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RP – HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ANALYSIS OF TENOFOVUIR IN PHARMACETICAL DOSAGE FORMS

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Abstract:

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of TENOFOVUIR in tablet dosage form. Isocratic elution at a flow rate of 1ml min^{-1} was employed on a symmetry C18 column at ambient temperature. The mobile phase consisted of Methanol:Acetonitrile:OPA 85:10:05 (v/v/v). The UV detection wavelength was at 260nm.Linearity was observed in concentration range of 5-35ppm. The retention time for TENOFOVUIR was 2.3 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of TENOFOVUIR in pharmaceutical dosage forms.

Key Words:

Tenofovir, HPLC, Development, 260nm.

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Introduction

Tenofovir disoproxil fumarate (TDF or PMPA[1]), marketed by Gilead Sciences under the trade name Viread, belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NRTIs), which block reverse transcriptase, an enzyme crucial to viral production.

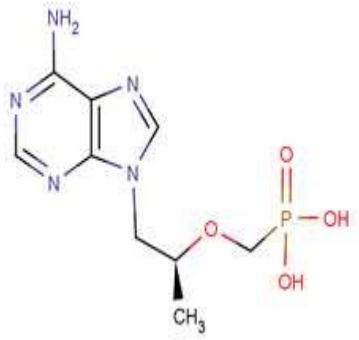


Figure 1: Stricture of Tenofovir.

Tenofovir disoproxil fumarate is a pro-drug form of tenofovir. Tenofovir is also available in a fixed-dose combination with emtricitabine in a product with the brand name Truvada for once-a-day dosing. Atripla, a fixed-dose triple combination of tenofovir, emtricitabine and efavirenz, was approved by the FDA on 12 July 2006 and is now available, providing a single daily dose for the treatment of HIV.

Tenofovir was discovered through a collaborative research effort between Antonín Holý at the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic (IOCB) in Prague, and Erik DeClercq, Rega Institute for Medical Research, Catholic University of Leuven, Belgium.Tenofovir was approved by the U.S. Food and Drug Administration (FDA) on October 26, 2001 for the treatment of HIV, and on August 11, 2008 for the treatment of chronic hepatitis B.[2][3]A difficult step in the manufacture of tenofovir is near the end, when the mixture is "like oatmeal, making it very difficult to stir," said Joseph Fortunak, who left Abbott Laboratories to teach at Howard. That slows the next reaction, a problem because the intermediary is highly unstable and decomposing, lowering the yield.

Fortunak's graduate student Adrian Williams tested different methods to improve this step. A catalyst, TBAB (tetrabutylammonium bromide) sped up the reaction and thinned the oatmeal-like mixture into something "like milk," Williams said. But unexpectedly, it made the product more stable, which substantially increased the yield. This lowered the cost by about 20%.[4]Tenofovir is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults. This indication is based on analyses of plasma HIV-1 RNA levels and CD4 cell counts in controlled studies of tenofovir in treatment-naive and treatment-experienced adults. There are no study results demonstrating the effect of tenofovir on the clinical progression of HIV. It also has activity against wild-type and lamivudine-resistant HBV.

The most common side effects associated with tenofovir include nausea, vomiting, diarrhea, and asthenia. Less frequent side effects include hepatotoxicity, abdominal pain, and flatulence.[5] Tenofovir has also been implicated in causing renal toxicity, particularly at elevated concentrations.[6]Tenofovir can cause acute renal failure, Fanconi syndrome, proteinuria

or tubular necrosis. These side effects are due to accumulation of the drug in proximal tubules. Tenofovir can interact with didanosine by increasing didanosine's concentration. It also decreases the concentration of atazanavir sulfate.Tenofovir may be measured in plasma by liquid chromatography. Such testing is useful for monitoring therapy and to prevent drug accumulation and toxicity in patients with renal or hepatic impairment.[7][8][9]

A 2006 trial by Family Health International gave either tenofovir or a placebo to 936 high-risk women in Cameroon, Ghana and Nigeria. While the results show signs that the tenofovir group contracted HIV at a reduced rate, the researchers cautioned against drawing conclusions from the study because the sample size was so small.[10] [11] In July 2010, a vaginal gel containing tenofovir was shown to reduce HIV infection rates by 39 percent in the CAPRISA 004 trial conducted in South Africa.[12]In July 2011, a placebo-controlled trial in Africa of daily tenofovir was shown to reduce HIV infection rates by 62%. [13]

Experimental Details:

Chemicals and reagents

All HPLC solvents used like Acetonitrile, Methanol, THF which are of HPLC grade were purchased from E.Merck

Instrumentation and analytical conditions: The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Inertsil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing.

Isocratic elution with Methanol,Acetonitrile,,OPA 85:10:05 (v/v/v) was selected with a flow rate of 1.3 ml min^{-1}.The detection wavelength was set at 260 nm with a runtime of 6 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Preparation of Stock, working standard solutions and Sample solutions:

100mg of TENOFOVUIR was weighed and transferred (working standard) into a 100ml volumetric flask. The diluent methanol was added and sonicated to dissolve it completely and made up to the mark with the same solvent. Further 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with diluent.

The contents were mixed well and filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. The calibration curve was plotted with the concentrations of the 35 to 5 ppm working standard solutions. Calibration solutions were prepared and analyzed immediately after preparation.

The formulation tablets of TENOFOVUIR were crushed to give finely powdered material. Powder equivalent to 10 mg of drug was taken in 10 ml of volumetric flask containing 5 ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 20 ppm.

Method Validation procedure:

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, robustness and system suitability.

Linearity

The developed method has been validated as per ICH guidelines (Zucman D, 2007). Working standard solutions of TENOFOVUIR in the mass concentration range of 5 ppm to 35 ppm was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of TENOFOVUIR was obtained by plotting the peak area ratio versus the applied concentrations of TENOFOVUIR. The linear correlation coefficient was found to be 0.999

S.NO	CONC ppm	AREA
1	5	54099.0
2	10	101366.4
3	15	162403.6
4	20	216217.3
5	25	264292.6
6	30	329940.3
7	35	367243.3

Table 1: Linearity of TENOFOVUIR

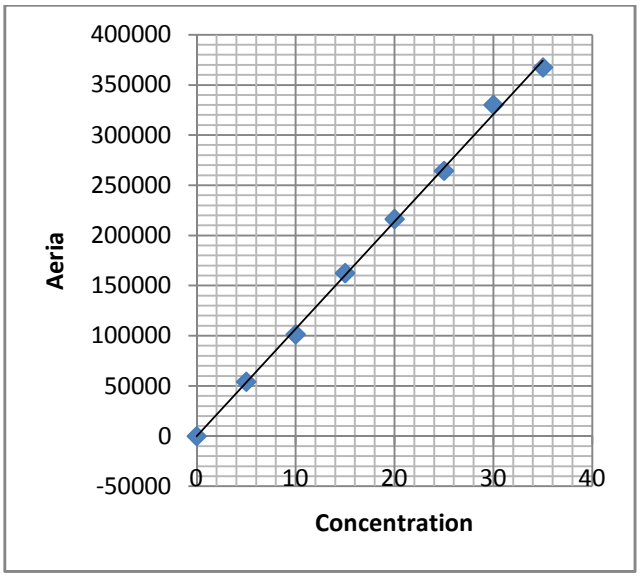


Fig 2: Calibration curve of TENOFOVUIR

Drug	TENOFOVUIR
Concentration range	35-5ppm
Slope (m)	1.8669
Intercept (b)	0.00098
Correlation coefficient	0.999
% RSD	0.50

Table.2 Linear Regression Data for Calibration curve

Precision

Repeatability of the method was checked by injecting replicate injections of 20 ppm of the solution for six times on the same day as intraday precision study of TENOFOVUIR and the RSD was found to be 0.43

Injection	**Concentration**	**Peak area**
1	35 ppm	363167.8
2	35 ppm	365748.1
3	35 ppm	367514.5
4	35 ppm	365546.3
5	35 ppm	367777.0
6	35ppm	364752.9

Table 3: Precision parameters of TENOFOVUIR

Accuracy:

The accuracy of the method was determined by calculating recovery of TENOFOVUIR by the method of standard addition. Known amount of TENOFOVUIR (10ppm, 20ppm and 30ppm) was added to a pre quantified sample solution and the amount of TENOFOVUIR was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of TENOFOVUIR was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Recovery	Conc. of sample	Recovery	% of recovery
50%	10ppm	9.98	99.8
100%	20ppm	20.04	100.1
150 %	30ppm	29.93	99.77

Table 4: Accuracy results of TENOFOVUIR

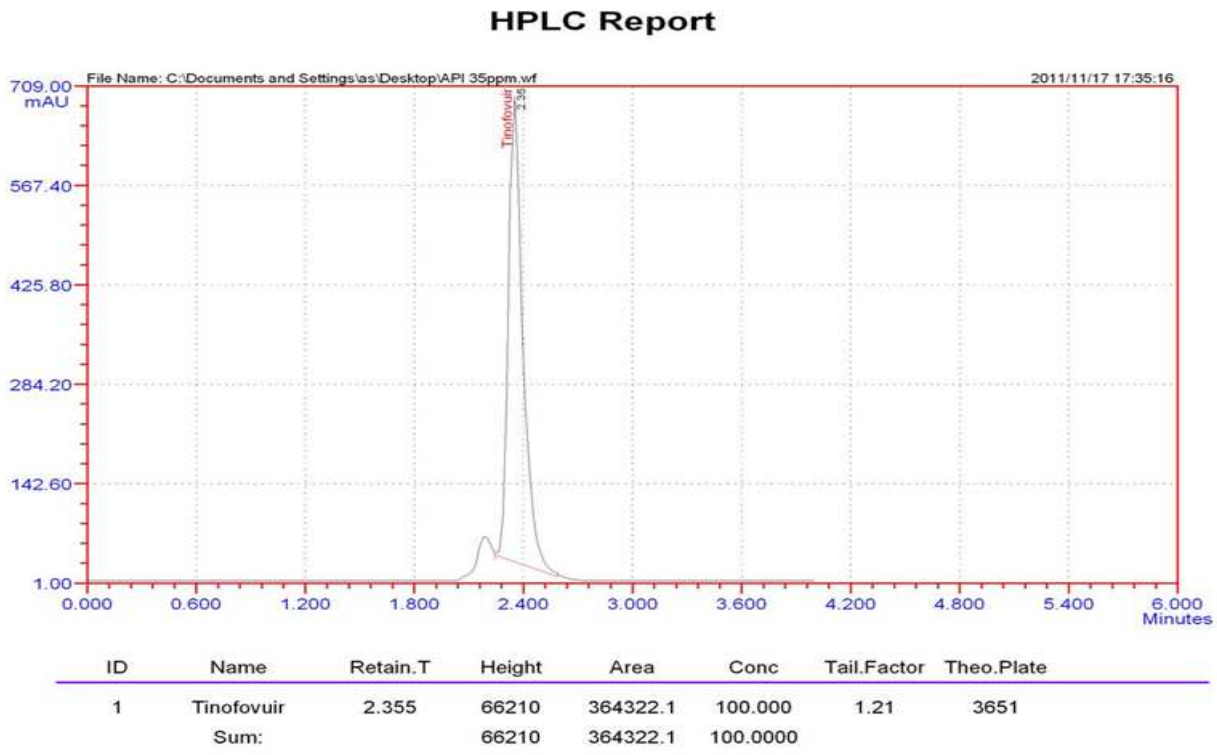


Figure 3: Typical chromatogram of TENOFOVUIR

Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug.

LOD and LOQ:

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 10 ng/ml and 35ng/ml respectively as per ICH guide-lines. Results are shown in table.

Parameter	Measured
LOD	10ng/ml
LOQ	35ng/ml

Table 5: Results of LOD and LOQ

Robustness:

To determine the robustness of the method, two parameters from the optimized chromatographic conditions were varied. Results of Robustness are shown in table 6.

Ruggedness:

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation.

Parameter	Modification	Peak Area	% of change
Mobile Phase	Methanlo: Acetonitrile : 0.1% OPA (80:15:5)	328964	0.29
P^H	5.6	328697	0.376
Wavelength	267	330974	0.33

Table 6: Robustness results

System Suitability Parameter:

System suitability tests were carried out on freshly prepared standard stock solutions of TENOFOVUIR and it was calculated by determining the standard deviation of TENOFOVUIR standards by injecting standards in six replicates at 6 minutes interval and the values were recorded.

Parameters	**Values**
λ max (nm)	260
Beer's law limit (µg/ml)	35 – 5ppm
Correlation coefficient	0.999
Retention time	2.3 min
Theoretical plates	367432
Tailing factor	1.22
Limit of detection	10ng/ml
Limit of quantification	35 ng/ml

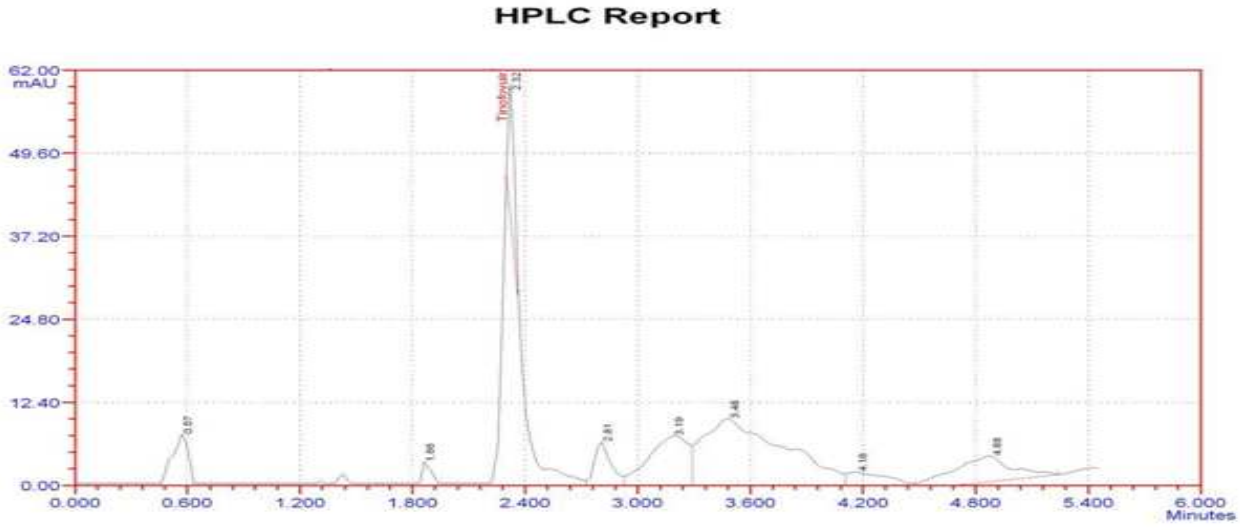
Table7: System suitability parameters of TENOFOVUIR

Results and Discussion

Optimization of the chromatographic conditions

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug TENOFOVUIR being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. The concentration of the methanol and Acetonitrile were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol,Acetonitrile,OPA 85:10:05 (V/V/v). The retention time of TENOFOVUIR was found to be 2.3 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability and validation parameters are given in Table 4. The high percentage of recovery of TENOFOVUIR was found to be 99.65 indicating that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of TENOFOVUIR in tablet formulation. The result for TENOFOVUIR was comparable with a

corresponding labelled amount (Table 5). The absence of additional peaks indicates no interference of the excipients used in the tablets.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		0.570	1312	11635.8	10.189	0.78	82
2		1.862	1112	12353.5	10.818	0.76	560
3	Tinofovuir	2.320	2050	4761.2	4.169	1.05	19893
2		2.807	1194	9748.1	8.536	1.25	2356
3		3.189	1173	19854.2	17.386	0.70	707
4		3.484	1341	42328.3	37.067	2.10	243
7		4.164	351	4837.1	4.236	2.91	1819
8		4.882	381	8676.9	7.598	1.00	916
	Sum:		8914	114195.0	100.0000		

Figure 5: Typical chromatogram of TENOFOVUIR Formulation

Formulation	**Tablet dosage**	**Sample concentration**	**Amount of drug estimated**	**% of TENOFOVUIR in Tablet**
TENTIDE	300 mg	30 ppm	29.73 ppm	99.06

Table 8: formulation results of TENOFOVUIR

Conclusion:

A validated RP-HPLC method has been developed for the determination of TENOFOVUIR in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of TENOFOVUIR in pharmaceutical dosage form.

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