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Stability Indicating Method Development and Validation of Tenofovir Disoproxil Fumarate in Bulk and its Tablet Dosage Form by HPTLC

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ABSTRACT - A novel stability – indicating high performance thin layer chromatography HPTLC assay method was developed and validated for quantitative determination of Tenofovir Disoproxil Fumarate in bulk drugs and in pharmaceutical dosage form in the presence of degradation products generated from forced degradation studies. The present study is completed by using precoated silica gel aluminium plate 60 F – 254, (20 × 10 cm) with 250 µm thickness, as stationary phase and the mobile phase consisted of n-butanol: acetic acid: water (4: 1: 1 v/v/v). The detection was carried out at the wavelength of 260 nm. Tenofovir Disoproxil Fumarate was subjected to stress conditions of hydrolysis (acid, base, neutral), oxidation, photolysis, and thermal degradation. Degradation was observed for Tenofovir Disoproxil Fumarate in acid, base and in oxidation conditions. The drug was found to be stable in the other stress conditions attempted. The degradation products were well resolved from the main peak. The developed method was validated with respect to linearity, range, precision, repeatability, LOD and LOQ, robustness, specificity, and recovery. The analysis of the marketed product and the forced degradation studies prove the stability-indicating power of the method.

Keywords: Tenofovir Disoproxil Fumarate, HPTLC, Degradation, Validation.

INTRODUCTION

Tenofovir Disoproxil Fumarate (TDF) chemically, 9-[(R)-2-[[bis[(Isopropoxycarbonyl) oxy]methoxy] phosphinyl] methoxy] propyl]adenine fumarate (1:1) (as shown in Fig.I.).Tenofovir disoproxilfumarate (TDF) is a prodrug form of tenofovir. TDF belongs to a class of antiretroviral drugs known as nucleoside reverse transcriptase inhibitor (NRTI), which block reverse transcriptase, a crucial viral enzyme in human immunodeficiency virus 1 (HIV-1) and hepatitis B virus infections.[1-3]It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system.[4]TDF is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults and pediatric patients 2 years of age and older.

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TDF is indicated for the treatment of chronic hepatitis B in adults and pediatric patients 12 years of age and older.[5]It was found that few analytical methods were available for the determination of TDF with combination by UV Spectrophotometry [6, 7] BY HPLC [8-11] AND by HPTLC.[12,13]

After thorough survey of literature there is not a single HPTLC method reported for the analysis of TDF by HPTLC.

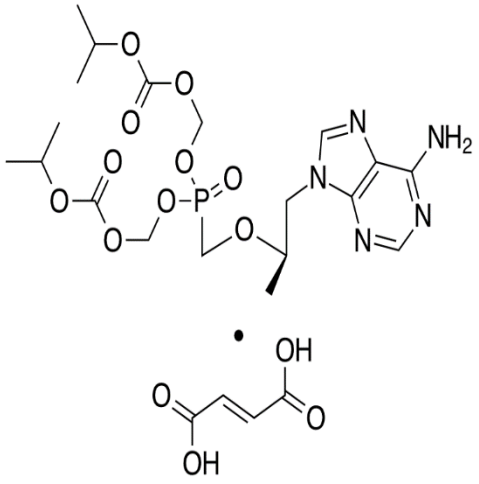

Fig.I. Structure of Tenofovir Disoproxil Fumarate (TDF).

MATERIALS AND METHODS

Chemicals and reagents

CiplaPvt.Ltd,Panvel, Maharashtra, India, generouslygifted pure TDF. Commercialtablets (Brand name: Tenvir of CiplaPvt. Ltd.) containing TDF (300 mg) was used for the study. All theother chemicals used were of analytical grade (E.Merck, India).

Solubility and stability studies

Solubility of TDF was checked in different organic solvents having wide range of polarity indices along with its stability in these solvents.

Selection of analytical wavelength

In order to determine the absorbance maxima, various concentrations of the drug from standard stock solution were spotted on the plate and developed. After developing, the plate was dried and then scanned for spectrum in the range of 190-400 nm (to get In situ spectra). Also by using UV spectrophotometer scanning of drug was carried out in the range of 200-400nm.

Instrumentation and Chromatographic Condition

Precoated silica gel aluminium plate 60 F – 254, (20 × 10 cm) with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai are used through the study. The plates were prewashed with methanol and activated at 110^{o}C for 5 min prior to chromatography. The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on using a Camag Linomat IV applicator (Switzerland). A constant application rate of 0.1 µl/s was employed and space between two bands was 6 mm.

The slit dimension was kept at 5 × 0.45 mm and 10 mm/s scanning speed was employed. The monochromator bandwidth was set at 20 nm with K 320 cut off filter, each track was scanned thrice and baseline correction was used. The mobile phase consisted of n-butanol: acetic acid: water (4: 1: 1 v/v/v) was used per chromatography. Linear ascending development was carried out in 20 x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). It was saturated (lined on the two bigger sides with filter paper that had been soaked thoroughly with the mobile phase) and the chromatoplate development was carried out in dark with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25^{o}C ± 2) at relative humidity of 60 % ± 5. The length of chromatogram run was 8 cm and approximately 20 min. Subsequent to the development, TLC plates were dried in a current of air in wooden chamber with adequate ventilation. The flow of air in the laboratory was maintained unidirectional (laminar flow, towards exhaust). Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode at 260 nm for all measurements and operated by CATS software (V 4.06, Camag). The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was via peak areas with linear regression.

Stock solutions

A. Standard stock Solutions

A stock solution of TDF (600 mg/ml) was prepared by dissolving 6gm of TDF accurately weighed in a 10 ml volumetric flask and was dissolved in 7 ml of HPLC grade methanol and volume was made up to the mark with HPLC grade methanol. The solution was further diluted as per requirement.

B. Sample stock Solutions

For preparing sample stock solution, twenty tablets were weighed (Tenvir of Cipla Pharmaceuticals Ltd. having label claim: 300 mg TDF per tablet) and their average weight was calculated (380.60 mg). The tablets were finely powdered and powder equivalent to 6000 mg of TDF was accurately weighed and transferred into a 10 ml volumetric flask containing 8 ml methanol (HPLC grade) and sonicated for 30 mins then made the volume up to the mark with same. The solutions were filtered through a 0.45 µm nylon filter. The solution was further diluted as per requirement.

Forced degradation studies

In order to determine whether the analytical method and assay were stability-indicating, TDFstandard stock solutions was stressed under various conditions to conduct forced degradation studies. The plates of degradation studies are developed by using predefined chromatographic conditions and scanned at 260 nm.

A. Acid degradation

To 5 ml of standard stock solutionand 5 ml of 0.01 N hydrochloric acid was added. The solution was kept for 15minutes at room temperature.The resultant solutions were applied on TLC plate in such a way that final concentration achieved was 600 ng spot^{-1} and the TLC plate was developed in optimized chromatographic conditions.

B. Alkali degradation

To 5 ml of standard stock solution and 5 ml of 0.01N sodium hydroxide was added. The solution was kept for 4 hours at room temperature.The resultant solution was applied on TLC plate in such a way that final concentration achieved was 600 ng spot^{-1} and the TLC plate was developed in optimized chromatographic conditions.

C. Neutral degradation

10 ml of standard stock solution of TDF was refluxed for 48 hours, the resultant solution was applied on TLC plate in such a way that final concentration achieved was 600 ng spot^{-1} and the TLC plate was developed in optimized chromatographic conditions.

D. Oxidative degradation

To 5 ml of standard stock solution5 ml of 6% hydrogen peroxide was added separately. The solution was kept for 24 hours at room temperature. The resultant solutions was applied on TLC plate in such a way that final concentration achieved was 600 ng spot^{-1} and the TLC plate was developed in optimized chromatographic conditions.

E. Tharmal degradation

50mg of the drug was spread in a borosilicate glass petri dish and placed in a hot air oven maintained at 60^{0}C for 48 hours, then the solution was prepared to achieve a final concentration of 600 ng spot^{-1}and the TLC plate was developed in optimized chromatographic conditions.

F. Photo degradation

50 mg of the drug was spread in a borosilicate glass petri dish and placed in a light cabinet (Thermo lab, India) and exposed to ≥200 W h m–2 UV irradiation at 320–400 nm, at 25°C, for 10 days. After removal from the light cabinet, sample was analyzed by making final concentration 600 ng spot^{-1} in optimized chromatographic conditions.

Validation of the stability indicating method

A. Linearity and range

Linearity of the method was determined with the standard stock solution. The standard stock solution was diluted in such that to obtain concentration of 120ng/µl, 240ng/µl, 360ng/µl, 480ng/µl, 600 ng/µl. This solution was applied on plate to get concentrations of 120 ng spot^{-1}, 240 ng spot^{-1}, 360 ng spot^{-1}, 480 ng spot^{-1}, 600 ng spot^{-1}respectively, each concentration is applied for six times for linearity study. The plates were developed using optimized mobile phase and applied optimized chromatographic conditions.

B. Precision

Precision of the method was determined with the standard stock solution. The standard stock solution was diluted in such that to obtain concentration of 120 ng/µl, 360ng/µl and 600ng/µl. This solution was applied on plate to get concentrations of 120 ng spot^{-1}, 360 ng spot^{-1}, &600 ng spot^{-1} respectively, each concentration is applied for six times on same day for intra-day precision study & three consecutive days for inter day precision study. The plates were developed using optimized mobile phase and applied optimized chromatographic conditions.The measurement of peak areas for TDF were expressed in terms of % RSD (relative standard deviation).

D. Limit of Detection and Limit of Quantitation

Limits of detection (LOD) and Limits of quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ. The LOD and LOQ were determined by measuring the magnitude of analytical background by injecting a blank and calculating the signal-to-noise ratio forby injecting a series of solutions until the S/N ratio 3 was obtained for the LOD and 10 for the LOQ. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. To determine the LOD and LOQ, serial dilutions of mixed standard solution of TDF was made from the standard stock solution.

E. Robustness of the method

To evaluate the robustness of a HPTLC method, few parameters were deliberately varied. The robustness of the method was studied, during method development at three different concentration levels of 120 ng/spot, 360 ng/spot and 600 ng/spot, by determining the effects of small variation, mobile phase composition (±0.1 %), amount of mobile phase (±5 %), time from spotting to chromatography (±20 min) and scanning time (±20 min). Each concentration was applied in six times and % RSD was calculated.

F. Specificity

To confirm the specificity of the proposed method, sample stock solution of marketed formulation of TDF in a concentration of 120, 360, 600 ng/spot, were spotted on TLC plate, developed and scanned as described earlier. The peak purity of TDF was assessed by comparing the spectra at three different levels i.e., peak start, peak apex and peak end positions of the spot.

G. Recovery

Recovery studies were carried out by applying the method to drug sample to which known amount of TDF corresponding to 50, 100 and 150 % of label claim had been added (standard addition method). At each level of the amount, six determinations were performed and the results obtained were compared with expected results.

H. Stability in sample solution

The sample stock solution stored at room temperature for 24h and three concentrations 120, 360, and 600 ng spot^{-1} for TDF were applied on HPTLC plate, after development the densitogram there was no additional peak found.

2.7 Analysis of the marketed formulation (ASSAY)

The sample stock solution was further diluted to obtain three different concentration levels of 120, 360 and 600 ng/µl respectively. One microliter of each sample solution was applied six times to the HPTLC plate to give concentration 120 ng spot^{-1}, 360 ng spot^{-1}, and 600 ng spot^{-1} for TDF. The plate was developed in the previously described chromatographic conditions. The peak areas of the spots were measured at 260 nm and concentrations in the samples were determined using multilevel calibration developed on the same plate under the same conditions using linear regression equation.

RESULT AND DISCUSSION

Solubility and stability studies

Solubility of TDF was checked in different organic solvents having wide range of polarity indices along with its stability in these solvents. It was found that TDF was freely soluble as well as stable in methanol & dimethylformamide. It is sparingly soluble in water.

Selection of analytical wavelength

The UV spectra of TDF scanned in UV spectrophotometer and in situ HPTLC (Fig.II), both shows λmax at 260 nm. Therefore 260 nm was selected as scanning wavelength for all study of TDF.

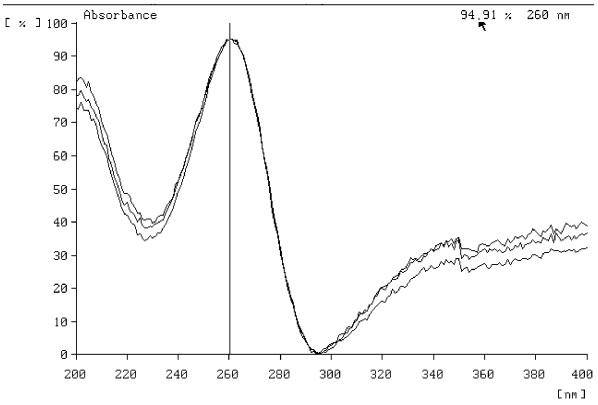

Fig.II. HPTLC spectra of TDF drug (λ_{max} 260 nm)

Optimization of the HPTLC method

Initially many different combinations of mobile phases were tried for the method development. tolune: ethyl acetate and Toluene: Ethyl acetate: methanol in different proportions were tried. It was found that in the toluene: ethyl acetate: methanol the movement of the drug was very less and poor resolution was observed. The mobile phase of n-butanol: acetic acid: water (4: 1: 1 v/v/v) gave compact spot of TDF with good peak shape. The R_f of TDF was 0.75 ± 0.03 (Figure 3). The mobile phase was also able to resolve all the degradent products at 260 nm wavelength. The R_f of all the degradation components were between 0.30 to 0.65. Therefore this mobile phase combination was chosen for the validation studies. The optimized saturation time for mobile phase was 30 min at room temperature (25°C ± 2) at relative humidity of 60 % ± 5 (Fig.III.).

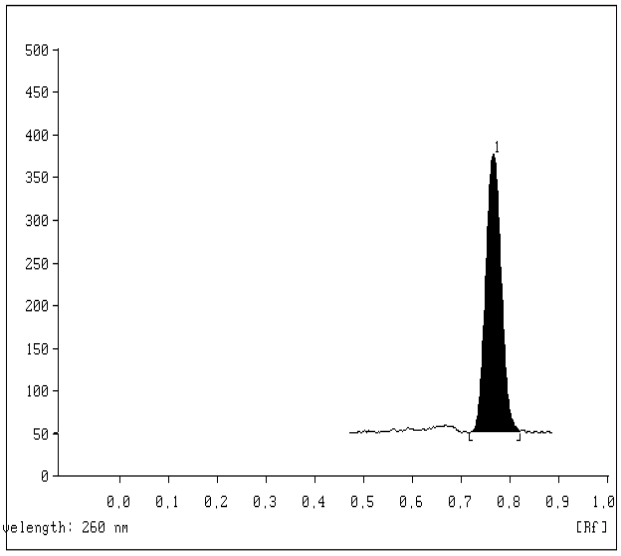

Fig.III. Densitogram of TDF standard 600 ng/spot

Forced degradation studies of TDF

HPTLC studies of samples obtained on stress testing of TDF under different forced conditions, was carried out using optimized mobile phase and scanning conditions.

A. Acid degradation

The drug was extremely labile in acidic conditions. Initially 1 N hydrochloric acid at room temperature was used but drug was totally degraded, so that the strength of acid was decreased gradually i.e. 0.1 N, 0.01N Then, 10-20 % degradation was observed with 0.01 N hydrochloric acid at room temperature for 15 min. Whereas a densitogram of acid-degraded TDF showed two degradation peaks, at R_f 0.61(% Area=7.54), R_f 0.43(% Area=5.24) and TDF at R_f0.73(% Area=87.54) (Fig.IV.).

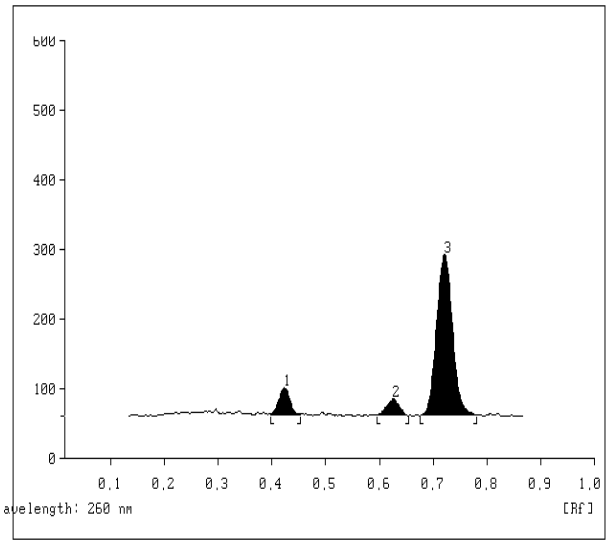

Fig.IV. Densitogram of acid degradation product 600 ng/spot. (Condition: 0.01 N hydrochloric acid at room temperature for 15 min.)

B. Alkali degradation

The drug was found to undergo alkaline degradation slower as compared to acid degradation. Initially 1 N sodium hydroxide at room temperature was used but drug was totally degraded, so by gradual decreasing the conditions at 0.01N NaOH at room temperature for 4 h, 10-20 % degradation was observed. Whereas a densitogram of base-degraded TDF showed two degradation peaks at R_f 0.45(% Area=12.55), at R_f 0.67(% Area=5.45) and TDF at (% Area=82.00) (Fig.V.).

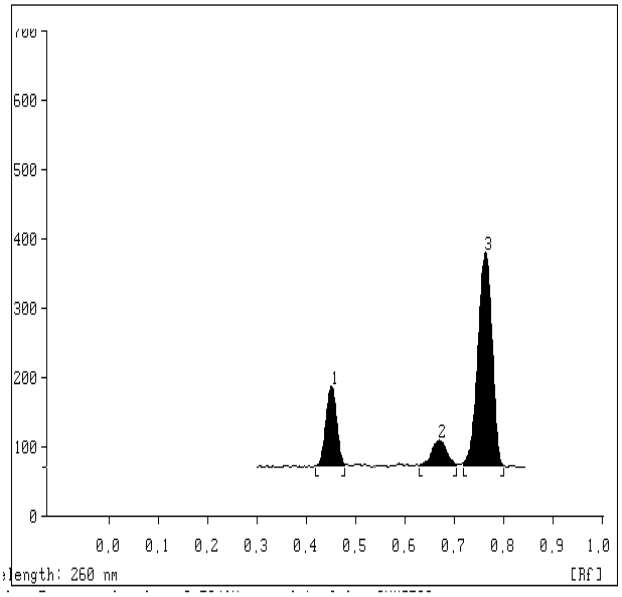

Fig.V. Densitogram of base degradation product 600 ng/spot. (Condition: 0.01 N sodium hydroxide at room temperature for 4 h.)

C. Neutraldegradation

No degradation was observed by refluxing the drug solution in water for 48 hours. Whereas a densitogram of neutral degraded TDF does not showed any degradation product only the peak area of TDF (98.38%) was reduced slightly.

D. Oxidative degradation

The drug was found to undergo oxidative degradation in 6 % H_2O_2. The reaction was carried out at room temperature for 24 h. The drug showed a degradation of around 10-20 %. Whereas a densitogram of oxidative-degraded TDF showed one major degradation peak, at *Rf 0.39* (% Area=11.75) and TDF at (% Area=88.25) (Fig.VI.).

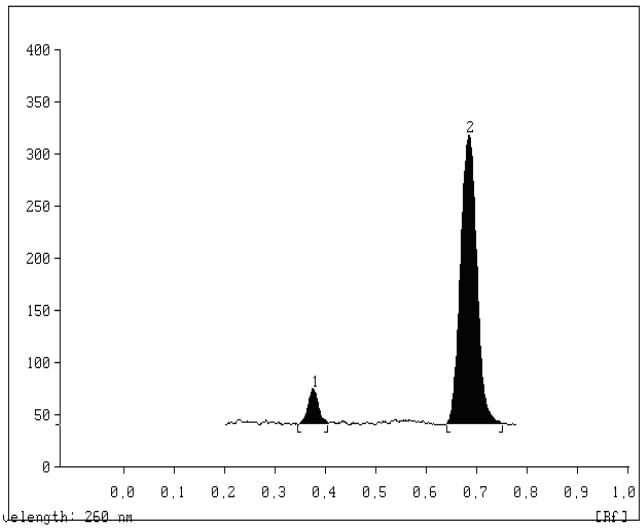

Fig.VI. Densitogram of hydrogen peroxide degradation product 600 ng/spot (Condition: 6 % hydrogen peroxide at room temperature for 24 h.)

E. Thermal Degradation

No degradation was observed in the drug treated at 60^0Cfor 48 hours. Whereas a densitogram of thermal degraded TDF does not showed any degradation product only the peak area of TDF (97.95%) was reduced slightly.

F. Photo Degradation

TDF was found to be stable to photo degradation as no degradation was seen after exposing drug to UV light for 10days. Peak area was found to be 98.87%.

Validation of the stability indicating method

The validation studies of the stability indicating method for TDF was carried out using optimized mobile phase and scanning conditions by HPTLC.

A. Linearity and range

For linearity and range of TDF by HPTLC, five concentrations 120 ng μl^{-1}, 240 ng μl^{-1}, 360 ng μl^{-1}, 480 ng μl^{-1}, 600 ng μl^{-1} were prepared from standard stock solution in optimized mobile phase. Each concentration was injected in six times. The TDF showed linear increase in area by increasing concentration in a range of 120 ng μl^{-1} to 600 ng μl^{-1}(Figure 7)with good correlation coefficient of (r^2=0.999). The average % RSD was in the acceptable limit (Table 1).

Table- 1: Data for TDF from linearity study.

Sr. No.	Concentration (ng $spot^{-1}$)	Average area	%RSD
1	120	651.66	1.65
2	240	1284	1.33
3	360	1778.33	0.52
4	480	2267.83	1.03
5	600	2911.66	1.21

Note: Each sample was applied six times (N=6).

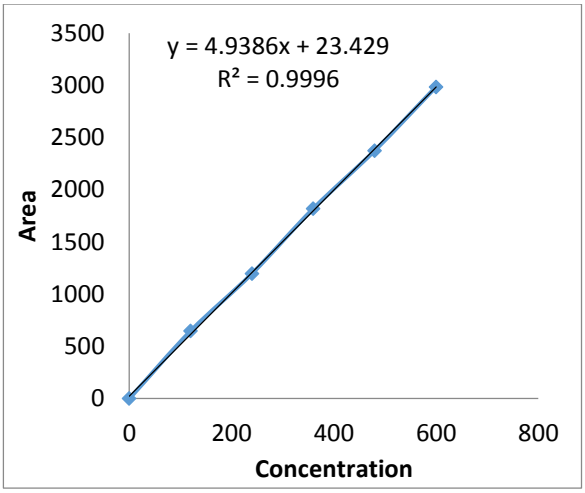

Fig.VII. Calibration curve for TDF by HPTLC.

B. Precision

The Intraday precision and Inter day precision experiments are shown in Table 2. The developed method was found to be precise as the RSD (%) values for Intraday precision and Inter day precision studies were < 2%, as recommended by ICH guideline.

Table- 2: Precision studies of proposed HPTLC method.

Concentration (ng/spot)	Measured concentration ± SD, RSD (%)	
	Intraday precision	**Inter day precision**
120	120.30 ± 1.46, 1.21	119.78 ± 1.23, 1.03
360	361.54 ± 4.81, 1.33	360.70 ± 5.14, 1.41
600	600.73 ± 2.13, 0.35	597.88 ± 4.53, 0.75

Note: Each sample was applied six times (N=6), and the result is expressed in Measured concentration ± SD, %RSD.

D. LOD and LOQ

The signal to noise ratios of 3:1 and 10:1 were considered as LOD and LOQ respectively. For HPTLC method the LOD and LOQ were found to be 40 ng $spot^{-1}$ and 100 ng $spot^{-1}$ respectively for TDF.

E. Robustness of the method

Robustness of the method was tested by small changes in parameters and the effects on the results were examined. The standard deviation of peak areas was calculated for

each parameter and % RSD was found to be less than 2%. The values of %RSD are (as shown in Table 3) indicates robustness of the HPTLC method.

Table- 3: Robustness testing of HPTLC method.

Parameter	SD of peak area	% RSD
Mobile phase composition (± 0.1 mL)	5.21	1.29
Amount of mobile phase (± 5%)	3.65	1.21
Time from spotting to chromatography (± 10 min.)	1.32	1.56
Time from chromatography to scanning (± 10 min.)	1.53	1.42

Note: Each sample was applied six times (N=6)

F. Specificity

The peak purity of TDF was assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9994 and r (M,E) =0.9998. Good correlation (r=0.999) was also obtained between standard and sample spectra of TDF.

G. Recovery Studies

Recovery studies of the drugs were carried out for the accuracy parameter. These studies were carried out at three levels i.e. multiple level recovery studies. Sample stock solutions in three 10 ml volumetric flasks in that 50 %, 100 % and 150 % of the standard drug solutions were added. Dilutions were made and recovery studies were performed. The average recovery of three levels was found that 99.799%, i.e.within the limits (Table 4).

Table- 4: Standard addition techniques for determination of TDF using HPTLC.

Drug	Label claim (mg/tablet)	Amt. Added (%)	Total amount (mg)	Amount recovered (mg)	Recovery (%)
TDF	300	50	450	450.7977	99.823
		100	600	601.7862	99.704
		150	750	750.9011	99.870

Note: Each sample was applied six times (N=6).

H. Stability in sample solution

Solution of three concentrations 120, 360, and 600 ng spot^{-1} for TDF were prepared from sample solution and stored at room temperature for 24h applied HPTLC plate, after development the densitogram there was no additional peak found.

Estimation of TDF from Pharmaceuticaldosage form (Assay)

Experimental results of the amount of TDF in tablets (using three concentrations i.e. 120 ng spot^{-1}, 360 ng spot^{-1}, and 600 ng spot^{-1}), expressed as percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any excipients, which are present in tablets. The drug content was found to be 98.99 % ± 0.33. Two different lots of commercially available TDF tablet were analyzed using the proposed procedures and the results are tabulated in Table 5.

Table- 5: Determination of TDF in commercial formulations by HPTLC.

Formulation (300 mg)	TDF found (mg per tablet)	
	Mean ± SD	Recovery (%)
1st Lot	**299.77 ± 1.03**	98.75
2nd Lot	299.17 ± 0.94	99.23

Note: Each sample was applied six times (N=6) and the result is expressed in Measured Mean concentration ± SD, %RSD.

CONCLUSION

The developed HPTLC method provide simple, accurate, reproducible and stability indicating for quantitative analysis for determination of TDF in pharmaceutical dosage form, without any interference from the excipients and in the presence of its acidic, alkaline, oxidative, dry and photolytic degradation products. The method was validated as per ICH guidelines. Statistical tests indicate that the proposed HPTLC method reduce the duration of analysis and appear to be equally suitable for routine determination of TDF in pharmaceutical dosage form in quality control laboratories, where economy and time are essential. This study is a typical example of development of a stability indicating assay, it is one of the rare studies where forced decomposition was done under all different suggested conditions and the degradation products were resolved. Hence it is proposed for the analysis of the drug

and degradation products in stability samples in industry. The method however, is not suggested to establish material balance between the extent of drug decomposed and formation of degradation products. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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REFERENCES

1. Emau P, Jiang Y, Agy M B, "Post-exposure prophylaxis for SIV revisited: Animal model for HIV infection" *AIDS Res Ther,* 2006, 3: 29.
2. Indian Pharmacopoeia, The Indian Pharmacopoeia Commission, Ghaziabad, Volume-III, 2007, 1782-1783.
3. Martindale: The Complete Drug Reference, Pharmaceutical Press, London, 2005, 34th Edition: 648, 655.
4. "WHO Model List of EssentialMedicines". *World Health Organization*. October 2013. Retrieved 22 April 2014.
5. Wlodawer A, Miller M, Jaskolski M, Sathyanarayana B K, Baldwin E, Weber I T, Selk L M, Clawson L, Schneider J, Kent S B, "Conserved folding in retroviral proteases: crystal structure of a synthetic HIV-1 protease." Science, 1989,11;245(4918):616-621.
6. Bojja S, Thimmaraju M K, NerellaRaghunandhan U V, "Spectrophotometric Method Development for Simultaneous Quantification of Tenofovir disoproxilfumarate and Lamivudine In slightly alkaline medium" International Journal of Pharmaceutical and Clinical Science, 2012; 2(1): 1-6
7. Behera A, Parida A, Meher A K, Sankar D G, Moitra S K, Chandra S S, "Development and Validation of Spectrophotometric method for determination of Emtricitabine and Tenofovir DisoproxilFumarate in Bulk and Tablet dosage form." International Journal of Pharm Tech Research, 2011, 3(3): 1874-1882.
8. Narendra D, Satyanarayana T, Ganga Rao B, "HPLC method development and validation for simultaneous estimation of tenofovir and emtricitabine in combined pharmacetuical dosge form." International Journal of Research in Pharmaceutical and Biomedical Sciences, 2012, 3 (1): 361-367.
9. N Appala R, Shabana B, "Simultaneous RP-HPLC Method for the Estimation of the Emtricitabine, Tenofovir DisoproxilFumerate and Efavirenz in Tablet Dosage Forms" *Research J. Pharm. and Tech., 2008, 1(4): 522-525*
10. Budagamlavanya, Perumalla H, Allumellu V, Dudipala P L, Maram R, "Simultaneous estimation of emtricitabine and Tenofovir disproxil fumerate by HPLC method", *Der Pharmacia Lettre, 2012, 4 (6):1855-1862*
11. VenkatReddiah C H, Rama Devi P, Mukkanti K, Srinivasu P, "Development and Validation of Stability Indicating HPLC Method for Combination Tablet Dosage Form of Efavirenz, Lamivudine and Tenofovir in Tablet" *International Journal of Pharmaceutical and Phytopharmacological Research,* 2012, 2(1): 40-45.
12. Chitlange S S, Kanthale S B, Choudhary B, Bhole R P "Stability Indicating HPTLC method for the Simultaneous estimation ofRilpivirin, Emtricitabine and Tenofovir in Bulk and CombinedPharmaceutical Dosage Form" Journal of Advanced Drug Delivery, 2014; 1(4); 157-172.
13. Rao J R, Gondkar S A, Yadav S S, "Simultaneous HPTLC–Densitometric analysis of Tenofovir and Emtricitabine in Tablet dosage form" International Journal of PharmTech Research, 2011, 3(3): 1430-1434