Journal of Young Pharmacists, 2023; 15(1):98-102.
www.jyoungpharm.org

Original Article

Simultaneous Analysis of Lamivudine and Dolutegravir Sodium in Formulation Using First Order Derivative Method

Sapna M. Rathod[1,*], Paresh U. Patel[2], Nisarg C. Patel[3]

[1]Department of Pharmaceutical Chemistry and Quality Assurance, APMC College of Pharmaceutical Education and Research, Himatnagar, Gujarat, INDIA.
[2]Department of Pharmaceutical Chemistry and Quality Assurance, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Gujarat, INDIA.
[3]Department of Pharmacognosy, APMC College of Pharmaceutical Education and Research, Himatnagar, Gujarat, INDIA.

ABSTRACT

Objective: This present study is focused on the development and validation of first order derivative spectrophotometric method for simultaneous quantification of Lamivudine and Dolutegravir sodium in dosage form. **Materials and Methods:** The solutions were prepared using distilled water as solvent and were scanned in UV region for first order derivative spectrum. The quantitation of drugs were done at 255.6 nm (ZCP (zero crossing point) of Dolutegravir Sodium) and 268.6 nm (ZCP of Lamivudine) over the concentration range 5–30 µg/ml and 1 – 30 µg/ml for Lamivudine and Dolutegravir Sodium respectively in tablet dosage form. **Results:** The Lamivudine and Dolutegravir Sodium showed linear response with a correlation coefficient of 0.9998 and 0.9999 correspondingly. The relative standard deviation for precision study was found less than 2 %. The accuracy study was determined to be between 98 and 102 % utilising standard addition method. The limit of detection for Lamivudine and Dolutegravir sodium was found 0.89 µg/ml and 0.28 µg/ml correspondingly. The limit of quantitation for Lamivudine and Dolutegravir sodium was found 2.70 µg/ml and 0.85 µg/ml correspondingly. **Conclusion:** The proposed approach, validated as per ICH Q2 (R1), was deemed to be accurate, repeatable and precise. The results of proposed method suggests that it can be effectively utilised for simultaneous quantitation of said drugs in formulation.

Keywords: Dolutegravir sodium, First order derivative, Lamivudine, Zero Crossing point.

Correspondence:
Sapna M. Rathod
Associate Professor, Department of Pharmaceutical Chemistry and Quality Assurance, APMC College of Pharmaceutical Education and Research, Himatnagar-383001, Gujarat, INDIA.
Email id: srathod456@gmail.com

Received: 20-06-2022 ;
Revised: 31-08-2022 ;
Accepted: 11-09-2022.

INTRODUCTION

Analytical methods are important in maintaining the quality and quantity of drug in drug products, as well as in the formulation development process, because they help to maintain the quality and efficiency of the drug product throughout the product development process until its final therapeutic use. AIDS is now the world's fourth leading cause of mortality. Anti-HIV medications come in a variety of classes and can be used as efficient preventative techniques to keep the virus from spreading across the community.[1-3]

Lamivudine (LAM) is a Nucleoside Reverse Transcriptase Inhibitor (NRTI) that prevents the virus from multiplying, slowing the spread of HIV infection in the body. Although the medicine does not cure HIV, it does help to reduce the severity of HIV-related illnesses.[4] The drug also used to treat Hepatitis B infection. Lamivudine (LAM) is a chemically 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one. It is official in Indian Pharmacopeia.[5]

Dolutegravir Sodium (DOL) is an inhibitor of integrase strand transfer. The medication binds to integrase, preventing HIV replication by blocking the DNA strand transfer process. It is chemically sodium;(3S,7R)-13-[(2,4-difluorophenyl) methylcarbamoyl]-7-methyl-9,12-dioxo-4-oxa-1,8-diazatricyclo[8.4.0.03,8]tetradeca-10,13-dien-11-olate.[6]

Both of these pharmaceuticals are available on the market alone and in conjunction with other medications. The USFDA authorised Lamivudine in conjunction with Dolutegravir Sodium on April 18, 2019.[7] The combo medicine used to manage HIV infection and improve the function of your immune system.

Literature survey revealed that there are RP–HPLC,[6,8-13] UPLC[14] and one HPTLC[15] method was available for this combination.

Derivative spectrophotometry is a valuable analytical technique for extracting qualitative and quantitative information from unresolved band spectra while also reducing the effects of baseline shifts and tilts. It entails computing and plotting one of

DOI: 10.5530/097515050403

a spectral curve's mathematical derivatives. UV spectroscopic approaches offer the benefit of being simple, quick, and inexpensive. The positive outcome of derivatisation leads to segregation of overlapped spectra, removal of background due to interfering substances in a sample, mixtures can be resolved completely, minor spectral signals can be detected, and sensitivity and specificity is improved. In comparison to the parent profile, derivative spectra provide a more characteristic profile; new maxima and minima arise, as well as Zero Crossing Point (ZCP).[16,17]

No derivative Spectroscopic method has been reported in the literature for the simultaneous quantification of Lamivudine and Dolutegravir sodium in tablet dosage form. As a result, the goal of this study was to develop and validate a first-order derivative spectrophotometric method for simultaneous quantitation of Lamivudine and Dolutegravir Sodium in dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

The LAM and DOL were received as a gift sample from Cipla Pharmaceuticals, Ltd, Mumbai. Tablet (DOVATO) comprising 300 mg LAM and 60 mg DOL was procured from local market. Methanol (S.D. Fine Chemicals Ltd., Mumbai, India) and Distilled water used was of high-purity.

Instrumentation

The absorbance of all the solutions was measured using a UV/Visible spectrophotometer (Shimadzu UV-1700) with a 2 nm spectral width, 0.5 nm wavelength sensitivity, and two matched quartz cells. UV probe software (version 2.33) was used to automatically obtain spectra and perform derivative operations. The work included the use of a Toshcon ultrasonic bath (Toshniwal Process Instrument pvt ltd.) and a Reptech analytical balance.

Stock solution preparation

The weighed amount (10 mg) of LAM and DOL were added to 10 ml individual volumetric flask. Required amount (5 ml) of methanol was added and subjected to sonication. Make up the volume with same solvent and the resultant solution represents 1000 µg/ml each of LAM and DOL.

Working standard solution preparation

Take 10 ml from the above (stock) solution in 100 ml volumetric flask. Distilled water was used as diluent and the resultant solution comprising of 100 µg/ml each of LAM and DOL.

Conditions

Measurement of derivative spectra

In a 10 mm cell, first-derivative UV spectra for LAM and DOL solutions were collected over the range 200-400 nm with distilled water in the reference cell. Each spectrum was recorded thrice. The zero-crossing points for the said drugs were recorded. By adjusting the concentrations of proposed drugs, characteristic wavelengths (ZCPs) were confirmed. The cell was replenished with fresh solution after each replicate measurement.

Method Validation

Linearity

Two series were prepared, namely Series A and Series B. Series A comprising of LAM encompassing the range of 5 – 40 µg/ml and Series B comprising of DOL encompassing the range of 1 – 30 µg/ml. The parameter was evaluated six times by preparing the fresh solutions at each determination. The regression equation was calculated at 255.6 nm and 268.6 nm for quantification of LAM and DOL correspondingly.[18]

Precision

Repeatability

The solution comprising 30 µg/ml LAM and 5 µg/ml DOL were examined repetitively for six times. The results were stated as % CV.

Intra–day and inter–day study

The solution comprising 10, 20 and 30 µg/ml LAM and 5, 10 and 15 µg/ml DOL were analysed thrice on similar day for intraday study and on three consecutive days for interday study. Every time fresh solutions of LAM and DOL were prepared and results were stated in terms of % CV.

Accuracy study

The quantity equivalent to 9.6, 12, 14 µg/ml LAM and 1.7, 2.1, 2.5 µg/ml DOL at 80, 100 and 120 % were added to the prequantified sample comprising of 12 µg/ml LAM and 2.1 µg/ml DOL. The analysis was done thrice at each level.

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

The parameter was assessed by substituting the standard deviation of y intercept and slope of calibration curve in the equation mentioned in ICH Q2 (R1) guideline.[18]

Assay of formulation

Weigh ten tablets (DOVATO) and calculate the average weight and pulverize into powder. Weigh Powder equivalent to 300 mg LAM in a 50 ml volumetric flask. Methanol was used a diluent

and subject to sonication. Using a whatman filter paper, strain the resulting solution. Aliquot 5 ml filtrate into 100 ml volumetric flask to generate a solution comprising 300 µg/ml LAM and 52.6 µg/ml DOL. Distilled water was used for making the volume. An appropriate dilution was made to generate a solution representing 12 µg/ml LAM and 2.1 µg/ml DOL. Distilled water was utilized as a diluent. For the study of LAM and DOL in formulation, absorbance was measured at the required wavelength. The assay was accomplished in triplicate by preparing the sample solution newly every time.

RESULTS

Derivative Spectrophotometric Method Development

The spectra show overlapping in the 220-320 nm range. For both LAM and DOL, derivative spectra of different orders were examined separately and simultaneously. The investigations revealed that the first derivative spectra of LAM and DOL were simple and provided accurate findings at a wavelength of 4 nm and a scaling factor of 10. DOL exhibit some absorbance in the first derivative spectrum at 268.60 nm (ZCP of LAM), whereas LAM exhibit some absorbance at 255.60 nm (ZCP of DOL) (Figure 1). For the quantitation of LAM and DOL in formulation, 255.60 nm and 268.60 nm were optimised respectively. At these two wavelengths, no interference of excipients was seen.

Method validation

Linearity

The calibration curve is plotted and shown in Figure 2. The correlation coefficients for LAM and DOL was found 0.9998 and 0.9999, implies the best linear response to analyte concentration. The outcomes of parameter are tabulated in Table 1.

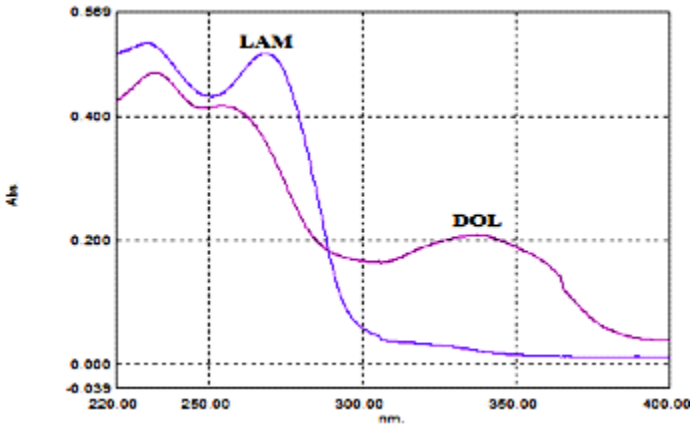

Figure 1: Overlay Zero Order absorption spectra of 10 µg/ml of LAM and DOL in distilled water in the range 220 – 400 nm.

Precision

Repeatability

The coefficient of variance for repeatability of solution comprising 30 µg/ml LAM and 5 µg/ml DOL were found 1.57 and 1.59 correspondingly. The percent CV value (Table 1) confirms that the suggested approach is precise.

Intra - day and inter – day study

The % CV value for intra–day study ranged from 0.53–1.61 % for LAM and 0.89–1.02 % for DOL. The % CV value for inter–day study ranged from 1.37–1.82 % for LAM and 0.99–1.59 % for DOL. The outcomes are tabulated in Table 1.

Accuracy

The percentage recovery ranged from 99.42–100.34 % for LAM and 98.78–99.46 % for DOL. The outcomes of the said parameter are tabulated in Table 2.

LOD and LOQ

The results of the said parameters were represented in Table 1.

Assay of formulation

The drug content was found 100.49 % for LAM and 99.79 % for DOL using proposed method with % CV of 1.37 % and 1.65 % respectively. The % CV value was found less than 2. The results of assay are represented in Table 1.

DISCUSSION

Literature survey revealed that there are chromatographic methods[6,8-15] available for this combination. No derivative Spectroscopic method has been reported in the literature for the simultaneous quantification of Lamivudine and Dolutegravir sodium in tablet dosage form. Though other sophisticated methods like HPLC, HPTLC are available for estimation, still analyst search for an economically viable method and the spectrophotometric methods fulfil this criterion. During the initial studies, the spectra show overlapping in the 220 - 320 nm range. This makes standard UV spectrophotometry impossible to use to determine LAM in the presence of DOL. However, the derivative spectrophotometry technique was chosen for the determination of proposed drugs since it might reduce broadband contributions from placebo and maybe overcome peak overlapping interference.[16]

The investigations revealed that the first derivative spectra of LAM and DOL were simple and provided accurate findings at a wavelength of 4 nm and a scaling factor of 10. For the simultaneous determination of the components, the first-order derivative spectrum had the maximum sensitivity and resolution. LAM exhibited zero crossing at 268.60 nm while DOL exhibited zero crossing at 255.60 nm (Figure 2). DOL exhibit some

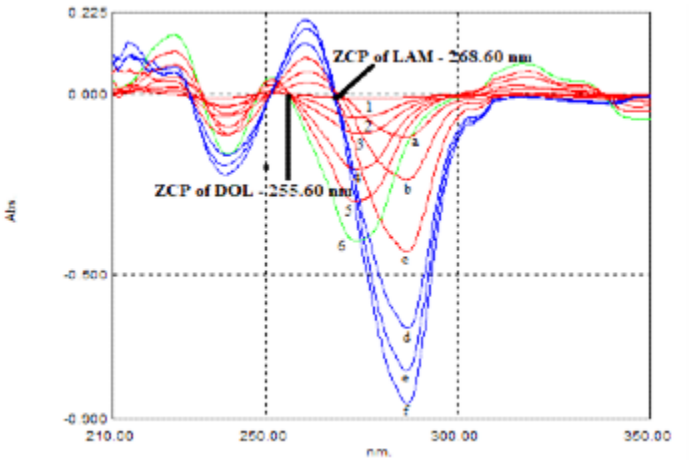

Figure 2: Overlain First Derivative spectra of a: 5, b:10, c: 20, d: 30, e: 35, f: 40 µg/ml of LAM and 1: 1, 2: 5, 3: 10, 4: 15, 5: 20, 6: 30 µg/ml of DOL.

absorbance in the first derivative spectrum at 268.60 nm (ZCP of LAM), whereas LAM exhibit some absorbance at 255.60 nm (ZCP of DOL). For the quantitation of LAM and DOL in formulation, 255.60 nm and 268.60 nm were optimised respectively. No interference from the excipients was observed. Two different series of solutions were established to estimate the linearity, Series A for LAM and Series B for DOL. The range was established using proposed method at six varied concentration of LAM (5 - 40 µg/ml) and DOL (1 – 30 µg/ml). The correlation coefficients for LAM and DOL was found 0.9998 and 0.9999, which suggest good linearity. Parameters like repeatability, precision and accuracy were necessary to be performed to validate the method.

The method validation parameters like precision and accuracy were performed according to ICH guideline Q2R1[18] and the results were found acceptable. For repeatability, predetermined concentration of LAM (30 µg/ml) and DOL (5 µg/ml) were performed six times. The % CV was found 1.57 and 1.59 correspondingly for LAM and DOL. Precision was performed in intraday and interday for the LAM and DOL in triplicate and each have achieved < 2.0 % RSD. Standard addition method was

Table 1: Summary of Validation parameters of First Derivative method for Quantitation of LAM and DOL.

Parameters	Lamivudine	Dolutegravir sodium
	ZCP of DOL (255.6 nm)	ZCP of LAM (268.6 nm)
Linearity (µg/ml)	5-40	1-30
Molar Absorptivity (1 $mole^{-1}$ cm^{-1})	676.57	4909.28
Sandell's sensitivity (µg/ cm^2/ 0.001 absorbance unit)	0.3388	0.0899
Regression equation, y = mx + c		
Slope	0.0030	0.0112
Intercept	0.0032	- 0.0001
Residual std deviation of regression line	0.00070	0.0009
Correlation Coefficient (R^2)	0.9998	0.9999
LOD (µg/ml)	0.89	0.28
LOQ (µg/ml)	2.70	0.85
Repeatability (% CV, n = 6)	1.57	1.59
Intra-day (n = 3), % CV	0.85 – 1.61	0.89 – 1.02
Inter-day (n = 3), % CV	1.37 - 1.82	0.99 – 1.59
Accuracy (Mean % recovery ± SD), % CV, (n = 3)	99.89 ± 0.459, 0.46	99.13 ± 0.660, 0.66
% Assay (Mean ± SD), % CV	100.49 ± 1.376, 1.38	99.79 ± 1.646, 1.64

Table 2: Recovery Data of LAM and DOL by First Derivative method.

Drug	Level (%)	Initial Quantity of Formulation (µg/ml)	Quantity spiked (µg/ml)	Final Quantity (µg/ml)	Quantity obtained (µg/ml), Mean, n = 3	% Recovery Mean ± SD, n = 3	% CV
LAM	80	12	9.6	21.6	21.68	100.34 ± 0.739	0.73
	100		12	24	23.86	99.42 ± 0.961	0.96
	120		14.4	26.4	26.38	99.93 ± 0.318	0.32
DOL	80	2.1	1.7	3.8	3.75	98.78 ± 1.797	1.78
	100		2.1	4.2	4.16	99.15 ± 1.428	1.42
	120		2.5	4.6	4.58	99.46 ± 1.404	1.41

incorporated to study the recoveries. This parameter was assessed by spiking the known concentrations of LAM and DOL to the pre-analysed sample at three levels (80, 100 and 120 %). The outcomes were in range of 99.42–100.34 % for LAM and 98.78–99.46 % for DOL. The assay was performed on DOVATO, with appropriate dilution representing 12 µg/ml LAM and 2.1 µg/ml DOL and scanning was done in triplicate. As the % CV value is less than 2 %, it can be inferred that the proposed approach can be utilised for QC of LAM and DOL in formulations.

CONCLUSION

The developed first derivative approach for LAM and DOL was carried out as per ICH guidelines and confirmed to be simple, precise, sensitive and accurate. The assay findings were found to be quite close to the formulation's label claim. The current findings show that the proposed method can be utilised for the simultaneous quantitation of said drugs in bulk and marketed formulations.

ACKNOWLEDGEMENT

The authors are pleased to Cipla Pharmaceuticals Ltd., Mumbai for providing sample of drugs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Skoog DA, West DM, Holler FJ, Crouch SR. Fundamental of analytical chemistry. 8th ed. Philadelphia: Saunders College Publishing. 2004;973-92.
2. Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale's pharmacology. 6th ed. New York: Churchill Livingstone. 2007;679-91.
3. AIDS, World Health Organization [cited Jun 5 2022] Available from: https://www.who.int/news-room/fact-sheets/detail/hiv-aids.
4. Howland RD, Harvey RA, Champe PC. Lippincott's illustrated reviews: pharmacology. 1st Indian ed. New Delhi: Wolters Kluwer Health (India) Pvt. Ltd. 2007;433-50.
5. Indian pharmacopoeia. Ghaziabad, India: Indian Pharmacopoeia Commission, Government of India, Ministry of Health and Family Welfare; 2007. p. 659-60.
6. Devanna N, Dudekula B, Ramachandraiah C. Development and validation for the simultaneous estimation of dolutegravir and lamivudine in drug product by RP-HPLC. Int J Res Pharm Nano Sci. 2017;6(4):173-80.
7. US Food and Drug Administration [cited Jun 8 2022]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/211994s000lbl.pdf.
8. Sowjanya B, Rambabu K. Development and validation for the simultaneous estimation of lamivudine and dolutegravir in drug product by RP-HPLC. Indo-. Am J P Sci. 2018;8(1):1237-44.
9. Dhanwate SS, Pachauri AD, Ghode PD, Khandelwal KD. Development and validation of analytical method for the estimation of lamivudine and Dolutegravir sodium in dosage form. JPharmSciRes. 2019;11(8):2886-90.
10. Noorbasha K, Nurbhasha S. A new validated stability-indicating RPHPLC method for simultaneous quantification of dolutegravir and lamivudine in bulk and pharmaceutical dosage form. Futur J PharmSci. 2020;6(39):1-10.
11. Godela R, G S. An effective stability indicating RP-HPLC method for simultaneous estimation of Dolutegravir and lamivudine in bulk and their tablet dosage form. Futur J Pharm Sci. 2020;6(1):9-17. doi: 10.1186/s43094-020-00026-0.
12. Rathod SM, Patel PU. Development and Validation of RP – HPLC method for estimation of lamivudine and Dolutegravir sodium in synthetic mixture. Res J Pharm Technol. 2020;13(6):2864-68. doi: 10.5958/0974-360X.2020.00510.7.
13. Mahaparale SP, Deshmukh JU. Analytical method development and validation for simultaneous estimation of dolutegravir and lamivudine in tablet dosage form by RP HPLC. Eur J Pharm Res. 2020;7(8):481-92.
14. Venkatesh P, Kulandaivelu U, Rao GK, Chakravarthi G, Alavala RR, Rajesh B. A novel UPLC-PDA stability indicating method development and validation for the simultaneous estimation of lamivudine and dolutegravir in bulk and its tablets. J Pharm Res Int. 2020;32(32):52-60. doi: 10.9734/jpri/2020/v32i3230933.
15. Dhanve A, Rao JR, Dhale C. Development and validation of stability indicating HPTLC method for simultaneous estimation of lamivudine and dolutegravir sodium in bulk and pharmaceutical dosage formulation. Int J Pharm Sci Res. 2018;9(11):4701-8.
16. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry. Part-II. New Delhi: CBS Publishers and Distributors. 1998;284-7.
17. Mendham J, Denney R, Barnes J, Thomas M, Sivasankar B. Vogel's textbook of quantitative chemical analysis. 6th ed. London: Pearson Education Ltd.; 2011;662-5: 37-56.
18. International conference on harmonization (ICH) Q2B, Validation of analytical procedures: Text and methodology Q2. Geneva. Vol. R1; 2005.

Cite this article: Rathod SM, Patel PU, Patel NC. Simultaneous Analysis of Lamivudine and Dolutegravir Sodium in Formulation Using First Order Derivative Method. J Young Pharm. 2023;15(1):98-102.