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A new RP-HPLC method for the determination of Tenofovir Disoproxil Fumarate in pure form and pharmaceutical formulation

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ABSTRACT

The present study aimed to develop the new method for the estimation and validation of tenofovir in pure form and in pharmaceutical dosage form by RP-HPLC. The chromatogram of tenofovir was developed through column (Hyper ODS2 C18), UV detection at 260 nm at a flow rate of 1.2 ml/min with Methanol and Phosphate buffer (90:10) as mobile phase. The method was validated by various validation parameters such as accuracy, precision, linearity, specificity as per the ICH guidelines. A linearity range and retention time of Tenofovir were found to be 20-110 µg/ml and 2.1 min respectively. The % RSD of the Tenofovir was found to be 0.7. The % recovery was obtained as 99.7% for standard and 96.32% for tablets. This method was simple, accurate, precise and sensitive. Hence, the developed method was employed for the routine analysis of Tenofovir in the pharmaceutical dosage form.

Keywords: Analytical method; Estimation; Tenofovir; HPLC.

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INTRODUCTION

Tenofovir disoproxil fumarate (TDF) is chemically 9-((R)-2- ((bis (((isopropoxycarbonyl) oxy] methoxy) phosphinyl) methoxy) propyl) adenine [1,2]. It is an antiretroviral drug used to treat AIDS and hepatitis-B. TDF (Figure 1) is an active form of tenofovir that exists as a foremost form due to its lesser oral bioavailability of tenofovir. TDF is available as a fixed-dose combination with numerous antiretrovirals such as Efavirenz, Cobicistat, Emtricitabine, Elvitegravir, Lamivudine, Nevirapine, and Rilpivirine[3-6]. Therefore, pharmaceutical analysis of TDF in bulk and in pharmaceutical dosage form by RP-HPLC is very important. There are very few analytical methods was reported for the estimation of tenofovir disoproxil such as sensitive determination of tenofovir in human plasma samples using RP-HPLC[7], development and validation of a sensitive LC-MS/MS method for the determination of TD in human plasma[8] . These methods are costly, time consuming as well as complicated relatively than a simple RP-HPLC method. The aim of the present study was to develop the new method for the estimation and validation of tenofovir in pure form and in pharmaceutical dosage form by RP-HPLC.

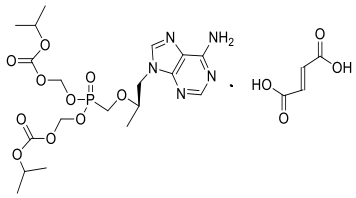

Figure 1: Structure of Tenofovir Fumarate

MATERIALS AND METHODS

Chemicals and reagents

Tenofovir disoproxil fumarate and its tablets (Viread) were procured from Cipla pharmaceuticals. HPLC grade Methanol and Water were purchased from Leonid Chemicals Pvt Ltd and Merck Specialty Pvt Ltd. All the other chemicals and reagents used were of AR grade and purchased from Finar chemicals limited and Fisher Scientific India Pvt Ltd.

Instrumentation

High performance liquid chromatographic system (Analytical-2230) consisting of a pump, an injector, a

Column **(**Hyper ODS2 C18) equipped with UV-Visible detector and A2000 data system software was used. Ultrasonic cleanser was used for sonication and Elico pH meter was used for adjusting the pH of the buffer.

Preparation of Tenofovir solution

Weighed accurately about 100 mg of Tenofovir into 100 ml volumetric flask, added with a minimum quantity of methanol, sonicated to dissolve and further diluted to 100ml with methanol. 1ml of this solution was diluted to 10 ml with methanol (100 µg ml^{-1}). Then it was filtered through 0.45µ PVDF membrane filter by discarding the first 5 ml of the filtrate.

Preparation of Buffer solution

Weighed accurately about 3.4 g of Potassium dihydrogen phosphate and transferred into a 500 ml volumetric flask. It was dissolved completely and the volume was made up to the mark with HPLC Water. Then the pH of the solution was adjusted to 5.0 with Glacial acetic acid. Finally, it was sonicated and filtered through 0.45µm PVDF membrane filter.

Method selection

Selection of wavelength[9]

Weighed accurately about 100mg of Tenofovir into 100ml volumetric flask and was dissolved in 100ml of HPLC methanol. 0.6ml of this solution was diluted to 10 mL with methanol (60µg ml^{-1}). Then it was filtered through 0.45µ PVDF membrane filter by discarding the first 5 ml of the filtrate. It was scanned on a UV-Visible spectrophotometer between wavelength ranges of 200 to 400 nm.

Preparation of mobile phase[10]

HPLC Methanol and Phosphate buffer (PH-5) were mixed in a 90:10 ratio and the resulting solution was sonicated on a sonicator for 30 min, then finally filtered through a 0.45µm membrane filter and used.

Preparation of standard drug stock solution

Standard drug stock solution of Tenofovir disoproxil fumarate was prepared by dissolving accurately 100 mg of the pure drug (Pharmaceutical grade) in 100 ml of HPLC grade Methanol to get 1mg/ml concentration. This solution was then sonicated, filtered and used to prepare further dilutions.

General procedure for construction of the calibration curve[11]

Aliquots of (0.2-1.5ml) the standard drug stock solutions (1mg/ml) were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with methanol. This solution was sonicated, filtered and 20µl of this solution was injected into HPLC and analyzed. The calibration curve was constructed from 20-150µg concentrations by plotting the peak area ratios of analyte versus the respective drug concentration.

Method development

Procedure for standard

An accurately weighed portion of 100mg of TDF was dissolved in 50mL of methanol into a 100 ml volumetric flask by sonication for 30 min with intermittent vigorous shaking. The final volume was made up to the mark with methanol to get a stock solution of 1mg/ml. This solution was filtered through 0.45 µm filter. Aliquots of (0.3-0.7ml) the standard drug stock solutions (1mg/ml) were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with methanol. All the concentrations were sonicated, filtered and 20µl of each solution was injected into the column. All measurements were repeated 6 times for each concentration.

Procedure for the tablets –standard addition method[12,13]

20 tablets were accurately weighed and powdered. An accurately weighed portion of powder equivalent to 100 mg of TDF was extracted in 50 ml of methanol into a 100 ml volumetric flask by sonication for 30 min with intermittent vigorous shaking. The final volume was made up to the mark with methanol to get a stock solution of 1mg/mL. This solution was filtered through 0.45 µm filter. Aliquots of (0.15-0.55 ml) the standard drug stock solutions (1mg/ml) were transferred into series of 10 ml volumetric flasks and 0.2ml of sample solution (tablets) was added to each flask and the volume was made up to the mark with methanol. All the flasks were sonicated, filtered and 20µl of each solution was injected into the column. All measurements were repeated 6 times for each concentration. The amount of Tenofovir per tablet was calculated from the calibration curve.

RESULTS AND DISCUSSIONS

Absorption spectra

The absorption spectra of the Tenofovir were measured in the range of 200-400nm against the blank solution as shown in Figure 2. Tenofovir shows maximum absorbance at 260 nm and it was selected as the detection wavelength for the HPLC investigation. Linearity, accuracy, precision, sensitivity and stability of the proposed methods were described and these developed methods applied to pharmaceutical preparations as tablets and obtained results were evaluated statistically.

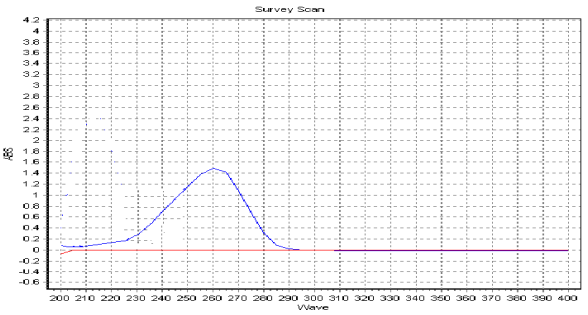

Figure 2: UV spectrum of Tenofovir

Table 1: Statistical data of the regression equations for the determination of TDF

Parameter	TDF
λ_{max}	260nm
Linearity range (µg/mL)	20-110
Regression equation (y)	-19198+20885x
Intercept (b)	-19198.6
Slope (a)	20885.7
Correlation coefficient (r)	0.999
SD	660516
LOD (µg /mL)	0.104
LOQ (µg /mL)	0.316

Optimized method

Satisfactory elution of the peak for Tenofovir was obtained with a solvent system of Methanol and Phosphate buffer of pH-5 adjusted with glacial acetic acid (90:10). Finally the method was optimized by selecting the mobile phase, HPLC Methanol: Phosphate buffer of pH-5 (90:10) due to its lower retention time and lower cost of solvents.

Linearity and range

Beer's law range, regression equation and correlation coefficient determined for the given method are shown in Table 1. A linear relationship was found between the Peak area at λmax and the concentration of the drug in the range of (20-110µg ml^{-1}) in the final measured volume of 10 ml. Regression analysis of Beer's law plots at λmax reveals a good correlation. The graph shows negligible intercept and is described by the regression equation, Y= aX + b (where *a* is the slope, *b* is the intercept and *X* is the concentration of the measured solution in µg ml^{-1}) obtained by the least-squares method. The calibration curve for the proposed methods is shown in Figure 3.

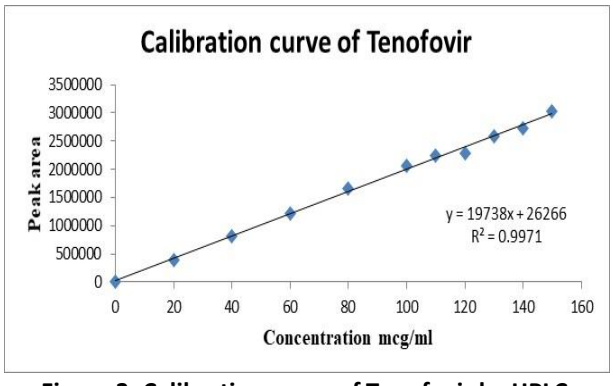

Figure 3: Calibration curve of Tenofovir by HPLC

Precision studies

For standard

The intra-day precision was determined by analyzing standard solutions of 30,50,70µg/ml (Figure 4) for six times on the same day while inter-day precision was determined by analyzing corresponding standards on the other day for 6 times and the results were shown in Table 2.

For tablet

The intra-day precision was determined by analyzing the tablet solutions of 35-75µg/ml, which were prepared by using the standard addition method for six times on the same day and the results were shown in Table 2.

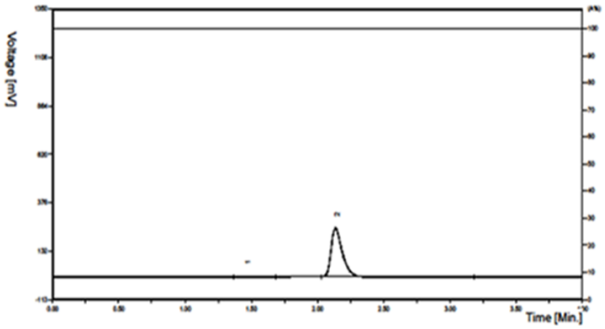

Figure 4: HPLC spectrum of Tenofovir

Table 2: Precision study of Tenofovir

Sl.no.	Concentration of Tenofovir (µg/ml)	Intraday precision peak area	Interday precision peak area
1.	30	632911.51	655475.68
		636302.65	590331.85
		610313.85	599643.68
		657647.92	644684.03
		632076.16	620312.42
		573104.47	618320.16
2.	50	991438.10	956091.89
		989633.22	942402.24
		961720.33	949925.62
		1004076.06	929423.79
		1010727.19	943264.03
		984621.29	938177.76
3.	70	1487887.15	1394955.95
		1431215.20	1407791.75
		1440358.49	1420146.69
		1429331.11	1398347.88
		1434687.78	1397881.67
		1433880.68	1430310.45

Validation of the methods

Procedure for the standard

Samples of standard TDF were prepared and tested at three levels (30, 50, 70 µg/ml) according to the proposed method. The complete set of validation assays were performed for the standard drug in intra and inter days. The precision and accuracy of the proposed method were tested by analyzing six replicates of the standard drug. The standard deviation, relative standard deviation, recovery and 95% confidence limits of the proposed method are recorded in Table 3 & 4. The average percent recoveries obtained were quantitative (93.96–100.7%), indicating the good accuracy of the method.[14]

Table 3: Tenofovir tablet analysis

Concentration (µg/ml)		Peak area	Average ± SD	%RSD
Standard	Sample			
15	20	668343.49 659959.55 660067.72 668958.57 675551.25 672815.87	667616.08 ± 6448.82	0.97
25	20	894270.69 892690.34 939895.73 871019.73 867807.49 878489.40	890695.56 ± 26447.72	2.97
35	20	1129817.70 1073399.67 1140276.12 1069100.42 1070303.49 1122652.51	1100924.99 ± 33357.18	2.97
45	20	1261498.08 1234830.97 1247823.91 1247707.88 1244232.05 1267695.88	1250631.46 ± 11969.26	0.96
55	20	1431897.31 1465355.78 1426710.96 1403244.45 1407260.23 1478436.77	1435484.25 ± 30538.71	2.13

Table 4: Analysis of TDF in bulk powder by HPLC (n=6) intraday

Method	Conc µg/ml		S.D	Recovery (%)	Precision[a] R.S.D (%)	Accuracy ER%	Cofidence limits[b] (95%)
	Taken	Found					
HPLC	30	30.61 30.77 29.52 31.81 30.57 30.16 28.00	1.30	100.7	4.3	0.7	28.846-31.574
	50	49.39 49.30 47.91 50.02 50.35 49.05	0.85	98.66	1.72	-1.34	48.438-50.222
	70	71.97 69.22 69.67 69.13 69.39 69.35	1.08	99.6	1.55	-0.31	68.647-70.913

n, number of determination, % R.S.D, %, percentage relative standard deviation; Er %, percentage relative error. [a]Mean of six determinations.[b]Confidence limit at 95% confidence level and five degrees of freedom

Table 5: Analysis of TDF in bulk powder by HPLC (n=6) interday

Method	µg/ml			S.D	Recov-ery (%)	Preci-sion[a] R.S.D (%)	Accuracy ER%	Cofidence limits[b] (95%)
	Taken	Added	Found					
HPLC	30	31.70 28.55 29.00 31.18 30.00 29.90	1.21	100.16	4.04	0.166	28.776-31.324	30
	50	47.63 46.95 47.32 46.30 46.99 46.74	0.46	93.96	0.97	-6.04	46.497-47.463	50
	70	69.50 70.14 70.75 69.67 69.64 71.26	0.70	100.22	1.007	0.228	69.417-70.903	70

Table 6: Evaluation of accuracy and precision of TDF tablets by standard addition method (n= 6)

Method	µg/ml			S.D	Recovery (%)	Precision[a] R.S.D (%)	Accuracy ER%	Cofidence limits[b] (95%)
	Taken	Added	Found					
HPLC	20	15	33.30 32.88 32.88 33.33 33.65 33.52	0.320	95.02	0.962	-4.97	32.924-33.596
		25	44.55 44.47 46.83 43.39 43.23 43.77	1.320	98.6	2.97	-1.4	42.985-45.755
		35	54.65 52.00 55.15 51.71 51.77 54.30	1.590	96.8	2.98	-3.16	51.592-54.928
		45	62.85 61.52 62.17 62.16 61.99 63.16	0.597	95.84	0.95	-4.15	61.674-62.926
		55	71.34 73.01 71.08 69.91 70.11 73.66	1.523	95.34	2.12	-4.65	69.912- 73.108

n, number of determination, % R.S.D, %, percentage relative standard deviation; Er %, percentage relative error. [a]Mean of six determinations.[b]Confidence limit at 95% confidence level and five degrees of freedom

Procedure for the tablet: The proposed method was applied to the determination of TDF in commercial tablets. The accuracy of the proposed method is evaluated by applying standard addition technique, in

which variable amounts of a previously analyzed portion of the standard drug were added to the formulation and the results are tabulated in Table 8. Six replicates determinations were made. Satisfactory results were obtained for drug and were in a good agreement with the label claims (Table 5 & Table 6). The results were reproducible with low R.S.D. values. The average percent recoveries obtained were quantitative (95.02–98.60%), indicating the good accuracy of the method. The results of analysis of the commercial tablets and the recovery study of drug suggested that there is no interference from any excipients which are present in tablets.

CONCLUSION

An efficient high performance liquid chromatographic method was developed and validated for the estimation of Tenofovir in pure form and in pharmaceutical formulation. The HPLC method was developed by using column (Hyper ODS2 C18), UV detection at 260 nm at a flow rate of 1.2 ml/min and isotonic composition of methanol and phosphate buffer (90:10) as mobile phase. The method was validated by using various validation parameters like accuracy, precision, linearity, specificity in an analytical solution. This method was rapid, simple and has great sensitivity and accuracy. The proposed method makes use of simple reagents, which an ordinary analytical laboratory can afford. Hence the method can also be applied for routine estimation of Tenofovir in the formulation. This work can be further extended to study the applicability of this method to determine Tenofovir in biological fluids.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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