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Article *in* World Journal of Pharmaceutical Research · October 2021

DOI: 10.20959/wjpr202011-18631

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HPLC Method Development And Validation For Estimation Of Tenofovir Alfenamide Hemifumarate In API And Tablet Dosage Form.

Corresponding Author*

Mrs. Parina Dobariya

Faculty of Pharmaceutical Sciences, Madhav University, Pindwara (Sirohi), Rajasthan

Email address:parinadobariya45@gmail.com

Abstract

Background: In the current study, a simple, improved, precise, rapid, and accurate reverse phase liquid chromatographic method was produced for the estimation of Tenofovir Alfenamide fumarate in tablet dosage form which is a nucleotide reverse transcriptase inhibitor (NRTI) and a novel ester prodrug of the antiretroviral Tenofovir. This method was developed by C18 column having 250 mm length, 4.6 mm internal diameter, 5µ particle size. Peak was observed in the mobile phase consist of Buffer (pH 3.5): Acetonitrile (50:50). The flow rate was 1ml/min. The estimation was carried out at 260 nm. The retention time observed for Tenofovir Alfenamide was 6.5 minutes.

Results: The validated method for Tenofovir Alfenamide Accuracy between 99.59-100.14 % and LOD and LOQ 0.11 and 0.33 µg/ml. Linearity was found in the range of 20-60 mcg/ml respectively.

Conclusion: The results of the analysis prove that the method is simple, improved, precise, accurate, and rapid for estimating the content of Tenofovir Alfenamide in bulk drug and tablet dosage form and it can be applied for routine analysis. The method was validated as per ICH guideline Q2R1. All validation parameters were found to be within the accepted range specified in ICH guideline Q2R1.

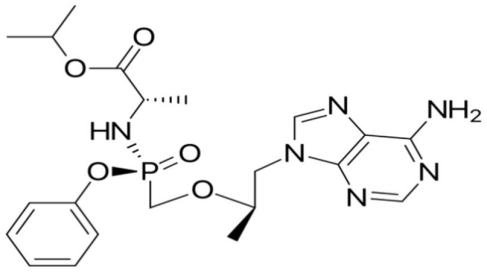
Keywords: Tenofovir Alfenamide Hemifumarate, RP-HPLC, ICH guideline Q2R1.

Background

Antiretroviral drugs which are used for manage of HIV \AIDS normally includes the use of anti retro viral drugs in an attempt to control HIV infection. Several classes of anti retro viral agents that act on different stages of HIV life cycle. The use of multiple drugs that act on different viral targets is known as (HAART) highly active antiretroviral therapy . Tenofovir Alafenamide is a nucleotide reverse transcriptase inhibitor and a novel prodrug of tenofovir. It is closely related to tenofovir disoproxil fumarate but has greater antiviral activity. Mainly used in the treatment of HIV infection and chronic hepatitis B. [1-3] IUPAC name of Tenofovir propan-2-yl(2S)-2-{[(S)-({[(2R)-1-(6-amino-9H-purin-9-yl) propan-2-yl]oxy} methyl) (phenoxy) phosphoryl] amino} propanoate with a molecular formula $C_{21}H_{29}N_6O_5P$. It is a White crystalline powder with melting point of Tenofovir 125ºC to 135ºC.[4-7] It is a product

of Gliend science brand name of Descovy strength of 200mg/5mg and 200 mg/25mg respectively.

Fig.1



Structure of Tenofovir Alfenamide Hemifumarate

Methods

Instruments

Shimadzu model LC-20AT instrument, series RP-HPLC system with UV detector. Phenomex column C18 column having 250 mm length, 4.6 mm internal diameter, 5µ particle size. Sonicator of Soltec-sonica ultrasonic cleaner (Spincotech Pvt Ltd) and analytical balance of Electronic analytical balance (AUX-200), Uni bloc- SHIMADZU.

Chemicals

The Tenofovir alfenamide standard was supplied by Gifted by Emcure Pharmaceutical Company. Acetonitrile, Potassium Dihydrogen Phosphate, Water HPLC grade was purchased from AR Grade, Merck.

HPLC condition

A chromatographic separation of drug was achieved using Phenomex column 250X 4.6mm, 5µ particle size C18 column with mobile phase of Buffer (pH 3.5): Acetonitrile (50:50) Drug was monitored at a detection wavelength of 260nm, the flow rate was 1ml/min and the injection volume was 20µL. The retention time was found to be Tenofovir alfenamide 6.5 minutes respectively.

Preparation of Mobile Phase

Buffer preparation:

6.8 gm Potassium dihydrogen phosphate buffer was transferred to 1000ml beaker and 800 ml water was added shacked to dissolve and volume was made up with water, pH 3.5 was adjusted with diluted o-Phosphoric acid.

Preparation of Standard Solution

TEN Standard stock solutions (200 µg/ml):

The Standard solution 20mg TEN of drug was dissolved in 100 ml volumetric flask. Diluted 100 ml with Methanol.

Take 1ml from TEN stock solution to make up to 10ml with methanol **(**TEN 20µg/ml).

Method Validation

System Suitability test Parameters

System suitability testing is an internal part of a liquid chromatographic method, and it is used to verify that the chromatographic method is able to produce good resolution between the peaks of interest with high reproducibility. The system suitability was determined by making six replicate injections from a freshly prepared standard solution of 50 µg/ml of TEN and 200 µg/ml and analyzing each solute for its retention time (Rt), Number of theoretical plates (N), resolution (RS) and tailing factor (T). The system suitability method acceptance criteria set in each validation run were: a %RSD<2%, Capacity factor > 2.0, tailing factor ≤ 2.0, and theoretical plates>2000.

Selectivity

It is the ability of the method to measure specifically the analyte of interest, in the presence of other components, such as impurities, degradation products, excipients that are expected to be present in the sample preparation.

Linearity and Range (n=5)

Aliquots of working standard solution (0.5, 0.75, 1.0, 1.25 and 1.5 ml) of TEN (200 µg/ml) were transferred to a series of 10 ml volumetric flask. The volume was adjusted up to the mark with Diluent to obtain obtain10, 15, 20, 25 and 30µg/ml of Tenofovir Alfenamide.

An aliquot of 20µl of each solution was injected under the operating chromatographic condition. Plot the calibration curve of area versus respective concentration and find out correlation co-efficient and regression line equation for TEN. Each response was an average of five determinations.

Precision

Intraday precision (n=3)

Intraday precision was determined by analyzing of TEN standard solutions in the range (10, 20, and 30 µg/ml). It was analysed three times on the same day and % RSD was calculated.

Interday precision (n=3)

Interday precision was determined by analyzing of TEN standard solutions in the range (10, 20, and 30 µg/ml). It was analyzed on three different successive and % RSD was calculated.

Repeatability (n=6)

Repeatability was determined by analyzing TEN test solutions having the concentration 20µg/ml TEN Measure six times. Calculate %RSD for TEN.

Accuracy (n=3)

The accuracy of the method was determined at 50%, 100% and 150% by calculating recoveries of TEN by the standard addition method. The Known amount of standard solutions of TEN was added to pre-quantified sample solution of TEN. Each solution was injected in triplicated, and the percentage recovery was calculated by measuring the peak areas and fitting these values into the regression equation of the respective calibration curves.

Limit of detection and Limit of Quantitation

LOD and LOQ of the drug were calculated using the following equations according to ICH guideline. LOD = 3.3 σ/s and LOQ = 10 σ/s, where σ is the SD of the response and S is the slope of the calibration curve.

Robustness

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by changing three small changes.

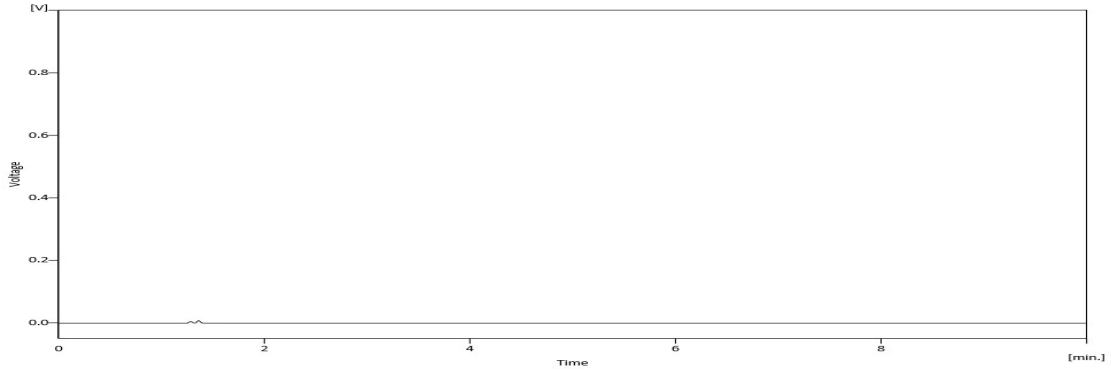
1) Different flow rate (i.e. +0.2 ml/min and -0.2 ml/min).
2) Different pH (i.e. +0.2pH and -0.2pH)
3) Solvent % in mobile phase (+2% solvent and -2% solvent in mobile phase.)

Results

The analytical method was found to be specific as there was no interference of any excipients or impurities which can be shown from figure 2-4. Overlay of linearity was shown in figure 5 and regression coefficient was found to be 0.9996, which is shown in figure 6 calibrations data are shown in table 1 and regression data is shown in table 2. The %RSD for repeatability was found to be 1.62 Tenofovir Alfenamide as mentioned in table 3. The %RSD for intraday precision was found to be 1.31-1.68 of Tenofovir Alfenamide mentioned in table 4. The %RSD for Interday precision was found to be 0.82-1.77 of Tenofovir Alfenamide mentioned in table 5.

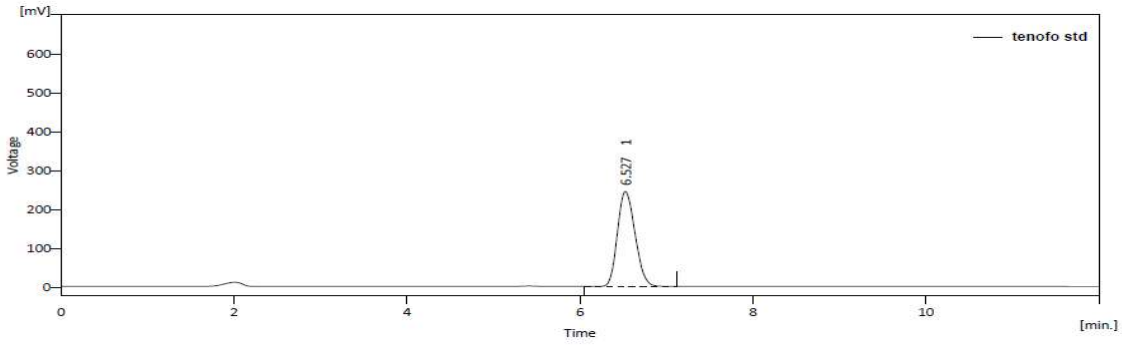
Mean percentage recovery of Tenofovir Alfenamide was found to be in the range of 99.59-100.14 % mentioned in table 6. The % RSD for robustness was found to be 0.27-0.96 % for Tenofovir Alfenamide as mentioned in table 7. Assay of Tenofovir Alfenamide was found in the range of 98.39 – 100.59 % mentioned in table 8.

Fig.2



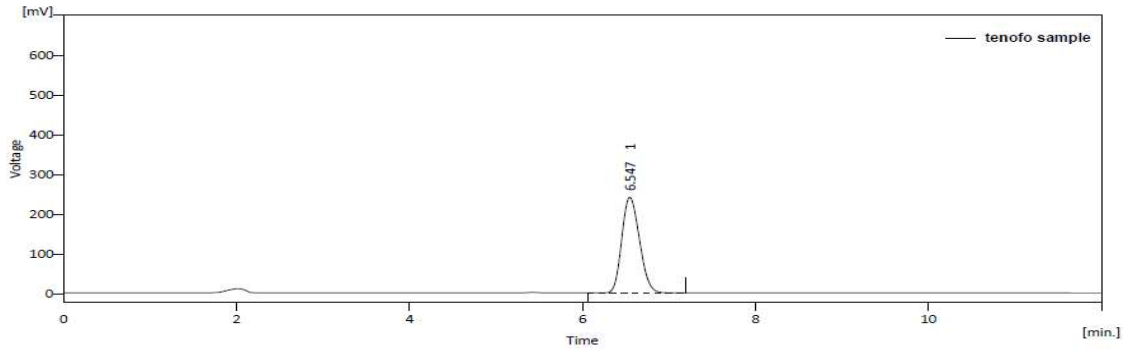
Chromatogram of blank

Fig.3



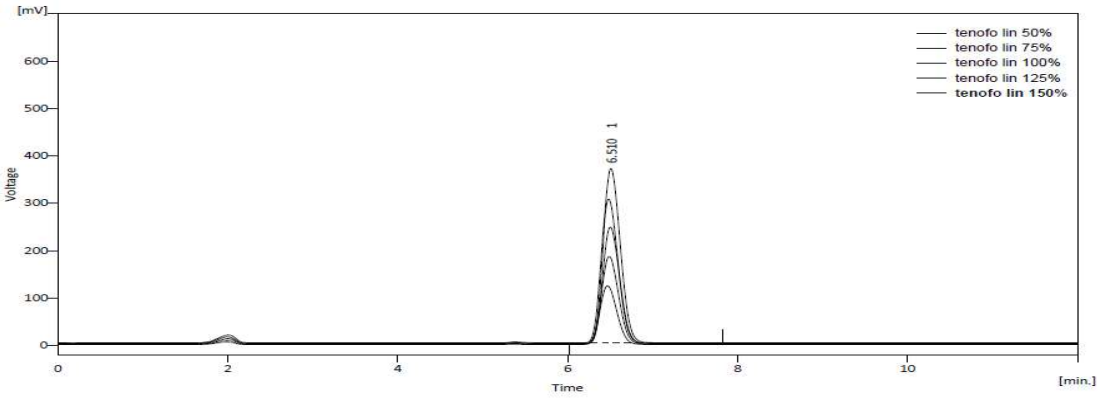
Chromatogram of standard solution of TEN

Fig.4



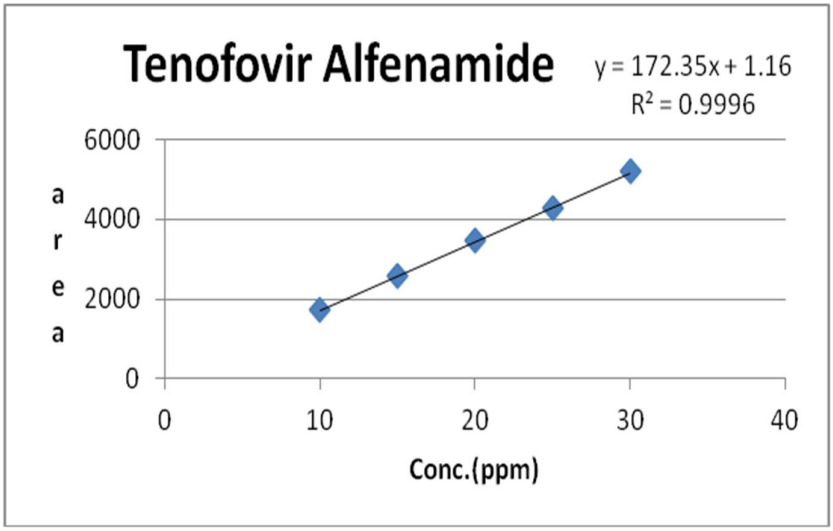
Chromatogram of Sample solution of TEN

Fig.5



Linearity overlay spectra of TEN

Fig.6



Calibration curve of TEN for HPLC

Table 1 Calibration data for TEN at 260 nm

Conc. (mcg/ml)	Mean Response
10	1723.13
15	2596.84
20	3456.91
25	4267.31
30	5196.67

Table 2 Data of regression analysis of TEN

Drug	Straight line equation of Calibration curve	Correlation Coefficient
Tenofovir	Y = 172.35x+ 1.16	0.9996

Observation

A method is linear with a range of 10-30 mcg/ml of Tenofovir Alfenamide of the standard solution.

A correlation coefficient for Tenofovir Alfenamide is 0.9996. The areas obtained were directly proportional to the concentration of the analyte in the sample. The method can, therefore, be termed as linear in the specified range.

Precision

Repeatability

The repeatability studies were carried out by measuring response for a single concentration for 6 times a day.

Intraday precision

Intraday precision was performed by analyzing three different concentrations within linearity range, three times a day (3*3 determination).

Table 3 Repeatability data for Tenofovir Alfenamide

Conc of Tenofovir Alfenamide (mcg/ml)	Area (n=6)
20	3419.74
	3444.73
	3424.16
	3354.32
	3393.26
	3519.96
Mean	3426.03
SD	55.55
% RSD	1.62

Table 4 Data for Intraday Precision for Tenofovir

Tenofovir Alfenamide		
Conc. (mcg/ml)	**Mean response**	**% RSD**
10	1722.48	1.68
20	3429.16	1.57
30	5155.21	1.31

Interday precision (n=3)

Interday precision was performed by analyzing three different concentrations within linearity range on different days.

Table 5 Data for Interday Precision for Tenofovir

Tenofovir		
Conc. (mcg/ml)	**Mean response**	**% RSD**
10	1698.41	1.77
20	3402.49	1.05
30	5119.05	0.82

Observation

Repeatability-The % RSD was found to be 1.62% for Tenofovir Alfenamide. The % RSD value was found to be less than 2.0, which indicates that the method is precise.

Intraday- The % RSD was found to be 1.31-1.68 % for Tenofovir Alfenamide. The % RSD value was found to be less than 2.0, which indicates that the method is precise.

Interday-The % RSD was found to be 0.82-1.77 % Tenofovir Alfenamide. The % RSD value was found to be less than 2.0, which indicates that the method is precise.

Accuracy

Accuracy of the method was confirmed by a recovery study from marketed formulation at three levels (50%, 100%, and 150%) of standard addition.

Table 6 Determination of accuracy of Tenofovir Alfenamide

Amount of TEN present(mcg/ml)	% Amount of std TEN added	Total amount of TEN present(mcg/ml)	Amount recovered mean (mcg/ml)	SD n=3	% Recovery
20	10	30	30.04	1.06	100.14
	20	40	39.84	1.03	99.59
	30	50	49.97	1.33	99.94

To develop a method and validation for estimation of Tenofovir Alfenamide Hemifumarate in bulk in combined tablet dosage form and to validate the developed method as per ICH guideline Q2 (R1).

Robustness

Change in flow rate, pH and Mobile phase

Table 7 Data of Robustness for TEN

			Mean	% RSD
TEN (20mcg/ml)	**Flow Rate**	0.9ml/min	35.91.23	0.53
		1.1ml/min	32.97.91	0.82
	Mobile phase	52:52	32.71.52	0.96
		48:48	3614.88	0.60
	pH	3.3	3606.12	0.78
		3.7	3255.37	0.27

Assay

Table 8 Data of Assay for TEN

TEN	
Amt of TEN present (µg/ml)	**Amt found in assay (%)**
20	98.39
	99.53
	100.59
Mean ± SD	1.09
% RSD	1.10

Summary of Validation parameter

Table 9 Summary of validation parameters

Parameter	TEN
Linearity range (n=5)	10-30µg/ml
Accuracy (%)	99.59-100.14
LOD (mcg/ml)	0.11
LOQ (mcg/ml)	0.33
Repeatability (n=6) %RSD	1.62
Intraday (n=3) %RSD	1.31-1.68
Interday (n=3) %RSD	0.82-1.77
Robustness(% RSD)	0.27-0.96
Assay	1.10

Discussion: Few methods like RP-HPLC were reported for the estimation of Tenofovir Alfenamide Hemifumarate in API and Tablet dosage form. In the present method, Tenofovir Alfenamide Hemifumarate was eluted at 6.5 min with a run time of 10 min. The present method was developed using Buffer (pH 3.5): Acetonitrile in the ratio of 60:40% v/v. The method was developed with the minimum or reduced amount of organic solvents as the mobile phase which results in a more sensitive and cost-effective method. The method was successfully validated according to ICH guidelines for all the parameters which were found within acceptance criteria. The developed method may be useful for routine analysis of Tenofovir Alfenamide Hemifumarate tablets.

Conclusion

The method was found to be simple, specific, accurate, economical and responsible. The method can be successfully applied for routine QC analysis. It reveals that RP-HPLC method was validated as per ICH guideline Q2R1 as all validation parameters were found within range.

Abbreviations

API	:	Active pharmaceutical Ingredient
TEN	:	Tenofovir Alfenamide Hemifumarate
RP-HPLC	:	Reverse phase High performance liquid chromatography
Rt	:	Retention time
RSD	:	Relative standard deviation
SD	:	Standard deviation

Availability of Data and material

The data for verification is provided with a Supplementary file and the rest of the data, if required, will be available upon request.

Competing of interest

The Author declares that they have no conflict of interest

Funding

There were no funding sources for this study.

Acknowledgement

It gives me immense pleasure to express my gratitude towards my guide Dr. Mrunal Shirsat for their encouragement and positive attitude towards work, which has instilled more confidence in me.

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