was given to each solution's mean peak area response value. The outcomes are displayed in Tables 7 and 8.

Table 7: Results of System Precision

Injections	Dolutegravir Area	Lamivudine Area
1	4561474	23665617
2	4536890	23590462
3	4580914	23329160
4	4526963	23647815
5	4577064	23557194
6	4578753	23615466
Mean	4560343	23567619
S. D.	23269.00407	123140.18011
% RSD	0.51	0.52

Table 8: Results of Method Precision

Injections	Peak response for Method precision	
	Dolutegravir	**Lamivudine**
1	99.26	98.96
2	97.94	97.18
3	98.09	98.77
4	99.01	98.12
5	97.10	96.85
6	98.93	97.15
Mean	97.84	98.39
S.D.	0.9018	0.824527
%RSD	0.922	0.838

Intermediate Precision

By conducting precision tests on various lab days, the method's intermediate precision was considered. Six injections of the standard preparation quantities of 30µg/mL of lamivudine and 05µg/mL of dolutegravir were injected into the HPLC, and the percentage RSD for the area of six replicates was calculated. Table 9 presented the findings.

Table 9: Results of Intermediate Precision

Injections	Peak response for Intermediate Precision	
	Dolutegravir	**Lamivudine**
1	97.10	96.86
2	99.41	97.03
3	98.17	99.29
4	96.95	98.40
5	97.80	97.90
6	99.61	98.83
Mean	98.17	98.05
S.D.	1.128685	0.9730
%RSD	1.150	0.992

Robustness

Three parameters were varied from the optimal chromatographic conditions: wavelength (± 3 nm), column temperature (±2 °C), and flow rate (± 0.1 mL/min) in order to assess method's robustness. The method has been found to be robust based on the robustness results. The outcomes are displayed in Tables 10, 11, and 12.

Table 10: Robustness results for change in wavelength (±3 nm)

Drug	Change in Wavelength (± 3 nm)	Retention time	Average peak area	Theoretical plates	Tailing Factor
Dolutegravir	272 nm	9.8 min	3625550	13245	1.18
	266 nm	9.8 min	4328575	13341	1.19
Lamivudine	272 nm	1.85 min	27373568	4537	1.22
	266 nm	1.85 min	20031425	4547	1.23

Table 11: Robustness results for change in flow rate (± 0.1mL/min)

Drug	Change in flow (± 0.1 mL/min)	Retention time	Average peak area	Theoretical plates	Tailing Factor
Dolutegravir	1.1 mL/min	8.89	3447380	13245	1.18
	0.9 mL/min	10.81	4314849	13341	1.19
Lamivudine	1.1 mL/min	1.68	21480786	4193	1.23
	0.9 mL/min	2.06	25966501	4985	1.23

Table 12: Robustness results for change in column temperature (± 2^0C)

Drug	Change in flow (± 2^0C)	Retention time	Average peak area	Robustness Theoretical plates	Tailing Factor
Dolutegravir	42 ^{0}C	9.82	4558610	13478	1.20
	38 ^{0}C	9.81	4553956	13379	1.24
Lamivudine	42 ^{0}C	1.86	23648701	4672	1.21
	38 ^{0}C	1.85	23657813	4581	1.25

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

The Openlab Ezchrom workstation was utilized to calculate LOD and LOQ using the signal-to-noise ratio approach. It was discovered that the LODs for dolutegravir and lamivudine were 0.08µg/ml and 0.37µg/ml, respectively. It was discovered that the LOQs for dolutegravir and lamivudine were 0.26µg/ml and 1.13µg/ml, respectively.

Assay

Weigh the 20 tablets, then determine the Dovato tablet's average weight. Crush the same 20 tablets in mortar pestle and mix the contents uniformly with butter paper. Weigh the powder substance (277.60 mg of powder material), which is equal to 25 mg of dolutegravir and 150 mg of lamivudine. After transferring it to a 100 mL volumetric flask, sonicate it for 15 minutes with intermittent shaking with adding 10 ml of DMSO. To make up the volume, use methanol. Filter the mixture using a suitable 0.45 µ syringe filter; discard 3–5 mL of the filtrate. Using mobile phase, further dilute the filtrate by 0.5 ml to 25 ml. (5 µg/mL of Dolutegravir, and 30 µg/mL of Lamivudine). The test findings were displayed in Table 10.

Table 10: Assay of Dolutegravir and Lamivudine

Drug name	Labelled claim (mg)	%Assay
Dolutegravir	50 mg	98.51
Lamivudine	300 mg	99.26

CONCLUSION

The currently established RP-HPLC method is fast, robust, accurate, simple, and specific for determining the dosage forms of dolutegravir and lamivudine in bulk and tablets. There were no interactions with the standard or sample with the mobile phase during the simple isocratic form of development that was used. Dolutegravir and lamivudine had respective retention durations of 9.8 and 1.8 minutes. For Lamivudine and Dolutegravir, accuracy was attained with 99.67% and 99.15%, in that order. With correlation coefficients of 0.9998 and 0.9999, respectively, dolutegravir and lamivudine demonstrated linearity in the concentration range of 2.5-7.5 µg/mL and 15-45 µg/mL at their respective maxima. Therefore, the planned method can be applied to routine analysis for quality control in any laboratory that does testing and quality control.

ABBREVIATIONS

RP-HPLC: Reverse Phase - High Performance Layer Chromatography; RT: Retention Time; A: Area; nm: Nanometer; µL: Microliter, °C: Degree Centigrade, ICH: International Conference on Harmonisation; LOD: Limit of detection; LOQ: Limit of quantification; RSD: Relative standard deviation.

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