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DETERMINATION AND VALIDATION OF TENOFOVIR DISOPROXIL FUMARATE BY RP-HPLC METHOD

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ABSTRACT

The main objective of the present work is to develop a new simple stability indicating RP-HPLC method for determination of Tenofovir Disoproxil Fumarate. A series of mobile phases were tried, among the various mobile phases Acetonitrile; Buffer containing 1.36 gm of Potassium dihydrogen phosphate in 1000 ml of Milli Q water, pH adjusted to 3.0 with Orthophosphoric acid in the ratio of 65:35 v/v was found to be an ideal mobile phase since it gave a good resolution and peak shapes with perfect optimization The flow rate was found to be optimized at 1 ml/min.The linearity and range was found to be in the range of 6 to120ppm. The correlation coefficient of Tenofovir Disoproxil Fumarate was found to be 0.99993244. The developed method was validated for accuracy, precision, and system suitability, solution stability, filter interference and robustness. The good percentage recovery of the sample clearly indicates the reproducibility and accuracy of the developed method. Similarly the % RSD value for precision was also found to be within the acceptable limit.

INTRODUCTION:

Chromatography is the method of separation that finds applications in all branches of science. It was first invented by Russian Botanist Mikhail Twsett. This technique was used separate various plant pigments like chlorophylls and xanthophylls by passing solutions of these compounds through a glass column packed with finely divided calcium carbonate. The separated species appeared as colored bands on the column hence the name of the process (Greek chroma meaning “color” and graphein meaning “writing”).

Tenofovir Disoproxil Fumarate: Tenofovir disoproxil fumarate is a nucleotide reverse transcriptase inhibitor (NtRTI) widely used in the treatment of human immunodeficiency virus (HIV-1) infection and chronic hepatitis B virus (HBV) infection. It is the fumarate salt of tenofovir disoproxil, an oral prodrug of tenofovir, which enhances bioavailability. Chemically, it is designated as (R)-9-[2-({bis [(iso-propoxy carbonyl) oxy] methoxy}phosphoryl)methoxy]propyl]adenine fumarate, with a molecular formula of $C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$ and a molecular weight of approximately 635.5 g/mol. The drug appears as a white to off-white crystalline powder and is freely soluble in water.

After oral administration, tenofovir disoproxil fumarate is rapidly absorbed and converted by esterases to tenofovir, which is subsequently phosphorylated intracellularly to its active metabolite, tenofovir diphosphate. This active form competitively inhibits viral reverse transcriptase by incorporating into viral DNA and causing chain termination, thereby preventing viral replication. Tenofovir shows activity against HIV-1, HIV-2, and hepatitis B virus. It has a long intracellular half-life, allowing once-daily dosing, and exhibits minimal interaction with cytochrome P450 enzymes.

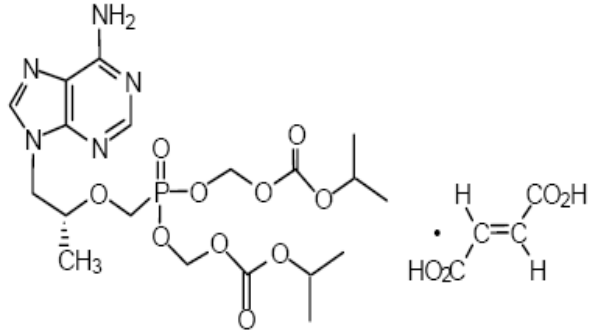


Fig 1: Structure of Tenofovir Disoproxil Fumarate

METHOD DEVELOPMENT:

A new HPLC method was developed and validated for the determination of Tenofovir Disoproxil Fumarate(300mg), the HPLC method was then validated to indicate that the analytical procedure used is suitable for intended use the parameters validated are Specificity, Linearity, LOD, LOQ, Precision, Accuracy, Range and Robustness. The Stability of drug substance in analytical solution was also ascertained and System suitability confirmed.

Selection of chromatographic method

Proper selection of the method depends upon the nature of the sample, molecular weight, and the solubility. Reverse phase chromatography technique was selected for initial separations from the knowledge of properties of the compound.

Selection of detection wavelength:

The sensitivity of method that uses UV detector depends upon the proper selection of wavelength. An ideal wavelength is that it gives maximum absorbance and good response for the drug and should not interfere with other peaks.

A UV spectrum of Tenofovir Disoproxil Fumarate was recorded by scanning by scanning between 400-200 nm.

Optimization of wavelength:

From the spectrum Tenofovir Disoproxil Fumarate showed maximum absorbance at 260. From this spectrum λmax, at 260 nm was selected for proper study, Fig-2.

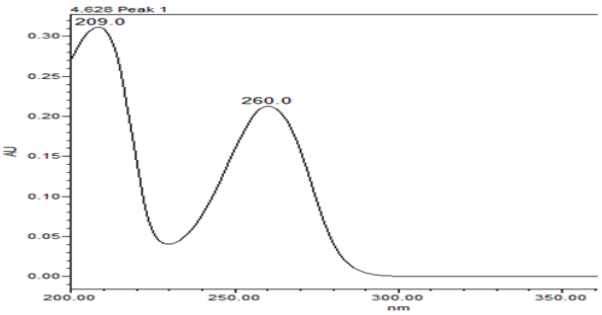


Fig 2: Selection of Wavelength

Optimised Chromatographic Conditions

After performing all the trails as mentioned in Table-1,the following conclusion has been derived,

The following conditions are being selected as Optimised condition.

Column: ACE C18 150x4.6mm, 5µ

Detector: Photodiode array detector (200-400nm)

Flow rate: 1ml/min

Column temperature: 30ºC

Sample temperature:25ºC

Injection volume:20 µL

Wavelength:260nm

Mobile phase preparation: The eluent composed of Acetonitrile; Buffer containing 1.36 gm of Potassium dihydrogen phosphate in 1000 ml of Milli Q water, pH adjusted to 3.0 with Orthophosphoric acid in the ratio of 65:35 v/v was used. Degassed by Sonication

Diluent: MilliQ water

VALIDATION OF HPLC METHOD FOR THE ESTIMATION OF TENOFOVIR DISOPROXIL FUMARATE :

HPLC method was developed for estimation of Tenofovir Disoproxil Fumarate by using Buffer. Acetonitrile (65:35) as mobile phase and Water as diluent. ACE (150 X 4.6 mm; 5µ) as the column, and absorbance was monitored at 260 nm. The developed method further was validated by using various parameters.

System Suitability

The system suitability parameter was carried out to verify that the analytical system was

working properly and could give accurate and precise results.

The standard solution preparation was injected five times into the chromatograph and chromatograms were recorded. The results obtained are given below.

Data Interpretation:

It is observed from the data tabulated above, that system suitability parameters are passed. The %RSD for Tenofovir Disoproxil Fumarate was found to be 0.08, hence it can be concluded that the system suitability parameter meets the requirement of method validation

Observation

Found Response, Plate count and symmetry of the TDF peak was satisfactory.

Found Retention Time for the TDF peak was satisfactory.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte in the presence of components that might be expected to be present, such as impurities, degradation products and matrix components.

Placebo Interference:

Sample solutions were prepared in duplicate with placebo equivalent to the amount present in the sample preparation and analyzed as per the test method

Observation: Chromatograms of placebo preparations are not showing any interference at the retention time of Tenofovir Disoproxil Fumarate.

Linearity:

The linearity of the analytical method was carried out to check its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatograms were recorded. The results are given in Table-5 and Fig-5.

Data Interpretation:

From the statistical treatment of the linearity data of Tenofovir Disoproxil Fumarate it is clear that response is linear between 5.952 µg/mL to122.446 µg/ml (from about 10% to 200%of target concentration). The correlation coefficient is greater than 0.9999.

Method Precision (Repeatability):

In method precision, a homogenous sample of a single batch was analyzed six times and was checked whether the method gave consistent results for a single batch.

The samples Tenofovir Disoproxil Fumarate were analyzed 6 times of the same batch as per analytical procedure. The %RSD was calculated.

Data Interpretation:

The % RSD of the Tenofovir Disoproxil Fumarate for six determinations was found to be 0.5 % which is well within the acceptance criteria limit and hence it is concluded that the method is precise.

Accuracy:

The accuracy of an analytical method was carried out to assess the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Five different levels of sample preparation (each in triplicates) were prepared by spiking known quantity of Rosuvastatin Calcium standard at 50%, 80%, 100%, 120% and 150% level into the placebo. The results are given in Table-7.

Data Interpretation:

From the mentioned results, it can be concluded that the recovery is well within the limit. Hence, the method is accurate

Bench top stability of standard and sample solutions:

The stability of standard and sample solution was performed by injecting the working standard and sample solutions into HPLC at initial and after 24 hours and compared with freshly prepared standard solution. The results are given in Table-8.

Data Interpretation:

The standard and sample solutions are stable for 24 hours at room temperature.

Bench top stability of mobile phase:

Stability of mobile phase is conducted by performing system suitability of standard on initial, after 1day and 4 days), areas were recorded and % RSD was calculated. The results obtained are presented in Table-9.

Data Interpretation:

The system suitability results were found to be within the limits for 5 days and the mobile phase was clear and no haziness was observed during the stability period.

From the above elaborate, it can be concluded that, the method is robust.

Impurities Interference:

The impurities interference was evaluated by performing individual impurities, sample and sample spiked with impurities with 1% level. The peak purity of Rosuvastatin peak in the chromatogram of the known impurities spiked sample solution was analyzed. The results of impurities interference was reported in Table-10 and Fig-7a-7c.

Conclusion:

From the above data, it can be concluded that the product of forced degradation do not interfere with the main peak.

Table-1 Different trails have been performed in the following way

S.No	Column	Mobile Phase	Rt (Min)	Tailing	Plate Count
1	**ACE-150X4.6mm, C18, 5µ**	**pH 4.0 phosphate buffer, B:ACN=65:35**	**4.6**	**1.0**	**10079**
2		pH 3.0 TEA buffer, B:ACN=55:45	3.7	1.0	10152
3		pH 3.0 TEA buffer, B:ACN=65:35	4.5	1.0	9985
4	INERTSIL ODS 3V150X4.6mm, C18, 5µ	pH 3.0 TEA buffer, B:ACN=55:45	3.7	1.1	7609
5		pH 4.0 phosphate buffer, B:ACN=65:35	4.5	1.1	7554
6		pH 4.0 phosphate buffer, B:ACN=55:45	3.5	1.1	7123

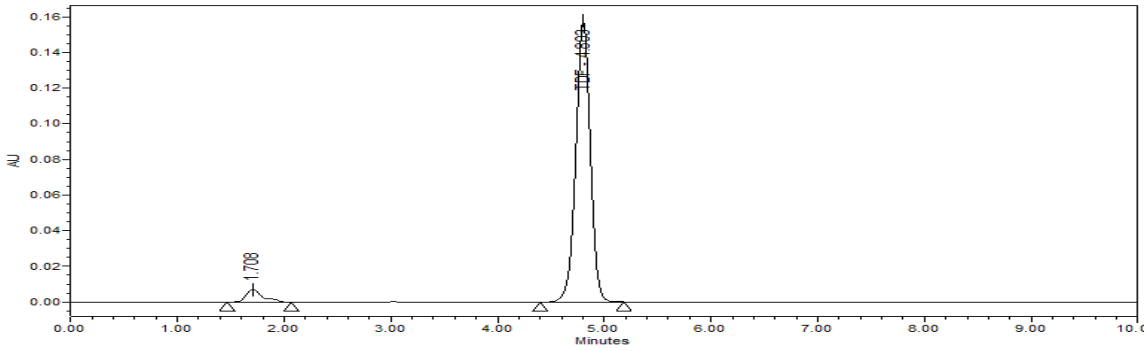


Fig- 3. Typical Chromatogram of System Suitability

System Suitability:

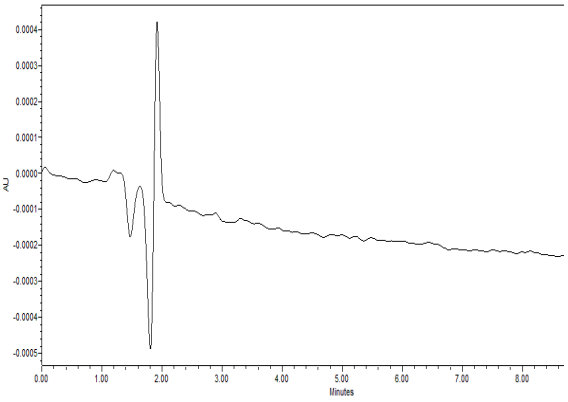
Table-2

Injection Number	Peak area
1	1497778
2	1497261
3	1495720
4	1494890
5	1496707
Mean	1496471.2
% RSD	0.08
Tailing Factor	1.0

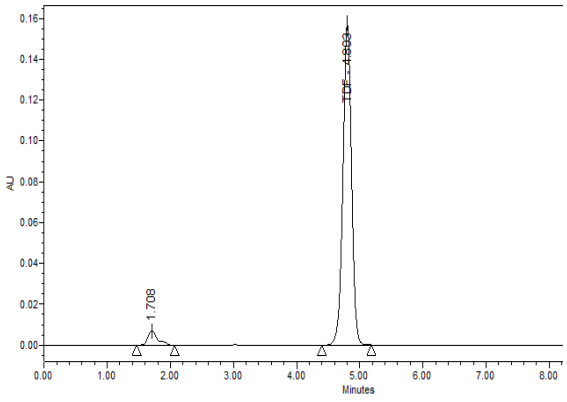
Table-3

Name	Retention Time	Area	Height	USP Tailing	USP Plate Count
TDF	3.766	1517521	158124	1.1	7609

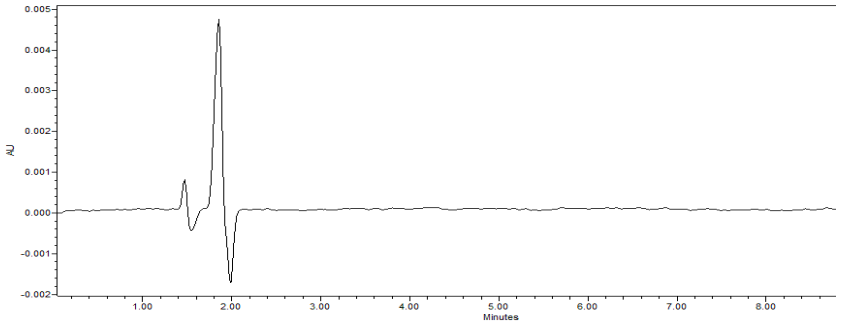
Fig-4. Chromatograms for Specificity of the Placebo Interference



4a. Typical Chromatogram of Blank



4b. Typical Chromatogram of Std solution

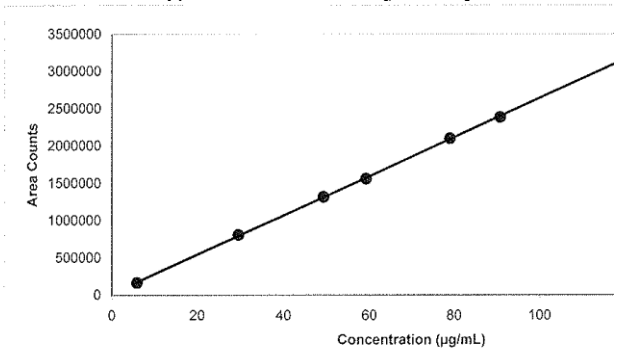


4c. Typical Chromatogram of Placebo

Table-5. Results of Linearity

S.No.	Concentration (µg/mL)	Tenofovir Disoproxil Fumarate Peak Area
1	5.952	166018
2	29.624	811913
3	49.374	1323723
4	59.248	1562696
5	78.998	2102478
6	90.847	2388414
7	122.446	3236113

Figure 5: Linearity Graph



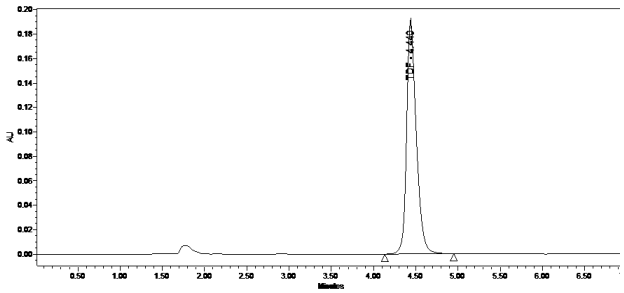


Fig-6: Typical Chromatogram of sample solution (Method precision)

Sample No.	Assay %
1	99.6
2	100.8
3	100.2
4	100.2
5	100.5
6	99.6
Mean (N=6)	100.2
%RSD(N=6)	0.5

Table 6. Results of Method Precision

Table-7 Results of Recovery

Sample No.	%Level (about)	"mg" added	"mg" recovered	% Recovery	Mean % Recovery	% RSD
1	50	150	146.02	98.8	98.7	0.2
2		150.4	146.47	98.8		
3		150.3	145.9	98.5		
1	80	239.5	237.72	100.7	100.4	0.3
2		240.2	237.71	100.4		
3		240.4	236.9	100.0		
1	100	299.1	290.98	98.7	99.0	0.3
2		299.7	293.14	99.3		
3		299.9	292.66	99.0		
1	120	359.9	361.31	101.9	100.7	1.1
2		361	355.44	99.9		
3		359.7	355.32	100.2		
1	150	450.1	449.51	101.3	99.5	1.6
2		450.5	436.58	98.3		
3		449.2	438.06	99.0		

Table-8. Results of Bench top Stability for Standard and Sample solutions

Time in Hours	Similarity factor of Std	%Assay		Difference from initial	
		Sample-1	Sample-2	Sample-1	Sample-2
Initial	NA	99.5	99.3	NA	NA
24	1.01	100.0	99.8	0.4	0.4

Table-9. Results of Mobile Phase Stability on Bench top

System suitability Parameters	Observed value		
	At Initial	After 2 days	After 5 days
The %RSD for Tenofovir Disoproxil Fumarate peak areas from five injections of standard solution	0.06	0.1	0.9
The tailing factor for Tenofovir Disoproxil Fumarate peak from the chromatogram of Standard solution	1.1	1.1	1.2

Table-10. Results of Impurities Interference

S.NO	Name of Impurity/Analyte	RT from individual injection	RT from impurities spiked sample solution
1	Tenofovir Impurity	1.416	1.419
2	Adenine Impurity	1.461	1.680
3	Monoester Impurity	1.871	1.863
4	Ethyl Impurity	3.459	3.460
5	Isopropyl Impurity	2.955	2.963
6	Carbonyl Impurity	14.250	ND
7	Dimer Impurity	2.086	2.082
8	Tenofovir Disoproxil Fumarate	NA	4.672

Fig-7. Chromatograms for Specificity of the Impurities Interference

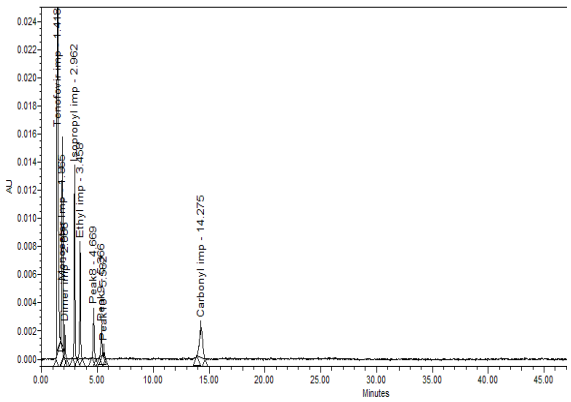


Figure-7a

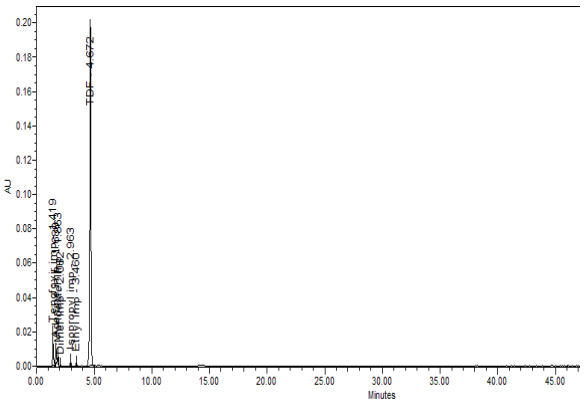


Figure-7b

7a. Typical Chromatogram of known impurities Blend solution
7b. Typical Chromatogram of sample solution spiked with known impurities

Table-11. Results of Forced Degradation studies

Stress conditions	% Degradation	Purity Angle	Purity threshold	Purity flag
Treated with 1N HCl solution for about 10 minutes on bench top	7.5	0.108	0.292	No
Treated with 1N NaOH solution for about 10 minutes on bench top	8.7	0.583	0.687	No
Treated with 30% H_2O_2 solution for about 60 minutes on bench top.	1.8	0.059	0.247	No
Treated with water at 60°C for about 60 minutes in a water bath.	6.9	0.119	0.251	No
Treated thermally at a temperature of 80°C for about 15 hours.	5.5	0.090	0.246	No

RESULTS:

- Comparison of the results obtained concludes that ACE column is showing a better plate count when compared with Inertsil ods-3V column.
- ACE column is giving a better peak symmetry when compared with Inertsil ODS-3V column.
- Phosphate buffer is having a long term bench top stability when compared with TEA buffer.
- Lower consumption of organic solvents is sufficient for the complete separation of all the impurities and degradants from drug peak.
- By using the specified chromatographic conditions all the known impurities have been separated in specificity and in degradation parameters. And the sample has passed the peak purity in every case. It is also observed that there is no placebo interference.

DISCUSSION

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- Lower consumption of organic solvents is sufficient for the complete separation of all the impurities and degradants from drug peak.
- By using the specified chromatographic conditions all the known impurities have been separated in specificity and in degradation parameters. And the sample has passed the peak purity in every case. It is also observed that there is no placebo interference.

CONCLUSION:

An efficient Reverse Phase High Performance Liquid Chromatographic method was developed and validated for the determination of Tenofovir Disoproxil Fumarate Tablets (300 mg). The RP-HPLC method was developed using ACE, 150 X 4.6 mm, 5µ column, detection carried out at 260 nm with flow rate of 1 mL/min. The mobile phase used, Buffer: Acetonitrile (65:35) and diluent used is Water. The method was validated by using various validation parameters like system suitability, specificity, linearity, precision, accuracy, solution stability, filter interference and robustness. All the validation parameters were found to be within the acceptance criteria.

A "Stability Indicating" Rap Id, Convenient, Accurate, Precise and Economical HPLC method has been developed for estimation of Tenofovir Disoproxil Fumarate in tablet dosage for. The assay provides a linear response across a wide range of concentrations (6 ppm to 120 ppm) and it utilizes a mobile phase which has a longer stability. The proposed method can be used for the routine analysis with "LESS RUN TIME" of TDF in drug substance and drug product without interference of excepients and impurities.

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