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Development and Validation of HPTLC Method for Estimation of Tenofovir Disoproxil Fumarate in Tablet Dosage Form.

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ABSTRACT:

A simple, rapid, precise and accurate HPTLC method for the estimation of Tenofovir disoproxil fumarate was developed and validated. The chromatograms were developed using a mobile phase of ethyl acetate : methanol : formic acid(7:2.5:0.5 %v/v) on pre-coated plate of silica gel GF aluminum TLC plate and quantified by densitometric absorbance mode at 266 nm. The R_f value for Tenofovir disoproxil fumarate was 0.78. The linearity of the method was found to be within the concentration range of 125-750 ng/spot. The lower limits of detection and quantification were found to be 7.90 ng/spot and 23.93 ng/spot. The method was also validated for precision, specificity and recovery. This developed method was used to analyze fixed-dose tablet (TENOF, Genix Pharma).

KEYWORDS: HPTLC, Tenofovir disoproxil fumarate.

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Introduction:

Tenofovir disoproxil fumarate, an anti retroviral agent (nucleoside reverse transcriptase inhibitor), (TNV) chemically it is 9-[(R)-2[[[bis [[(isopropoxy carbonyl)oxy]- methoxy]phosphinyl]methoxy]propyl]adenine fumarate (1:1). having molecular formula C₁₉H₃₀N₅O₁₀P .C₄H₄O₄ and a molecular weight 635.52. It is official in IP'2007 and IP' 2010.

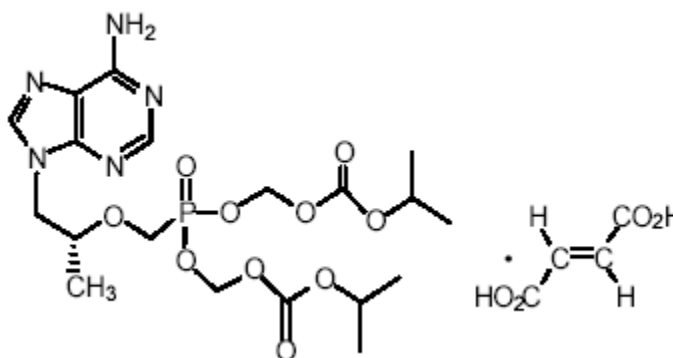


Figure 1. Structure of Tenofovir disoproxil fumarate

Tenofovir disoproxil fumarate has been reported to be quantified individually or in combination by spectrophotometric methods ^[1-4] and HPLC ^[5-9]. The literature survey reveals that here are analytical methods available for determination of Tenofovir disoproxil fumarate from biological matrices with combination of other

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antiviral drugs by RP-HPLC [10–12]. Chandra P. et al. [13] had reported HPTLC method for simultaneous estimation of lamivudine and tenofovir disoproxil fumarate in pharmaceutical dosage form using Merck HPTLC aluminum plates of silica gel 60 F254, (20 × 10 cm) with 250 micrometer thickness as a stationary phase and chloroform: methanol: toluene (8:2:2, v/v/v) as Mobile phase with detection at 265 nm. In this study, we report the HPTLC method for the analysis of Tenofovir disoproxil fumarate using a solvent system of ethyl acetate : methanol : formic acid(7:2.5:0.5 %v/v)

EXPERIMENTAL

Chemicals and reagents

Pure Tenofovir disoproxil fumarate powder was kindly gifted by Aurobindo Pharmaceuticals Ltd., Hyderabad, India. Commercial tablets (TENOF, Genix Pharma) containing Tenofovir disoproxil fumarate (300mg) were used for the study. ethyl acetate, formic acid and methanol used were of analytical grade (E.Merck, Mumbai, India). All the other chemicals used were also of analytical grade (E.Merck, India).

Instrumentation and conditions

HPTLC plates pre-coated with silica gel GF on aluminum TLC plate, (10cm X 10cm) were from Merck. Densitometry was carried out with a CAMAG TLC Scanner3, fitted with win-CATS 1.4.0 planar chromatography manager software. Samples were applied to the HPTLC plates using the spray-on technique of CAMAG LINOMAT V under nitrogen gas flow, and developed in a CAMAG 10cm X 10cm twin trough chambers.

Standard preparation

TNV (100mg) was accurately weighed and transferred into 100 mL volumetric flask, and dissolved in methanol. The volume was made up to the mark with methanol. The resulting stock solution was further 4 times diluted with methanol to get the final concentration of 250 mcg/mL TNV which was used for calibration purpose.

Preparation of sample solution

For analysis of tablet dosage form, twenty tablets, each containing 300 mg of TNV, were weighed and their average weight was calculated. The tablets were finely powdered and powder equivalent to 100 mg TNV was accurately weighed and transferred in to 100 mL volumetric flask. 60 mL methanol was added to it and shaken for 30 minutes. The volume was made up to the mark with methanol. The solution was sonicated for 30 min, filtered through the Whattmann No.41 filter paper. This solution was further diluted with methanol to get the same concentration as that of the final standard solution.

Chromatographic conditions

TNV reference standard solution was prepared using

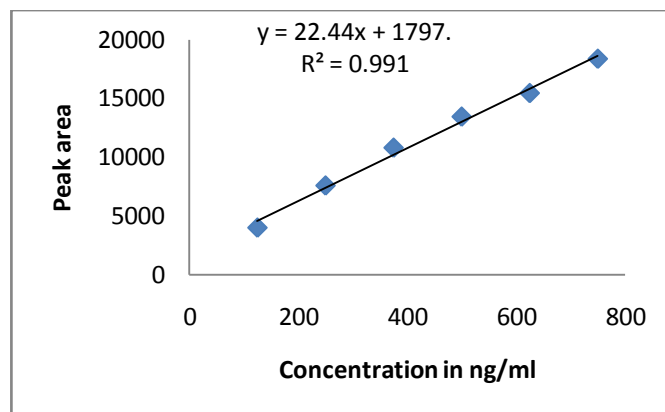


Figure 2. Calibration curve of Tenofovir disoproxil fumarate

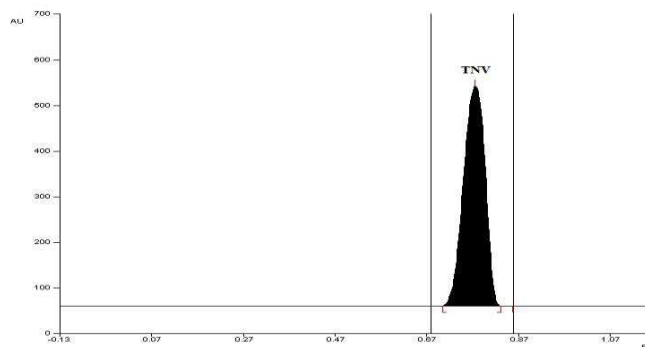


Figure 3. HPTLC chromatogram of Tenofovir disoproxil fumarate (750 ng/spot) standard with corresponding R_f at 266 nm

methanol as solvent. From the prepared standard solution, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 μ L aliquots were applied to the HPTLC plates as spot bands of 6mm using LINOMAT V. Application positions were at least 15mm from the sides and 10mm from the bottom of the plates. Mobile phase components were mixed prior to use and the development chamber was left for saturation with mobile phase vapors for 10min before each run. Development of the plate was carried out by the ascending technique to a migration distance of 7cm. Then the plates were dried on a hot plate. All the analysis was carried out in a laboratory with temperature control (20–24°C). Densitometry scanning was done in absorbance mode at 210 nm using a deuterium lamp. The slit dimensions were set at 6mm x 0.30 mm, the scanning speed of 10mm/s, and the data resolution at 100 mm/step. Single wavelength detection was performed since the main components were only analyzed.

Method validation

The developed method was validated as per the International Conference on Harmonization (ICH) [14,15] guidelines with respect to linearity and range, specificity, precision, accuracy, limit of detection and limit of quantification.

RESULTS AND DISCUSSION

Linearity and range

A stock standard solution (1000 mcg/mL) containing TNV was prepared in methanol and 4 times diluted. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 μ L aliquots of the diluted standard solution was applied to the HPTLC plate to deliver 125, 250, 375, 500, 625 and 750 ng of TNV per spot. This was done in triplicate and repeated for three days. For each concentration, the applied spot bands were evenly distributed across the plate to minimize possible variation along the silica layer. The results are indicated in Table 1.

Table 1 . Linearity results

Components	Concentration range (ng/spot)	Equation for regression line	R ²
Tenofovir disoproxil fumarate	125-750	$y = 22.44x + 1797$	0.991

Precision

The repeatability (intra-daysprecision) is expressed as percentage relative standard deviations (%RSD) for the TNV at the concentrations of 250, 375 and 500 ng/spot, their % RSD values were 0.89, 1.67 and 0.72, respectively, and for the time-different intermediate precision (inter-daysprecision) the % RSD values were 0.95, 1.36 and 1.56 respectively. The pooled repeatability precision was 1.09 for TNV concentrations and the pooled time-different intermediate precision was 1.29. The %RSD levels of intra-day and inter-day precision were less than 2.0 in all cases, which indicated that there were no significant variations in the analysis of TNV at the concentrations, which are shown in Table 2.

Accuracy

The accuracy was assessed by the methodological recovery. The recovery of the method was calculated by comparing the determined concentration of spiked samples to the theoretical concentrations. The mean percentage recovery for each compound was calculated at each concentration level and reported with its standard deviation. The intra-day and inter-day percentages of accuracy obtained for TNV at the concentrations of 250, 375 and 500 ng/spot are shown in Table2. The % recoveries of intra-day were $98.75 \pm 1.87\%$, $99.13 \pm 1.65\%$ and $101.34 \pm 1.39\%$ respectively. The mean recovery for all the concentration levels was $99.74 \pm 1.63\%$. The % recoveries of inter-day were $97.48 \pm 1.75\%$, $100.52 \pm 1.48\%$ and $98.86 \pm 1.94\%$ respectively. The mean recovery for all the concentration levels was $98.95 \pm 1.29\%$. (Table 2).

Limits of detection and quantification

The limit of detection was found by the equation $LOD = 3.3 \times \sigma/s$ as well as limit of quantitation was found by the equation

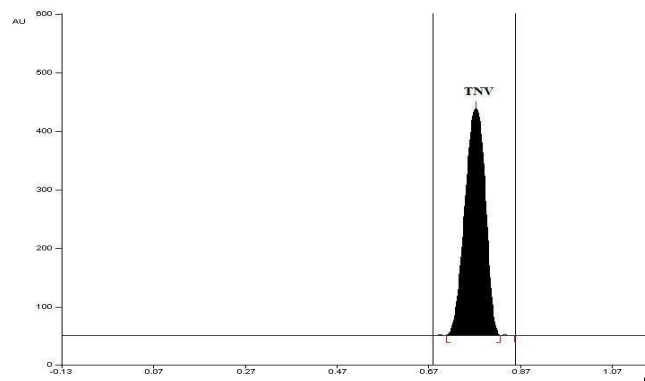


Figure.4. HPTLC chromatogram of Tenofovir disoproxil fumarate (500 ng/spot) tablet sample with corresponding R_f at 266 nm

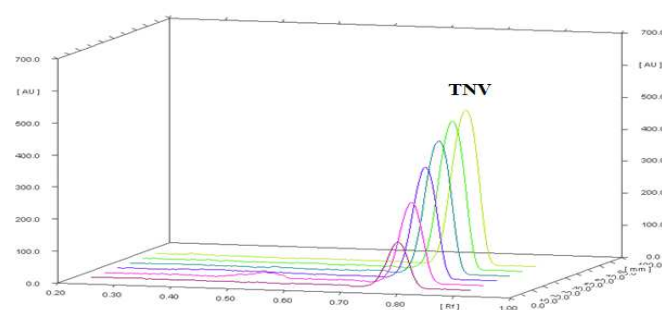


Figure.5. 3D Chromatogram showing peaks of Tenofovir disoproxil fumarate standards in different concentrations

$LOQ = 10 \times \sigma/s$. The limit of detection was found to be 7.90 ng/spot. The limit of quantification was found to be 23.93 ng/spot.

Specificity

The chromatogram of the solution, which was not spiked with TNV, did not show any spot, while the chromatogram of the solution of the tablet matrix spiked with TNV showed clear, compact and well- separated peak of TNV (Fig. 3 & 4). Moreover, from Fig. 3 & 4, it can be seen that no other peaks were eluted besides the active compound. The method was therefore considered to be specific.

Results of analysis of tablet formulation

Analysis of samples of marketed antiretroviral tablet containing TNV 300mg was carried out and the amounts recovered were expressed as a percentage amount of the label claims. The percentage recoveries in intra-day as well as interday precision were found to be $99.74 \pm 1.63\%$ and $98.95 \pm 1.29\%$ respectively, and these values are complying with the assay specifications for active drugs in the United States of Pharmacopoeia (90.0–110.0%), which are required to be met by most drug formulations.

Conclusion

A quick, precise and accurate method based on normal-phase HPTLC has been developed for routine analysis of TNV in fixed-dose tablets. The method was validated for linearity, precision, accuracy and specificity. It has the advantage over HPLC methods in general. It consumed less than 35mL of mobile phase per run (8 samples per plate), whereas HPLC methods would consume more than 50mL per runs of similar number of samples. If we consider the time from sample preparation to densitometric evolution for one plate, the new method took an average of 1h, whereas HPLC methods would generally take more than 2h for the same number of samples. It is cheap, quick and does not use chloroform, therefore suitable for routine analysis of TNV in fixed-dose tablets. When compared with the reported HPLC method, the developed HPTLC method is both time and cost effective for the determination of TNV in bulk and tablet dosage form.

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