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Research Article

A VALIDATED RP-HPLC METHOD FOR ESTIMATION OF TENOFOVIR DISOPROXIL FUMARATE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, economic, accurate, reverse phase isocratic HPLC method was developed for quantitation of Tenofovir disoproxil fumarate in tablet dosage form. The quantification was carried out using Reverse phase column Inertsil ODS-3 (150×4.6 mm), 5µm. The detection was carried out at the wavelength of 260nm. The elution was achieved isocratically with a mobile phase comprising a mixture of Sodium dihydrogen orthophosphate buffer pH 2.3 and Methanol (49:51v/v). The flow rate was 1.0 mL/min. The procedure was validated as per ICH rules for accuracy, precision, detection limit, linearity, reproducibility, and quantitation limit. The linearity concentration range was 50-300 µg/mL with the correlation coefficient of 0.9995. The percentage recovery for Tenofovir disoproxil fumarate was found to be 99.98-100.14. Limit of detection and limit of quantitation values were found to be 0.28 µg/mL and 0.85 µg/mL. The developed method was successfully applied to estimate the amount of Tenofovir disoproxil fumarate in tablet formulations.

Keywords: RP-HPLC, Tenofovir disoproxil fumarate, Pharmaceutical dosage forms

INTRODUCTION

Tenofovir disoproxil fumarate (TDF) is a fumaric acid, salt of bis-isopropoxycarbonyloxy methyl ester derivative of tenofovir. Chemically it is 9-[(R)-2-[[bis [[isopropoxy- carbonyl) oxy] methyl] phosphinyl] methoxy] propyl] adenine fumarate[1-2] (Fig 1). TDF is the first nucleotide analog approved for HIV-1 treatment. Tenofovir is a nucleotide reverse transcriptase inhibitor[3] used in combination with other antiretrovirals for the treatment of HIV infections. TDF remains in cells for longer periods of time than many other antiretroviral drugs, thereby allowing for once-daily dosing.

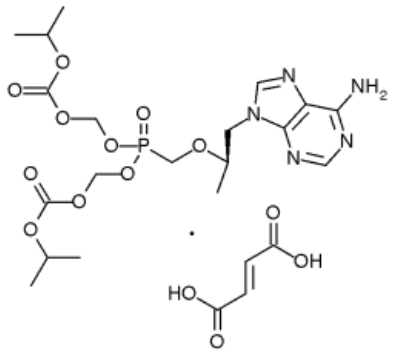

Fig 1: Chemical structure of Tenofovir Disoproxil Fumarate

Literature survey reveals that there are several reports describing the determination of Tenofovir in plasma using HPLC coupled with fluorescence and UV detection[4-8]. Liquid chromatography coupled with tandem mass spectrometry were also reported[9-11], Spectrophotometric[12]. The focus of present study is to develop and validate a rapid, stable, and economic high performance liquid chromatographic method for quality control of TDF in tablet dosage form.

EXPERIMENTAL

Chemicals and Reagents

A reference standard sample of TDF was obtained from Macleods Pharmaceuticals Ltd and commercial dosage form containing the studied drug were purchased Cipla Ltd. HPLC-grade methanol, Orthophosphoric acid and water were HPLC grade purchased from E. Merck, Mumbai, India. All the other chemicals and reagents used were of AR grade and purchased from S.D. Fine Chemicals, Mumbai, India.

HPLC Instrumentation and Chromatographic Conditions

Chromatography was performed with Jasco, Japan equipment comprising a PU-2089 plus quaternary pump, degasser and a photo diode array detector (Jasco MD-2010 Plus). A Rheodyne injector fitted with a 20 µL loop was also used and data were recorded and evaluated by use of Chrompass software. The detector wavelength was set at 260nm. The chromatographic separations were performed at ambient temperature on an Inertsil ODS-3 (150mm X 4.6 mm), 5µm. The mobile phase was a mixture of Sodium dihydrogen orthophosphate buffer pH 2.3 and Methanol (49: 51 v/v) filtered and degassed for 30 mins prior to use and flowing at the rate of 1.0ml/min and run time of 13 min. Analytical Balance (Sartorius) and pH meter (Lab India) were used.

Preparation of standard solutions

About 5 mg of TDF reference/ working standard was accurately weighted and dissolved in 50 mL of methanol in the volumetric flask to get a concentration of 100µg/ mL. From this stock solution, suitable dilutions were made to get the concentrations of 50-300 µg/ mL and filtered through 0.45µ filter before use. 20 µL of each solution was injected into the column. All measurements were repeated five times for each concentration. The calibration curve was constructed by plotting the peak area ratios of analyte versus the respective drug concentration.

Sample preparation

20 tablets were accurately weighed and powdered. An accurately weighed portion of powder equivalent to 5 mg of TDF was extracted in 25 ml of Methanol in to a 50 ml volumetric flask by means of sonication for 25 min with intermittent vigorous shaking. The final volume was made up to 50 mL with methanol to get a stock solution of 100µg/mL. This solution was filtered through 0.45 µm nylon filter discarding first few mL of the filtrate and 20 µL of this solution was injected into the HPLC system.

RESULT AND DISCUSSION

Method Validation

The analytical method was validated for linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), specificity, robustness and ruggedness, in accordance with ICH guidelines[13].

System suitability

System suitability is an integral part of method development and is used to ensure adequate performance of chromatographic system. Retention time (t_R), number of theoretical plates (N), tailing factor (T), peak asymmetry (A_T) were evaluated for five replicate injections of the drug at a concentration of 100 µg/ mL. The results are given in Table 1. A typical chromatogram of TDF in standard and formulation is given in Fig 3 and Fig 4.

Table 1: Results From System Suitability Parameters

Sr.no	Parameters	Values*
1	Retention Time (t_R)	9.437
2	Theoretical Plates (N)	4848.99
3	Tailing factor (T)	1.09
4	Asymmetric Factor(As)	1.11
5	LOD µg/ mL	0.28
6	LOQ µg/ mL	0.85

***Mean ±SD from six determinations**

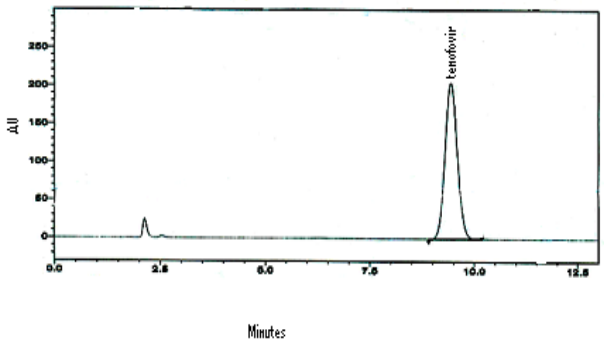

Fig 3: HPLC Chromatogram of Tenofovir Disoproxil Fumarate (standard)

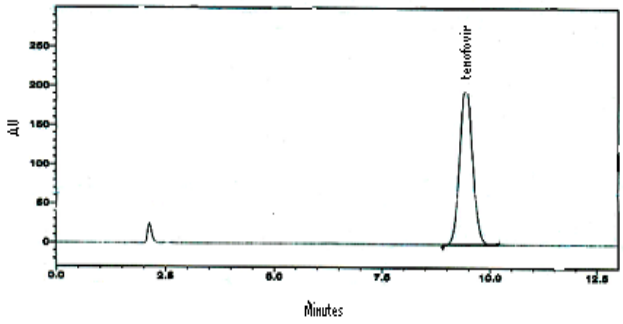

Fig 4: HPLC Chromatogram of Tenofovir Disoproxil Fumarate (formulation)

Linearity and range

Linearity was studied by preparing standard solutions at different concentrations from 50-300µg/ mL, plotting a graph of concentration against peak area, and determining the linearity by least-squares regression. The result shows that excellent co-relation exists between peak area and concentration of drugs within the concentration range and the results are given in Table 2 and Fig2.

Table 2: Linearity Range Of Tenofovir Disoproxil Fumarate

Sr.no	Concentration(µg/mL)	Peak area (mv)
1	50	681166.3
2	75	1021750
3	100	1362333
4	125	1772916
5	150	2043600
6	200	2724665
7	300	4074544

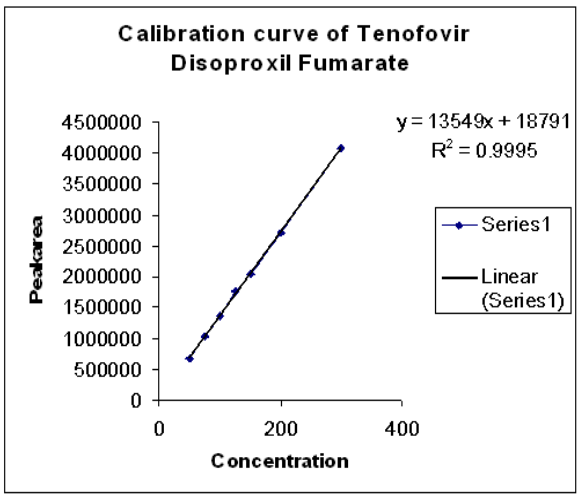

Fig 2: Graph for linearity

Accuracy

Accuracy of the methods were checked by recovery studies by standard addition method known amount of standard TDF was added to pre-analyzed sample and subjected it to the proposed high performance liquid chromatographic method. These studies were carried out at three different levels i.e., (80%, 100% and 120%). The recovery studies were carried out and the % recovery and standard deviation of the % recovery were calculated and presented in Table 3.

Table 3: Amount Of Tenofovir Disoproxil Fumarate In Tablet Dosage Form By Hplc

Formulation in mg	Labeled amount in mg	Recovered amount in mg	% Recovery
Tenvir 300	300	299.42	99.80

***Each value is the average of five determinations**

Precision

The intra-day precision was determined by analyzing standard solution of 100 µg/ mL for six times on the same day while inter-day precision was determined by analyzing corresponding standards daily for 6 days over a period of one week. The values of percentage relative standard deviation (%R.S.D) for intra and inter-day variation are given in Table 4.

Table 4: Results From Precision Studies

Sr.no	Concentration(µg/mL)	Intraday precision (Area)	Interday precision (Area)
1	100	1378456	1362243
2	100	1360456	1358496
3	100	1362987	1360542
4	100	1365432	1362977
5	100	1363524	1362528
6	100	1362333	1362492
Mean		1365531.33	1361546.33
S.D		6535.26	1714.83
%RSD		0.4786	0.1259

Sensitivity

The sensitivity of measurement of TDF by use of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantitation (LOQ). The (LOD) and (LOQ) were calculated by use of formulae LOD = 3.3σ/S and LOQ =10 σ/S where σ is the standard deviation of intercept of calibration plot and S is the average of slope of corresponding calibration plot. The limit of detection (LOD) was 0.28 µg/ mL and limit of quantitation (LOQ) was 0.85µg/mL.

Table 5: Results From Accuracy Studies

Brand	Label Claim (mg)	Initial amount (µg/mL)	Amount added (µg/mL)	Amount Recovered (µg/mL)	Recovery± SD* (%)	%RSD
Tenvir 300	300	100	50	150.21	100.14 ± 0.11	0.108
		100	100	199.97	99.98 ± 0.23	0.228
		100	150	250.12	100.04 ± 0.14	0.142

***Mean ±SD from six determinations**

Ruggedness and Robustness

The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of TDF. Robustness was determined by changing the mobile phase flow rate to 1.2 and 0.8 mL/min, the organic composition of the mobile phase was varied from 48 and 50% and pH was varied from 2.1and 2.5. There was no significant change in the retention time of TDF when mobile phase composition, flow rate and pH of mobile phase slightly. The low value of the RSD (Table 7) indicates the method is robust.

Table 7: Results From Robustness Studies

Condition	Modification	Mean Area ± SD*	%RSD	Mean RT± SD*(min)
Mobile phase composition (Buffer: Methanol)(v/v)	50:50	1376441 ±2995	0.217	9.405±0.049
	49:51	1378765 ± 5777	0.418	9.480±0.054
	48:50	1380786±6779	0.490	9.398±0.061
	1.2	1384132±12492	0.902	7.601± 0.152
Mobile phase flow rate ($mLmin^{-1}$)	1	1377115±2087	0.152	9.702±0.144
	0.8	1384109 ± 4937	0.356	10.456± 0.229
	2.1	1389111 ± 5500	0.395	9.4727±0.048
Change in buffer pH	2.3	1379442 ± 6078	0.440	9.4497±0.009
	2.5	1384206 ± 4946	0.357	10.370±0.032

***Mean ±SD from six determinations**

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst. The ruggedness of the method was assessed by comparison of intra-day and inter-day results for assay of TDF performed by two analysts in the same laboratory. The results of ruggedness testing are reported in (Table 6).

Table 6 :Results From Ruggedness Studies

Sample	Label Claim(mg)	Analyst I		Analyst II	
		Amount found (mg)	Recovery ± SD* (%)	Amount found (mg)	Recovery ± SD* (%)
Tenvir 300	300	300.15	100.05 ±0.21	300.42	100.14±0.16

***Mean ±SD from six determinations**

CONCLUSION

The proposed HPLC method was found to be simple, rapid, precise, accurate and sensitive for the determination of TDF in pharmaceutical dosages forms. Hence, this method can easily and conveniently adopt for routine analysis of TDF in pure and its pharmaceutical formulations.

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