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Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Lamivudine as Bulk Drug and in Pharmaceutical Tablet Dosage Form

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Sandeep. B. Swami, Padmanabh B. Deshpande*, Saurabh Jadhav, Dipak Supe

All India Shri Shivaji Memorial Society's College of Pharmacy, Department of Pharmaceutical Quality Assurance, Kennedy Road, Near RTO, Pune-411001, India.

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ABSTRACT

A new simple, economical, sensitive RP-HPLC method involving UV detection has been developed and validated for the determination and quantification of Lamivudine as a bulk drug and in the tablet dosage form. Chromatographic resolution of the drug was carried out on a Sun Q C18 (250 x 4.6 mm) column using filtered and degassed mixture of Methanol: Water (90: 10, v/v) as mobile phase at a flow rate of 1.0 ml $min^{-1,}$ and effluent were monitored at 271 nm. The drug was found susceptible to all the analyzed stress conditions after performing stress degradation studies. The developed method was validated in terms of linearity, precision, accuracy, and specificity, the limit of quantification, and the limit of detection. A linear response was observed in the concentration range 5-30 µg mL^{-1}. The developed method has been successfully applied for the estimation of the drug in the tablet dosage form. The developed method can be used for the quantification of the drug in the dosage form, bulk drug as well as for routine analysis in quality control laboratories.

1.0 INTRODUCTION

Lamivudine, chemically, 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3- oxathiolan-5-yl]-1, 2-dihydro pyrimidine-2-one is an antiretroviral drug that acts by inhibiting nucleoside reverse transcriptase and hence used for the treatment of HIV / AIDS and chronic Hepatitis B at low dose. [1, 2]

Literature survey revealed that various analytical methods such as UV Spectrophotometric have been reported for the determination of Lamivudine either as a single drug or in combination with other drugs in human plasma and pharmaceutical preparations. [3-8] Analytical methods representing the RP-HPLC determination of Lamivudine either as a single drug or in combination with other drugs in human plasma and pharmaceutical preparations were also found in the literature. [9-23] Analytical methods demonstrating the method development and validation for Lamivudine in pharmaceutical dosage forms by HPTLC were also reported in the literature. [24-27]

To the best of our knowledge, no reports were found in the literature for the analysis of Lamivudine in pharmaceutical tablet dosage form by stability-indicating reverse phase high performance liquid chromatographic (RP-HPLC) method. This work describes the development of a simple, precise, accurate, and economic stability-indicating RP-HPLC procedure for the determination of Lamivudine as a bulk drug and in the tablet dosage form by the International Conference on Harmonisation Guidelines. [28, 29]

2.0 MATERIALS AND METHODS

2.1 Chemicals and reagents

Pharmaceutical grade working standard Lamivudine was obtained as a gift sample from Hetero Labs Ltd., Hyderabad, India. Pharmaceutical tablet dosage form Lamivir-150 tablets labeled to contain 150 mg was purchased from a local pharmacy. Methanol (AR grade) was procured from LOBA Chemie Pvt. Ltd. Mumbai, India.

2.2 Instrumentation and chromatographic conditions

The chromatographic resolution was achieved by the use of the JASCO HPLC system equipped with Model PU 2080 Plus pump, Rheodyne sample injection port (20 µL), MD 2010 PDA detector, and Borwin- PDA software (version 1.5). The study was performed using Sun Q C18 (250 x 4.6 mm) column and a mixture containing methanol: water (90: 10,

v/v) as mobile phase at the flow rate of 1 ml min^{-1}. Detection was carried out with a UV detector at a wavelength of 271 nm.

2.3 Preparation of standard stock solution

The standard stock solution was prepared by dissolving 10 mg of drug in 10 mL methanol to get the concentration of 1000 µg mL^{-1} which was diluted further with mobile phase to acquire final concentration of 100 µg mL^{-1}.

2.4 Analysis of tablet formulation

Analysis of tablet formulation was performed to estimate the content of Lamivudine by using a commercial brand of tablet namely Lamivir-150. Twenty tablets were weighed and powdered. A quantity of tablet powder equivalent to 10 mg was transferred to a 100 mL volumetric flask containing 50 mL of methanol and the contents were sonicated for 15 min. The solution was filtered using Whatman paper No. 41 and the volume was made up to the mark with mobile phase to obtain the final concentration of 10 µg mL^{-1}. After setting the chromatographic conditions, the tablet sample solution was injected, a chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curve. The % assay was found to be 101.58 ± 1.39 (mean ± S.D.).

2.5 System suitability

The system suitability was assessed by six replicate injections of the standard Linagliptin having a concentration of 10 µg mL^{-1}. The resolution, peak asymmetry, number of theoretical plates, and height equivalent to a theoretical plate (HETP) were calculated. The values obtained demonstrated the suitability of the system for the analysis of the drug. The results obtained are represented in Table 1.

Table No. 1: System suitability parameters

Sr. No.	Parameters	Lamivudine
1	Theoretical plates	3646.11
2	HETP (cm)	0.0096
3	Resolution	3.13
4	Asymmetry factor	1.88

2.6 Stress degradation studies

Stress degradation studies were performed to check the intrinsic stability of the bulk drug. The study was carried out by exposing the drug to different stress conditions for a different period as recommended by ICH. A stressed sample at high concentration (20 µg mL^{-1}) was injected and multi-wavelength scanning was done to search for peaks of a degradation product. The hydrolytic studies were carried out by treatment of stock drug solution separately with 2 N HCl and 0.1 N NaOH at room temperature for 4h. The acid and alkali stressed samples were neutralized with NaOH and HCl, respectively to furnish the final concentration of 20 µg mL^{-1}. The drug was treated with water at room temperature for 2 h for neutral hydrolysis. Oxidative degradation was performed by treating standard drug solution with 3 % H_2O_2 at room temperature for 2 h and was diluted with mobile phase to obtain 20 µg mL^{-1} solution. Thermal degradation was performed by keeping the solid drug in an oven at 60ºC for 24 h. The solid drug powder was exposed to UV light up to 200-watt h square $meter^{-1}$ to check photolytic degradation. Thermal and photolytic samples were diluted with mobile phase to get the concentration of 20 µg mL^{-1}.

3.0 RESULTS AND DISCUSSION

3.1 Method development and optimization

To develop and optimize the stability-indicating RP-HPLC method which would be capable to give the satisfactory resolution of Lamivudine, initial trials performed using different columns and mobile phases were tried to separate and resolve spots of lamivudine from its impurities and other excipients present in the formulation. The optimized method involved a mixture of methanol: water (90: 10, v/v) which gave a satisfactory resolution of the drug with a well-defined and symmetrical peak. Detection was performed at 271 nm. The retention time (t_R) was found to be 2.85 min (Figure 1).

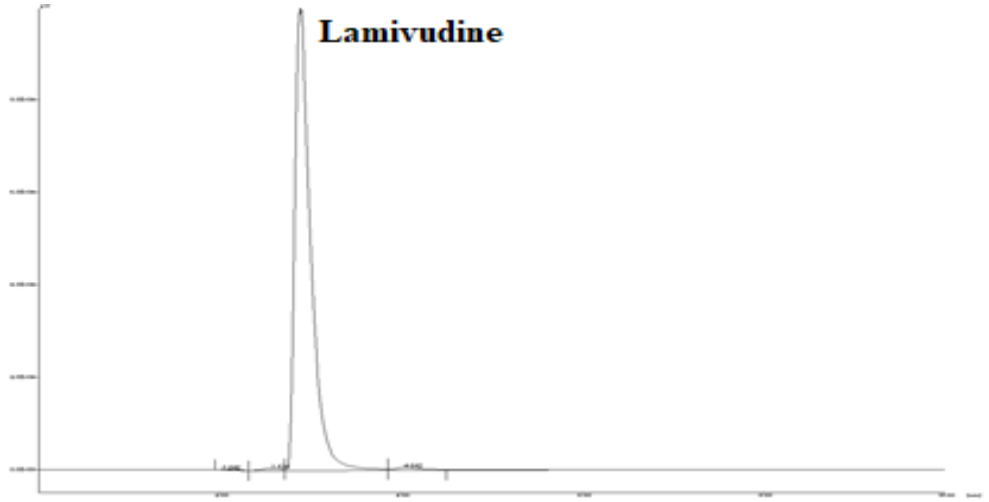

Figure No. 1: Chromatogram of standard drug solution (10 µg mL^{-1}, 2.85 min)

3.2 Result of stress degradation studies

The stress degradation results indicated that the drug was found to be susceptible to all the analyzed stress conditions. The drug was found to be more prone to oxidative degradation in comparison to other stress conditions. The drug was also found light-sensitive as significant degradation was observed under photolytic stress conditions. Figures 2 and 3 denote the dendrograms of acid and alkali hydrolytic degradation while Figures 4-6 symbolize the dendrograms of oxidative, thermal, and photolytic degradation, respectively. The findings of degradation studies along with % degradation and % recovered are represented in Table 1.

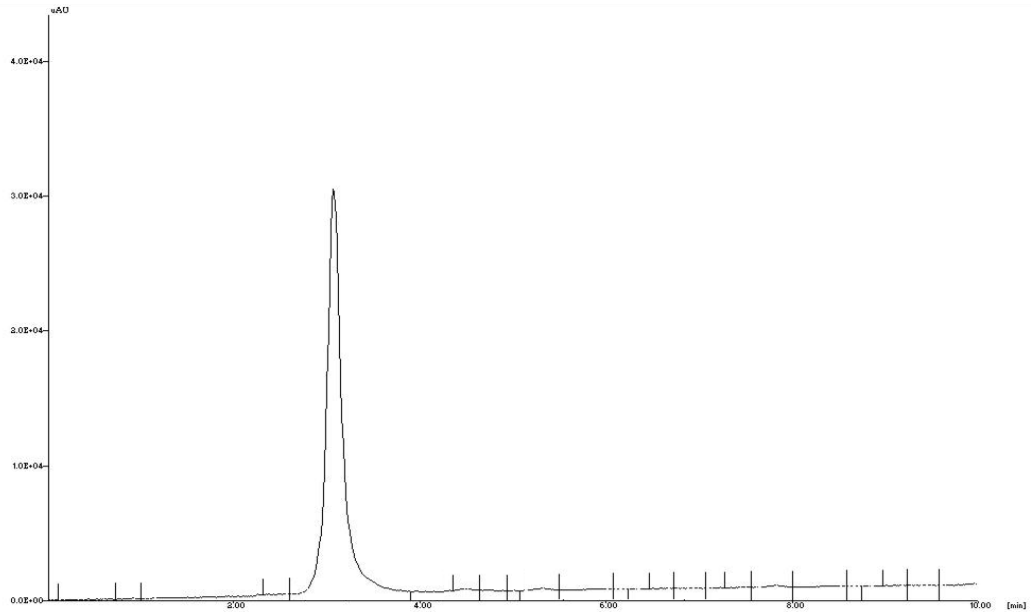

Figure No. 2: Chromatogram after treatment with 2 N HCl, Kept at RT for 4 h

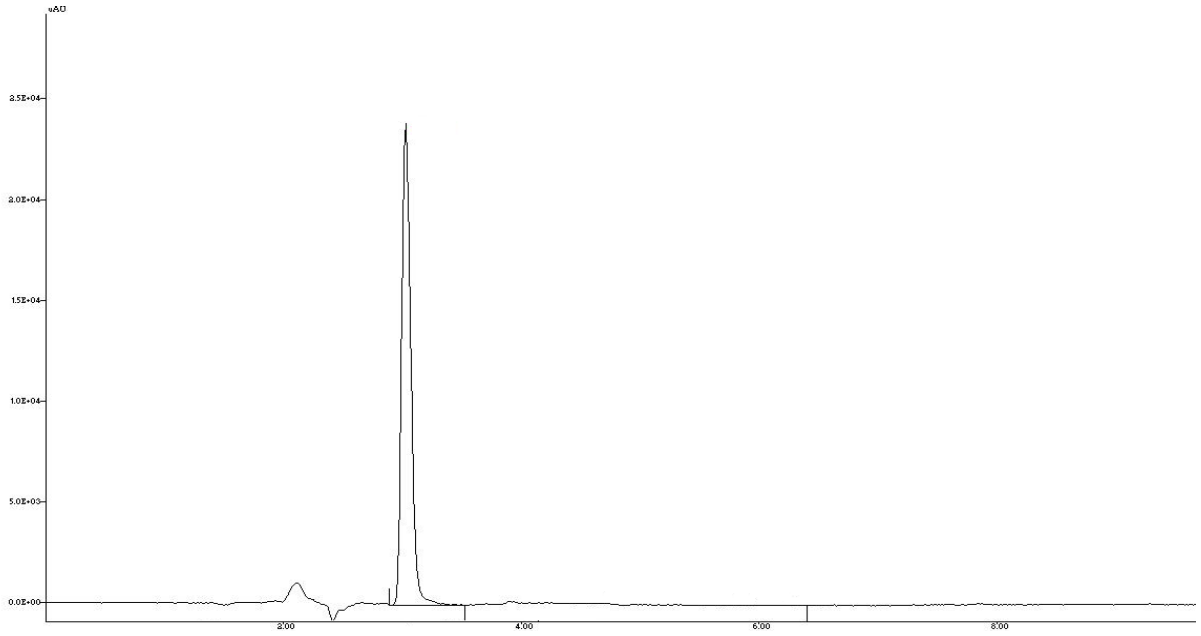

Figure No. 3: Chromatogram after treatment with 0.1 N NaOH, Kept at RT for 4 h

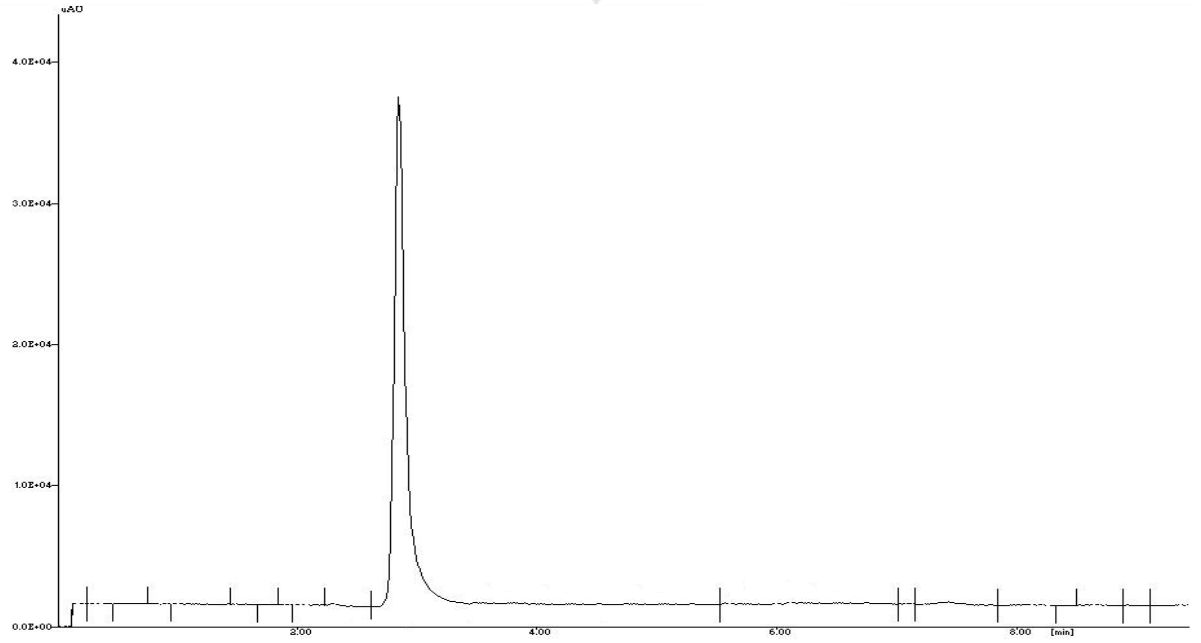

Figure No. 4: Chromatogram after treatment with 3% H_2O_2, Kept at RT for 2 h

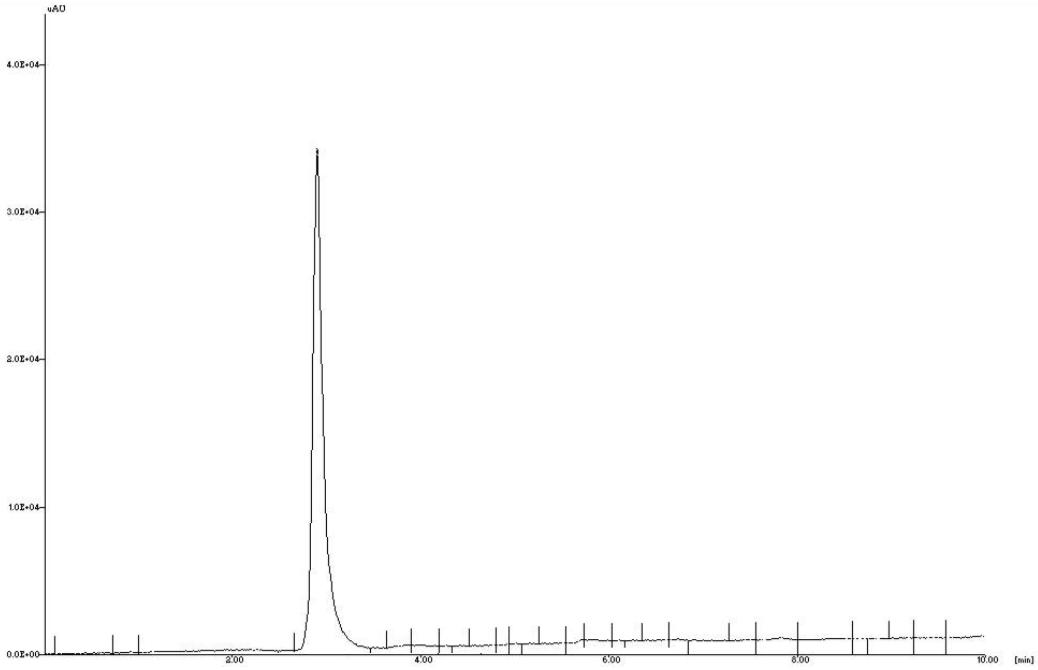

Figure No. 5: Chromatogram after exposure to heat at 60ºC for 24 h

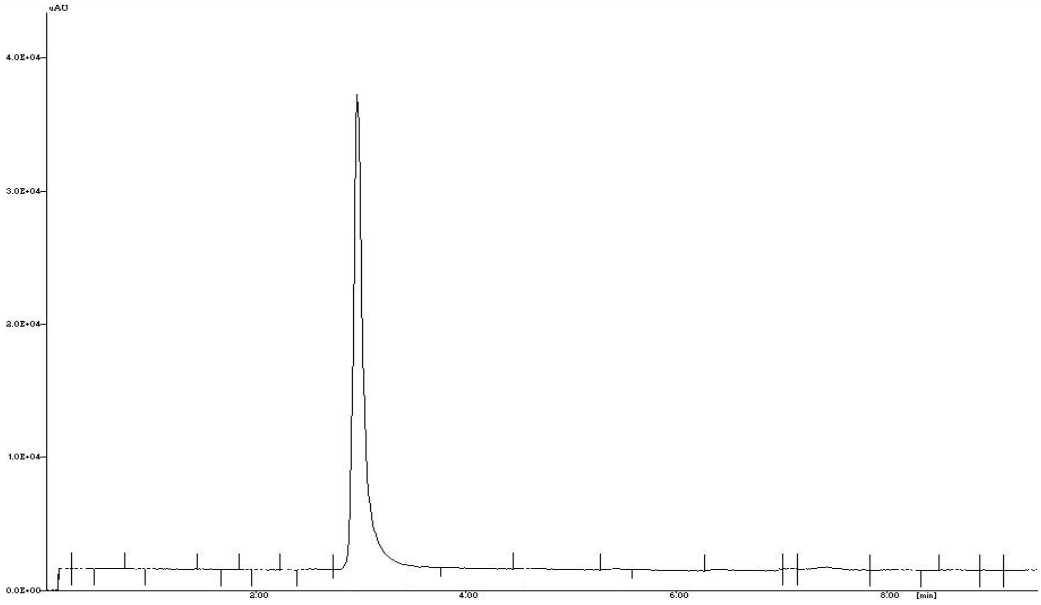

Figure No. 6: Chromatogram after exposure to UV light

Table No. 2: Data for stress degradation studies

Sr. No.	Stress conditions	% Recovery	% Degradation
1	Acid/ 2 N HCl/ Kept at RT for 4 h	84.03	15.97
2	Alkali/ 0.1 N NaOH/ Kept at RT for 4 h	85.04	14.96
3	Oxidative/ 3% H_2O_2 / Kept at RT for 2 h	87.01	12.99
4	Thermal degradation/ 60ºC for 24 h	79.12	20.88
5	Photolysis	80.54	19.46

3.3 Analytical method validation

The method has been validated according to the guidelines of ICH Q2 (R1) for parameters such as linearity, Intraday, and interday precision, accuracy, the limit of detection, the limit of quantification, and robustness.

3.3.1 Linearity

For the preparation of the calibration curve, volumes 0.5, 1, 1.5, 2, 2.5, and 3 mL from standard solution (100 µg mL^{-1}) were withdrawn and diluted with mobile phase to get the range of 5-30 µg mL^{-1}. The developed method was found to be linear in the concentration range 5-30 µg mL^{-1} with a high correlation coefficient. The linear regression equation was found to be y = 46736x + 41339 with a correlation coefficient (R^2) value of 0.995. The calibration curve obtained by the plot of concentration vs peak area is depicted in Figure 7.

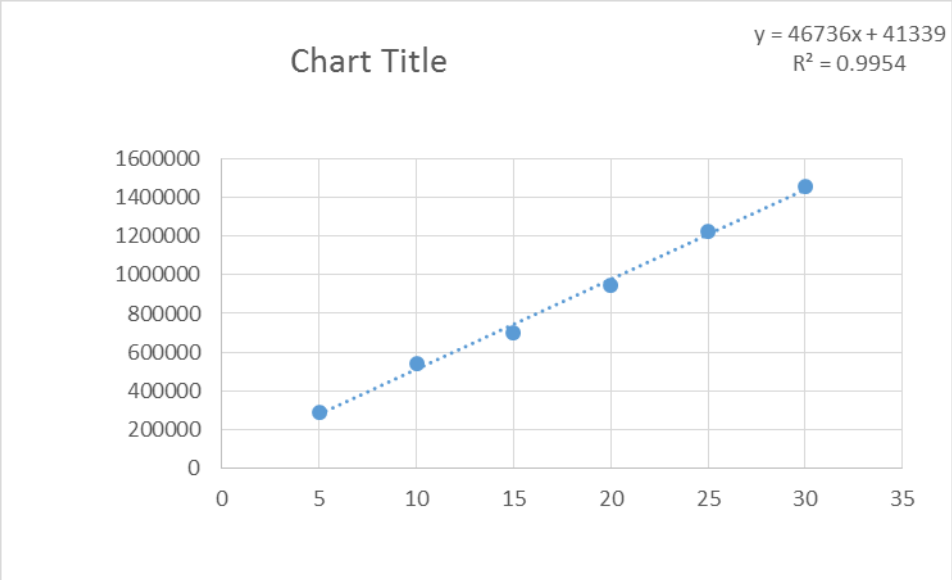

Figure No. 7: Calibration curve of Lamivudine

3.3.2 Precision

The method was subjected to intraday and inter-day precision studies. Precision was evaluated by injecting three different concentrations (10 µg mL^{-1}, 15 µg $mL^{-1}$and 20 µg mL^{-1}) of standard solution within linearity range in three replicates on the same day and three consecutive days. Intra-day variation, as R.S.D. (%), was found to be in the range of 0.76 to 1.22. Interday variation, as R.S.D. (%) was found to be in the range of 0.54 to 1.27. The method was found to be precise as % R.S.D. was less than 2 %.

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

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ values were found to be 1.38 µg mL^{-1} and 4.19 µg mL^{-1}, respectively.

3.3.4 Accuracy

Recovery studies were performed to check the accuracy of the developed method by standard addition method which involved addition standard drug solution to pre-analyzed sample solution at three different levels 80 %, 100%, and 120 %. Sample concentration 10 µg mL^{-1} from tablet solution was used. The drug concentrations were calculated from the linear regression equation. The results of the recovery studies showed that the developed method is accurate for the estimation of a drug in tablet formulation.

Table No. 3: Recovery study

Drug	Concentration taken (µg mL^{-1})	Concentration added (µg mL^{-1})	Concentration found (µg mL^{-1})	% Recovery	% R.S.D.*
Lamivudine	10	8	17.64	98.02	1.06
	10	10	19.28	96.43	0.90
	10	12	23.73	101.79	0.54

*Average of three determinations, R.S.D. is the relative standard deviation.

3.3.5 Robustness

Robustness was carried out by doing small and deliberate changes to optimized method parameters such as a change in mobile phase composition (± 1% methanol), flow rate (± 0.1 mL min^{-1}), and wavelength (± 1 nm). The areas of peaks of interest remained unaffected by small changes of the operational parameters which indicated the robustness of the method.

4.0 CONCLUSION

A simple, precise, accurate, reproducible, and stability-indicating RP-HPLC method without interference from the excipients has been developed and validated for the determination of Lamivudine as a bulk drug and in the tablet dosage form. The developed method can be used for quantitative analysis of Lamivudine in the pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic. As the method is stability-indicating one it may be extended to study the degradation kinetics of the drug.

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6.0 REFERENCES

1. Indian Pharmacopoeia 2010, Volume II, 6th ed. Government of India, Ministry of Health and Family Welfare. Ghaziabad, India: The Indian Pharmacopoeia Commission, p.1337-1338, p.2071-2072.
2. https://www.drugbank.ca/drugs/DB01098. (Accessed on 20/08/2021).
3. Anandakumar K, Anusha K, Kokilavani V, Sangeetha VP, Saranya R. 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. 2017; 5: 129-137.
4. Nasir I, Musa A, Garba M. Spectrometric method for determination of lamivudine from methanolic extract of pure and tablet dosage form. Nig Journ Pharm Sci. 2014; 13: 28-34.
5. Sonar KV, Sapkale P, Jadhav A, Deshmukh T, Patil S. Development and validation of UV spectroscopic method for estimation of lamivudine in tablet dosage form. Int J Curr Pharm Res. 2017; 9(6): 86-89.
6. Ratan K, Jadhav VB, Jain NP, Kawade DM, Kashid GA. Spectrophotometric analytical method development and validation for simultaneous estimation of a lamuvidine and stavudine in solid dosage form. Innovational Journal of Chemistry. 2016; 1: 33-38.
7. Bhadauria R, Gupta R. Development and validation of UV spectrophotometric method for simultaneous estimation of lamivudine and tenofovir disproxil fumarate in the combined dosage form. Journal of Drug Delivery and Therapeutics. 2019; 9: 1156-1159.
8. Yadavalli R, Yellina H, Velayudhankutty S, Eapen SC, Mary J. UV spectrophotometric absorption correction method for the simultaneous estimation of efavirenz, lamivudine and zidovudine in tablet dosage forms. The Pharma Innovation Journal. 2013; 2(2): 174-179.

9. Ozkan SA, Uslu B. Rapid HPLC assay for lamivudine in pharmaceuticals and human serum. J Liq Chromatogr Rel Technol. 2002; 25(9): 1447–1456.
10. Alebouyeha M, Amini H. Rapid determination of lamivudine in human plasma by high-performance liquid chromatography. J Chromatogr B. 2015; 975: 40-44.
11. Vikram Singh A, Natha LK, Pani NR. Development and validation of analytical method for the estimation of lamivudine in rabbit plasma. Journal of Pharmaceutical Analysis. 2011; 1(4): 251-257.
12. Jayaseelan S, Suresh S, Satishkumar G, Sekar V, Perumal P. Bioanalytical method development and validation of lamivudine by RP-HPLC method. International Journal of Chem Tech Research. 2010; 2(2): 1-9.
13. Zheng JJ, Wu ST. High-performance liquid chromatographic assay for the determination of 29-deoxy-39-thiacytidine (lamivudine) in human plasma. J Chromatogr B. 2001; 761; 195-201.
14. Alnouti Y, White CA, Bartlett MG. Determination of lamivudine in plasma, amniotic fluid, and rat tissues by liquid chromatography. J Chromatogr B. 2004; 803: 279-284.
15. Bahrami G, Mirzaeei S, Kiani A, Mohammadi B. High-performance liquid chromatographic determination of lamivudine in human serum using liquid–liquid extraction: application to pharmacokinetic studies. J Chromatogr B. 2005; 823: 213-217.
16. Kano EK, Serra CH, Koono EEM, Andrade SS, Porta V. Determination of lamivudine in human plasma by HPLC and its use in bioequivalence studies. International Journal of Pharmaceutics. 2005; 297: 73-79.
17. Hoetelmans RMW, Meenhorst PL, Mulder JW, Beijnen JH. Quantitative determination of (2)-29-deoxy-39-thiacytidine (lamivudine) in human plasma, saliva and cerebrospinal fluid by high-performance liquid chromatography with ultraviolet detection. J Chromatogr B. 1998; 713: 387-394.
18. Noorbasha K, Nurbhasha S. A new validated stability-indicating RP-HPLC method for simultaneous quantification of dolutegravir and lamivudine in bulk and pharmaceutical dosage form. Future Journal of Pharmaceutical Sciences. 2020; 6(39): 1-10.
19. Wagh PJ, Pai N. Development and validation of high performance liquid chromatographic method for simultaneous determination of lamivudine and zidovudine from pharmaceutical preparation. Indian drugs. 2002; 30(12): 654-657.
20. Nandi U, Das A, Roy B, Choudhury H, Gorain B. Development and validation of an HPLC-UV method for simultaneous determination of zidovudine, lamivudine, and nevirapine in human plasma and its application to pharmacokinetic study in human volunteers. Drug Test Analysis. 2012; 1-7.
21. Uslu B, Ozkan SA. Determination of lamivudine and zidovudine in binary mixtures using first derivative spectrophotometric, first derivative of the ratio-spectra and high-performance liquid chromatography-UV methods. Analytica Chimica Acta. 2002; 466: 175-185.
22. Shaik K, Subramaniam S, Muthuraman MS, Sivasubramanian A. RP-HPLC analytical method development and validation for lamivudine and zidovudine in pharmaceutical dosage forms. Int J of PharmTech Res. 2013; 5(3): 1321-1331.
23. Govindrao K, Vadrevu S, Naga Valli K. Development and validation of RP-HPLC method for simultaneous estimation of lamivudine and zidovudine in bulk. Int J Curr Pharm Res. 2016; 8(1): 28-33.
24. Palani V, Daggumati M. Development and validation of a normal-phase HPTLC method for the simultaneous analysis of lamivudine and zidovudine in fixed-dose combination tablets. Journal of Pharmaceutical Analysis. 2012; 2(2): 152-155.
25. Nyamweru BC, Kaale E, Mugoyela V, Chambuso M. Development and validation of an HPTLC-densitometric method for simultaneous analysis of lamivudine, tenofovir disoproxil fumarate, and efavirenz in tablets. Journal of Planar Chromatography. 2013; 26(3): 226-231.
26. Gu Y, Zheng B, Sherma J. Development and quantification of HPTLC method for dolutegravir lamivudine, and tenofovir disproxil fumarate in a combination pharma product using a model process published earlier for transfer of minilab TLC screening method of HPTLC- densitometry. Acta Chromatographica. 2020; 32(3): 199-202.
27. Gopani M, Patel RB, Patel MR, Solanki AB. Development of a new high performance thin layer chromatographic method for quantitative estimation of lamivudine and zidovudine in combined tablet dosage form using quality by design approach. J Liq Chromatogr Rel Technol. 2014; 37: 2420-2432.
28. The International Conference on Harmonization, Q2 (R1), Validation of Analytical Procedure: Text and Methodology, 2005.

29. The International Conference on Harmonization, Q1 (B), Stability Testing: Photostability Testing of New Drug Substances and Products: Text and Methodology, 2005.
30. Indian Pharmacopoeia 2010, Volume II, 6th ed. Government. of India, Ministry of Health and Family Welfare. Ghaziabad, India: The Indian Pharmacopoeia Commission, p.1337-1338, p.2071-2072.
31. https://www.drugbank.ca/drugs/DB01098. (Accessed on 20/08/2021).
32. Richman, D. D. 2006. Antiviral drug resistance. Antiviral Res. 71: 117–121
33. AIDSinfo. 2007. Lamivudine//Sidovudine. Available at http://www.aidsinfo. nih.gov/DrugsNew/DrugDetailT.aspx? MenuItem¼Drugs&int id¼285& Search¼Off&ClassID¼&TypeID¼#FA1.
34. AVERT. 2007. Introduction to HIV//AIDS Treatment. Available at http:// www.avert.org/introtrt.htm
35. Pharmainfo.net. 2006. Methods of Estimation of Multi-component Formulations: A Review. Available at http://www.pharmainfo.net/exclusive/reviews/methods_ of_estimation_of_multi-component_formulations:_a_review/
36. R4drdfrG.Deepali, M. Elvis; UV Spectrophotometric method for assay of the Anti Retroviral Agent Lamivudine in API And in its Tablet formulation; *j of young Pharmacist*;(2010); Vol. 2(4).p.417-419.
37. *K.Anandakumar, K.Anusha, V.Kokilavani, V.P.Sangeetha, R Saranya, M.Jambulingam and J.Ramesh; method development and validation of uv-spectroscopic method for the determination of lamivudine as an active pharmaceutical ingrediant and in tablet dosage form; *Int Journal of Pharmaceuticals and Health care Research*;(2017); Vol. 05; p.129 – 137.
38. A. Biksham babu, G.Ramu, C.H. Murali Krishna , S. Brahma Reddy,and C. Rambabu*; Spectrophotometric Determination of Lamivudine in Pure and Tablet Form; E-Journal of Chemistry ; (2012);Vol 9(2);p.569-575.
39. Nasir I*, Musa A, Garba M; Spectrometric method For determination of Lamivudine from methanolic extract of pure and tablet dosage form ; *Nigerian J of Pharma Sci*.; (2014); Vol 13;p28-34.
40. Pimpodakar N.V*, Mahajan N.S, Jadhav R.L, Nalawade R.S, Kuchekar B.S; New Spectrometric method For Estimation of Lamivudine In Bulk And Pharmaceutical Dosage Forms; *Int j chem sci*; (2008); Vol.6(2);p.688-692.
41. G. Krishnamoorthy*, N.Gayathri, M.Gnanaraja, R.senthamarai; Assay of Lamivudine in pharmaceutical preparation by Spectrophotometric method.; *Asian J.of Pharma analysis*;(2012)Vol.2(3);p.77-78.
42. K.V. Sonar*, P.Sapkale, A.Jadhav, T.Deshmukh, S.Patil, P.Murkute; development and validation of UV spectroscopic method for estimation of lamivudine in tablet dosage form; *Int J Curr Pharm Res*, (2017)Vol 9,(6), p.86-89.
43. Kranti Ratan*, Jadhav V.B, Jain N.P, Kawade D.M, Kashid G.A; spectrophotometric analytical method development and validation for simultaneous estimation of a lamuvidine and stavudine in solid dosage form; *Innovational Journal of Chemistry ;* (2016) Vol1;p.33-38.
44. Bhadauria R, Gupta R*; Development and validation of UV Spectrophotometric method for simultaneous estimation of Lamivudine and Tenofovir Disproxil Fumarate in the combined dosage form; *Journal of Drug Delivery & Therapeutics*. (2019); Vol.9(4-s):p.1156-1159.
45. Manikanta Kumar A, B.Naga Sandhya*, Mahesh Nasare, V.V.L.N Prasad, Prakash.V.Diwan.; Development and validation of UV Spectrophotometric method for simultaneous estimation of Lamivudine and Efavirenz in the Pharmaceutical dosage form; *J. Adv. Pharm. Edu. & Res*; (2012);Vol.2(4); p.210-214.
46. Serife Evrim Kepekci Tekkeli; Extractive Spectrophotometric Method for the Determination of Lamivudine and Zidovudine in Pharmaceutical Preparations Using Bromocresol Purple*; Journal of Chemistry;* (2013);p.1-5.
47. R.sharma, K.Mehta; Simultaneous Spectrophotometric Estimation of tenofovir disoproxil fumarate and lamivudine in three component formulation containing efavirenz; Indian j of pharma sci. (2010;) Vol.72(4), p.527-530.
48. Yadavalli R, Yellina H, S.Velayudhankutty, S.C. Eapen, J.Mary; UV Spectrophotometric Absorption Correction Method for the Simultaneous Estimation of Efavirenz, Lamivudine and Zidovudine in Tablet Dosage Forms*; The Pharma Innovation - Journal* (2013)Vol. 2(2), p.174-179.
49. Severino P.Santana, Maria Silva, Simone S. Simões*; simultaneous spectrophotometric determination of lamivudine and zidovudine in fixed dose combinations using multivariate calibration*; Quim. Nova*, (2011)Vol. 34(5), p.859-863.

50. S.A.Ozkan a & B.Uslu ; rapid HPLC assay for lamivudine in pharmaceuticals and human serum*; J. LIQ. CHROM. & REL. TECHNOL.*, (2002);Vol.25(9), p.1447–1456.
51. M. Alebouyeha, H. Amini ; Rapid determination of lamivudine in human plasma by high-performance liquid chromatography; */ J. Chromatogr. B* ;(2015)Vol.975;p.40–44.
52. A.Vikram Singha, L. K. Natha , N. R. Pani; Development and validation of analytical method for the estimation of lamivudine in rabbit plasma; *Journal of Pharmaceutical Analysis;*(2011);Vol. 1(4);p.251-257.
53. S.Jayaseelan, S. Suresh, G. Satishkumar, V. Sekar, P. Perumal;Bioanalytical Method Development And Validation of Lamivudine By RP-HPLC Method.; *International Journal of Chem Tech Research*;(2010); Vol.2(2),p.1-9.
54. J. J. Zheng , S.T. Wu, T. A. Emm; High-performance liquid chromatographic assay for the determination of 29-deoxy-39-thiacytidine (lamivudine) in human plasma; *Journal of Chromatography B*, (2001); Vol.761; p.195–201.
55. Y. Alnouti, C. A. White, M. G. Bartlett∗; Determination of lamivudine in plasma, amniotic fluid, and rat tissues by liquid chromatography; *Journal of Chromatography B*, (2004) Vol.803 p.279–284.
56. G.Bahrami ,∗, S. Mirzaeei, A. Kiani , B. Mohammadi; High-performance liquid chromatographic determination of lamivudine in human serum using liquid–liquid extraction; application to pharmacokinetic studies*; Journal of Chromatography B*, (2005) Vol.823 p.213–217.
57. E.K. Kano, C.H. Serra, E.E.M. Koono, S.S Andrade, V. Porta ∗;Determination of lamivudine in human plasma by HPLC and its use in bioequivalence studies*; International Journal of Pharmaceutics* ; (2005); Vol.297;p.73–79.
58. R. M.W. Hoetelmans, M. Profijt, P. L. Meenhorst, J. W. Mulder, * J. H. Beijnen; Quantitative determination of (2)-29-deoxy-39-thiacytidine (lamivudine) in human plasma, saliva and cerebrospinal fluid by high-performance liquid chromatography with ultraviolet detection; *Journal of Chromatography B*, (1998) Vol.713 ;p.387–394.
59. K. Noorbasha* and S. Nurbhasha; A new validated stability-indicating RP-HPLC method for simultaneous quantification of dolutegravir and lamivudine in bulk and pharmaceutical dosage form*; a Future Journal of Pharmaceutical Sciences* (2020) Vol.6(39);p.1-10.
60. Wagh P.J, Pai.N; Development and validation of high performance liquid chromatographic method for simultaneous determination of lamivudine and zidovudine from pharmaceutical preparation; *Indian drugs*;(2002);Vol30(12);p.654-657.
61. U. Nandi, A. Das, B. Roy, H.Choudhury, B. Gorain and T. Pal; Development and validation of an HPLC-UV method for simultaneous determination of zidovudine, lamivudine, and nevirapine in human plasma and its application to pharmacokinetic study in human volunteers; *Drug Test. Analysis* ;(2012);p1-7.
62. B. Uslu, S. A. Özkan∗;Determination of lamivudine and zidovudine in binary mixtures using first derivative spectrophotometric, first derivative of the ratio-spectra and high-performance liquid chromatography–UV methods; *Analytica Chimica Acta* ;(2002)Vol466; p.175–185.
63. K. Shaik, S. Subramaniam, M.S. Muthuraman and A. Sivasubramanian*; RP-HPLC Analytical Method Development And Validation For Lamivudine And Zidovudine In Pharmaceutical Dosage Forms; *Int J of PharmTech Res.;* (2013); Vol. 5(3); p.1321-1331.
64. K.Govindrao; S. Vadrevu; K.Naga Valli; Development And Validation Of RP-HPLC Method For Simultaneous Estimation Of Lamivudine And Zidovudine In Bulk; *Int J Curr Pharm Res* (2016); Vol.8(1); p.28-33.
65. V. Palani, M. Daggumati; Development and validation of a normal-phase HPTLC method for the simultaneous analysis of Lamivudine and Zidovudine in fixed-dose combination tablets*; Journal of Pharmaceutical Analysis;* (2012); Vol2(2);p.152-155.
66. M.S. Baig*, M. H. Dehghan; Stability-Indicating HPTLC Method development and validation for Analysis of Lamivudine and Tenofovir from its Tablet Dosage Form *Journal of Medicinal Chemistry and Drug Discovery* ;(2015); p.463-474.
67. B.C. Nyamweru, E. Kaale*, V. Mugoyela, and M. Chambuso; Development and Validation of an HPTLC–Densitometric Method for Simultaneous Analysis of Lamivudine, Tenofovir Disoproxil Fumarate, and Efavirenz (LTE) in Tablets; *Journal of Planar Chromatography* ; (2013) Vol.26(3);p.226–231.

68. A. Dhanve, J.R. Rao * and C. Dhale; development and validation of stability indicating HPTLC method for simultaneous estimation of lamivudine and dolutegravir sodium in bulk and pharmaceutical dosage formulation; IJPSR,(2018); Vol. 9(11):p.4701-4708.
69. Y. Gu, B. Zheng, J. Sherma; Development and quantification of HPTLC Method for dolutegravir Lamivudine, and Tenofovir disproxil Fumarate in a combination pharma product using a Model process Published Earlier for Transfer of Minilab TLC screening Methid of HPTLC- Densitometry.; Acta Chromatographica; (2020);Vol.32(3); p.199-202.
70. M. Gopani a, R. B. Patel, M. R. Patel & A. B.Solanki; development of a new high-performance thin-layer chromatographic method for quantitative estimation of lamivudine and zidovudine in combined tablet dosage form using quality by design approach*; Journal of Liquid Chromatography & Related Technologies*,(2014); Vol.37:p.2420–2432.
71. P. Chandra, A. S. Rathore, L. Sathiyanarayanan, K. R. Mahadik*; Application of high-performance thin-layer chromatographic method for the simultaneous determination of lamivudine and tenofovir disoproxil fumarate in pharmaceutical dosage form; *J. Chil. Chem. Soc.* .(2011),Vol 56,(2);p.702-705 .
72. G.Habte , A.Hymete & A.M.I.Mohamed; Simultaneous Separation and Determination of Lamivudine and Zidovudine in Pharmaceutical Formulations Using the HPTLC Method*; Analytical Letters*,(2009) Vol.42: p.1552–1570.