RESEARCH ARTICLE

Stability Indicating Technique and Validation for Dolutegravir, Lamivudine and Tenofovir Disoproxil Fumarate by UPLC

Gampa Nagamalleswari[1], MS Uma Shankar[2*]

[1]*Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRMIST, Kattankulathur, Tamil Nadu, India.*
[2]*Department of Pharmaceutics, SRM College of Pharmacy, SRMIST, Kattankulathur, Tamil Nadu, India.*

Received: 08th December, 2023; Revised: 09th January, 2024; Accepted: 18th February, 2024; Available Online: 25th March, 2024

ABSTRACT

Objective: To simultaneously estimate lamivudine, tenofovir, and dolutegravir in substance and tablet dosage form," a technologically sophisticated approach was developed for ultra-performance liquid chromatography (UPLC) stability indicating that is modest, accurate, and specific.

Methods: We were able to isolate each of the three active components using a state-of-the-art stability-indicating approach. Waters X-Bridge C8 column, 100 x 3.0 mm, 3.5 µm, 0.5 mL/min flow rate, and monitoring at 260 nm were used for the chromatographic behavior, which was shown by a mobile phase consisting of acetonitrile and 0.1% formic acid in an 80:20 ratio.

Results: A mean retention time of 0.879 minutes was recorded for lamivudine, 1.571 minutes for tenofovir, and 0.607 minutes for dolutegravir. "The degrees of linearity for lamivudine, tenofovir, and dolutegravir were determined to be 20 to 120 µg/mL, 20 to 120 µg/mL, and 3.4 to 20.4 µg/mL, respectively.

Conclusion: Combination tablet dosage forms of lamivudine, tenofovir, and dolutegravir were accurately and reliably estimated using the suggested technique, which underwent validation in terms of linearity, range, accuracy, precision, specificity, and robustness. Stability tests were also conducted.

Keywords: Lamivudine, Tenofovir, Dolutegravir, UPLC, Stability indicating method, Validation.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.1.02

How to cite this article: Nagamalleswari G, Shankar MSU. Stability Indicating Technique and Validation for Dolutegravir, Lamivudine and Tenofovir Disoproxil Fumarate by UPLC. International Journal of Drug Delivery Technology. 2024;14(1):9-13.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Medications such as lamivudine, which1-[(2R, 5S)-2-(hydroxyl methyl)-1, 3-oxathiolan-5-yl]"-1, 2-dihydropyrimidin-2-one. Reverse transcriptase lamivudine is effective against hepatitis B, HIV-1, and HIV-2. A lower dosage of lamivudine, as shown in Figure 1(a) for HIV therapy, has been used to treat chronic hepatitis B. Both the histological staging of the liver and the seroconversion of e-antigen-positive hepatitis B are improved by it.[1-3]

Tenofovir's bis-isopropoxy carbonyl oxy methyl ester derivative is the fumaric acid salt of tenofovir disoproxil fumarate [Figure 1(b)]. 9-[(R)-2- [[(isopropoxcarbonyl)-oxy] methoxy] phosphinyl] methoxy] propyl] is its chemical formula. fumarate of adenine.[4-7]

A recently discovered human immunodeficiency virus (HIV) integrase inhibitor is dolutegravir (DTG), [Figure 1(c)]). DTG is an INSTI that inhibits the viral integrase enzyme's two-step process of genome integration into host cell DNA.[8] Unlike ritonavir, which is needed for cytochrome P450 3A4 inhibition, DTG selectively blocks this step. By chelating Mg2þ ions at the active site, DTG prevents the binding of the integrase viral DNA complex to host cell DNA, the same as the two authorized INSTIs raltegravir (RAL) and elvitegravir (EVG).[9] Interrupting the viral replication cycle by blocking integration renders HIV-1 incapable of replicating. The effectiveness of DTG in reducing viral load quickly and increasing CD4+ cell count was shown in phase II studies.[10] The literature[11-13] highlights various approaches currently popular for separately estimating TDF, LMV, and DTG.

According to the literature, tenofovir disoproxil fumarate can be measured in plasma using high-performance liquid chromatography (HPLC),[14] plasma liquid chromatography–mass spectrometry (LC-MS/MS),[15] and human peripheral blood mononuclear cell methods.[16] Lamivudine can also be estimated in human serum using HPLC.[17-19] It has been reported that both lamivudine and tenofovir disoproxil fumarate can be measured simultaneously using reversed-phase high performance liquid chromatography (RP-HPLC),[20,21] high-performance thin layer chromatography (HPTLC)[22,23] and LC-MS/MS.[24,25]

**Author for Correspondence: umashans@srmist.edu.in*

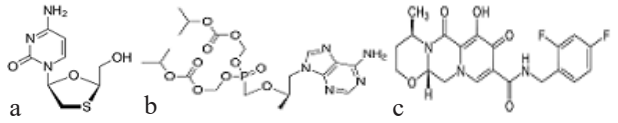

Figure 1: (a) Lamivudine (b)Tenofovir (c) Dolutegravir

Unfortunately, we are unaware of any validated forced degradation experiments conducted on pharmaceutical formulations, including lamivudine, tenofovir, and dolutegravir, for simultaneous ultra performance liquid chromatography (UPLC) estimates. The goal was to develop an analytical forced degradation approach that could rapidly, selectively, and sensitively estimate the combined dose form of lamivudine, tenofovir, and dolutegravir using UPLC. In this study, we developed a simple, dependable, and repeatable UPLC technique that has been statistically verified using recovery, accuracy, and precision.

MATERIAL AND METHODS

Materials

The pharmaceutical grade working standards of lamivudine, tenofovir, and dolutegravir were obtained as a gift sample from Richer Pharmaceuticals (Prasanth Nagar, Hyderabad, India). Fixed dosage tablet combination containing 300 mg lamivudine, 300 mg tenofovir and 50 mg dolutegravir was purchased from local market Hyderabad, India. Acetonitrile, Ortho phosphoric acid, formic acid HPLC grade purchased from SD Fine Chem., Mumbai. Milli Q water was used.

Chromatographic conditions

Agilent1290 Infinity II LC System consisting of pump, Auto sampler, Auto injector, VWD & photo diode array detector, thermostatic column compartment connected with Empower 2 software connected with a Waters X-Bridge C8 100 x 3.0 mm, 3.5 µm.

Mobile phase

The correct proportions of acetonitrile to 0.1% formic acid are 80:20. Passed through a 0.45 µ membrane filter paper forfiltration. Consistent with 0.5 mL/min, the mobile phase flow rate was maintained. A 5 µL injection volume was used for detection, while the column temperature was maintained at 35°C.

Standard solution preparation

Accurately evaluate and transfer 50 mg of dolutegravir, 300 mg of lamivudine and 300 mg of tenofovir disoproxil fumarate working standards into a 100 mL clean dry volumetric flask, add diluent and sonicate to dissolve it completely and make up around the mark with the identical solvent. Further, pipette 5 mL of the above stock solution into a 50 mL volumetric flask and dilute it to the mark with diluent.

Preparation of Sample Solution

About 20 pills of average weight; take 20 tablets daily. After crushing the pills into powder, put 764 mg of the sample into a 100 mL volumetric flask. Mix with 70 mL of diluent, sonicate for 15 minutes to dissolve the ingredients, and then dilute the volume with more diluent.

Using diluent, further dilute 5 mL of the aforementioned solution into a 50 mL volumetric flask.

RESULT AND DISCUSSION

Method Development and Optimization of Chromatographic Conditions

It takes experimenting with various buffer pH-conditions and solvent proportions, such as acetonitrile, to get a successful separation between the three components. For best results, use an 80:20 ratio of acetonitrile to 0.1% formic acid. Measure the flow rate at 0.5 mL/min and read the detection at 260 nm (Figure 2). Figure 3 displays the chromatogram of the improved standard combination. Tabulated in Table 1 are the system suitability characteristics for the optimized standard mixture chromatogram, including retention duration, asymmetry, resolution, and theoretical plates.

Method Validation

System suitability

In order to validate the procedure, it is necessary to assess the system's appropriateness by measuring factors such as the tailing factor, theoretical plates, resolution, and %RSD for duplicate injections. The findings, shown in Table 1, were well within the parameters. The system suitability chromatogram is shown in Figure 3.

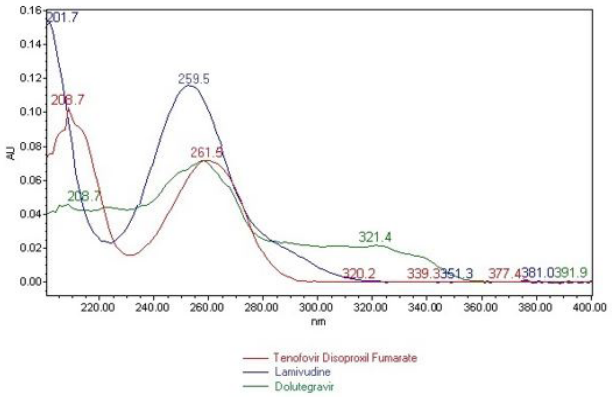

Figure 2: UV spectra of three drugs

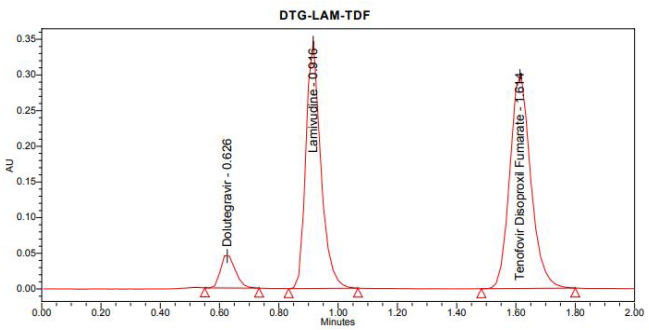

Figure 3: System suitability chromatogram

Table 1: System suitability results

Parameters	*Results*			*Limits*
	Lamivudine	*Tenofovir*	*Dolutegravir*	
RSD of peak area	1.12	1.49	.737	<2.0 for n≥6
RSD of retention time	0.814	0.194	0.502	<1.0 for n ≥6
USP tailing factor (T)	1.19	1.22	1.10	T<2
USP plate count (N)	13264	12702	3315	>2000
USP resolution (R)	3.02	6.15		R > 2

Table 2: Accuracy data

Parameter (%)	*Amount added (µg)*	*Amount recovered (µg)*	*%recovery*	*Mean %recovery*
Lamivudine				
50 level	40	40.05	100.12	100.12
100 level	80	79.95	99.93	99.93
150 level	120	120.15	99.87	99.87
Tenofovir				
50 level	40	40.12	100.3	100.3
100 level	80	80.05	100.06	100.06
150 level	120	120.09	100.07	100.07
Dolutegravir				
50 level	6.8	6.78	99.70	99.70
100 level	13.6	13.5	99.26	99.26
150 level	20.4	20.3	99.50	99.50

Specificity

The absence of peaks at the retention periods of lamivudine, tenofovir, and dolutegravir in the placebo chromatogram and the results of the degradation experiments indicating that there was no interference with degradants, together with peak purities for the sample solution >0.99, suggest that the technique is specific, all point to its reliability.

Accuracy

In order to ensure that the expected approach would work as intended, researchers conducted recovery trials in which a known quantity of pure drug concentrations were spiked into placebo at three distinct levels (50, 100, and 150%). Percentage of recovery was used to determine accuracy. Table 2 displays the findings.

Precision

At three different levels, namely repeatability, reproducibility, and intermediate precision, the accuracy was assessed using six duplicate injections of 60, 60, and 10.2 µg/mL of lamivudine, tenofovir, and dolutegravir, respectively. Precision was reported as a percentage of RSD and is shown in Table 3.

Linearity and range

The linearity was tested by measuring the standard solutions of lamivudine, tenofovir, and dolutegravir at different concentrations ranging from 25 to 150%. Table 4 displays an overview of the parameters.

Table 3: Precision studies

Parameter			
	Lamivudine	*Tenofovir*	*Dolutegravir*
Repeatability			
RSD of retention time	0.814	0.194	0.502
RSD of peak area	1.11	1.48	0.737
Reproducibility			
RSD of retention time	0.795	0.181	0.51
RSD of peak area	1.15	1.49	0.729
Intermediate precision			
RSD of retention time	0.80	0.185	0.48
RSD of peak area	1.09	1.41	0.725

Detection limit and Quantitation limit

When estimating detection limit (DL), we took into account a signal-to-noise ratio of 3:1 and, for quantitation limit (QL), 10:1. As for lamivudine, the limit of detection and quantitation are 2.45 and 7.434 mg/mL, tenofovir, 2.338 and 7.087 mg/mL, and dolutegravir, 0.443 and 1.342 mg/mL, respectively.

Robustness and ruggedness

When modest, intentional modifications were made to the flow rate, mobile phase composition, and column temperature

Table 4: Regression equation parameters

Parameter	*Lamivudine*	*Tenofovir*	*Dolutegravir*
Linearity range (mg/mL)	20–120	20–120	3.4–20.4
Correlation co-efficient	0.9994	0.9997	0.9986
Slope	27167	25849	8955.4
Y-intercept	31943	3033.7	2881.4

Table 5: Assay results

Drug	*Labelled amount (mg/tab)*	*Amount found (mg/tab)*	*% of Assay*
Lamivudine	300	299.5	99.83
Tenofovir	300	300.10	100.03
Dolutegravir	50	49.85	99.7

at 100% test concentration, the method's resilience was confirmed. The suggested method's robustness was tested using the same sample under different conditions, including different columns, analysts, instruments, and labs.

Standard solution stability

At room temperature, the standard solution was subjected to stability tests every 24 and 48 hours. Theory plates, tailing factors, retention duration, and resolution, which are all appropriate system parameters, did not alter much. Consequently, the conventional solution remains stable for at least 48 hours at room temperature.

Mobile phase stability

The mobile phase was subjected to room temperature stability tests at 24 and 48 hours intervals. Retention duration, resolution, theoretical plates, peak regions, and tailing parameters all remained unchanged. That is why the mobile phase won't change even after 48 hours at room temperature.

Analysis of marketed sample

Table 5 summarizes the findings of an investigation of the tablet dosage forms of lamivudine, tenofovir, and dolutegravir using the suggested approach, which yielded values ranging from 99 to 101%.

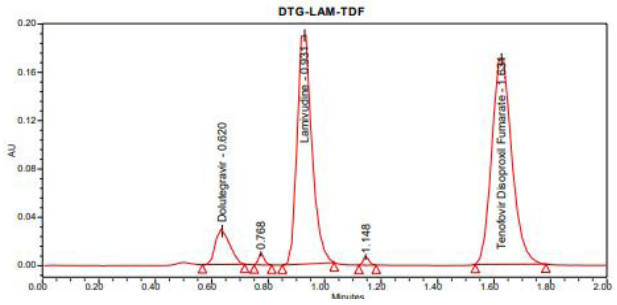

Figure 4: Acid degradation study

Forced degradation and stability-indicating tests

- *Stock solution*

To make a standard solution with concentrations of 900 mg/mL lamivudine, 900 mg/mL tenofovir, and 150 mg/mL dolutegravir, measure out 100 mL of each drug and add 90 milligrams of each to separate 100 mL volumetric flasks. Dissolve the drugs in 60 mL of diluent. Table 6 provides a summary of the findings.

- *Acidic degradation*

After 10 mL of 1.0 N HCl and 6 mL of stock solution were heated in a water bath at 80°C for about 24 hours, the mixture was diluted with 50 mL of water. Use a 0.22 micron membrane filter to strain the mixture (Figure 4).

- *Alkali degradation*

Mix 10 mL of 1.0 N NaOH with 6 mL of stock solution. Place in a water bath and maintain at 800C for about 72 hours. Once cooled, add 50 mL of diluent to make the final volume. Pass the mixture through a membrane filter with a pore size of 0.22 microporosity.

- *Oxidative degradation*

Mix 5 mL of 3% H_2O_2 with 6 mL of stock solution; in a water bath, maintain at 800°C for about 48 hours; then, add 50 mL of diluent to adjust volume. Use a 0.22 micron membrane filter to strain the mixture.

- *Thermal degradation*

The material that was subjected to ultraviolet light was forcibly transformed. Gather the specimen on the first, third, fifth, and

Table 6: Results of forced degradation study

Stress condition	*%Assay of active ingredient*					
	Lamivudine	*%Degradation*	*Tenofovir*	*%Degradation*	*Dolutegravir*	*%Degradation*
Acid	85.1	14.9	86.2	13.7	85.7	14.3
Alkali	84	16	85.5	14.4	84.9	15.1
Peroxide	86.5	13.5	88.6	11.3	89.5	10.5
Reduction	84.2	15.8	84.9	15	83.8	16.2
Thermal	88.9	11.1	89.1	10.8	87.4	12.6
Photo	99	1	97.9	2	99.1	0.9
Hydrolysis	98.5	1.5	98.8	1.1	98.7	1.3"

tenth day. Fill a 100 mL volumetric flask with a balanced tablet powder that is equal to 320 mg of lamivudine. Add 60 mL of diluent, stir to combine, and then add 6 mL more to get it up to 50 mL; finally, strain the mixture through a 0.45-micron filter. Put into chromatography as an injection.

CONCLUSION

In conclusion, the developed UPLC stability indicating technique offers a reliable and efficient method for the simultaneous estimation of lamivudine, tenofovir, and dolutegravir in substance and tablet dosage forms. Through meticulous method development and optimization, the study successfully isolated each active component, achieving a mean retention time for each compound. Validation parameters including linearity, range, accuracy, precision, specificity, and robustness were thoroughly evaluated, demonstrating the method's effectiveness and reliability. Additionally, forced degradation studies confirmed the stability-indicating nature of the approach, providing insights into potential degradation pathways. Overall, the proposed UPLC method presents a promising tool for pharmaceutical quality control, ensuring accurate assessment and monitoring of these critical antiretroviral agents.

REFERENCES

1. R. Stephen, W.H. David, Antiretroviral agents, in: J.G. Hardman, L.E. Limbird, A.G. Gilman (Eds.), Goodman & Gilman's The Pharmacological Basis of Therapeutics, 10th ed., mgraw- Hill Professional, New York; 2001.p 1358-1360, 1372.
2. S. Budavari (Ed.), The Merck Index, 13th ed., Merck and Co., Inc, Whitehouse Station. NJ, USA; 2001. p1573.
3. S.C. Sweetmann, Martindale the Complete Drug Reference, 34th ed., The pharmaceutical press, London; 2005.p 648-655.
4. R. Stephen, Wh David, Antiretroviral agents, in: J.G. Hardman, L.E. limbrid, A.G. Gilman (Eds.), Goodman & Gilman's the Pharmacological Basis of Therapeutics, 10th ed., mgraw-Hill professional, New York; 2001. p1358-1360 1372.
5. S. Budawari, The Merck Index, 14th ed., Merck and Co. Inc, Whitehouse Station. NJ; 2006.p 927-1573.
6. Martindale: The Complete Drug Reference, 34th Edition, Pharmaceutical Press, London;2005 p 648-655.
7. H.W. Rang, M.M. Dale, J.M. Ritter, P.K. Moore, Pharmacology, fifth ed., Churchill Livingstone, Edinburgh; 2005.p 660.
8. Y. Pommier, A. Johnson, C. Marchland, Nat. Rev. Drug Discov. 4 (2005) 236 [PubMed: 15729361].
9. C. Katlama, R. Murphy, Opin. Investig. Drugs 21 (2012) 523.
10. A. Karunakaran, K. Kamarajan, V. Thangarasu, Development and validation of first-derivative spectrophotometric method for the simultaneous estimation of lamivudine and tenofovir disoproxil fumerate in pure and in tablet formulation, Der Pharm. Lett. 2010;2(5): 221-228.
11. C. Bennetto-Hood, G. Tabolt, P. Savina, E.P. Acosta, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 2014; 225-232.
12. P. Hamrapurkar, M. Phale, N. Shah, Quantitative estimation of efavirenz by high performance thin layer chromatography, J. Young Pharm. 2009;1: 356-366.
13. S. Sentenac, C. Fernandez, A. Thuillier, P. Lechat, G. Aymard, Sensitive determination of tenofovir in human plasma samples using reversed phase liquid chromatography, J. Chromatogr. B. 2003; 793 (2): 317-324.
14. T. Delahunty, L. Bushman, C.V. Fletcher, Sensitive assay for determining plasma tenofovir concentrations by LC/MS/MS, J. Chromatogr. B 2006; 830: 6-12.
15. T. King, L. Bushman, J. Kiser, P.L. Anderson, R. Michelle, T. Delahunty, C.V. Fletcher, Liquid chromatography-tandem mass spectrometric determination of tenofovir-diphosphate in human peripheral blood mononuclear cells, J. Chromatogr. B.2006; 843 (2, 7) :147-156.
16. Marc Schuman, Serge Schneider, Christine Omes, Robert Wennig, Leon Fundira, Jean-Claude Tayari, Vic Arendt, HPLC analysis of generic antiretroviral drugs purchased in Rwanda, Bull. Soc. Sci. Med.2005;3: 317-325.
17. Gholamreza Bahrami, Shahla Mirzaei, Amir Kiani, Bahareh Mohammadi, High- performance liquid chromatographic determination of lamivudine in human serum using liquid-liquid extraction; application to pharmacokinetic studies, J. Chromatogr. B 2005; 823 (2): 213-217.
18. A. Sibel, Uslu Ozkan Bengi, Rapid HPLC assay for Lamuvidine in pharmaceutical and human serum, J. Liq. Chromatogr. Relat. Tech. 2002; 25 (9): 1447-1456.
19. Eunice Kazue Kano, Cristina Helena dos Reis Serra, Eunice Emiko Mori Koono, Simone Schramm, Determination of Lamuvidine in human plasma by HPLC and its use in bioequivalence studies, Pharm. Biomed. Anal. 2006; 4 (3): 761-765.
20. K. Hymavathi, D. Mahesh Babu, Patan Afroz, Development and validation of RP-HPLC method for the simultaneous estimation of tenofovir disproxil fumerate and Lamuvidine in combined dosage form, IJAPBS 2012; 2 (3): 144-148.
21. Sravan kumar chopperla, B. Vijaykumar, D. Gouri Shankar Dr, Method development and validation of RP-HPLC method for the simultaneous estimation of tenofovir disproxil fumerate and Lamuvidine in combined dosage form, IJPRD .2013; 4 (11): 110-118.
22. M. Balaji, I. Srikanth Reddy, V. Ashok Kumar, C. Ulaganathan, S. Muneer, Method development and validation of HPTLC method for quantitative estimation of tenofovir disproxil fumerate and Lamuvidine in combined dosage form, IJPRD .2011 4; 6: 023-029.
23. Shah DA, Bhatt KK. Development and validation of a stability-indicating UPLC method for simultaneous estimation of Dolutegravir, Lamivudine, and Tenofovir Disoproxil Fumarate in bulk and pharmaceutical dosage form. Journal of Liquid Chromatography & Related Technologies. 2020; 43(7-8): 233-242. doi:10.1080/10826076.2019.1674735.
24. Patel B, Patel S. Stability-indicating UPLC method development and validation for simultaneous estimation of Dolutegravir, Lamivudine, and Tenofovir Disoproxil Fumarate in combined dosage form. Analytical Chemistry Letters. 2021; 11(4): 745-756. doi:10.1080/22297928.2020.1802149.
25. Nandi S, Banerjee D, Sengupta P, et al. Development and validation of a stability-indicating UPLC method for the simultaneous estimation of Dolutegravir, Lamivudine, and Tenofovir Disoproxil Fumarate in pharmaceutical dosage form. Biomedical Chromatography. 2022; e5305. doi:10.1002/bmc.5305.