ejpmr, 2021,8(10), 353-359

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

VALIDATED STABILITY INDICATING HPTLC METHOD FOR ESTIMATION OF LAMIVUDINE AS BULK DRUG AND IN PHARMACEUTICAL TABLET DOSAGE FORM

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Article Received on 28/07/2021 **Article Revised on 18/08/2021** **Article Accepted on 08/09/2021**

ABSTRACT

A simple, precise and accurate stability-indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for the estimation of Lamivudine in pharmaceutical tablet dosage form. Chromatographic resolution of drug was achieved on aluminium-backed plates pre-coated with silica gel G60 F_{254} using Chloroform: Methanol: Formic acid (8: 2: 0.1, v/v/v) as the mobile phase. The drug was resolved satisfactorily with Rf value 0.38 ± 0.02. Densitometric analysis of was carried out in the absorbance mode at 271 nm. Forced degradation studies were conducted to check the stability of drug as per ICH guidelines. Lamivudine was found susceptible to all the analyzed stress conditions. Linear regression analysis showed good linearity with respect to peak area in the concentration range of 300-1800 ng band^{-1}. The developed method has been successfully applied for the estimation of Lamivudine in tablet dosage form.

KEYWORDS: Lamivudine, HPTLC, Stability indicating method, Method development, Validation.

INTRODUCTION

Lamivudine, chemically, 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3- oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one is antiretroviral drug which 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 has been reported for determination of Lamivudine either as 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 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 best of our knowledge, no reports were found in the literature for analysis of Lamivudine in pharmaceutical tablet dosage form by stability-indicating high performance thin layer chromatographic (HPTLC) method. This work describes development of simple, precise and accurate stability indicating HPTLC procedure for determination of Lamivudine as bulk drug and in tablet dosage form in accordance with International Conference on Harmonisation Guidelines.[28, 29]

MATERIALS AND METHODS

Chemicals and reagents

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

Instrumentation and chromatographic conditions

Chromatographic separation of the drug was performed on silica gel 60 F_{254} (10 cm × 10 cm with 250 µm layer thickness) Merck TLC plates using a CAMAG Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band under nitrogen stream with a 6 mm of band width using Camag 100 µL sample syringe (Hamilton, Switzerland). A constant application rate of 0.1 µL sec^{-1} was employed.

Linear ascending development was carried out in 10 ×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using chloroform: methanol: formic

Acid (8: 2: 0.1, v/v/v) as mobile phase. The mobile phase was saturated in the (CAMAG) twin trough TLC chamber for 20 min before chromatogram development at room temperature. After development, TLC plates were removed and dried. A Camag TLC scanner III with winCATS software version 1.4.2 was used for densitometric evaluation.

Selection of Analytical Wavelength for Densitometry Evaluation

After chromatographic development, the TLC plate was scanned over the wavelength range of 200-400 nm. Lamivudine exhibited maximum absorbance at about 271 nm and hence was selected as the analytical wavelength for further analysis.

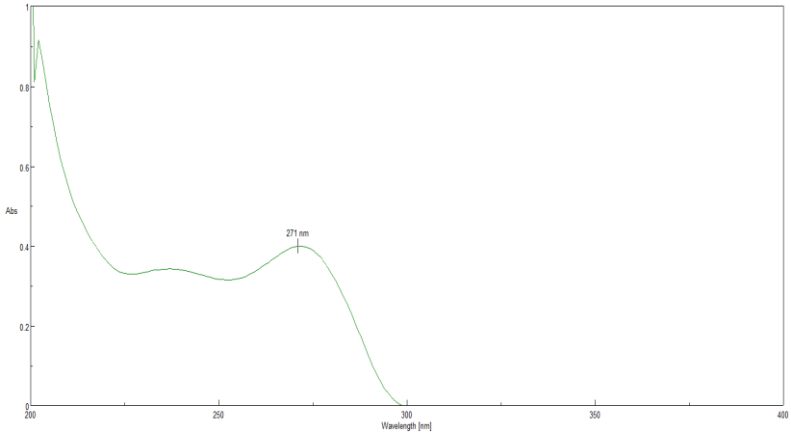

Fig: 1. UV absorption spectrum of Lamivudine.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving accurately weighed 10 mg of the drug in 10 mL of methanol to get solution having concentration 1000 ng μL^{-1} which was diluted further using methanol to acquire final working standard concentration 300 ng μL^{-1}.

Tablet formulation analysis

Analysis of tablet formulation was performed to estimate the content of Lamivudine by using commercial brand of tablet namely Lamivir-150. Twenty tablets were weighed and powdered. A quantity of tablet powder equivalent to 30 mg was transferred to 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 methanol to obtain the final concentration of 300 ng $band^{-1}$. Two µL volume of this solution was applied on TLC plate to obtain final sample concentration of 600 ng $band^{-1}$. After chromatographic development peak areas of the bands were measured at 271 nm and the amount of drug present in sample was estimated from the calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies

Stress degradation studies were performed to check intrinsic stability of bulk drug. The study was carried out by exposing drug to the different stress conditions for different time span as recommended by ICH. A stressed sample at high concentration (1000 ng μL^{-1}) was spotted and multi wavelength scanning was done to search for peaks of 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 1200 ng $band^{-1}$. The drug was treated with water at room temperature for 2h 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 methanol to obtain 1200 ng $band^{-1}$ solution. Thermal degradation was performed by keeping solid drug in oven at 60ºC for 24 h. The solid drug powder was exposed UV light up to 200 watt h square $meter^{-1}$ to check photolytic degradation. Thermal and photolytic samples were diluted with methanol to get concentration of 1200 ng $band^{-1}$.

RESULTS AND DISCUSSION

Method development and optimization

In order to develop and optimize stability indicating HPLTC method which would be capable to give the satisfactory resolution of Lamivudine, initial trials involving neat solvents of differing polarity were used. Different combinations of solvent systems containing various ratios of toluene, methanol, acetic acid, chloroform, ethyl acetate, glacial acetic acid and formic acid (data not shown) were tried to separate and resolve spot of lamivudine from its impurities and other excipients present in formulation. The optimised method involved mixture of chloroform: methanol: formic acid (8: 2: 0.1, v/v/v) which gave satisfactory resolution of drug with well-defined and symmetrical peak. Densitometric detection was performed at 271 nm. The retention factor (Rf) was found to be 0.38 ± 0.02 (Fig. 2).

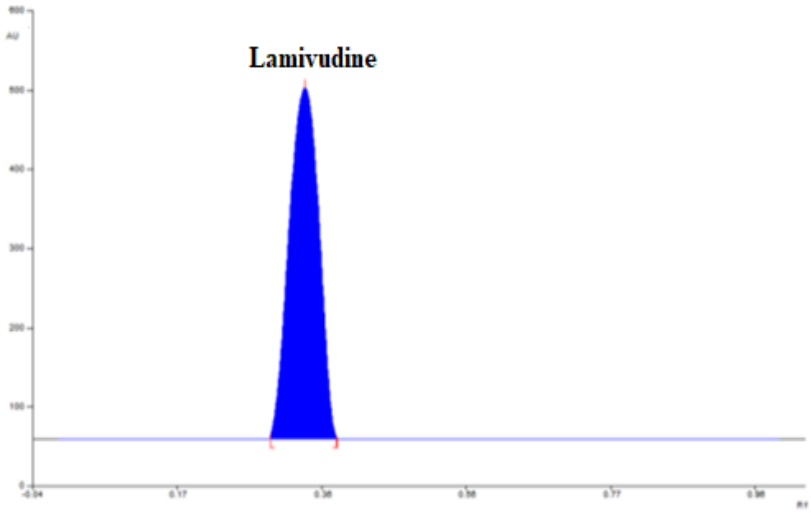

Fig: 2. Representative densitogram of standard solution of Lamivudine.

(1200 ng band^{-1}, Rf= 0.38 ± 0.02)

The stress degradation results indicated that drug was found to be susceptible to all the analysed stress conditions. The drug was found to 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. Fig. 3 and 4 represents the densitograms of acid and alkali hydrolytic degradation, while Fig. 5-7 denotes the densitograms of oxidative degradation, thermal degradation and photolytic degradation, respectively. The findings of degradation studies are represented in Table 1.

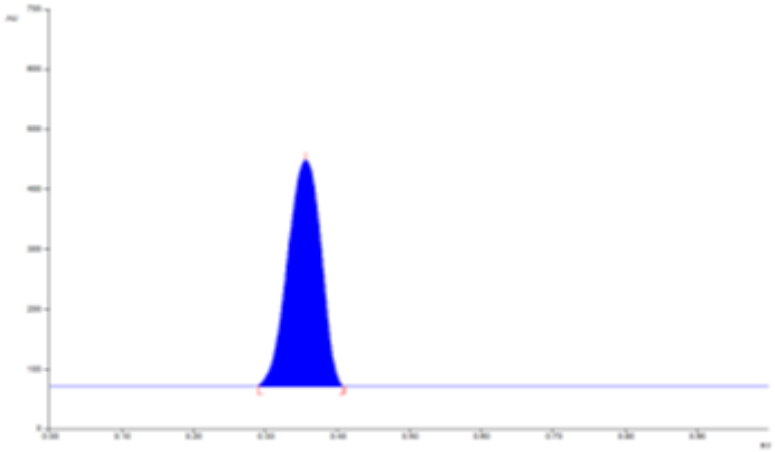

Fig. 3: Densitogram after treatment with 2 N HCl for 4 h.

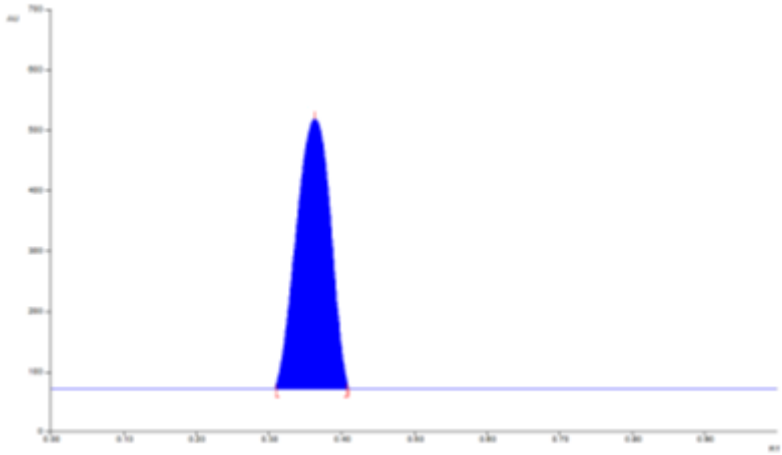

Fig: 4. Densitogram obtained after treatment with 0.1 NaOH.

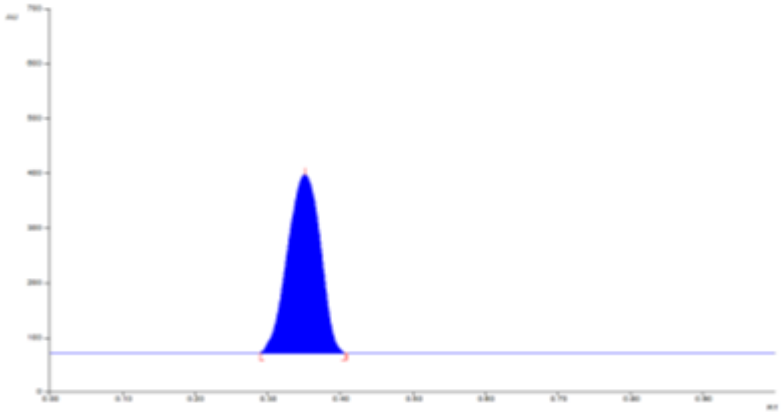

Fig: 5. Densitogram of peroxide treated sample.

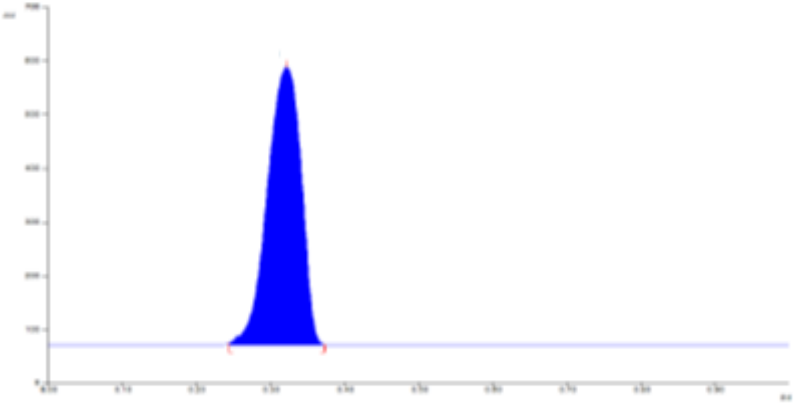

Fig: 6. Densitogram after exposure of drug at 60ºC for 24 h

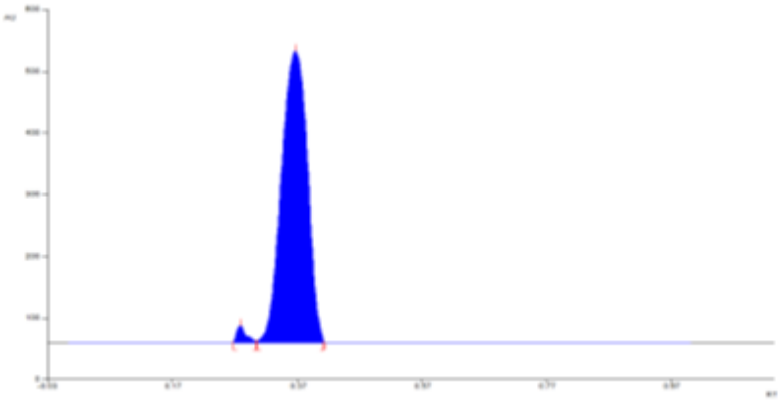

Fig. 7: Densitogram obtained after photolysis.

Table 1: Summary of stress degradation studies

Sr. No.	Stress conditions	% Recovery	% Degradation
1.	Acid/ 2 N HCL/ Kept at RT for 4 h	82.34	17.65
2.	Alkali/ 0.1 N NaOH/ Kept at RT for 4 h	86.74	13.25
3.	Neutral/ H_2O/ Kept at RT for 2 h	89.68	10.31
4.	Oxidative/ 3% H_2O_2 / Kept at RT for 2 h	78.29	21.70
5.	Thermal Degradation/ 60ºC for 24 h	87.30	12.69
6.	UV Degradation	80.08	19.91

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, limit of detection, limit of quantification, and robustness.

Linearity

For the preparation of calibration curve, volumes 1, 2, 3. 4, 5 and 6 µL from standard solution (300 ng μL^{-1}) were spotted on TLC plate to get the range of 300-1800 ng $band^{-1}$. The developed method was found to be linear in

the concentration range 300-1800 ng band^{-1} with high correlation coefficient. The linear regression equation was found to be y = 9.6941x + 2844.1 with correlation coefficient (R^2) value of 0.998. A 3D densitogram obtained is represented in Fig. 8 and calibration curve obtained by plot of concentration vs peak area is depicted in Fig. 9

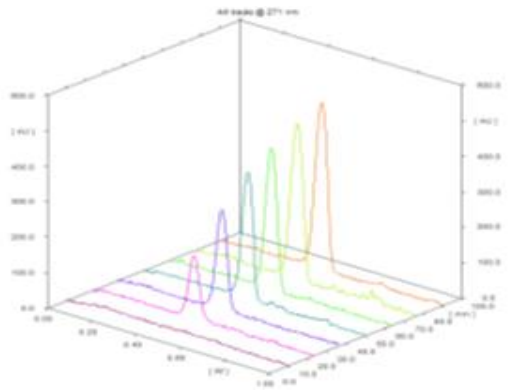

Fig: 8. 3D densitogram in concentration range 300-1800 ng band^{-1}

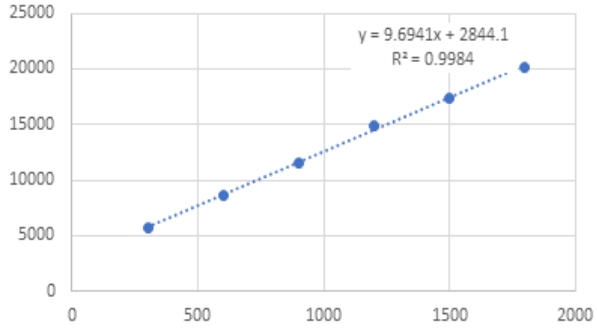

Fig: 9. Calibration curve of Lamivudine.

Table 2: Recovery studies.

Drug	Concentration taken (ng band^{-1})	Concentration added (ng band^{-1})	Concentration found (ng band^{-1})	% Recovery	% R.S.D.*
Lamivudine	600	480	1089.00	100.83	1.41
	600	600	1203.21	100.26	1.36
	600	720	1320.49	100.03	1.47

*Average of three determinations

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 27.27 ng band^{-1} and 82.64 ng band^{-1}, respectively.

Robustness

Robustness was carried out by doing small and deliberate changes to optimised method parameters such as change in mobile phase composition (± 1% methanol), saturation time (± 10 min) and wavelength (± 1 nm). The areas of peaks of interest remained unaffected by small changes of the operational parameters which indicated robustness of the method.

Precision

The method was subjected to intraday and inter-day precision studies. Precision was evaluated by applying three different concentrations (600 ng band^{-1}, 900 ng band^{-1} and 1200 ng band^{-1}) of standard solution within linearity range in three replicates. Intra-day variation, as R.S.D. (%), was found to be in the range of 1.18 to 1.58. Interday variation, as R.S.D. (%) was found to be in the range of 1.05 to 1.19. The method was found to be precise as % R.S.D. was less than 2 %.

Accuracy

Recovery studies were performed to check accuracy of developed method by standard addition method which involved addition standard drug solution to pre-analysed sample solution at three different levels 80 %, 100% and 120 %. Sample concentration 600 ng band^{-1} from tablet solution was used. The drug concentrations were calculated from linear regression equation. The results of the recovery studies showed that developed method is accurate for estimation of drug in tablet formulation.

CONCLUSIONS

A simple, precise and accurate stability-indicating HPTLC-densitometric method without interference from the excipients has been developed and validated for the estimation of Lamivudine as bulk drug and in pharmaceutical tablet dosage form. The results obtained for validation parameters were well within the limits as specified by ICH guidelines. The developed method can be used for quantitative analysis of drug in pharmaceutical tablet dosage form as well as for routine analysis in quality control laboratories.

ACKNOWLEDGEMENT

The authors express their gratitude to Hetero Labs Ltd., Hydrabad, India for providing sample of Lamivudine. Thanks are also extended to Dr. A. R. Madgulkar,

Principal, A.I.S.S.M.S. College of Pharmacy, for providing necessary facilities to carry out research work.

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.