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Validated HPTLC Method for the Determination of Tenofovir as Bulk Drug and in Pharmaceutical Dosage Form

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Abstract

A simple, accurate, precise and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of Tenofovir in tablet dosage forms. The method employed TLC aluminium plates precoated with silica gel 60 F 254 as the stationary phase. The mobile phase used was a mixture of (Chloroform: Methanol 8.5: 1.5v/v). The detection of spot was carried out at 270nm. The calibration curve was found to be linear between 200 to 1200 ng mL^{-1} with regression coefficient of 0.9994. The proposed method can be successfully used to determine the drug content of marketed formulation. The accuracy of the proposed method was determined by recovery studies and found to be 98.39 to 101.19 %. The proposed method is applicable to routine analysis of Tenofovir in bulk and pharmaceutical formulations. The proposed method was validated according to various ICH parameters like linearity, accuracy, precision, specificity, limits of detection, limits of quantification, range and solution stability.

Key words: Tenofovir, validation, ICH guidelines, HPTLC.

Introduction

Tenofovir is chemically [(2R)-1-(6-aminopurin-9-yl) propan-2-yl] oxymethyl phosphonic acid figure 1. It is a white crystalline powder used as antiretroviral agents, for the treatment of HIV infection. It has an empirical formula of $C_9H_{14}N_5O_4P$ and molecular weight of 287.2123. Tenofovir belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NtRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people[1]. Food and drug administration granted approval to market viread (TENOFOVIR) for the treatment of chronic hepatitis B[2]. Literature survey reveals that very few analytical methods has been established for the estimation of tenofovir and emtricitabine in bulk and in tablet dosage form by spectrophotometric method[3], Simultaneous determination of emtricitabine and Tenofovir by area under curve and dual wavelength spectrophotometric method[4], relevance of a combined UV and single mass spectrometry detection for the determination of tenofovir in human plasma by HPLC in therapeutic drug monitoring[5], segmented polyurethane intravaginal rings for the sustained combined delivery of antiretroviral agents dapivirine and tenofovir[6], simultaneous quantification of a non-nucleoside reverse transcriptase inhibitor efavirenz, a nucleoside reverse transcriptase inhibitor emtricitabine and a nucleotide reverse transcriptase inhibitor tenofovir in plasma by liquid chromatography positive ion electrospray tandem mass spectrometry[7], RP-HPLC method for the determination of tenofovir in pharmaceutical formulations and spiked human plasma[8], spectrophotometric determination of tenofovir disoproxil fumarate after complexation with ammonium molybdate and picric acid[9], quantitative analysis of tenofovir by titrimetric, extractive ion-pair spectophotometric and charge-transfer complexation methods[10].

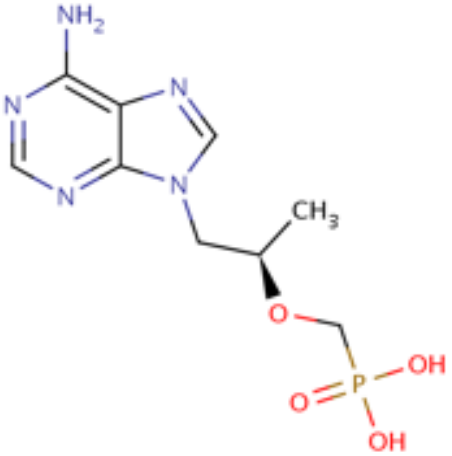


Figure-1
Chemical structure of Tenofovir

The objective of this work was to develop a new, simple, economic, rapid, precise, and accurate HPTLC method for quantitative analysis of tenofovir, and to validate the method in accordance with ICH guideline[11].

Material and Methods

Pure standard of tenofovir (assigned purity 99.98%) was obtained as a gift sample from Ranbaxy labs Pvt. Ltd, Gurgaon, India. The gift sample was used as standard without further purification. Silica gel 60 F 254 TLC plates (20x10cm) were used as stationary phase. All chemicals and reagents used were of analytical grade and obtained from qualigens. Commercial pharmaceutical preparation (viread) which was claimed to contain 300mg of tenofovir is used in analysis. The chemical structure and purity of the sample obtained was confirmed by TLC, IR, melting point studies.

Equipment: The instrument used in the present study was camag linnomat V- automatic sample applicator, hamilton syringe (100µl), camag TLC scanner 3, camag twin trough chamber of appropriate size, analytical weighing balance (Shimadzu AX 200), sonicator (model SONICA 2200MH) were used throughout the experiment. Camag wincats software was used for acquisition, evaluation and storage of chromatographic data.

Preparation of Standard Solution: A stock solution of drug was prepared by dissolving 100 mg of pure tenofovir in a 100 ml volumetric flasks containing sufficient amount of methanol to dissolve the drug, sonicated for about 15 min and then made up to volume with methanol (1 mg/ml). A standard solution was prepared by dilution of the stock solution with methanol to give in concentration of 100µg/ml. Further dilutions were made with methanol to give a solution in concentration range of 200-1200ng/ml.

Procedure for Sample Solution (From Formulation): Twenty tablets were weighed accurately and powdered. An amount of the powder equivalent to 300 mg of tenofovir (content of one tablet) was dissolved in sufficient amount of methanol to dissolve the drug, sonicated for about 15 min. and then filtered into a 100 ml volumetric flask trough 0.45 µm membrane filter. The residue was washed 3 times with 10 ml of methanol and then the volume was completed to 100 ml with the same solvent. Make further dilutions with methanol to obtain a stock solution of 10µg/ml. An aliquot of this solution (1 ml) was transferred to a 10 ml volumetric flask and made up sufficient volume with the methanol to give an expected concentration of 1 µg/ml.

Prewashing of TLC plates: HPTLC was performed on 20 cm × 10 cm precoated silica gel 60 F 254 TLC plates. The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack has been opened. The non-volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid possible interference from such impurities in quantitative analysis, plates were prewashed with methanol dried and activated for 30 min. at 110 C, with the plates being placed between two sheets of glass to prevent deformation of the aluminum during heating.

Procedure: A methanolic solution of tenofovir (1 mg/ml) was prepared. This solution was further diluted with methanol to yield a solution containing 1µg/ml. Different concentrations of tenofovir in a concentration range of 200-1200ng/ml were applied on plates as 8 mm bands, 8 mm apart and 1 cm from edge of the plate, by means of camag linomat V automatic sample applicator fitted with 100 µl hamilton syringe. A methanol blank was applied to parallel track. The mobile phase, chloroform: methanol 8.5: 1.5v/v was poured into the twin trough glass chamber and the glass chamber left to equilibrate for 10 min at 25 ± 2^0 C. After that the plate was placed in camag twin trough glass chamber. After development, the plate was removed from the chamber, dried in current of hot air, and scanned at 270 nm, using a deuterium lamp, by means of camag TLC scanner III densitometer. Densitograms were obtained by HPTLC of Tenofovir at various concentrations. This method was followed for all quantitative analysis. The Wincats software was used for data acquisition and processing of the plate. Peak height and peak area were integrated for the entire track. The calibration curve was established by plotting the obtained peak area on ordinate against corresponding concentration on abscissa.

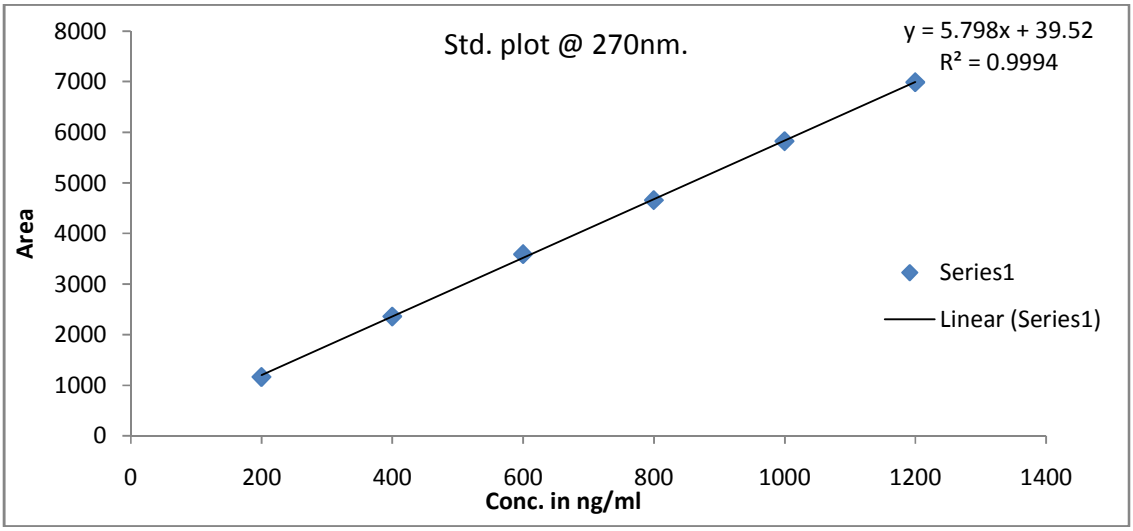
Validation of Analytical Method: Validation of an analytical method is process to establish by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

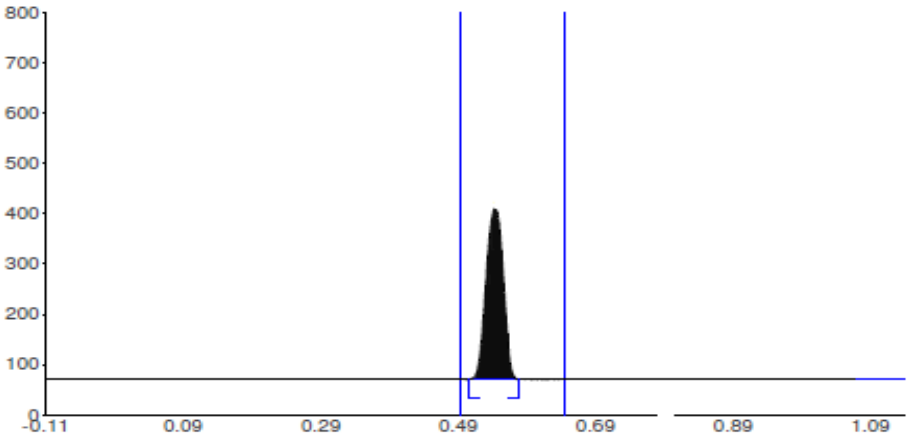
Typical analytical parameters used in validation area:

Linearity, Accuracy, Precision, Specificity Limit of detection, Limit of quantification Range, Solution stability

Linearity: Acceptance criteria: Coefficient of correlation (r2) should be greater than 0.998

Procedure: A stock solution of drug was prepared by dissolving 100 mg of pure tenofovir in a 100 ml volumetric flasks containing sufficient amount of methanol to dissolve the drug, sonicated for about 15 min and then made up to volume with methanol (1 mg/ml). A standard solution was prepared by dilution of the stock solution with methanol to give in concentration of 100µg/ml. Further dilutions were made with methanol to give a solution in concentration range of 200-1200ng/ml (graph-1 and chromatogram-1)





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.51	2.0	0.54	340.7	100.00	0.58	0.9	6989.9	100.00

Graph-1
Chromatogram-1

Results and Discussion

Correlation coefficient (r^2) for Tenofovir was found to be 0.9994, indicating the linearity and the method is linear between the concentrations of 200-1200ng/ml with Rf value 0.54±0.01.

Accuracy: The accuracy is the closeness of the measured value to the true value of the sample. To evaluate the accuracy of the method, known amount of pure drug was added to the previously analyzed solution containing pharmaceutical formulation and the mixture was analyzed by the proposed method and the recoveries were calculated. Accuracy was found out by recovery study from prepared solution (three replicates) with standard solution, of the label claim. Aliquots of 0.1 ml, 0.3ml and 0.5 ml of sample drug (Tenofovir) solution of 10µg/ml were pipetted into each of three volumetric flasks. To this 0.3 ml of standard drug (Tenofovir) solution of 10µg/ml was added to each volumetric flask respectively. The volume was made up to 10 ml with methanol. The range of recovery studies were found between 99.39 to 101.19 %. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients table 1.

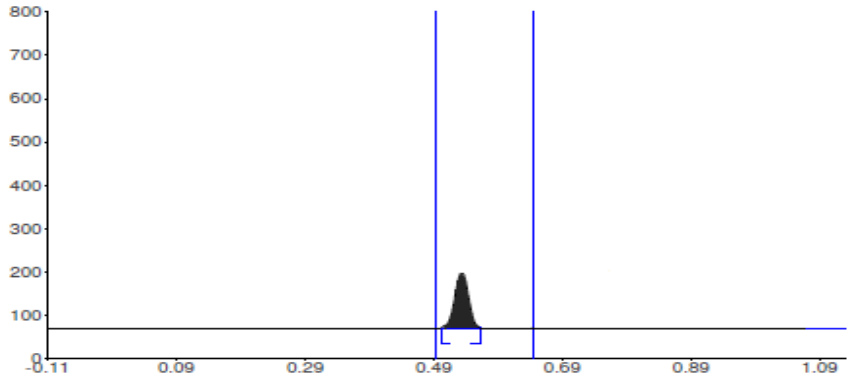
Table-1

*Average of three readings

Conc. taken in ng/ml (A)	Std addition in ng/ml (B)	Total drug conc. in ng/ml (A+B)	Peak Area*	% Recovery	Average	% RSD
100	300	400	2316.8	98.39	99.79333	1.402911
300	300	600	3580.9	99.8		
500	300	800	4709.8	101.19		

Table-2

S.No.	Area Response
1	1092.6
2	1108.3
3	1115.9
4	1095.2
5	1098.1
Average	1102.02
S.D	8.748806
R.S.D	0.793888



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.50	1.8	0.54	165.2	100.00	0.57	0.2	2388.92	100.00

Chromatogram-2

Result: The percentage recovery by the proposed method was ranging from 98.39 to 101.19% indicating no interference of the tablet excipients with drug under analysis.

Precision: Precision is measure of repeatability or reproducibility and it was determined by injecting 5 times the expected operating range concentration. The chromatograms were recorded to determine mean standard deviation and relative standard deviation table-2

Acceptance criteria: RSD < 2.0% for peak area.
Result: From the above analytical data it is observed that RSD for the assay is 0.793 which indicates that the method is precise and reproducible.

Specificity: Specificity is the ability to assess the analyte in the presence of components that may be expected to be present in the sample matrix (USP 2004). For demonstrating the specificity of the method for drug formulation the drugs was spiked and observe the chromatogram (chromatogram no.2).

Result: The excipients used in different formulation products did not interfere with the drug peak and thus, the method is specific for tenofovir.

Limits of detection and quantification: The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio of 2:1 or 3:1. The lower limit of detection for tenofovir is 4.123ng/ml in reference material and formulation. Limit of quantification (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10:1 can be taken as LOQ of the method. The LOQ values were found to be 12.494ng/ml for raw material and formulations.

Range: The specific range derived from the linearity studies. The range was calculated from the linearity graph. From the lower to higher concentration between which the response is linear, accurate and precise. Acceptance criteria: RSD < 2.0 The range for tenofovir was found to be 200-1200 ng/ml.

Solution Stability: The solution stability of the standard and sample prepared in methanol was studied for 5 days at bench top. The solution under study was compared with freshly prepared standard solution, the samples were found to be stable for period of more than 72 hours.

Conclusion

The proposed HPTLC method is found to be accurate, precise, linear, stable, specific, and simple, for quantitative estimation of Tenofovir in raw material and pharmaceutical formulations. Hence the present HPTLC method is suitable for routine assay of Tenofovir in raw materials and in pharmaceutical formulations in the quality control laboratories.

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