Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [8] July 2023 : 86-93
©2023 Academy for Environment and Life Sciences, India
Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

First Derivative UV Spectroscopic Method for Simultaneous Estimation of Dolutegravir and Lamivudine in Fixed Dose Combination

Ceema Mathew*, SahithiPoorna M, Shashikala Metri
GokarajuRangaraju College of Pharmacy, Osmania University, Bachupally, Hyderabad-90, Telangana, India.

ABSTRACT
A derivative spectroscopic method is developed for the simultaneous quantification of Dolutegravir (DTG) and Lamivudine (3TC). Simultaneous quantification of DTG and 3TC depends on the amplitude measurements at the zero crossing wavelengths of each drug. Since Dolutegravir and lamivudine presented good solubility and suitable zero crossing point for both the drugs, water and acetonitrile in 1:1 ratio is selected as the solvent for the stock solution and subsequent dilutions. Two wavelengths, 365 nm (zero crossing point (ZCP) for 3TC) and 262 nm (ZCP for DTG) were selected for the quantification of DTG and 3TC respectively. The first derivative amplitude-concentration plots were linear over the range of 2–10 µg/ml and 6–30 µg/ml with detection limits of 0.568 and 0.713 µg/ml and quantification limits of 1.704 and 2.178 µg/ml for DTG and 3TC respectively. The percentage recovery was within the range between 98.2-101.25 for DTG and 3TC, it was 100.08-100.37. The % RSD for accuracy and precision of the method was <2%. The proposed method is simple, accurate and precise so that one can successfully apply it to the routine quality control analysis of studied drugs in fixed-dose formulations, available as tablets.
Keywords: *Dolutegravir, Lamivudine, first derivative UV spectroscopy, zero crossing point, antiretroviral agent*

Received 04.05.2023 Revised 16.06.2023 Accepted 29.07.2023

INTRODUCTION

Lamivudine, an antiretroviral drug, is effective in preventing and treating AIDS. It can be used for hepatitis B, when other options are ineffective. The IUPAC name of lamivudine is 2',3'-dihyroxy-3'thiacytidine 4-Amino-1- [(2R,5S)-2- (hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2- dihydropyrimidinone. Molecular formula is C8H11N3O3S. Lamivudine is a synthetic nucleoside analog with antihepatitis B (HBV) and HIV activities. Intracellular phosphorylation of Lamivudine leads to the active metabolites, lamivudine triphosphate (L-TP) and lamivudine monophosphate (L-MP). In HIV, after incorporating the nucleoside analogue into viral DNA, L-TP inhibits HIV-1 polymerase (RT) via DNA chain termination. In HBV, incorporating L-MP by HBV polymerase into viral DNA leads to termination of the DNA chain. L-TP is a weak inhibitor of alpha and beta mammalian DNA polymerases and mitochondrial polymerase DNA.(1,3). Dolutegravir is an antiretroviral agent used for HIV or AIDS prevention and treatment. (4R,12aS)-N-(2,4- difluoro benzyl)-7-hydroxy-4- methyl-6,8-dioxo-3,4,6,8,12,12a- hexahydro-2H-pyrido [1',2':4,5] pyrazino [2,1-b] [1,3] oxazine-9- carboxamide is its IUPAC name. The molecular formula and molecular weight of Dolutegravir is: C20H19F2N3O5 and 419.38gm/mol, respectively. Dolutegravir is strand-transfer integrase (INSTI) inhibitor, that is orally active, with activity against human immunodeficiency virus type 1 (HIV-1) infection. It binds to the integrase site, an HIV enzyme, which forces the conversion of viral genetic material into human chromosomes at a faster rate. Figure 1 represents the chemical structure of Dolutegravir and Lamivudine.(2,3)The concomitant use of dolutegravir and Lamivudine reduces the risk of acquired immunodeficiency syndrome (AIDS) and HIV-related illnesses such as cancer or serious infections by reducing the HIV load in blood circulation. The fixed-dose combination is available as a tablet formulation with 300 mg of Lamivudine and 50 mg of Dolutegravir.(4) Since both the drugs are used either alone or as double or triple combination with other anti-retroviral agents, there is a vast array of literature regarding the analysis of the drugs. Hence, the analytical methods reported are grouped as those for Lamivudine alone or in combination with other drugs, dolutegravir alone or in combination with other drugs, or for both the mentioned drugs.The methods available for Lamivudine, either alone or in a combined form with other drugs, include HPLC (5), HPTLC(6), UPLC(7,8), UV(9,10), and LC-MS(11,12). The methods available for dolutegravir, either alone

or in a combined form with other drugs, include HPLC (13), HPLC and HPTLC(14), UPLC(15-17), UV(18), and LC-MS(19,20). The methods available for Lamivudine and dolutegravir, either together or in a combined form with other drugs, include HPLC (21-28) and UPLC(29).A thorough analysis of the litearture revealed the absence of UV spectroscopic methods for Lamivudine and dolutegravir in combination and hence took an attempt to develop a derivative spectroscopic strategy.

MATERIAL AND METHODS

Instruments and chemicals

A double beam UV-Visible spectrophotometer (Shimadzu, 1800) having UV-probe software was used for the analysis. The samples of DTG and 3TC were procured from Hetero drugs ltd, India as gratis samples. The fixed-dose formulation, namely DTG tablets, were purchased from the nearby pharmacy.

Analytical method development and validation

Optimization of solvent and derivatization parameters

Since the simultaneous analysis of a binary mixture by derivative spectroscopy depends on the availability of zero crossing points (ZCPs) for both drugs, solvent selection is an important criterion. For this investigation, different solvents were tried, and the zero crossing points (ZCPs) were available for both drugs when the solvent was acetonitrile and water in a 1:1 ratio. Standard solutions of DTG (10 µg/ml) and 3TC (10 µg/ml) prepared in the above solvent were scanned in the UV range to obtain the respective zero-order spectra; Later, it was converted into first derivative spectra selecting $\Delta\lambda$ = 16 nm and scaling factor = 10. The overlapped spectra of DTG and 3TC revealed the ZCP for both the drugs at which the derivative amplitude can be measured for each of them.

Method validation

After method optimization, it was validated for accuracy, precision, linearity, LOD, and LOQ as per ICH guidelines which are detailed below.

Calibration graphs for dolutegravir and lamivudine

Standard solutions of dolutegravir (1000 µg/mL) and lamivudine (1000 µg/mL) were further diluted with the solvent to get working standard solutions of analytes in the concentration range of 2-10 µg/mL and 6-30 µg/mL of dolutegravir and lamivudine, respectively. Then the solutions were scanned in the range of 200-400 nm using a spectrophotometer and tyhe resulting zero order spetra were converted to the first-derivative spectra. The values of the first-derivative absorbance for dolutegravir and lamivudine were obtained using five different concentrations by measuring each concentration against solvent blank at the chosen wavelength of 365 nm and 262 nm for dolutegravir and lamivudine, respectively. This process was done three times, each time analyzing fresh samples. Calibration graphs were prepared with the values of first-derivative absorbance versus corresponding concentrations of the drugs. The regression equation and the correlation coefficients were calculated.

Accuracy

Standard addition method was employed to determine the accuracy of the method by means of calculating recoveries of dolutegravir and lamivudine. Tablet powder equivalent to 10 mg of dolutegravir was spiked separately with 80%, 100% and 120% of dolutegravir and lamivudine standard drug solutions. The amount of dolutegravir and lamivudine was estimated by measuring derivative responses at the appropriate wavelength (262 nm for lamivudine and 365 nm for dolutegravir). The recovery was verified by estimation of drugs in triplicate preparations at each specified concentration level.

Precision

The intra-day precision of the proposed first-derivative spectrophotometric method was performed by estimating the corresponding response three times on the same day for three different concentrations of dolutegravir (4, 6, 8 µg/mL) and lamivudine (12, 18, 24 µg/mL). Similarly, the inter-day precision of the proposed first derivative spectrophotometric method was performed using the same concentrations of dolutegravir and lamivudine, and the corresponding responses were recorded nine times on three different days. The results of both intra-day and inter-day precision were reported in terms of relative standard deviation (% RSD).

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of quantification (LOQ) and the limit of detection (LOD) and of dolutegravir and lamivudine was determined by way of the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines.

$$\mathbf{LOD = 3.3\ \sigma/S}$$

σ = standard deviation of the response

S = slope of the calibration grpah of the analyte

The quantitation limit (LOQ) may be expressed as:

$$\mathbf{LOQ = 10\ \sigma/S}$$

σ = standard deviation of the response
S = slope of the calibration graph of the analyte.

Determination of dolutegravir and lamivudine in their combined dosage form (assay)

Twenty tablets of DOVATO, each containing 50 mg of dolutegravir and 300 mg of lamivudine, were weighed, and the average weight was determined. A quantity of tablet powder equivalent to 30 mg of lamivudine was transferred to a volumetric flask of 100 mL capacity. The solvent was added to this volumetric flask and sonicated for 15 min, and the volume was diluted, filtered using whatmann filter paper. One mL filtrate was transferred into a volumetric flask of 10 mL capacity, and the volume was diluted upto the mark with the solvent to yield a final concentration of 5 µg/mL of dolutegravir and 30 µg/mL of lamivudine. Derivative amplitudes of these solutions were determined and substituted into the regression equation of each drug for the estimation of dolutegravir and lamivudine.

RESULTS AND DISCUSSION

Analytical method development

Derivative spectroscopy can be used with little error for the quantification of one analyte (dolutegravir/lamivudine) whose peak is obscured by a large overlapping peak of another analyte. Figure 2shows overlay zero-order spectra of the standard solution of dolutegravir and lamivudine. It was observed that dolutegravir and lamivudine contribute significantly at their corresponding λmax value for absorbance. Hence, the graphical derivative method was used to estimate dolutegravir and lamivudine in the presence of each other.

Selection of zero crossing point and fixing the derivative parameters

The first derivative spectrum of DTG had zero absorbance at 262 nm, where 3TC gave a good derivative response, while the first derivative spectrum of 3TC had zero absorbance at 365 nm, where DTG gave a good derivative response. Therefore, 262 nm and 365 nm were selected for estimation of 3TC and DTG, respectively, and it is shown in Figure 3. For a smooth derivative spectrum of each drug, Δλ = 16 nm and scaling factor = 10 were selected by trial and error.

Analytical method Validation:

Calibration plot for dolutegravir and lamivudine

Dolutegravir and lamivudine showed a linear relationship between concentration (µg/mL) and derivative absorbance. Dolutegravir and lamivudine were linear in the range of 2-10 µg/mL and 6-30 µg/mL, respectively. The correlation coefficient value (R2) obtained from the linear regression analysis for dolutegravir and lamivudine was 0.999 and 0.9996, respectively, which indicated the linearity of the method. From figure 4, it was observed that with the increase in dolutegravir concentration, the derivative response at 365 nm was increased. Similarly, the derivative response for lamivudine at 262 nm was increased with the increase in its concentration. The regression equation for dolutegravir and lamivudine is shown in the calibration graph in figure 5.

Accuracy results of DTG and 3TC:

For the accuracy determined by the standard addition method, three different levels (80%, 100% and 120%) of standards were spiked to commercial tablets in triplicate. The mean of percentage recoveries and % RSD values was calculated and reported in the table. The % recoveries of dolutegravir and lamivudine were found to be in the range 98-101% and 100.08 – 100.3%, respectively, which are satisfactory.

The percentage recoveries of the drugs were computed from the corresponding regression equations obtained in the linearity studies and tabulated in Table 2. The %recoveries of DTG and 3TC were found to be in the range of 97.96–99.33% and 100.62 – 101.30%, respectively, which are satisfactory.

Precision

The repeatability of the method was determined by intra-day (***n*** = 3) analysis of three standard solutions of dolutegravir and lamivudine at the concentration of 4, 6 & 8 µg/mL and 12, 18 & 24 µg/mL, respectively. Intermediate precision determination was done by the inter-day (***n***=9) analysis of three standard solutions of dolutegravir and lamivudine at the concentration of 4, 6 & 8 µg/mL and 12, 18 & 24 µg/mL, respectively and reported in the table. The % RSD of repeatability and inter-day analysis was <2.0 for both the drugs. These statistical data were indicative of good precision.

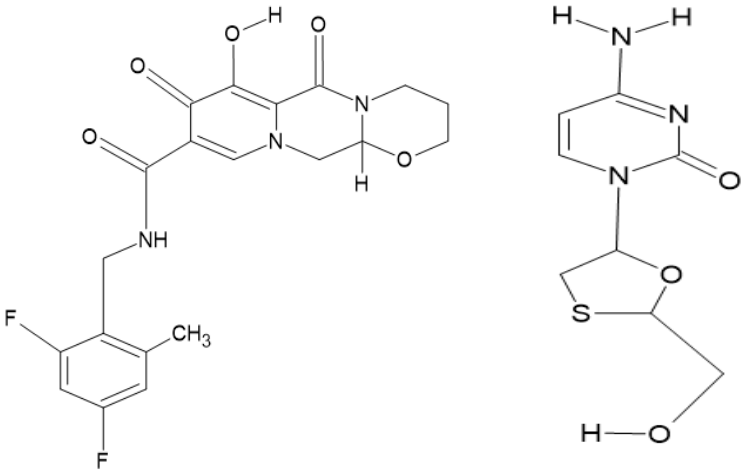
Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection and LOQ of dolutegravir was found to be 0.568 µg/mL and 1.704 µg/mL, respectively. For lamivudine, LOD was found to be 0.713 µg/mL, and LOQ was found to be 2.178 µg/mL.

Analysis of commercial tablets (assay)

The assay of commercially available tablets (DOVATO) containing 50 mg of dolutegravir and 300 mg of lamivudine was done. The results obtained for dolutegravir and lamivudine were compared with the corresponding labelled amounts and reported in table 3. The amount of dolutegravir and lamivudine

found was to be 50.84 mg and 298.43 mg, respectively. %Assay was found to be 101.68 % and 99.47% for dolutegravir and lamivudine, respectively.



a) b)

Fig 1. Chemical structure of a) Dolutegravir and b) Lamivudine

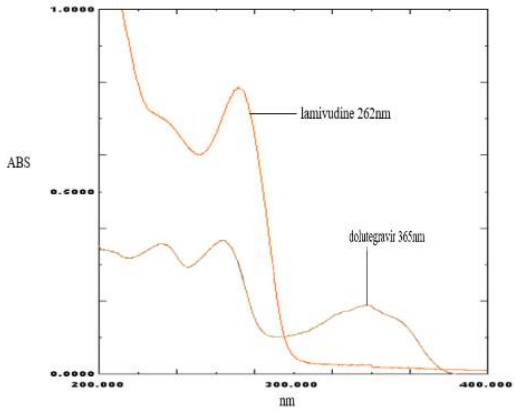


Fig 2: Zero order overlay spectra of dolutegravir (10 µg/mL) and lamivudine (10 µg/mL) in ACN: water

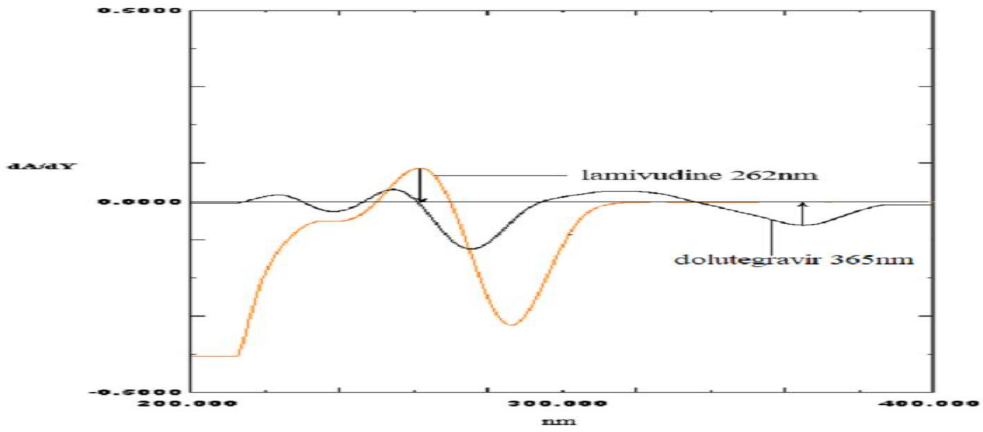


Fig 3: First order UV overlay spectra of dolutegravir (10 µg/mL) and lamivudine (10 µg/mL) in ACN: water

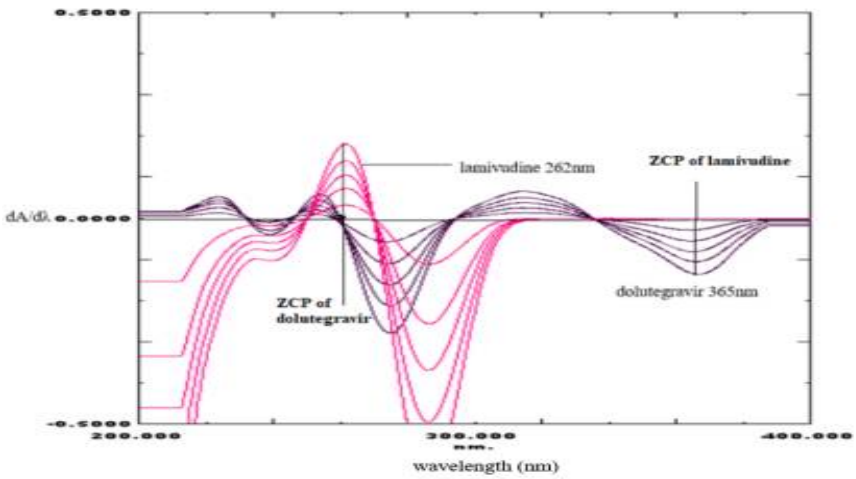
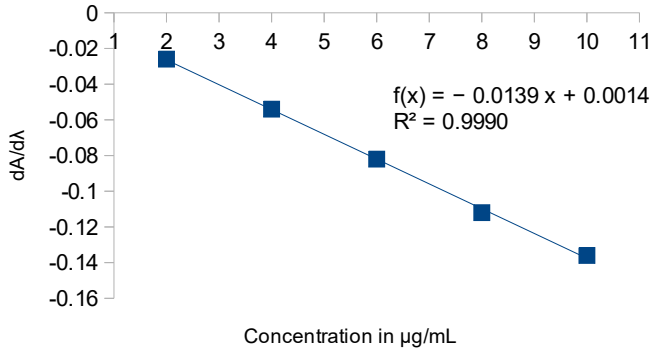
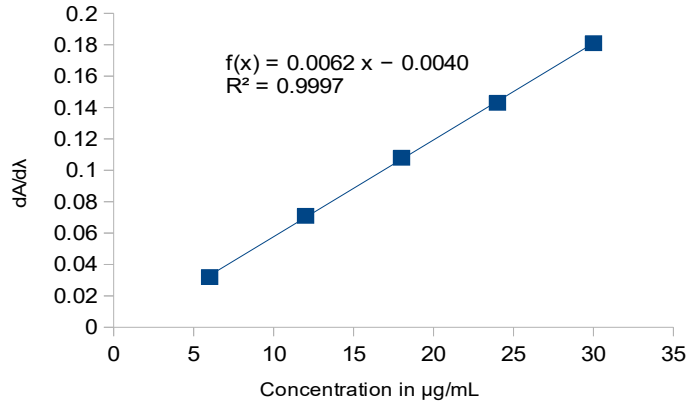


Fig 4: **First order UV overlay spectrum of dolutegravir (2-10µg/ml) and lamivudine (6-30 µg/ml)**



a)



b)

Fig 5. Linearity graph of a) Dolutegravir and b) Lamivudine

Table 1: Accuracy data of DTG and 3TC (recovery studies)

Brand	Spiking level (%)	Drug	Theoretical content (mg)	Amount found (mg)(n=3)	Recovery (%)	%RSD
DTG	80	DTG	3.6	3.54± 0.026	98.2	0.7
		3TC	21.6	21.68 ± 0.081	100.37	0.5
	100	DTG	4.0	4.05±0.02	101.25	0.49
		3TC	24.0	24.09 ± 0.102	100.37	0.98
	120	DTG	4.4	4.42 ± 0.015	101	0.33
		3TC	26.4	26.41± 0.035	100.08	1.33

Acceptance Criteria: % RSD should not be more than 2

Table 2: Precision data of DTG and 3TC

Drug name	Theoretical concentration (µg/mL)	Intra-day (n=3)		Inter-day (n=9)	
		Concentration found (µg/mL) AM±SD	% RSD	Concentration found (µg/mL) AM±SD	% RSD
Dolutegravir	4	4.07 ± 0.0378	0.93	4.06 ± 0.045	1.108
	6	5.96 ± 0.0655	1.098	5.89 ± 0.078	1.27
	8	8.09 ± 0.0916	1.132	8.05± 0.0665	0.826
Lamivudine	12	12.06 ± 0.036	0.297	12.1 ± 0.035	0.290
	18	18.05 ± 0.040	0.223	18.09± 0.052	0.284
	24	24.04 ± 0.051	0.316	24.07 ± 0.032	0.135

Acceptance Criteria: % RSD should not be more than 2

Table 3: Analysis of commercial tablets (assay of dolutegravir and lamivudine)

Drug name	Brand name	Label claim (mg)	Amount found(mg) AM ± SD	% ASSAY	% RSD
DOLUTEGRAVIR	DOVATO	50	50.84±0.6919	101.68	1.36
LAMIVUDINE		300	298.43±0.6132	99.47	0.20

CONCLUSION

The first-order UV-derivative method for simultaneous estimation of dolutegravir and lamivudine was developed using acetonitrile and water in a 1:1 ratio as the solvent and validated as per ICH guidelines. Its a simple, sensitive, economical method for simultaneous estimation of dolutegravir and lamivudine. The linearity range for dolutegravir and lamivudine was observed in 2-10 µg/mL and 6-30 µg/mL, respectively. The method validation was performed using ICH guidelines. The parameters such as linearity, accuracy, and precision were satisfactory, as indicated by low %RSD (<2). Detection limits of DTG and 3TC were found to be 0.568 µg/mL and 0.713 µg/mL, respectively, whereas Quantification limits were found to be 1.704 µg/mL and 2.178 µg/mL.Dolutegravir and lamivudine content was estimated in combined marketed tablets. The assay values were in good agreement with their label claim, evidenced by % assay values of 101% and 99.12%, respectively. The validation results obtained in this study demonstrated that the developed first-order UV derivative method could be utilised for the quality control of the said drugs in pharmaceutical dosage forms. The proposed method has some advantages as neither requires sophisticated instruments like HPLC or HPTLC nor costly reagents or solvents.

ACKNOWLEDGEMENTS

The authors are thankful to the management of GokarajuRangarju college of Pharmacy for the lab facilities.

Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

Author contributions: Concept – CM, SP, SM; Design – CM., SP.; Supervision – CM.; Materials – SM; Data Collection and/or Processing – SP,CM; Analysis and/or Interpretation – CM, SM; Literature Search – SP, SM; Writing – CM.; Critical Reviews – CM., SP, SM
CM: CEEMA MATHEW,SP: SAHITHI POORNA M, SM: SHASHIKALA METHRI

Funding: No funds received

Ethics statement: Not applicable

Informed Consent: Not applicable

REFERENCES

1. PubChem. Lamivudine [Internet]. Nih.gov. [cited 2022 Aug 20]. Available from: https://pubchem .ncbi.nlm.nih.gov /compound/Lamivudine.
2. PubChem. Dolutegravir [Internet]. Nih.gov. [cited 2022 Aug 20]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Dolutegravir.
3. Haribabu, Y., Nihila, K., Sheeja,V.K., & Akhil, M.B. (2021). Review on Analytical Methods for Determination of Lamivudine, Dolutegravir and Tenofovir, Disoproxil Fumarate in Fixed Dose Combination. International Journal of Pharmaceutical Sciences Review and Research; 71(1): 21-35.

4. AHFS Patient Medication Information [Internet]. [cited 2022 Aug 20].Available from: https://medlineplus.gov/druginfo/meds/a619043.html
5. Dubbaka A, Sireesha D, Bakshi V.(2016). Analytical method development and validation for the simultaneous estimation of lamivudine and tenofovir disoproxil fumarate by RP-HPLC method. MOJ proteombioinform. 4(5):306-309. DOI: 10.15406/mojpb.2016.04.00134.
6. Bizimana, T., Kayumba, P.C., Védaste, K., Egide, K., & Kaale, E. (2016). Development of high-performance thin layer chromatography for simultaneous analysis of lamivudine and tenofovir disoproxil fumarate. Rwanda j,.3(1): 6-10. DOI: 10.4314/rj.v3i1.1F
7. Dubey, S.O., & Duggirala, M. (2018). Simultaneous estimation of lamivudine, abacavir and dolutegravir by UPLC method. Int. J. App. Pharm.,10(1): 46-52.
8. Kurmi, M., Jayaraman, K., Natarajan, S., Kumar, G.S., Bhutani, H. & Bajpai, L. (2020). Rapid and efficient chiral method development for lamivudine and tenofovir disoproxil fumarate fixed dose combination using ultra-high performance supercritical fluid chromatography: A design of experiment approach. Journal of Chromatography A., 16;1625:461257. doi: 10.1016/j.chroma.2020.461257.
9. Mali, A.S., Salunke, P.A., Barhate, S.D., & Bar, M.M. (2015). Simultaneous Determination of Lamivudine and Tenofovir Disoproxil Fumarate by UV Spectrophotometric Method. JMSCR., 03(04): 5227–5236.
10. Ananda Kumar, K., Anusha, K., Kokilavani, V., Sangheetha, V.P., Saranya, R., Jambulingham, M. & Ramesh, J. (2017). Method development and validation of uv -spectroscopic method for the determination of lamivudine as an active pharmaceutical ingredient and in tablet dosage form. International Journal of Pharmaceuticals and Health care Research., 5 (04): 129–137.
11. Waitt, C., Diliiy Penchala, S., Olagunju, A., Amara, A., Else, L., Lamorde, M., et al. (2017). Development, validation and clinical application of a method for the simultaneous quantification of lamivudine, emtricitabine and tenofovir in dried blood and dried breast milk spots using LC–MS/MS. J Chromatogr B AnalytTechnol Biomed Life Sci,. 1060: 300–7.
12. Pereira, A.S., Kenney, K.B., Cohen, M.S., Hall, J.E., Eron, J.J., Tidwell, R.R. & Dunn, J.A.(2000). Simultaneous determination of lamivudine and zidovudine concentrations in human seminal plasma using high-performance liquid chromatography and tandem mass spectrometry. Journal of Chromatography B: Biomedical Sciences and Applications., 742(1): 173-83.
13. Venkarnarayana, M. & Jyothi, N.S. (2020). Development of Validation and Stability Indicating Method of Anti-HIV Dolutegravir Drug and its Related Impurities by Using RP-HPLC. J. Chromatogram. Sep. Technol., 11: 426.DOI: 10.35248/2157-7064.20.11.426
14. Bhavar, G.B., Pekamwar, S.S., Aher, K.B., Thorat, R.S., & Chaudhari, S.R. (2016). 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.,84(2): 305–20.
15. Wang, X., Penchala, S.D., Amara, A., Else, L., McClure, M., &Boffito, M. (2016). A validated method for quantification of dolutegravir using ultra performance liquid chromatography coupled with UV detection. Therapeutic drug monitoring.,38(3): 327-31.
16. Kalariya, P.D., Patel, P.N., Srinivas, R. &Talluri, M.K. (2015). Quality by design-based development of a selective stability-indicating UPLC method of dolutegravir and characterization of its degradation products by UPLC-QTOF-MS/MS. New journal of chemistry., 39(8): 6303-14.
17. Khaleel, N. Sk. A.R. (2019). Stability-indicating RP-UPLC method for the simultaneous determination of dolutegravir and rilpivirine in bulk and pharmaceutical dosage form. Der Pharmacia Lettre., 11 (2): 29-39.
18. Balasaheb, B.G., Balasaheb,A.K., Subhash, T.R., Jijabapu, K., & Sudhakar, P.S. (2015). Development and validation of UV spectrophotometric method for estimation of dolutegravir sodium in tablet dosage form. Malaysian Journal of Analytical Sciences., 19(6): 1156-63.
19. Sykes, C., Blake, K., White, N., Schauer, A.P., Guzman, B.B., Cottrell, M.L., Tamraz, B., &Kashuba, A.D. (2018). Development and validation of an LC-MS/MS assay for the quantification of dolutegravir extracted from human hair. Analytical and bioanalytical chemistry., 410(29): 7773-81.
20. Patel, S.H., Ismaiel, O.A., Mylott, Jr. W.R., Yuan, M., Hauser. K.F., & McRae. M. (2019). Simultaneous determination of intracellular concentrations of tenofovir, emtricitabine, and dolutegravir in human brain microvascular endothelial cells using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Analyticachimicaacta., 1056: 79-87.
21. Nekkala, K., Kumar, V.S., & Ramachandran, D. (2017). Development and validation for the simultaneous estimation of lamivudine, tenofovirdisproxil and dolutegravir in drug product by RPHPLC. Journal of Pharmaceutical Sciences and Research., 9(9): 1505.
22. Godela, R,,Sowjanya, G. (2020). An effective stability indicating RPHPLC method for simultaneous estimation of Dolutegravir and Lamivudine in bulk and their tablet dosage form. *Futur J Pharm Sci*., 6(1): 1-9.
23. Jagadabi, V., Kumar, P.V.N., Pamidi, S., Ramaprasad, L.A., &Nagaraju, D. (2018). A stability-indicating hplc method for the determination of potential impurities in a new fixed dose combination of dolutegravir, lamivudine and tenofovir disoproxil fumarate tablets used in the first line treatment of HIV-1 infection. Int Res J Pharm., 9(5): 65–74.
24. Rao, N.M., & Sankar, D.G. (2015). Development and validation of stability-indicating HPLC method for simultaneous determination of Lamivudine, tenofovir, and Dolutegravir in bulk and their tablet dosage form. *Futur J Pharm Sci.*, 1(2): 73-77.

25. Noorbasha, K. &Nurbhasha, S. (2020). A new validated stability indicating RP-HPLC method for simultaneous quantification of dolutegravir and lamivudine in bulk and pharmaceutical dosage form. *Futur J Pharm Sci.* 6,39. https://doi.org/10.1186/s43094-020-00060-y
26. Pal, N., Rao, A.S., & Ravikumar, P. (2016). Simultaneous HPLC method development and validation for estimation of Lamivudine, Abacavir and Dolutegravir in combined dosage form with their stability studies. Asian journal of chemistry., 28(2): 273 - 276.
27. Kalpana, T., Rajeswari, T.R., & Ganji, R.R. (2017). Development and validation of analytical method for determination of dolutegravir sodium, lamivudine and tenofovir disoproxil fumarate using reverse phase High Performance Liquid Chromatography. pharma chem., 9(8): 117–27.
28. Mastanamma, S., Jyothi, J.A., Saidulu, P., & Varalakshmi, M. (2018). Development and validation of RP-HPLC method for the simultaneous estimation of Lamivudine, Tenofovir Alafenamide and Dolutegravir bulk and their combined dosage form. Pharmaceutical Methods., 9(2): 49-55.
29. Venkatesh, P., Kulandaivelu, U., Rao, G.K., Chakravarthi, G., Alavala, R.R., & Rajesh, B. (2020). A Novel UPLC-PDA Stability Indicating Method Development and Validation for the Simultaneous Estimation of Lamivudine and Dolutegravir in Bulk and Its Tablets. Journal of Pharmaceutical Research International.,32(32):52-60.DOI:10.9734/jpri/2020/v32i3230933.

CITATION OF THIS ARTICLE

Ceema Mathew*, SahithiPoorna M, ShashikalaMetri. First Derivative Uv Spectroscopic Method for Simultaneous Estimation of Dolutegravir and Lamivudine In Fixed Dose Combination. Bull. Env.Pharmacol. Life Sci., Vol 12 [8] July2023: 86-93.