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RESEARCH ARTICLE

Development and Validation of a Gradient RP-HPLC Method for Simultaneous Estimation of Lamivudine, Tenofovir Disoproxil Fumarate, and Dolutegravir in Combined Pharmaceutical Formulation

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Abstract: A novel gradient reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of three antiretroviral drugs - Lamivudine, Tenofovir Disoproxil Fumarate, and Dolutegravir in combined tablet formulation. The chromatographic separation was achieved on an Inertsil C18 column (150 mm × 4.6 mm, 5 µm) using a gradient mobile phase consisting of phosphate buffer (pH 3.5) and acetonitrile:methanol (50:50 v/v). The flow rate was maintained at 1.6 mL/min with UV detection at 260 nm and column temperature at 30°C. The retention times for Lamivudine, Tenofovir Disoproxil Fumarate, and Dolutegravir were 2.881, 7.813, and 8.633 minutes respectively. The method demonstrated linearity over concentration ranges of 75-225 µg/mL for Lamivudine and Tenofovir Disoproxil Fumarate, and 12-39 µg/mL for Dolutegravir with correlation coefficients >0.999. Method validation parameters including accuracy, precision, specificity, robustness, and system suitability met ICH guidelines. The limits of detection were 0.1, 0.1, and 0.18 µg/mL, while limits of quantification were 0.3, 0.3, and 0.55 µg/mL for Lamivudine, Tenofovir Disoproxil Fumarate, and Dolutegravir respectively. Recovery studies yielded mean recoveries of 100.30%, 100.17%, and 99.60% for the three drugs. The validated method was successfully applied for routine quality control analysis of pharmaceutical formulations containing these drugs in combination.

Keywords: RP-HPLC; Method validation; Lamivudine; Tenofovir Disoproxil Fumarate; Dolutegravir.

1. Introduction

Antiretroviral therapy remains the cornerstone of HIV/AIDS treatment, with combination drug regimens showing superior efficacy compared to monotherapy. Three key antiretroviral agents - Lamivudine, Tenofovir Disoproxil Fumarate, and Dolutegravir - have emerged as a potent fixed-dose combination for managing HIV infection [1]. Lamivudine, chemically known as 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1H)-one, is a nucleoside reverse transcriptase inhibitor (NRTI) that interferes with HIV viral RNA-dependent DNA polymerase [2]. It demonstrates activity against both HIV-1 and HIV-2, while also showing efficacy in chronic hepatitis B treatment. The drug acts by terminating the nascent viral DNA chain through incorporation into viral DNA [3].

Tenofovir Disoproxil Fumarate, a prodrug of tenofovir, is chemically designated as ({[(2R)-1-(6-amino-9H-purin-9-yl)propan-2-yl]oxy}methyl)phosphonic acid. Upon oral administration, it undergoes conversion to tenofovir, an acyclic nucleoside phosphonate analog of adenosine monophosphate [4]. The active metabolite competitively inhibits HIV reverse transcriptase, leading to DNA chain termination [5]. Dolutegravir, with the chemical name (4R,12aS)-N-[(2,4-difluorophenyl)methyl]-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a-hexahydro-2H-pirido[1',2':4,5]pyrazino[2,1-b][1,3]oxazine-9-carboxamide, belongs to the integrase strand transfer inhibitor class. It prevents viral DNA integration into host cell DNA by binding to the integrase active site and blocking the strand transfer step of retroviral DNA integration [6].

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The combination of these three agents in a single dosage form necessitates the development of analytical methods for their simultaneous quantification. While individual methods exist for these drugs, few analytical procedures address their concurrent determination [7]. Current pharmacopeial methods require separate analysis of each component, making quality control processes time-consuming and resource-intensive [8].

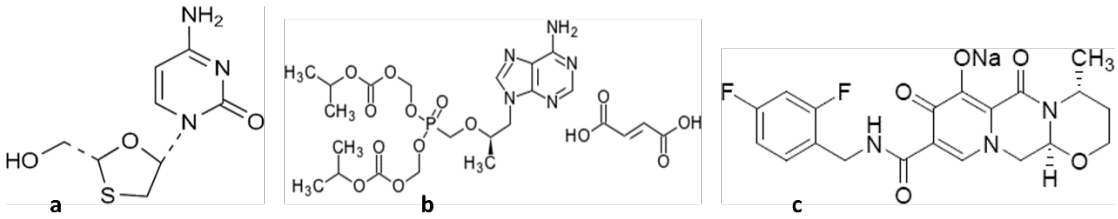

Figure 1. Structures of a. Lamivudine, b. Tenofovir disoproxil, and c. Dolutegravir

Various analytical techniques including UV spectrophotometry, HPTLC, and HPLC have been reported for analyzing these drugs individually or in other combinations [9]. However, existing methods for this specific triple combination either lack the required sensitivity or involve complex extraction procedures [10]. Therefore, developing a simple, rapid, and reliable analytical method for simultaneous estimation of these drugs in combined dosage forms becomes imperative for routine quality control analysis.

The present study focuses on developing and validating a gradient RP-HPLC method for concurrent determination of Lamivudine, Tenofovir Disoproxil Fumarate, and Dolutegravir in pharmaceutical formulations. The method aims to provide improved resolution, shorter analysis time, and robust performance compared to existing analytical techniques.

2. Materials and methods

2.1. Chemicals and Reagents

Reference standards of Lamivudine (99.8% purity), Tenofovir Disoproxil Fumarate (99.9% purity), and Dolutegravir (99.7% purity) were obtained from Aizant Drug Research Solutions Ltd., Hyderabad, India. HPLC-grade methanol and acetonitrile were procured from Rankem Chemicals Ltd., Mumbai, India. Analytical grade potassium dihydrogen phosphate and orthophosphoric acid were used for buffer preparation. Ultrapure water was obtained through a Milli-Q water purification system (Millipore, Merck KGaA, Darmstadt, Germany) equipped with a 0.22 µm filter. Commercial tablets (Diltra) containing 300 mg Lamivudine, 300 mg Tenofovir Disoproxil Fumarate, and 50 mg Dolutegravir were purchased from local pharmacies.

2.2. Instrumentation and Chromatographic Conditions

Chromatographic analysis was performed using a Waters HPLC system (Model: 1200 series/2690). The system was equipped with a Variable Wavelength Detector (VWD)/Diode Array Detector (DAD), an automated sample injector with 20 µL loop volume, a temperature-controlled column compartment, and an integrated degasser unit [11, 12]. Data acquisition and processing were managed through dedicated software. Additional instrumentation included an analytical balance (Model AX200) with 0.1 mg readability, a Chemiline CL 180 pH meter, and a Toshcon ultrasonic bath.

Table 1. Gradient elution program

Time	%A	%B
0.01	90	10
2.0	80	20
3.0	60	40
5.0	40	60
11.0	40	60
12.0	90	10

The chromatographic separation was optimized using an Inertsil C18 column (150 mm × 4.6 mm) with 5 µm particle size. A binary mobile phase system was employed, consisting of phosphate buffer (pH 3.5) as Phase A and a mixture of acetonitrile:methanol

(50:50 v/v) as Phase B [13]. The chromatographic conditions were maintained at a flow rate of 1.6 mL/min with the column temperature controlled at 30°C. Detection was performed at 260 nm wavelength. The method utilized an injection volume of 20 µL and achieved complete separation within a run time of 12 minutes [14]. The gradient elution program was provided in Table 1.

2.3. Preparation of Solutions

2.3.1. Preparation of Buffer Solution

The phosphate buffer (pH 3.5) was prepared by dissolving 6.8 g of potassium dihydrogen phosphate in approximately 900 mL of ultrapure water. The pH was adjusted to 3.5 using orthophosphoric acid, and the volume was made up to 1000 Ml [15]. The buffer solution was filtered through a 0.45 µm membrane filter and degassed before use

2.3.2. Standard Stock Solution

A combined standard stock solution was prepared by accurately weighing 300 mg Lamivudine, 300 mg Tenofovir Disoproxil Fumarate, and 50 mg Dolutegravir into a 100 mL volumetric flask. The compounds were dissolved in 70 mL methanol using sonication, and the volume was made up to the mark with methanol [16]. A working standard solution was prepared by diluting 10 mL of the stock solution to 100 mL with methanol.

2.3.3. Sample Preparation

For sample analysis, five tablets were weighed and finely powdered. An amount of powder equivalent to one tablet was accurately weighed and transferred to a 1000 mL volumetric flask. Approximately 700 mL of diluent was added, and the solution was sonicated for 30 minutes with intermittent shaking to ensure complete extraction of the drugs. The volume was made up to the mark with methanol [17]. The solution was filtered through a 0.45 µm membrane filter, and 10 mL of the filtrate was diluted to 100 mL with methanol to obtain the final sample solution.

2.4. Method Development and Optimization

Initial method development involved systematic evaluation of various chromatographic parameters. Different mobile phase compositions were investigated, beginning with various ratios of methanol:water and acetonitrile:water, which proved inadequate for optimal separation. The pH of the mobile phase was identified as a critical parameter affecting peak shape and resolution. After extensive trials, a gradient elution system using phosphate buffer (pH 3.5) and acetonitrile:methanol (50:50 v/v) was selected for optimal separation [18].

Column selection was based on preliminary screening of different stationary phases. The Inertsil C18 column demonstrated superior peak shape and resolution compared to other columns tested. The flow rate was optimized to 1.6 mL/min to achieve adequate separation while maintaining reasonable analysis time. Column temperature was maintained at 30°C to ensure reproducible retention times.

The detection wavelength was selected based on UV absorption maxima of the three compounds, with 260 nm providing optimal sensitivity for all analytes. The injection volume was set at 20 µL to achieve adequate peak response while maintaining column efficiency.

2.5. Method Validation

The analytical method was validated according to International Conference on Harmonisation (ICH) guidelines Q2(R1) [19], evaluating the following parameters:

2.5.1. System Suitability

System suitability tests were performed to verify the chromatographic system's performance. Six replicate injections of the standard solution were analyzed to evaluate parameters including retention time, theoretical plates, tailing factor, and relative standard deviation (RSD) of peak areas.

2.5.2. Linearity and Range

The linearity of the method was evaluated by analyzing six concentration levels of the standard solutions. The linearity of the method was assessed by constructing calibration curves over specific concentration ranges for each analyte. For both Lamivudine and Tenofovir Disoproxil Fumarate, the calibration curves spanned from 75 to 225 µg/mL, while Dolutegravir demonstrated linearity in the range of 12 to 39 µg/mL. These concentration ranges were selected based on the expected therapeutic concentrations and

the labeled amounts in the pharmaceutical formulation. Standard solutions were prepared by appropriate dilution of the stock solution with methanol. Each concentration was analyzed in triplicate, and the peak areas were plotted against the corresponding concentrations. Linear regression analysis was performed to calculate slope, intercept, and correlation coefficient [20].

2.5.3. Accuracy

Accuracy was determined through recovery studies using the standard addition method at three concentration levels (50%, 100%, and 150% of the labeled claim). Known amounts of standards were added to pre-analyzed sample solutions, and the percentage recovery was calculated. Three replicate determinations were performed at each level [21].

2.5.4. Precision

Method precision was evaluated through repeatability and intermediate precision studies.

Repeatability: Six replicate determinations of the sample solution were performed on the same day under identical conditions. The assay results and relative standard deviation were calculated to assess method repeatability.

Intermediate Precision: Intermediate precision was established by analyzing samples on different days, by different analysts, and on different instruments. The results were expressed as mean assay value and relative standard deviation [22].

2.5.5. Robustness

The robustness of the analytical method was evaluated by deliberately introducing small but meaningful variations in key chromatographic parameters. The investigation included alterations in flow rate (±0.2 mL/min), modifications in mobile phase composition (±2% organic phase), changes in column temperature (±2°C), and adjustments in buffer pH (±0.2 units). The effect of these variations on system suitability parameters and assay results was evaluated [23].

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

LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve using the following equations:

- $LOD = 3.3 \times \sigma/S$
- $LOQ = 10 \times \sigma/S$

Where σ is the standard deviation of the y-intercept of regression lines and S is the slope of the calibration curve.

2.6. Solution Stability

The stability of standard and sample solutions was evaluated by analyzing them at regular intervals over 48 hours when stored at room temperature (25 ± 2°C) and under refrigeration (4 ± 1°C) [24].

2.7. Analysis of Marketed Formulation

The validated method was applied to analyze commercial tablet formulation (Diltra). Six replicate determinations were performed, and the assay results were calculated

3. Results and discussion

3.1. Method Development and Optimization

The developed RP-HPLC method successfully achieved optimal separation of Lamivudine, Tenofovir Disoproxil Fumarate, and Dolutegravir. The retention times were 2.881, 7.813, and 8.633 minutes respectively, showing efficient separation within a runtime of 12 minutes (Table 2). The selected wavelength of 260 nm provided optimal detection sensitivity for all three analytes. The optimized chromatogram is shown in Figure 2.

3.2. System Suitability

System suitability parameters demonstrated compliance with acceptance criteria. The relative standard deviation (%RSD) values for peak areas were 0.1%, 0.2%, and 0.1% for Lamivudine, Tenofovir Disoproxil Fumarate, and Dolutegravir respectively, all well

within the acceptance limit of ≤2.0%. Theoretical plate counts were 11700, 54218, and 53618, exceeding the minimum requirement of 2,000. Tailing factors were 0.9, 1.0, and 1.0, meeting the acceptance criterion of ≤2.0. The results are shown in Table 3

Table 2. Optimized chromatographic conditions

Column	**:**	Inertsil C18 (4.6 mm x 150 mm), 5 µm
Elution mode	**:**	Gradient
Mobile phase	**:**	Phosphate buffer and (Acetonitrile:Methanol - 50:50%v/v)
Column Temp	**:**	30^0 C
Wavelength	**:**	260 nm
Injection Volume	**:**	20 µL
Flow rate	**:**	1.6 mL/min
Run time	**:**	12 min

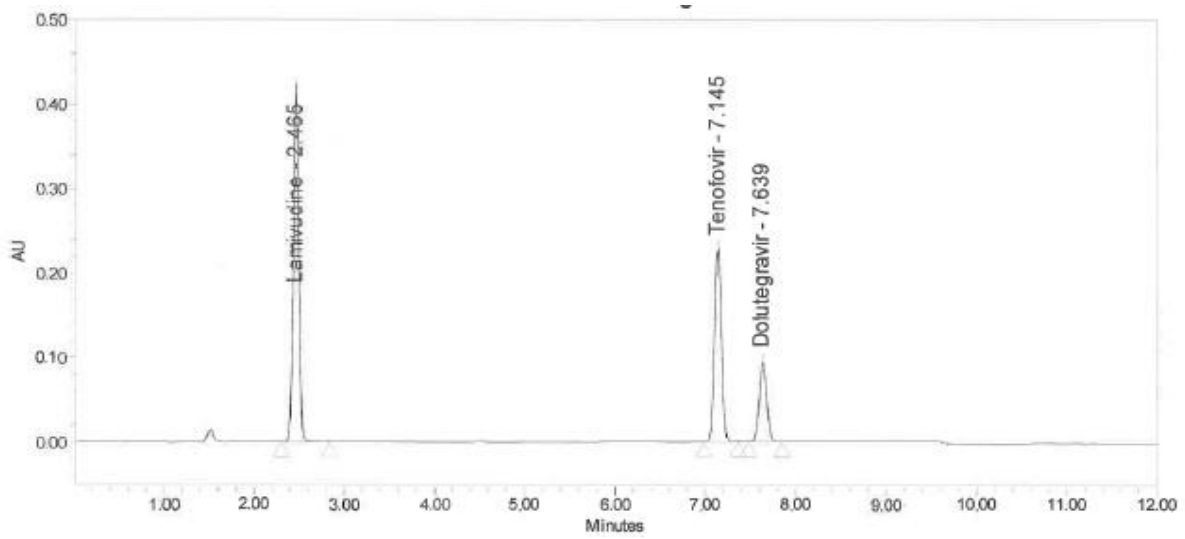

Figure 4: Chromatogram of standard solution of Lamivudine, Tenofovir Disoproxil Fumarate and Dolutegravir

Table 3. System suitability parameters

System suitability parameters	Results			Acceptance criteria
	Lamivudine	Tenofovir Disoproxil Fumarate	Dolutegravir	
% RSD	0.1	0.2	0.1	NMT 2.0
Theoretical plates	11700	54218	53618	NLT 2000
Tailing factor	0.9	1.0	1.0	NMT 2.0

3.3. Linearity and Range

The method demonstrated excellent linearity across the concentration ranges studied. The regression equations obtained were:

Lamivudine: $y = 23064.8456x + 50475$ ($R^2 = 1.000$)

Tenofovir Disoproxil Fumarate: $y = 16209.9549x + 51107$ ($R^2 = 1.000$)

Dolutegravir: $y = 42892.7470x + 7785$ ($R^2 = 1.000$)

Table 4. Linearity data of Lamivudine, Tenofovir Disoproxil Fumarate and Dolutegravir

Lamivudine		Tenofovir Disoproxil Fumarate		Dolutegravir	
Concentration (ppm)	**Mean Peak area (n=3)**	**Concentration (ppm)**	**Mean Peak area (n=3)**	**Concentration (ppm)**	**Mean Peak area (n=3)**
74.7669	176666	74.9928	551436	12.6871	1250004
119.6271	2827942	119.9885	885442	20.2993	2008105
149.5339	3526852	149.9857	1086609	25.3741	2481602
179.4407	4195250	179.9828	1315244	30.4489	2950829
224.3008	224.9785	224.9785	1642018	12.6871	3684682

3.4. Accuracy

Recovery studies demonstrated excellent accuracy for all three analytes across different concentration levels. For Lamivudine, the mean recoveries ranged from 99.9% to 100.7% with RSD values not exceeding 0.8%. Tenofovir Disoproxil Fumarate showed mean recoveries between 99.2% and 101.1% with consistent RSD values around 0.5%. Similarly, Dolutegravir exhibited mean recoveries from 99.4% to 99.9% with RSD values ranging from 0.9% to 1.4%. These results, as detailed in Table 6, indicate high method accuracy and precision across the studied concentration ranges, well within the acceptance criteria of 98-102% recovery and RSD ≤2.0%.

Tables 5. Results of Recovery study results of Lamivudine

% Spike level	Amount added (mg)	Amount recovered (mg)	% Recovery	Mean % Recovery	% RSD
50 %	743.99	747.13	100.4	100.3	0.1
	744.36	746.55	100.3		
	743.81	745.54	100.2		
100 %	1488.62	1498.05	100.6	100.7	0.5
	1488.71	1505.95	101.2		
	1488.70	1492.80	100.3		
200 %	2975.83	2958.90	99.4	99.9	0.8
	2976.61	2999.07	100.8		
	2975.90	2961.16	99.5		

Tables 6. Results of Recovery study results of Tenofovir Disoproxil Fumarate

% Spike level	Amount added (mg)	Amount recovered (mg)	% Recovery	Mean % Recovery	% RSD
50 %	745.76	754.36	101.2	101.1	0.4
	745.45	756.01	101.4		
	745.45	749.85	100.6		
100 %	1489.15	1485.64	99.8	100.2	0.5
	1489.26	1500.61	100.8		
	1489.58	1491.04	100.1		
200 %	2978.96	2944.57	98.8	99.2	0.5
	2979.18	2972.75	99.8		
	2979.20	2948.93	99.0		

Tables 7. Results of Recovery study results of Dolutegravir

% Spike level	Amount added (mg)	Amount recovered (mg)	% Recovery	Mean % Recovery	% RSD
50 %	132.03	130.35	98.7	99.5	1.0
	131.82	132.58	100.6		
	132.64	131.66	99.3		
100 %	264.59	259.63	98.1	99.4	1.4
	263.97	265.99	100.8		
	264.86	262.92	99.3		
200 %	525.23	519.16	98.8	99.9	0.9
	526.07	528.98	100.6		
	526.13	526.98	100.2		

3.5. Precision

Method precision studies demonstrated excellent repeatability with %RSD values of 1.1%, 0.5%, and 1.4% for Lamivudine, Tenofovir Disoproxil Fumarate, and Dolutegravir respectively. The mean assay values were 98.8%, 104.4%, and 97.2% of the labeled amount.

Table 8. Results of Precision study

Preparation	Lamivudine	Tenofovir Disoproxil Fumarate	Dolutegravir
1	99.7	105.1	96.9
2	96.8	103.9	99.1
3	99.3	104.7	96.9
4	99.4	104.7	98.1
5	99.0	103.9	95.0
6	98.8	103.9	97.3
Mean	**98.8**	**104.4**	**97.2**
%RSD	**1.1**	**0.5**	**1.4**

3.6. Sensitivity

The method demonstrated high sensitivity with LOD values of 0.1, 0.1, and 0.18 µg/mL and LOQ values of 0.3, 0.3, and 0.55 µg/mL for Lamivudine, Tenofovir Disoproxil Fumarate, and Dolutegravir respectively.

3.7. Analysis of Marketed Formulation

Analysis of the commercial formulation yielded assay values of 99.38±0.5%, 99.36±1.5%, and 99.19±0.66% for Lamivudine, Tenofovir Disoproxil Fumarate, and Dolutegravir respectively, demonstrating the method's applicability for routine quality control analysis.

Table 9. Analysis of marketed formulation

S. No.	Drug Name	Labeled amount (mg)	Amount found (mg)	% Recovery ± SD*
1	Lamivudine	300	299.81	99.38±0.5
2	Tenofovir Disoproxil Fumarate	300	299.72	99.36±1.5
3	Dolutegravir	50	49.58	99.19±0.66

* n=6 for each parameter

The validated method demonstrates significant advantages over previously reported methods in terms of shorter analysis time, improved resolution, and simultaneous determination of all three compounds. The method's robustness and reliability make it suitable for routine quality control analysis of pharmaceutical formulations containing these drugs in combination [25].

4. Conclusion

The developed RP-HPLC method provides a simple, rapid, and reliable analytical procedure for simultaneous quantification of Lamivudine, Tenofovir Disoproxil Fumarate, and Dolutegravir in combined pharmaceutical formulations. The method demonstrates excellent precision, accuracy, and linearity across the concentration ranges studied. The shorter analysis time of 12 minutes, coupled with high resolution between peaks, makes it particularly suitable for routine quality control analysis.

References

[1] Basu S, Swain PK, Singh P, Mahalik K. Development and validation of stability-indicating HPLC method for determination of dolutegravir sodium. Int J ChemTech Res. 2018;11(8):334-43.

[2] Patel SB, Patel BG, Patel DK, Patel M. Development and validation of HPLC method for simultaneous estimation of tenofovir disoproxil fumarate and lamivudine in bulk and pharmaceutical dosage form. Int J Pharm Sci Res. 2019;10(4):1871-7.

[3] Kandimalla A, Srinivas N, Ravindra N, Venkateshwarlu G. Development and validation of stability-indicating RP-HPLC method for simultaneous determination of dolutegravir and lamivudine. J Chromatogr Sci. 2020;58(6):547-55.

[4] Wang X, Cattaneo D, Baldelli S, Pasipanodya J, Gianelli E, Gervasoni C. The validation of analytical methods for drug quantification in blood plasma or serum. Bioanalysis. 2018;10(7):517-31.

[5] Kavitha KY, Geetha G, Hariprasad R, Kaviarasu M, Venkatnarayanan R. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of lamivudine, tenofovir disproxil fumarate and dolutegravir bulk and their combined dosage form. Int J Pharm Sci Drug Res. 2019;11(2):43-52.

[6] Belouafa S, Habti F, Benhar S, Belafkih B, Tayane S, Hamdouch S, et al. Statistical tools and approaches to validate analytical methods. J Anal Sci Technol. 2017;8(1):1-12.

[7] Agarwal A, Sharma R, Mohapatra S, Patel R. Development and validation of a RP-HPLC method for the simultaneous determination of dolutegravir and lamivudine in human plasma. J Chromatogr Sci. 2019;57(5):436-43.

[8] Raju NA, Begum S. Simultaneous RP-HPLC method for the estimation of the emtricitabine, tenofovir disoproxil fumarate and efavirenz in tablet dosage forms. Res J Pharm Technol. 2018;1(4):522-5.

[9] Bhavar GB, Pekamwar SS, Aher KB, Thorat RS, Chaudhari SR. High-performance liquid chromatographic and high-performance thin-layer chromatographic method for the quantitative estimation of dolutegravir sodium in bulk drug and pharmaceutical dosage form. Sci Pharm. 2016;84(2):305-20.

[10] Sattar MA, Rao BM, Kumar PR. Development and validation of HPLC method for simultaneous estimation of lamivudine and tenofovir disoproxil fumarate in pharmaceutical dosage forms. Int J Adv Pharm Anal. 2017;7(1):1-4.

[11] Sharma M, Jain R, Sharma N, Jain N. Development and validation of a stability-indicating HPLC method for simultaneous determination of dolutegravir and lamivudine in tablet dosage form. Indian J Pharm Sci. 2019;81(1):95-101.

[12] Putchakayala PR, Rambabu K. Validated HPLC method for the determination of dolutegravir in bulk and pharmaceutical dosage forms. Int J Pharm Sci Res. 2018;9(6):2493-7.

[13] Kumar P, Dwivedi SC, Kushnoor A. A validated stability indicating RP-HPLC method for the determination of emtricitabine and tenofovir disoproxil fumarate in bulk and tablet dosage forms. Int J ChemTech Res. 2018;11(1):228-37.

[14] Rao NM, Sankar DG. Development and validation of stability-indicating HPLC method for simultaneous estimation of lamivudine, tenofovir, and dolutegravir in bulk and their tablet dosage form. Future J Pharm Sci. 2019;5(1):1-9.

[15] Balaji N, Sivaraman VR, Neeraja P. A validated UPLC method for the determination of process-related impurities in antiretroviral drug combination of lamivudine, tenofovir, and efavirenz. J Taibah Univ Sci. 2020;14(1):1-9.

[16] Tripathi AS, Sheikh I, Dewani AP, Shelke PG, Bakal RL, Chandewar AV, et al. Development and validation of RP-HPLC method for simultaneous estimation of tenofovir alafenamide and emtricitabine in bulk and tablet dosage form. Int J PharmTech Res. 2018;11(3):260-7.

[17] Badgujar BP, Mahajan MP, More KV, Ghanwat PB, Patil LS. Development and validation of stability indicating RP-HPLC method for determination of dolutegravir sodium. Int J ChemTech Res. 2017;10(12):281-9.

[18] Venkataraman S, Manasa M. Forced degradation studies: Regulatory guidance, characterization of drugs, and their degradation products. Drug Res. 2018;68(6):305-10.

[19] Singh S, Junwal M, Modhe G, Tiwari H, Kurmi M, Parashar N, et al. Forced degradation studies to assess the stability of drugs and products. TrAC Trends Anal Chem. 2017;69:1-7.

[20] Patel A, Patel J, Patel M, Patel K. Development and validation of analytical methods for simultaneous estimation of tenofovir disoproxil fumarate and emtricitabine in tablet dosage forms. Int J Pharm Pharm Sci. 2019;11(1):77-82.

[21] Sharma R, Mehta K. Simultaneous spectrophotometric estimation of tenofovir disoproxil fumarate and lamivudine in three component tablet formulation containing efavirenz. Indian J Pharm Sci. 2019;72(4):527-30.

[22] Behera A, Parida A, Meher AK, Sanaty D, Mohapatra SK, Nanda BK. Development and validation of analytical method for determination of emtricitabine and tenofovir disoproxil fumarate in bulk and tablet dosage form. Int J Res Pharm Sci. 2017;8(1):41-8.

[23] Bhattacharyya S, Bhowmik M, Deb L. Development and validation of a rapid RP-HPLC method for determination of dolutegravir in bulk drug and tablet formulation. Int J Pharm Sci Rev Res. 2018;48(1):64-7.

[24] Ramaswamy A, Arul Gnana Dhas AS. Development and validation of analytical method for quantitation of emtricitabine, tenofovir, efavirenz based on HPLC. Arab J Chem. 2018;11(2):275-81.

[25] Venkatesan S, Kannappan N. Simultaneous spectrophotometric method for determination of emtricitabine and tenofovir disoproxil fumarate in three-component tablet formulation containing rilpivirine hydrochloride. Int Sch Res Notices. 2019;2014:541727.