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Development and Validation of Stability Indicating HPTLC-Densitometric Method for Simultaneous Estimation of Lamivudine and Tenofovir Disoproxil Fumarate in Combined Tablet Dosage Form

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ABSTRACT

The present work describes development and validation of a new simple, accurate and precise stability-indicating high performance thin layer chromatographic (HPTLC) method for the determination of Lamivudine and Tenofovir disoproxil fumarate as bulk drug and in combined tablet dosage form. Since stability testing is major step in the development of new drug as well as formulation, stress degradation studies were carried out according to ICH guidelines. HPTLC plates precoated with silica gel 60 F_{254} were used as the stationary phase and chromatographic separation was achieved by using Toluene: Methanol (8: 2, v/v) as mobile phase. Densitometric detection was carried out at 270 nm. The retardation factor was found to be 0.33±0.02 and 0.55±0.02 for Lamivudine and Tenofovir, respectively. The suitability of this HPTLC method for quantitative estimation of both drugs was proved by validation in accordance with requirements of ICH guidelines Q2A (R1). The developed method has been effectively applied for the estimation of drugs in combined tablet dosage form.

INTRODUCTION

Lamivudine (LAM), chemically, 4-amino-1-[(2*R*, 5*S*)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl] pyrimidin-2-one is an antiretroviral medication used to prevent and treat HIV/AIDS [1]. Tenofovir disoproxil fumarate(TEND), chemically, [[(2*R*)-1-(6-amino purin-9-yl) propan-2-yl] oxy methyl-(propan-2 yloxy carbonyloxymethoxy) phosphoryl] oxymethyl propan-2-yl carbonate; but-2-enedioic acid is used to treat chronic hepatitis B and to prevent and treat HIV/AIDS [2].

Extensive literature survey revealed that different analytical methods have been reported for quantitative analysis of LAM and TEND either as single drug and/or in combination with other drugs. Analytical methods such as UV spectrophotometry [3-11], reverse phase high performance liquid chromatography (RP-HPLC) [12-18] and high performance thin layer chromatography (HPTLC) [19-25] were available in the literature for determination of LAM and TEND either as single drug or in combination with other drugs in human plasma and in pharmaceutical dosage form.

One HPTLC method reported by Baig MS*et al*., [26] stating the stability-indicating HPTLC method development and validation for analysis of lamivudine and tenofovir from its tablet dosage form. The disadvantages of this method are use of chloroform as mobile phase, which is no longer recommended, and Rf value that is too low (Lamivudine Rf 0.06) for precise densitometric analysis. In the study reported in this paper a simple, accurate, economical, and reproducible stability representing procedure has been established and validated. The method does not use a chloroform-containing mobile phase, and appropriate Rf values enable more precise densitometric analysis of lamivudine and tenofovir.

MATERIALS AND METHODS

Chemical and reagents

Analytical pure working standards LAM and TEND were obtained as a gift sample from Cipla Ltd., (Mumbai Maharashtra). TENVIR L tablets labeled to contain 300 mg of LAM and 300 mg of TEND were procured from the local market. Toluene and Methanol (both AR grade) were purchased from LOBA CHEME Pvt Ltd., India.

Instrumentation and chromatographic conditions

Chromatographic resolution of the drugs was achieved by use of Merck TLC plates precoated with silica gel 60 F_{254} (10 ×10 cm with 250 µm layer thickness) from E. MERCK, Darmstadt, Germany, using a CAMAG Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with 8 mm width using CAMAG 100 µL sample syringe (Hamilton, Switzerland). Linear ascending development was carried out in 10×10 cm twin-trough glass chamber (CAMAG Muttenz, Switzerland) by using Toluene: Methanol (8: 2, v/v) as mobile phase. The saturation of mobile phase was done for 20 minutes in the chamber at room temperature. Mobile phase was allowed to run upto 8 cm. Densitometric scanning was performed in CAMAG TLC scanner III at 270 nm operated by winCATS software (version 1.4.3). Deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm was used as a radiation source.

Preparation of standard stock solutions

Standard stock solution of LAM and TEND were prepared by dissolving 5 mg of each drug in 10 mL of methanol separately to get concentration of 500 µg mL^{-1} from which 1 mL was further diluted to 10 mL to get stock solution of 50 ng μL^{-1} of LAM and TEND, respectively.

Tablet formulation analysis

Commercial brand of tablet formulation TENVIR L containing 300 mg of LAM and 300 mg of TEND was used to determine the content of each drug present in formulation. For this, twenty tablets were weighed accurately and finely powdered. A tablet powder equivalent to 5 mg was weighed and transferred to a 10 mL volumetric flask having about 6 mL of methanol. The contents were sonicated for 15 min, filtered and volume was made up to the mark with methanol. From the above solution, 1 mL of solution was diluted using same solvent to achieve final concentration of 50 ng μL^{-1} for LAM and TEND. Two microlitre volume of this solution was applied on TLC plate to obtain final sample concentration of 100 ng $band^{-1}$ for both drugs. After chromatographic development peak areas of the bands were measured at 270 nm and the amount of each drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies of bulk drugs

Stress degradation studies were carried out to provide evidence on how stability of drugs varies under the influence of variety of conditions like acid and base hydrolysis, oxidation, thermal and UV light as mentioned in ICH Q1A (R2) and Q1B guidelines. The stress degradation studies were carried out at initial drug concentration of 500 µg mL^{-1} of both drugs in methanol. The hydrolytic studies were carried out by mixing the drug solutions of LAM and TEND with 0.1 N HCl and 0.1 N NaOH and the resulting solutions were kept at room temperature for 1 h to achieve degradation within the acceptable limit. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 250 ng $band^{-1}$ of both the drugs. The oxidative degradation was carried out in 3 % H_2O_2 and the sample was diluted with methanol to obtain solution having concentration 250 ng $band^{-1}$ of both the drugs. Thermal stress degradation was performed by keeping the solid drugs individually in oven at 45ºC for a period of 3 d. Photolytic degradation studies were carried out by exposing both drugs individually to UV light up to 200-watt h square $meter^{-1}$. Thermal and photolytic samples were diluted with methanol to get the concentration of 250 ng $band^{-1}$ of both the drugs.

RESULTS AND DISCUSSION

Optimization of method

The objective of developing HPTLC method was to accomplish the satisfactory resolution of drugs from each other and also from their degradation products. Primarily, many method trials were performed using different mobile phases in order to obtain better separation and to achieve better resolution of both drugs. Finally, the mobile phase comprising of toluene: methanol (8: 2, v/v) was selected as optimum which gave acceptable resolution of both drugs with symmetrical peak shape. Densitometric scanning was carried out at 270 nm. The retardation factor (Rf) was found to be 0.33±0.02 and 0.55±0.02 for LAM and TEND, respectively. Representative densitogram of mixed standard solution of both drugs is shown in Figure 1.

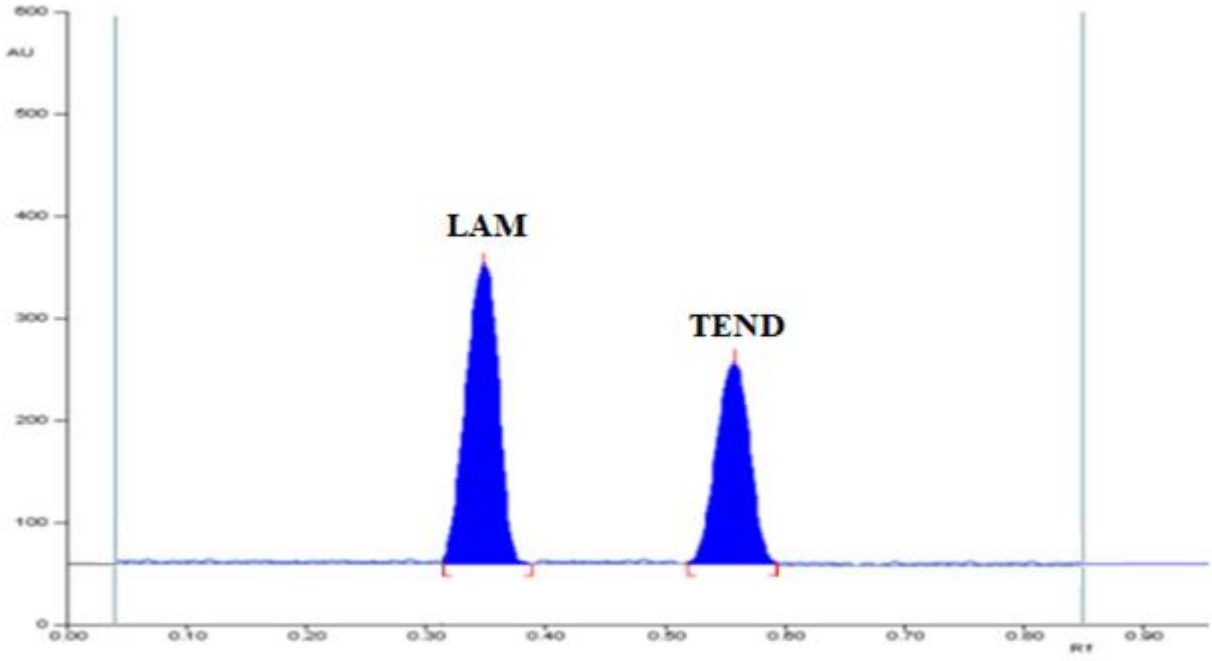


Fig. 1: Representative densitogram of mixed standard solution LAM (150 ng band^{-1}, Rf = 0.33±0.02) and TEN (150 ng band^{-1}, Rf = 0.55±0.02)

Stress degradation studies

The results of stress degradation demonstrated the proneness of both the drugs to hydrolytic, oxidative stress conditions and stability under thermal and photolytic stress conditions. Significant degradation in the densitograms was observed with appearance of degradation products at Rf values 0.20 and 0.72 for LAM and TEND, respectively under acid hydrolysis. LAM was found to undergo 25.91 % degradation with appearance of peak for degradation product at Rf 0.17 while TEND showed 17.68 % degradation without appearance of peak for degradation product after alkaline hydrolysis. Marked degradation was observed for LAM without appearance of peak for degradation product and TEND with appearance of peak for degradation product at Rf 0.79 after peroxide degradation. Figures 2 and 3 shows the densitograms of acid and alkali hydrolytic degradation, while Figure 4 shows the densitogram of oxidative degradation. Peak purity results greater than 995 indicate that peaks for both drugs are homogeneous in all stress conditions tested. The findings of degradation studies are represented in Table 1.

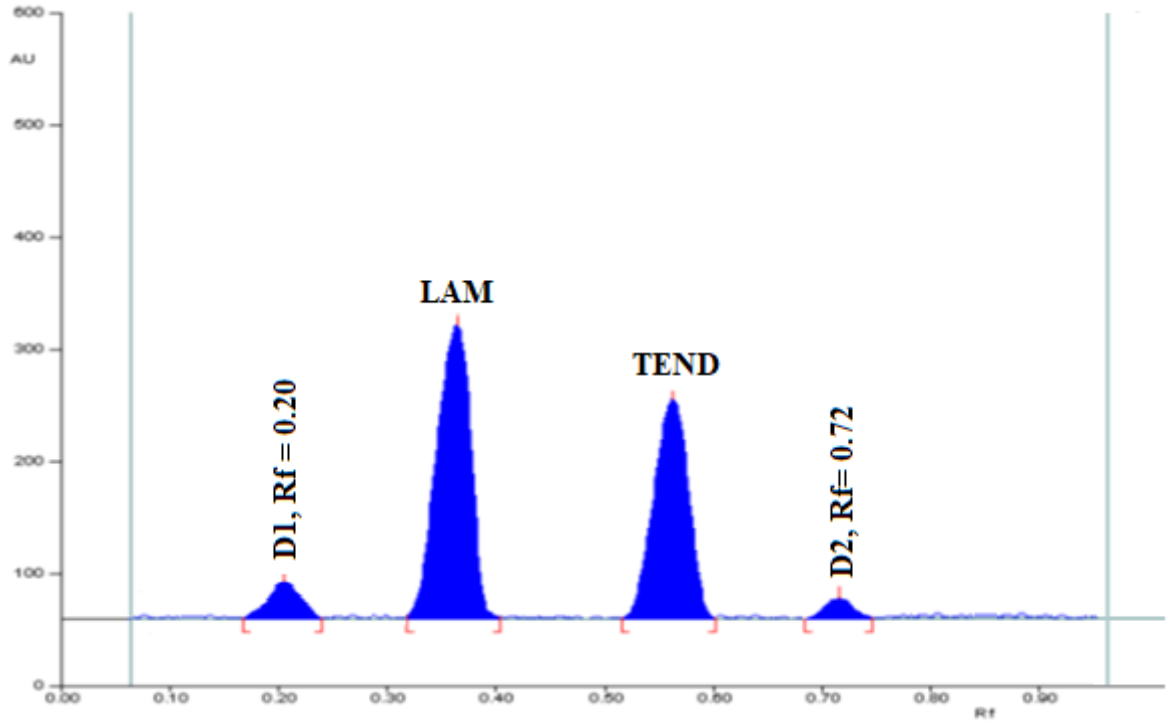


Fig. 2: Densitogram of mixed standard solution of LAM (D1, Rf = 0.20) and TEND (D2, Rf = 0.72) after acid degradation

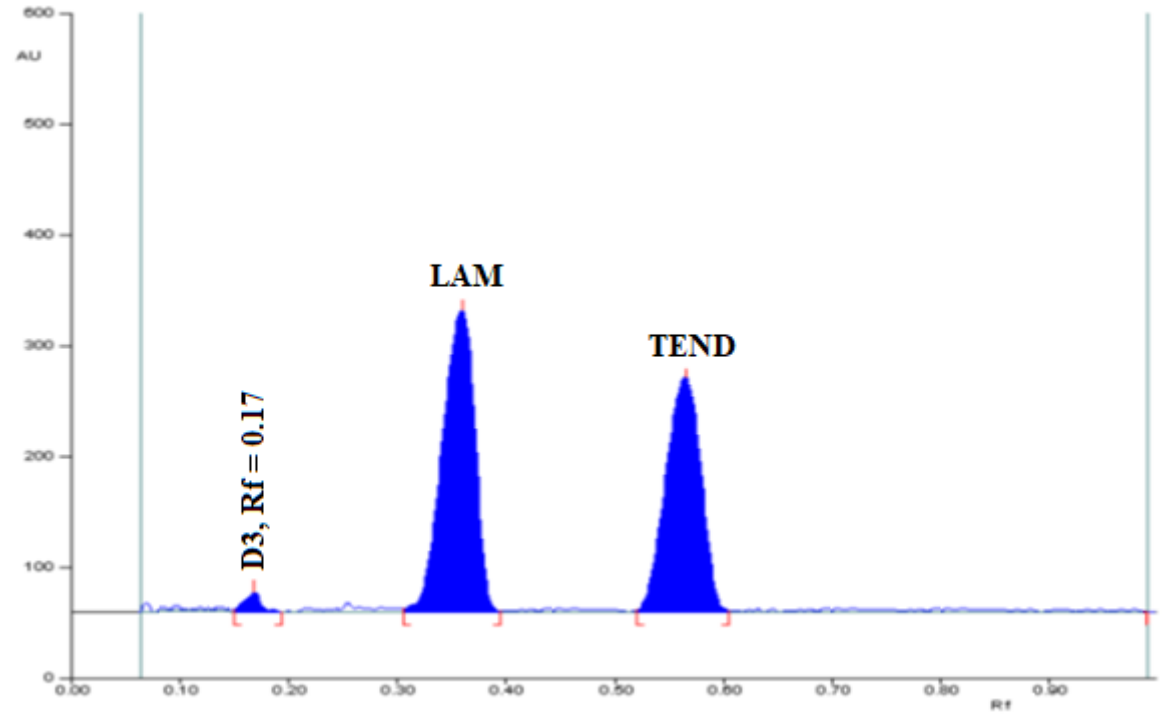


Fig. 3: Densitogram of mixed standard solution of LAM (D3, Rf = 0.17) and TEND after base degradation

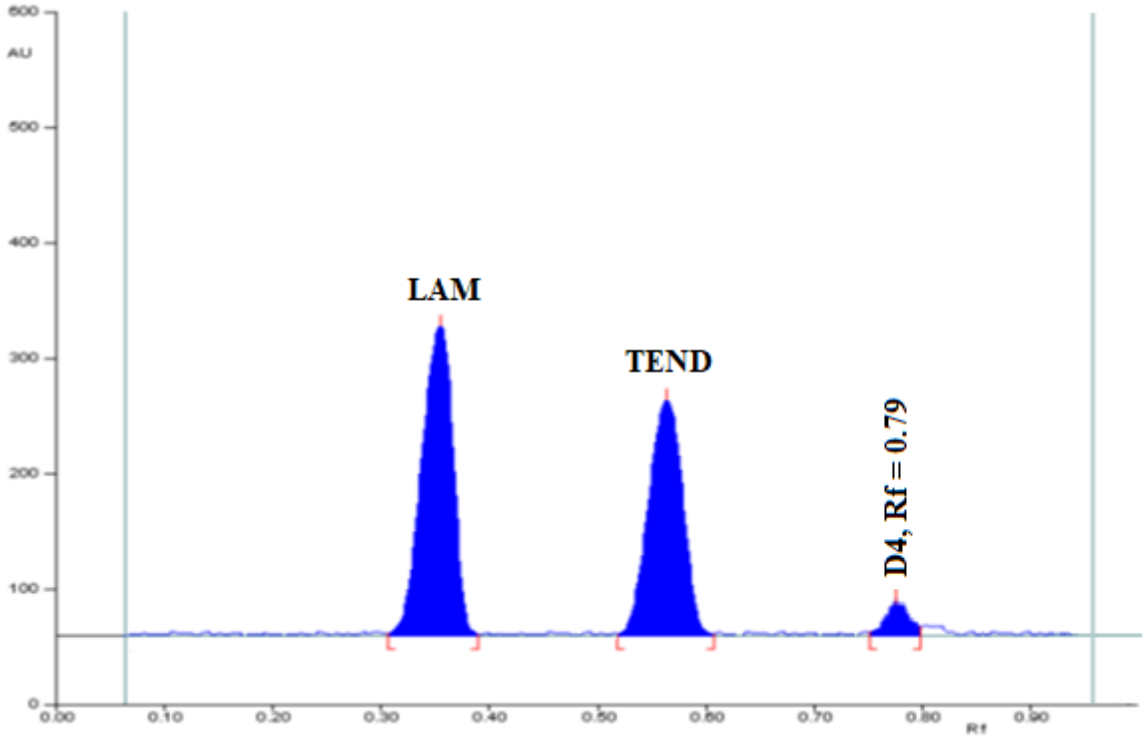


Fig. 4: Densitogram obtained for LAM and TEND (D4, Rf = 0.79) after peroxide induced degradation

Table 1: Data of forced degradation studies of LAM and TEND

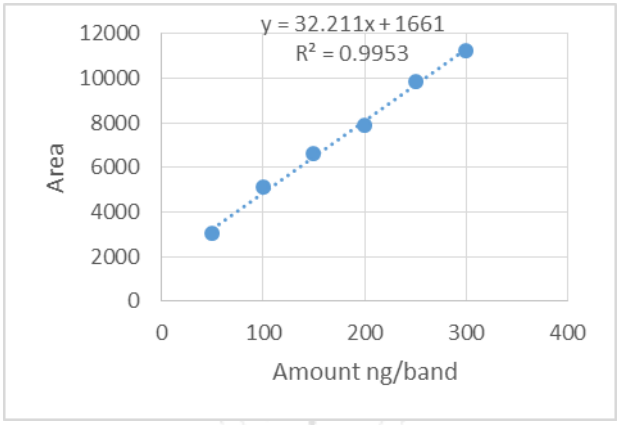
Stress conditions/ duration	LAM		TEND	
	% Assay	% degradation	% Assay	% degradation
Acidic / 0.1 N HCl/ Kept at RT for 1 h	79.63	20.37	77.92	22.08
Alkaline /0.1 N NaOH/ Kept at RT for 1 h	74.09	25.91	82.32	17.68
Oxidative /3 % H_2O_2/ Kept at RT for 1 h	86.36	13.64	76.60	23.40
Dry heat/ 45ºC/ 3 d	98.36	-----	98.21	-----
Photolysis	99.02	-----	99.63	-----

Method Validation

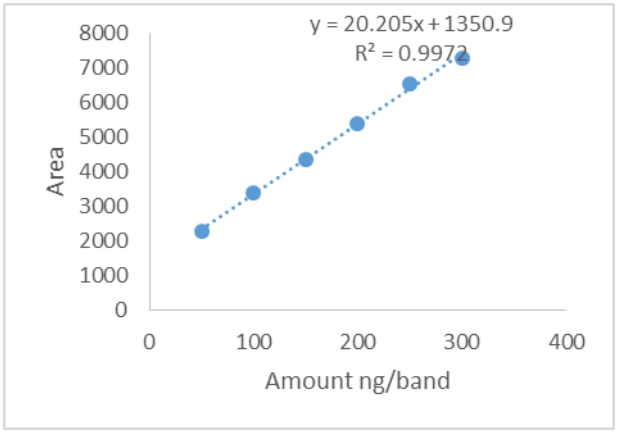
The developed method was validated with respect to linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness in accordance with ICH guidelines [27, 28].

Linearity

The standard stock solutions of LAM and TEND (50 ng μL^{-1}each) were applied by overspotting on TLC plate to obtain the concentration in the range 50-300 ng $band^{-1}$ for both the drugs. Results were found to be linear in the concentration range indicated above. The linear regression equation was found to be y = 32.211x + 1661 and y = 20.205x + 1350.9 with correlation coefficient (R^2) value of 0.995 and 0.997 for LAM and TEND respectively. The calibration curves and 3 D spectra obtained for LAM and TEND are represented in Figure 5 and 6, respectively.



(a)



(b)

Fig. 5: Calibration curve for (a) LAM (b) TEND

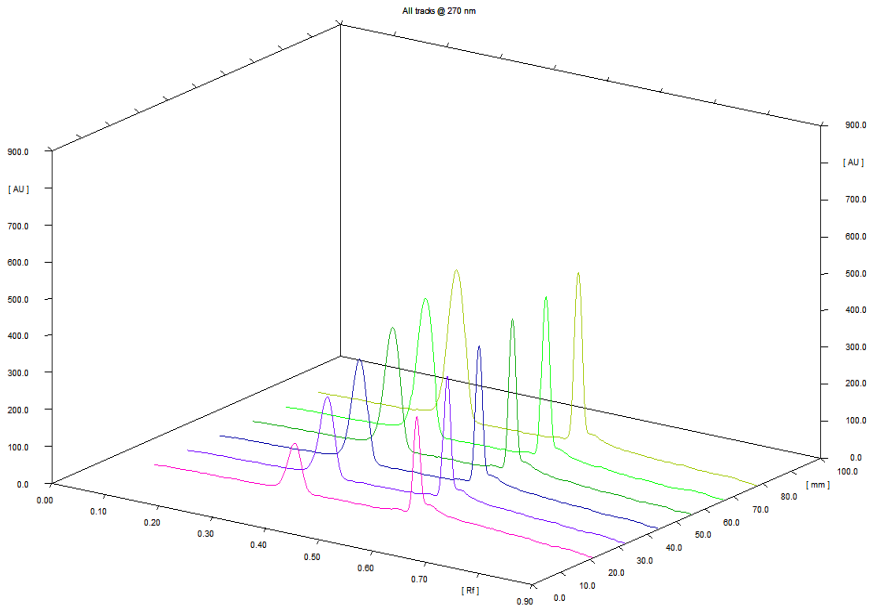


Fig. 6: 3D densitogram of LAM and TEND in the range 50-300 ng band^{-1}

Precision

Precision was evaluated in terms of intra-day and inter-day precisions. Intra-day precision was determined by analyzing three replicates of standard solutions of LAM and TEND within the linearity range on same day and percentage R.S.D was calculated. The percentage R.S.D was found to be in range of 0.62 to 1.00 for LAM and 0.87 to 1.90 for TEN. Inter-day precision was determined by analyzing three replicates of standard solutions of LAM and TEND within the linearity range on three consecutive days and percentage R.S.D was calculated. The percentage R.S.D was found to be in range of 0.83 to 1.44 for LAM and 0.59 to 1.73 for TEN.

Table 2: Intra-day precision studies

Drug	Applied concentration (ng band^{-1})	Average area	Recovered Concentration (ng band^{-1})	% R.S.D.*
LAM	50	3269	49.92	1.00
	100	4924	101.30	0.62
	150	6523	150.94	0.63
TEND	50	2361	49.99	1.55
	100	3374	100.12	0.87
	150	4394	150.61	1.90

*Average of three determinations

Table 3: Inter-day precision studies

Drug	Applied concentration (ng band^{-1})	Average area	Recovered Concentration (ng band^{-1})	% R.S.D.*
LAM	50	3274	50.07	1.00
	100	4887	100.15	0.62
	150	6444	148.48	0.63
TEND	50	2372	50.53	1.55
	100	3370	99.93	0.87
	150	4365	149.17	1.90

*Average of three determinations

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

LOD and LOQ were calculated using formulas LOD= 3.3 σ/S and LOQ= 10 σ/S; where σ = standard deviation of lowest response and S = slope of calibration curve. The LOD and LOQ was found to be 8.05 and 32.16 for LAM and 6.56 and 29.59 for TEN respectively.

Accuracy

Accuracy of developed method was checked by performing recovery studies by standard addition method. It involved addition of standard drug solution to pre-analyzed sample solution at three different levels 80, 100 and 120%. Basic concentration of sample chosen was 100 ng band^{-1}for both drugs. The results of recovery studies indicated accuracy of method for estimation of drugs in combined tablet dosage form.

Table 4: Recovery studies

Drug	Concentration taken (ng band^{-1})	Amount added (ng band^{-1})	Amount found (ng band^{-1})	% Recovery±R.S.D.*
LAM	100	80	179.13	99.51±1.02
	100	100	197.47	98.73±0.51
	100	120	217.41	98.82±0.45
TEND	100	80	178.92	99.40±0.38
	100	100	201.34	100.67±0.72
	100	120	219.19	99.63±1.10

*Average of three determinations

Robustness

Robustness of the method was determined by making deliberate changes in the saturation time, time from spotting to development and time from development to scanning and the effect on Rf values and peak areas was noted. The Rf values and areas of peak of interest remained unaffected which indicated robustness of method for both drugs.

Table 5: Robustness data

Parameters	% R.S.D*	
	LAM	TEND
Saturation time (± 5 min)	1.68	1.81
Development time (0, 5 and 10 min)	1.73	1.49
Scanning time (0, 5 and 10 min)	1.45	1.85

* Average of three determinations

CONCLUSION

Stability-indicating HPTLC densitometric method without interference from the excipients has been developed and validated for the simultaneous determination of Lamivudine and Tenofovir disoproxil fumarate as bulk drug and in combined tablet dosage form. The developed method is simple, sensitive, precise, accurate and reproducible. The method does not use a chloroform-containing mobile phase, and appropriate Rf values enable more precise densitometric analysis of lamivudine and tenofovir as compared to reported method. The developed method can be used for quantitative analysis of these drugs in pharmaceutical dosage form.

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REFERENCES

1. https://en.wikipedia.org/wiki/Lamivudine (Accessed on 02-05-2021)
2. https://en.wikipedia.org/wiki/Tenofovir_disoproxil (Accessed on 02-05-2021)
3. Shanti P, Prasad G, Hari P, Haque A, Sainath G and Bakshi V. Method development and validation of lamivudine in bulk and pharmaceutical dosage form using UV-Visible spectrophotometric method. International Journal of Innovative Pharmaceutical Sciences and Research. 2018; 6(1): 92-98.
4. Sonar K, Sapkale P, Jadhav A, Deshmukh T, Patil S and Murkute P. Development and validation of UV spectroscopic method for estimation of lamivudine in tablet dosage form. IntJCurr Pharm Res. 2017; 9(6): 86-89.
5. Kumar A, Jaishri R and Jambulingam M. Method development and validation of UV-spectroscopic method for determination of lamivudine as an active pharmaceutical ingredient and in tablet dosage form. Int JPharmHCare Res. 2017; 5(4): 129-137.
6. Rao S, Balaji M, Reddy H, Roa K and Krishna R. Development of UV spectrophotometric method for quantitative estimation of tenofovir disoproxil fumarate in bulk and pharmaceutical dosage form. Asian J Research Chem. 2012; 5(3): 422-424.
7. Rele R and Tiwatane P. UV spectrophotometric estimation of tenofovir disoproxil fumarate by area under curve method in bulk and pharmaceutical dosage form. Asian J Research Chem. 2020; 13(1): 7-11.
8. Rajavel P. Analytical method development and validation of tenofovir by UV spectrophotometry. Asian Journal of Research in Pharmaceutical Sciences and Biotechnology. 2016; 4(4): 88-94.
9. Asnani A, Mohurle S and Pratyush K. Development and validation of UV spectrophotometric method for estimation of lamivudine and tenofovir disoproxil fumarate in combined dosage form using quality by design approach. Int J Pharm Sci and Res. 2021; 12(6): 3306-3315.
10. Anees M and Baig M. Determination of lamivudine and tenofovir in pharmaceutical dosage form by area under curve and multicomponant UV spectrophotmetric method. Int J Curr Microbiol App Sci. 2015; 4(2): 756-763.
11. Sharma R and Mehta K. Simultaneous spectrphotometric estimation of tenofovir disoproxil fumarate and lamivudine in three component tablet formulation containing efavirenz. Ind J Pharm Sci. 2010; 72(4): 527-530.
12. Kumari A, Katakam P, Nagoji K and Rao M. RP-HPLC method for the estimation of lamivudine in bulk and pharmaceutical dosage form. Asian J Chem. 2007; 19(4): 2642-2646.
13. Singh A, Nath L and Pani N. Development and validation of analytical method for the estimation of lamivudine in rabbit plasma. J Pharm Anal. 2011; 1(4): 251-257.
14. Kano A, Serra C, Koona E, Andrade S and Porta V. Determination of lamivudine in human plasma by HPLC and its use in bioequivalence studies. International Journal of Pharmaceutics. 2005; 297(1-2): 73-79.
15. Kumar M, Saravanan D, Maheswaran A and Divakar P. A new RP-HPLC method for the determination of tenofovir disoproxil fumarate in pure form and pharmaceutical formulation. International Journal of Research in Pharmaceutical Sciences and Technology. 2020; 2(1): 1-6.
16. Kandagal P, Manjunatha D, Seetharamappa J and Kalanur S. RP-HPLC method for the determination of tenofovir in pharmaceutical formulations and spiked human plasma. Pharmaceutical Analysis. 2007; 41(4): 561-570.
17. Karunakaran A, Kamarjan K and Thangarasu V. A validated RP-HPLC method for simultaneous estimation of lamivudine and tenofovir disoproxil fumarate in pure and tablet dosage form. Eurasian J Anal Chem. 2012; 7(2): 56-66.
18. Adhao V, Chaudhari S, Ambhore J, Sangolkar S, Thenge R, Cheke R and Patil A. reverse phase liquid chromatography assisted protocol for simultaneous determination of lamivudine and tenofovir disoproxil fumarate in combined medication used to control HIV infection. Fut J Pharm Sci. 2021; 7(90): 1-11.
19. Swami S, Deshpande P, Jadhav S and Temgire S. Validated stability indicating HPTLC method for estimation of lamivudine as bulk drug and in pharmaceutical tablet dosage form. European Journal of Pharmaceutical and Medical Research. 2021; 8(10): 353-359.
20. Habte G and Hymete A. Simultaneous separation and determination of lamivudine and zidovudine in pharmaceutical formulations using HPTLC method. Pharmaceutical Analysis. 2009; 42: 1552-1570.

21. Dhanve A, Rao J and Dhale C. Development and validation of stability indicating HPTLC method for simultaneous estimation of lamivudine and dolutegravir sodium in bulk and pharmaceutical dosage formulations. International Journal of Pharmaceutical Sciences and Research. 2018; 9(11): 4701-4708.
22. Havele S and Dhaneshwar S. Stress studies of tenofovir disoproxil fumarate by HPTLC in bulk drug and pharmaceutical formulation. The Scientific World Journal. 2012: 1-6.
23. Mardia R, Suhagia B, Pasha T, Chauhan S and Solanki S. Development and validation of HPTLC method for estimation of tenofovir disoproxil fumarate in tablet dosage form. Journal of Pharmaceutical Science and Bioscientific Research. 2012; 2: 73-76.
24. Bizimana T, Kayumba P, Vedaste K, Egide K and Kaale E. Development of high-performancethin layer chromatography for simultaneous analysis of lamivudine and tenofovir disoproxil fumarate. Rwanda Journal. 2016; 3(1): 1-5.
25. Gu Y, Zeng B and Sherma J. Development of quantitative HPTLC methods for dolutegravir, lamivudine and tenofovir disoproxil fumarate in combination pharmaceutical product using a model process published earlier for transfer of minitab TLC screening methods to HPTLC-densitometry. Acta Chromatographica. 2020; 32(3); 199-202.
26. Baig M and Dehghan M. 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: 463-474.
27. The International Conference on Harmonization, Q2 (R1), Validation of Analytical Procedure: Text and Methodology, 2005.
28. The International Conference on Harmonization, Q1 (B), Stability Testing: Photostability Testing of New Drug Substances and Products: Text and Methodology, 2005.