WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 16, 614-626. Research Article ISSN 2277–7105

STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION BY HPLC FOR LAMIVUDINE, TENOFOVIR AND DOLUTEGRAVIR IN TABLET DOSAGE FORM

Swati Gondaliya[1], Janki Patel[*2], Ragin Shah[3], Divyakant Patel[4] and Hiren Doshi[5]

[1]Research Scholar, Swarnim Startup and Innovation University.

[2]Associate Professor, Department of Pharmaceutical Quality Assurance, Sharda School of Pharmacy, Pethapur, Gandhinagar.

[3]Professor and Principal Department of Quality Assurance, Arihant School of Pharmacy, Adalaj, Gandhinagar.

[4]Principal, Sharda School of Pharmacy, Pethapur, Gandhinagar.

[5]Professor, Dept. of Pharmaceutical Chemistry, Pro Vice Chancellor, Swarnim Startup and Innovation University, Gandhinagar.

Article Received on
21 June 2025,

Revised on 11 July 2025,
Accepted on 01 August 2025

DOI: 10.20959/wjpr202516-37879

***Corresponding Author**
Janki Patel
Associate Professor, Department of Pharmaceutical Quality Assurance, Sharda School of Pharmacy, Pethapur, Gandhinagar.

ABSTRACT

For the simultaneous measurement of dolutegravir, lamivudine, and tenofovir disoproxil fumarate in tablet dosage form, a stability-indicating HPLC method was created and verified. The goal of the study was to develop a straightforward, reliable, accurate, and repeatable procedure for regular stability and quality control tests. Using a Perkin Elmer C18 column (150 mm × 4.6 mm, 5 µm) and a gradient mobile phase made up of methanolic orthophosphoric acid solution (mobile phase B) and ammonium acetate buffer (mobile phase A), chromatographic separation was accomplished. With no interference from diluent, placebo, or known contaminants, the technique showed specificity and successfully separated the analyte peaks from excipients and degradation products. The method's capacity to indicate stability was confirmed by forced degradation studies conducted under acidic, basic, oxidative, thermal, and photolytic conditions. These investigations revealed considerable degradation under alkaline and oxidative stress. After evaluation, it was determined that the method validation parameters—specificity, linearity (50–150%), precision, accuracy, robustness,

solution stability, and filter compatibility—met ICH Q2 (R2) requirements. The approved technique can be used for regular stability testing and quality control of fixed-dose combination tablets that contain tenofovir, lamivudine, and dolutegravir.

KEYWORDS: Lamivudine, Tenofovir, Dolutegravir, HPLC method, Forced degradation study.

INTRODUCTION

Pharma companies have made a huge investment in research and development of a new drug. This research work tends to focus on the new medicines for the critical diseases that are very important to current world so that maximum people get benefited by this research work. Various anti-viral drugs like HIV medicines play very crucial role in the treatment all over the world. Hence a simple, efficient, accurate HPLC method of their assay and related substance is need of an hour to ensure maximum safety, effectivity and bio availability to patients. Based on Literature review, market studies and demands, following drug formulations are selected for research topic Lamivudine, Tenofovir and Dolutegravir tablets. Chemically Lamivudine is (4-amino-1-[(2R, 5S)-2- (hydroxymethyl)-1,3-oxathio-lan-5-yl]-1,2dihydropyrimidine-2-one) (3TC) [1]. Tenofovir is chemically known as propan-2-yl-(2S)-2-{[(S)({[(2R)-1-(6-amino-9H-purin-9-yl) propan-2-yl] oxy} methyl) (phenoxy)phosphoryl] amino} propanoate.[2] Dolutegravir is known as (4R,12aS)-9-{[(2,4-difluorophenyl) methyl] carbamoyl} -4-methyl6,8-dioxo-3,4,6,8,12,12a- Hexahydro-2H-pyrido [1',2':4,5] pyrazino[2,1-b] [1,3] oxazin-7-olate, is a novel integrase stand transfer inhibitor active against Human Immunodeficiency Virus. Dolutegravir (DTG) is active against HIV type 1 (HIV-1) and also has some in vitro activity against HIV type 2 (HIV-2).[3,4] Chromatography is a potent analytical technique widely utilized by modern chemists for its ability to quantitatively analyse multiple components in a single procedure. Dolutegravir, Lamivudine and Tenofovir disoproxil fumarate belongs to a group of antiretroviral medicines, indicated for the treatment of HIV-1 infection in adults and children. HIV (Human Immunodeficiency Virus) is a virus that attacks the immune system and destroys the white blood cells, making individual susceptible to other infections. Dolutegravir, Lamivudine and Tenofovir disoproxil fumarate tablet is a combination of three drugs, namely: Dolutegravir (integrase inhibitor), Lamivudine (nucleoside reverse transcriptase inhibitor), and Tenofovir disoproxil fumarate (nucleotide reverse transcriptase inhibitor). Dolutegravir increases the immune cells that help to fight infections in the body and decreases the amount of HIV in the blood. HPLC is a

powerful tool in pharmaceutical analysis, enabling precise quantification and identification of compounds in diverse samples.[5]

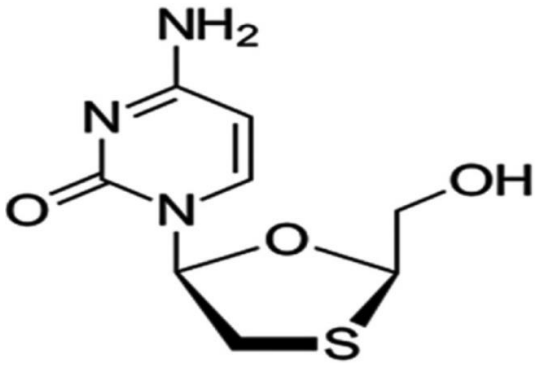

Fig: Chemical Structure of lamivudine.[6]

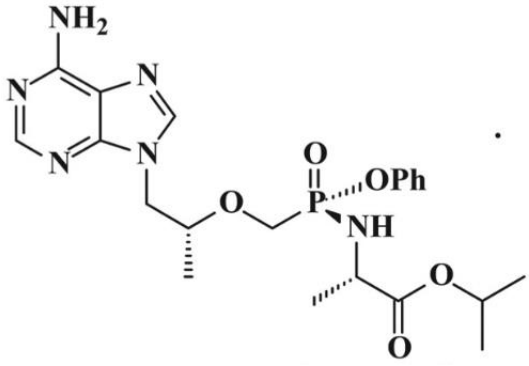

Fig: Chemical Structure of Tenofovir.[7]

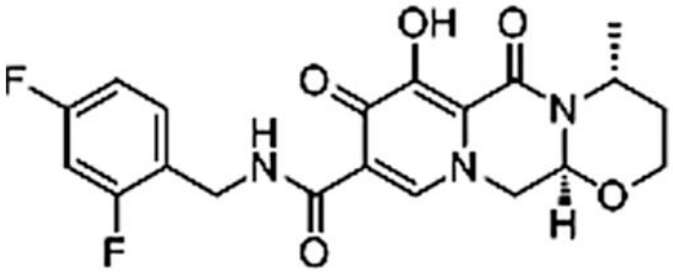

Fig: Chemical Structure of Dolutegravir.[8]

MATERIALS AND METHODS

Dolutegravir sodium and Lamivudine was received as a gift sample from Laurus labs. Emcure Pharmaceutical Ltd gifted a sample of Tenofovir Disoproxil Fumarate. Viropil tablet is marketed formulation of Emcure Pharmaceutical Ltd. Containing Dolutegravir (50 mg), Lamivudine (300 mg), Tenofovir Disoproxil Fumarate (300 mg). AR Grade ammonium acetate, ortho phosphoric acid, HPLC grade methanol, Milli-Q Water was purchased from merk chemicals.

Chromatographic condition

Column used for the work was Perkin Elmer C18,150mm x 4.6 mm,5 µ). For better separation gradient mode was used. 260 nm Wavelength, Flow rate was 1.0 mL/minute. Column oven temperature was maintained at 47°C and Sample cooler temp was 8°C. Injection volume was 15 µL. All chromatogram was running up to 15 minutes. Gradient system was used as shown in table.

Time (Min)	% Mobile phase A	% Mobile phase B
0	50	50
2.0	50	50
6.0	30	70
9.0	30	70
9.1	50	50
12.1	50	50

Preparation of buffer: Accurately weigh and dissolve 7.7 g ammonium acetate in 1000 mL of water and add 5 mL of glacial acetic acid and sonicate to dissolve. Filter through 0.45 µ nylon membrane filter and degas.

Preparation of mobile phase A: Use buffer as a mobile phase A.

Preparation of mobile phase B: Mix 1 mL of orthophosphoric acid with 1000 mL of methanol.

Preparation of diluent: Prepare a mixture of buffer solution: methanol in the ratio of 50:50 %v/v. Sonicate to degas.

Preparation of blank solution: Use diluent as blank solution.

Preparation of Dolutegravir: standard stock solution: Accurately weigh and transfer about 21 mg of Dolutegravir Sodium standard into a 100 mL clean and dry volumetric flask. Add about 50 mL of methanol and sonicate for 5 minutes. Add about 30 mL of buffer solution and sonicate to dissolve. Allow the solution to attain room temperature. Dilute to volume with buffer solution and mix well. (200 ppm Dolutegravir).

Preparation of Lamivudine and Tenofovir Disoproxil standard stock solution: Accurately weigh and transfer about 75 mg of Lamivudine standard and about 75 mg of Tenofovir Disoproxil Fumarate standard into 50 mL clean and dry volumetric flask. Add about 25 mL of methanol and sonicate to dissolve. Allow the solution to attain room temperature. Dilute to volume with buffer solution and mix well. (1500 ppm Lamivudine & 1500 ppm Tenofovir Disoproxil Fumarate)

Preparation of standard solution: Transfer 5 mL of Dolutegravir standard stock solution and 4 mL of Lamivudine and Tenofovir Disoproxil standard stock solution into 50 mL clean and dry volumetric flask. Dilute to volume with diluent and mix well. (20 ppm Dolutegravir, 120 ppm Lamivudine and 120 ppm Tenofovir Disoproxil Fumarate)

Preparation of sample solution: Accurately weigh 20 tablets and determine the average weight of tablets. Accurately weigh and transfer 5 intact tablets into 500 mL clean and dry volumetric flask. Add about 150 mL of buffer and allow to stand for about 20 minutes to disperse. Add accurately 250 mL of methanol and sonicate for 45 minutes at temperature below 25°C with intermittent shaking. Allow the solution to attain room temperature. Dilute to volume with buffer solution and mix well. Centrifuge the resulting solution at 3000 rpm for 15 minutes. Dilute 4 mL of the supernatant solution into 100 mL clean and dry volumetric flask. Dilute to volume with diluent and mix well. Filter this solution through 0.45 µ Nylon (MDI, SY25NN Nylon-66) filter by discarding first 5 mL of filtrate. (20 ppm Dolutegravir, 120 ppm Lamivudine and 120 ppm Tenofovir Disoproxil Fumarate).

Method Validation: The objective of the analytical procedure, appropriate performance characteristics and associated criteria and appropriate validation tests. A validation study is designed to provide sufficient evidence that the analytical procedure meets its objectives. These objectives are described with a suitable set of performance characteristics and related performance criteria, which can vary depending on the intended use of the 51 analytical procedure and the specific technology selected.[9]

Forced degradation Study[10-12]: A forced degradation study is an essential step in the design of a regulatory compliant stability program for both drug substances and products, and was formalized as a regulatory requirement in ICH Guideline. In a typical study, relevant stress conditions are light, heat, humidity, hydrolysis (acid / base influence) and oxidation or even a combination of described parameters. If it is necessary to form degradation products, the strength of stress conditions can vary due to the chemical structure of the drug substance, the kind of drug product, and product specific storage requirements. purity verification chromatographic peak of active pharmaceutical ingredient in the product, provides information about possible degradation routes of a certain product, evaluation of the factors that may interfere in any way in the drug stability and critical analysis of the drug degradation profile.

RESULT AND DISCUSSION

Assay method development: Stability Indicating Assay method development for Dolutegravir, Lamivudine and Tenofovir disoproxil fumarate tablet using HPLC was initiated to achieve two-mile stones during developmental stage before optimization of the method. First were all the three peaks of API, in the given formulation, should be well separated and

second was the three peaks of API, in the given formulation, should be pure and free from any interference due to diluent, placebo and other impurities.

Assay method Optimization: The method was optimized using Column: Inertsil ODS 3V,150 mm x 4.6 mm,5 µ with a flow rate of 1.0 mL/Minutes. Buffer solution was used as mobile phase A and diluted 1 mL of OPA acid to 1000 mL with methanol was used as mobile phase B. Buffer (as above) and methanol in the ratio of 50:50 v/v was used as diluent.

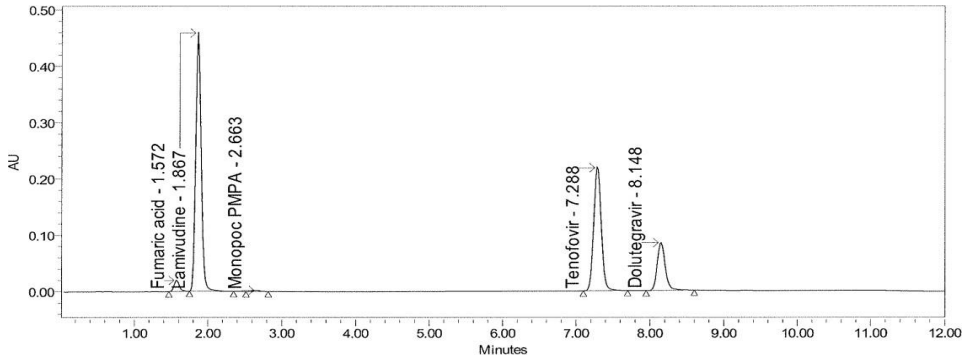

Fig. Chromatogram of standard solution.

No interference observed due to blank solution and Placebo solution at retention times of Dolutegravir, Lamivudine and Tenofovir Disoproxil peak. All main peaks are well resolved from each other. All the three peaks Dolutegravir, Lamivudine and Tenofovir Disoproxil peaks peak found pure when injected in individual, standard mixture and sample mixture Hence further trials were taken to inject one of the major degradations known impurity of Tenofovir DF which seen to be generated in sample solution on stability. This is done to check if impurity gets well separated from main peaks. Mono-poc pmpa, got well resolved from main peaks. Fumaric acid sample was injected for identification of peak RT in sample. Fumaric acid peak was identified and found well resolved from main peaks. As results looks promising in initial developmental stage, this trial to be evaluated for forced degradation study and based on FD data method was finalized.

Forced Degradation Study

Sample	Degradation condition	% Degradation		
		Dolutegravir	Lamivudine	Tenofovir Disoproxil
Acid	1ml,0.2NHCl_1Hrs.RT	2.3	1.1	3.8
Base	1ml,0.2N NaOH_1Hr.RT	4.5	2.9	18.4
Peroxide	1ml, 30% RT	2.3	15.9	25.1
Thermal	600C,1Hr water bath	1.2	0.2	2.1
Photo	1.2 million lux hours and 200-watt hrs./m2	0.0	0.0	0.0

Observation and Conclusion of forced degradation study

Significant degradation of Dolutegravir, Lamivudine and Tenofovir Disoproxil observed in Alkaline hydrolysis and oxidative condition. Peak purity of main components passes in all conditions. Mono POC PMPA impurity found as major degradation product in all forced conditions. Based on the above observations it is concluded that the method is specific at forced degradation studies.

Method Validation

Specificity- Blank, Placebo, Standard, sample, along with individual impurity solutions were prepared as per methodology and injected in to given chromatographic conditions with PDA detector for identification and main peaks were evaluated for peak purity.

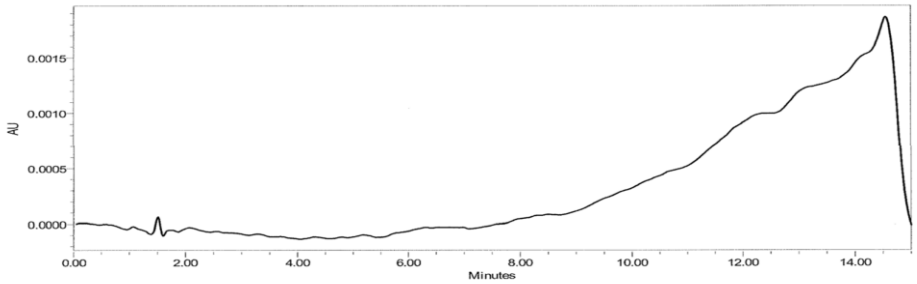

Fig. Chromatogram of blank solution.

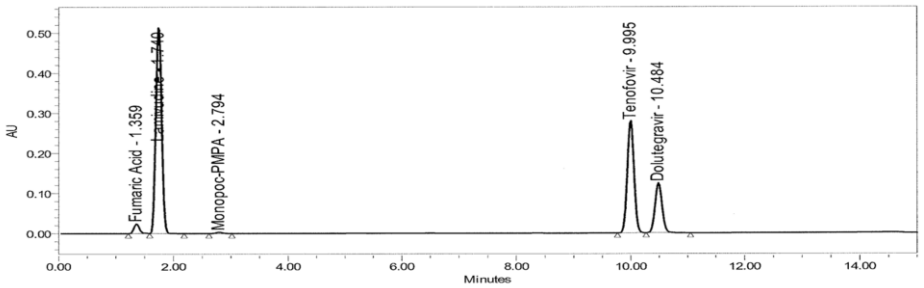

Fig: Chromatogram of standard solution.

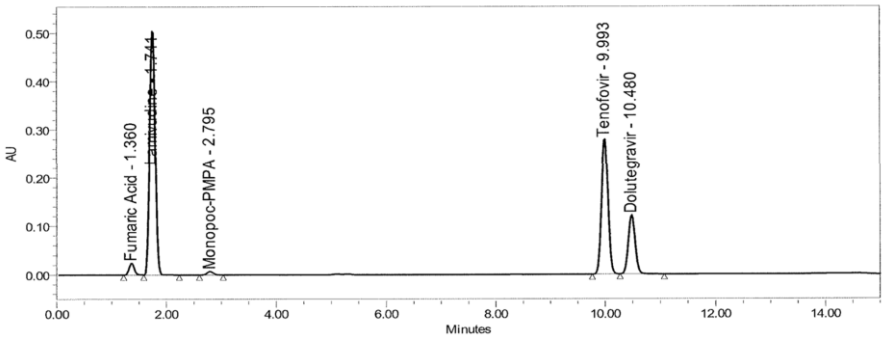

Fig: Chromatogram of sample solution.

Observation and Conclusion of Specificity

No interference was observed due to blank solution, placebo solution and known impurities at the retention time of Dolutegravir, Lamivudine and Tenofovir Disoproxil peaks. Peak of Dolutegravir, Lamivudine and Tenofovir Disoproxil found pure in standard and sample solution which conforms the method is specific.

Linearity

Standard stock solution was diluted at 5 different level ranging from 50%, to 150% of Assay concentration and injected to given chromatography to check linearity of method. Calibration curve for all drugs is showed in following figure.

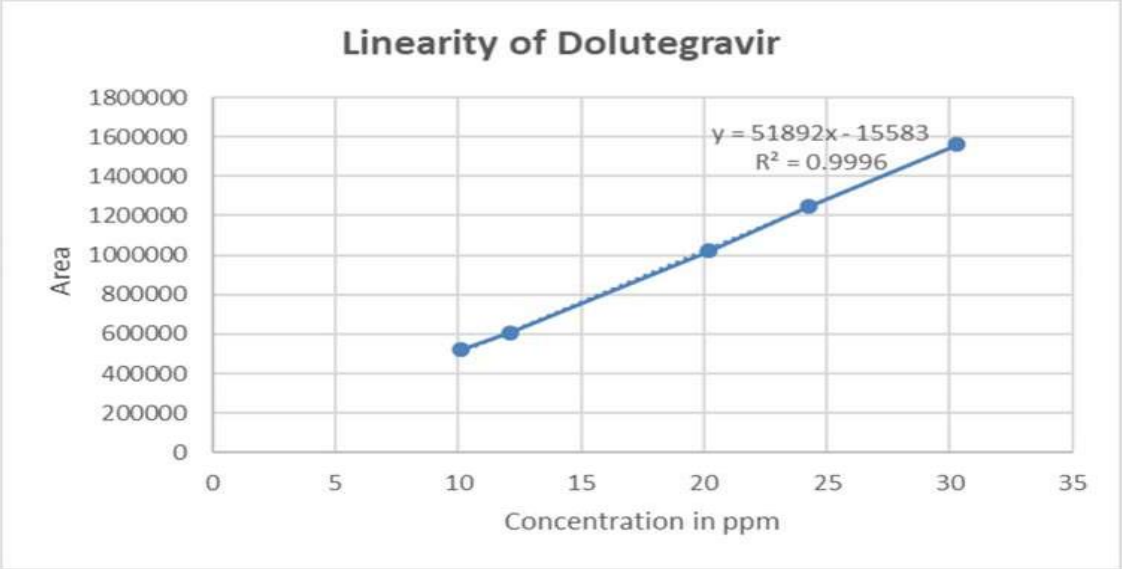

Fig: Calibration curve of Dolutegravir

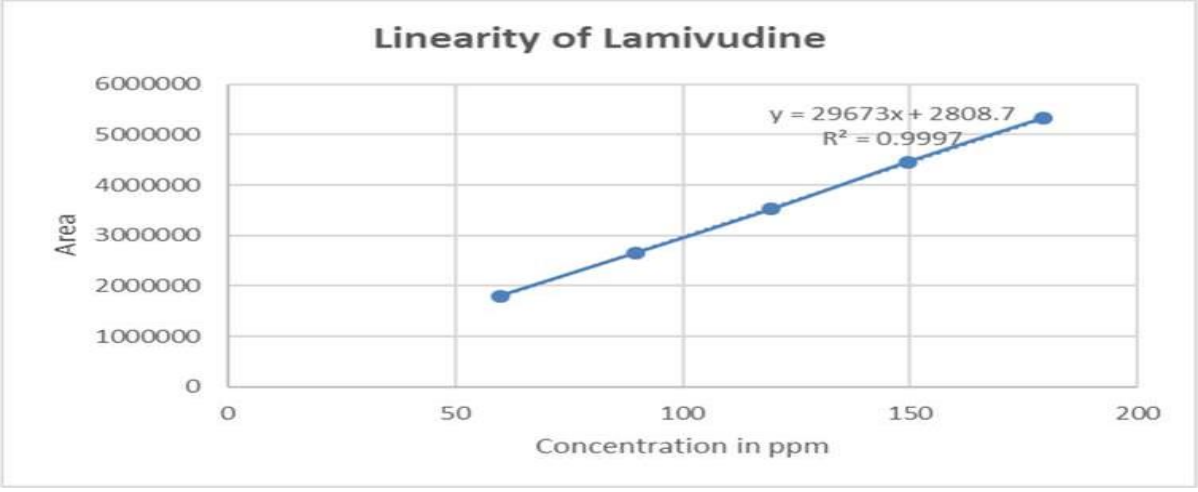

Fig: Calibration curve of Lamivudine

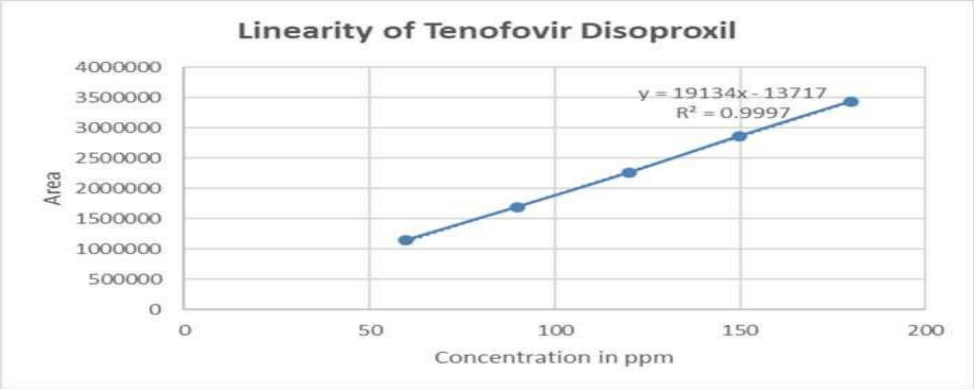

Fig: Calibration curve of Tenofovir Disoproxil

Conclusion of precision: In all the three drugs, Acceptance criteria pass in the given range and hence Proposed Assay method is linear in the range of 50% to 150% of assay concentration.

Precision

System Precision: Standard and Sample were prepared as per methodology and same sample was injected 5 times to check system's precision.

Injection	Lamivudine	Tenofovir Disoproxil	Dolutegravir
1	3640051	2311735	1066629
2	3641159	2312035	1066106
3	3640196	2309403	1065738
4	3637004	2307177	1065312
5	3642378	2306947	1065999
%RSD	0.1	0.1	0.0
Tailing factor	1.07	1.04	1.06
Theoretical plates	1324	32181	33035

Method Precision

Six samples of same batch of tablets were prepared on single day, and injected using same chromatography like same mobile phase, same diluent, same column. % Mean Assay and % RSD are mentioned below.

Sample	Dolutegravir	Lamivudine	Tenofovir Disoproxil
1	97.3	99.3	98.1
2	98.0	99.3	97.9
3	98.4	99.4	98.1
4	98.5	99.5	97.5
5	97.2	98.7	96.7
6	97.1	99.2	97.2
% Mean Assay	97.7	99.2	97.6
% RSD	0.65	0.27	0.59

Intermediate Precision

Another set of six samples were prepared on different day, different instrument and same make column with different lot and examined as below.

Sample	Dolutegravir	Lamivudine	Tenofovir Disoproxil
1	99.2	100.1	99.4
2	98.2	100.2	98.6
3	98.6	100.6	99.2
4	99.0	100.3	99.2
5	98.9	100.0	98.9
6	98.1	99.5	101.2
% Mean Assay	98.7	100.1	99.4
% RSD	0.44	0.38	0.92
% Mean Assay of 12 Sample	98.2	99.7	98.5
% RSD of 12 Sample	0.71	0.57	1.21

Conclusion of precision: System Precision/ System suitability complies as per acceptance criteria and % RSD meets acceptance criteria.

Accuracy

Standard stock solution was spiked at 50%, 100% and 150% of assay concentration along with placebo and % recovery was calculated on triplicate sample at each concentration.

Dolutegravir					
Level	**Recovery sample-1**	**Recovery sample-2**	**Recovery sample-3**	**% Mean Recovery**	**% RSD**
50% level	100.73	100.60	100.44	100.58	0.14
100% level	100.64	100.19	99.10	99.97	0.79
150% level	99.80	98.98	99.07	99.28	0.45
Lamivudine					
Level	**Recovery sample-1**	**Recovery sample-2**	**Recovery sample-3**	**% Mean Recovery**	**% RSD**
50% level	100.92	100.03	101.15	100.69	0.58
100% level	101.19	100.37	99.97	100.50	0.61
150% level	101.12	100.55	100.21	100.62	0.45
Tenofovir Disoproxil					
Level	**Recovery sample-1**	**Recovery sample-2**	**Recovery sample-3**	**% Mean Recovery**	**% RSD**
50% level	98.87	99.47	99.26	99.20	0.30
100% level	100.37	99.76	99.49	99.87	0.45
150% level	100.54	100.39	99.85	100.25	0.36

OBSERVATION AND CONCLUSION

% Recovery and % RSD at each level meets acceptance criteria. Hence, it shows that proposed method to determine Assay is Accurate.

Robustness

Parameter	Value	Dolutegravir	Lamivudine	Tenofovir Disoproxil
		% RSD		
Change in flow rate	0.9 mL/min	0.69	1.08	1.06
(Original flow rate: 1.0 mL/min) (± 0.1 mL/minute)	1.1 mL/min	0.641	1.13	1.07
Wavelength	258nm	0.61	0.27	0.57
(Original wavelength: 260nm) (± 2nm)	262 nm	0.62	0.23	0.57
Change column Temperature	44°C	0.60	0.45	1.22
(Original temperature: 47°C) (± 3°C)	50°C	0.60	0.451	.06

Stability of Analyte in Solution

Drugs	Time	Standard solution		Sample solution	
		% Result	% Recovery	% Assay	Absolute difference between the result value of that time point and its initial value
Dolutegravir	Initial	99.04	100.00	99.17	0.00
	24 Hrs.	101.89	102.88	100.57	1.40
Lamivudine	Initial	99.40	100.00	100.14	0.00
	24 Hrs.	99.57	100.17	98.64	1.50
Tenofovir Disoproxil	Initial	99.02	100.00	99.36	0.00
	24 Hrs.	97.79	98.76	96.64	2.72
Observation	Standard and Sample solution are stable up to 24 Hrs. at 8°C.				

Filter Study

Dolutegravir		
Sample	% Assay	% Absolute Diff.
Unfiltered	102.2	0.0
Mdi Nylon-66, 0.45µ filter, 1ml discard	99.9	2.3
Mdi Nylon-66, 0.45µ filter, 3ml discard	100.3	1.9
Mdi Nyon-66, 0.45µ filter, 5 ml discard	100.6	1.6
Mdi Nylon-66, 0.45µ filter, 7ml discard	99.8	2.4
Lamivudine		
Sample	% Assay	% Absolute Diff.
Unfiltered	102.2	0.0
Mdi Nylon-66, 0.45µ filter, 1ml discard	101.8	0.4
Mdi Nylon-66, 0.45µ filter, 3ml discard	102.1	0.1
Mdi Nyon-66, 0.45µ filter, 5 ml discard	102.0	0.2
Mdi Nylon-66, 0.45µ filter, 7ml discard	102.0	0.2
Tenofovir Disoproxil		
Sample	% Assay	% Absolute Diff.
Unfiltered	97.3	0.0
Mdi Nylon-66, 0.45µ filter, 1ml discard	98.6	1.3
Mdi Nylon-66, 0.45µ filter, 3ml discard	98.9	1.6
Mdi Nyon-66, 0.45µ filter, 5 ml discard	98.8	1.5
Mdi Nylon-66, 0.45µ filter, 7ml discard	98.6	1.3
Sample	% Assay	% Absolute Diff.
Observation & Conclusion:	Based on above data Millipore Millex Nylon 0.45µ filter and Mdi Nyon-66, 0.45µ filter suitable for analysis. Mdi Nyon-66, 0.45µ filter considered for intended used with initial 5 mL volume discard.	

CONCLUSION

A robust, specific, and accurate stability-indicating HPLC method was successfully developed and validated for the simultaneous estimation of Dolutegravir, Lamivudine, and Tenofovir Disoproxil Fumarate in tablet dosage form. The method effectively separated the active pharmaceutical ingredients from each other, as well as from degradation products and known impurities. It demonstrated excellent specificity, precision, linearity across 50–150% assay concentrations, and accuracy with recovery values within acceptable limits. Forced degradation studies confirmed that the method can reliably detect degradation under stress conditions, particularly alkaline and oxidative environments, without interference. Additionally, the method proved to be robust under varied chromatographic conditions and the analytes were stable in solution up to 24 hours. Filter compatibility was also confirmed. Overall, the method is suitable for routine quality control and stability analysis of combination antiretroviral tablets.

REFERENCE

1. Joshi, Anjali, and Moji Christianah Adeyeye. "Reversed Phase LC-UV method development and validation for simultaneous determination of three antiretrovirals: lamivudine, zidovudine, nevirapine and possible degradants in a fixed dose pharmaceutical product." Journal of Pharmaceutical Technology and Drug Research, 2012; 1.1: 4.
2. Kokkirala, Tej Kumar, and Duvvuri Suryakala. "RP-HPLC method development and validation for the estimation of Emtricitabine, Bictegravir and Tenofovir alafenamide in bulk and pharmaceutical dosage form." Journal of Taibah University for Science, 2019; 13(1): 1137-1146.
3. Balasaheb, Bhavar Girija, et al. "Development and validation of UV spectrophotometric method for estimation of dolutegravir sodium in tablet dosage form." Malaysian Journal of Analytical Sciences, 2015; 19(6): 1156-1163.
4. Castellino, S., Moss, L., Wagner, D. & et. Al, Metabolism, excretion, and mass balance of the HIV-1 integrase inhibitor dolutegravir in humans. Antimicrobial Agents and Chemotherapy, 2013; 57: 3536 - 3546.
5. Janki Patel, Ankit Chaudhary, Divyakant Patel, Urvi Trivedi, Mugdha Dhimar. "An Overview of The Analytical Strategy for HPLC Method Development." International Journal of Research and Analytical Reviews, 2023; 10(3): 855-866.

6. Ahmad, Sufiyan Ahmad Raees, et al. "Analytical method development and validation for the simultaneous estimation of abacavir and lamivudine by reversed-phase high-performance liquid chromatography in bulk and tablet dosage forms." Pharmacognosy Research, 2018; 10(1): 92.
7. Zhang, Zhenzhen, et al. "Development of a validated HPLC method for the determination of tenofovir disoproxil fumarate using a green enrichment process." Analytical Methods, 2015; 7(15): 6290-6298.
8. Hou, Xixi, et al. "Synthesis and antitumor activity of dolutegravir derivatives bearing 1, 2, 3-triazole moieties." BMC chemistry, 2024; 18(1): 97.
9. Guideline, I. H. T. "Validation of analytical procedures Q2 (R2)." ICH: Geneva, Switzerland, 2022; (1).
10. Singh, Saranjit, et al. "Forced degradation studies to assess the stability of drugs and products." TrAC Trends in Analytical Chemistry, 2013; 49: 71-88.
11. Brummer, Hildegard. "How to approach a forced degradation study." Life Sci. Technol. Bull, 2011; 31: 1-4.
12. C Kogawa, Ana, and Herida RN Salgado. "Impurities and forced degradation studies: a review." Current Pharmaceutical Analysis, 2016; 12(1): 18-24.