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ANALYTICAL METHOD DEVELOPMENT AND VALIDATIONFORTHEESTIMATIONOFTENOFOVIRN DISOPROXIL FUMARATE BY USING RP-HPLC

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Abstract:

A simple, precise, and accurate Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method was developed for the quantitative estimation of Tenofovir Disoproxil Fumarate (TDF) in tablet dosage form. The separation was carried out using an ODS C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of Acetonitrile, Methanol, and Water in the ratio of 5:50:45 (v/v/v), along with 1 mL of 0.1% Ortho Phosphoric Acid (OPA). The flow rate was maintained at 1.0 mL/min with UV detection at 260 nm and an injection volume of 20 µL.

The retention time of TDF was found to be approximately 5.92 minutes. The method showed excellent linearity in the concentration range of 0.4–0.6 mg/mL (80%–120%) with a correlation coefficient (R^2) of 0.9999. The assay of the marketed formulation demonstrated 99.12% of the labeled amount of TDF, falling within the ICH acceptance range of 97.0%–102.0%. System suitability parameters such as theoretical plate count, tailing factor, and %RSD were within acceptable limits, confirming method reliability.

Hence, the proposed RP-HPLC method is sensitive, accurate, and reproducible, and can be successfully applied for routine quality control analysis of Tenofovir Disoproxil Fumarate in pharmaceutical dosage forms.

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INTRODUCTION:

Pharmaceutical Analysis is an important part of drug development and quality control. It uses principles from chemistry, physics, and biology to identify and measure drugs, either qualitatively (what it is) or quantitatively (how much there is).

There are several types of analytical methods used in pharmaceuticals:

- Spectral methods like UV, IR, NMR, and Mass Spectroscopy, which measure how drugs absorb or emit light.
- Chromatographic methods like HPLC, TLC, and GC, which separate components based on how they move through a medium.
- Electroanalytical techniques like potentiometry and conductometry, which use the electrical properties of drugs.
- Radioactive and physical methods which measure radiation or physical properties like melting or weight loss.
- Titrimetric methods, which involve adding one solution to another until a reaction is complete.

High-Performance Liquid Chromatography (HPLC) is one of the most commonly used methods because it is fast, accurate, and works well even for complex drug mixtures. HPLC separates drug components using high pressure and allows for both qualitative and quantitative analysis.This technique is widely used to test the purity and concentration of drugs, especially in tablets and other dosage forms.Analytical method development and validation play a crucial role in the pharmaceutical industry, particularly for ensuring the quality, safety, and efficacy of drug substances and drug products. Among the various analytical techniques, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is widely used due to its high precision, accuracy, sensitivity, and reproducibility.

Tenofovir Disoproxil Fumarate (TDF) is a prodrug of tenofovir, classified as a nucleotide reverse transcriptase inhibitor (NtRTI), and is commonly used in the treatment of HIV-1 infection and chronic hepatitis B. It is a water-soluble ester prodrug that enhances the oral bioavailability of tenofovir. Monitoring the concentration of TDF in bulk and pharmaceutical formulations is essential to ensure consistent drug delivery and therapeutic effectiveness.

Due to its increasing therapeutic use and importance, there is a need to develop a reliable, robust, and validated analytical method for the estimation of Tenofovir Disoproxil Fumarate. The International Council for Harmonisation (ICH) guidelines mandate that any analytical method used for drug analysis must be validated for parameters such as accuracy, precision, linearity, specificity, robustness, LOD, and LOQ.

METHOD AND METHODOLOGY:

Materials and Instruments Used for HPLC Method

Instrument/Material	Specification/Details
HPLC System	Shimadzu SPD20A
HPLC Software	LC Solutions
HPLC Column	Octadecylsilane C18 (250 mm × 4.6 mm, 5 µm)
Detector	Spectrophotometric Detector
Analytical Balance	Shimadzu Electronic Balance
pH Meter	Shimadzu
Ultrasonicator	Digital, 2500 mL, Serial No: 04110114961
Syringe	TLD-25–S
Acetonitrile	HPLC Grade
Water	HPLC Grade
Methanol	HPLC Grade
Glassware	Borosilicate (JSGW)

Materials and Instruments Used for UV Method

Instrument/Material	Specification/Details
UV System	Systronics PC-based Double Beam Spectrophotometer (2202)
UV-VIS Spectrophotometer	Systronics Spectrophotometer 118
Acetonitrile	HPLC Grade
Water	HPLC Grade
Cuvettes	Quartz
Glassware	Borosilicate (JSGW)

Analytical Method Development and Optimization of Chromatographic Conditions

1. Selection of Column:

Based on the literature review, the Zorbax SB-Aq column (250 × 4.6 mm, 5 µm) was selected. This column was chosen after evaluating various system suitability parameters including column efficiency, retention time, tailing factor, and peak symmetry. It

provided satisfactory peak shape and resolution for the analyte.

2. Selection of Wavelength:

The wavelength was selected by scanning the UV spectrum of Tenofovir Disoproxil Fumarate between 200–400 nm. From the UV spectrum, 260 nm was identified as the optimal wavelength for analysis.

3. Selection of Mobile Phase:

Different compositions were tested based on literature and solubility data to achieve better resolution. The mobile phase selected was a mixture of Acetonitrile (ACN), Methanol (MeOH), and Water, with the addition of 1 mL Orthophosphoric Acid (OPA) to enhance separation of impurities.

4. Selection of Mode of Separation:

Given the polar nature of the drug, reverse-phase HPLC (RP-HPLC) was selected due to its simplicity, robustness, and suitability for separating polar compounds.

5. Selection of Flow Rate:

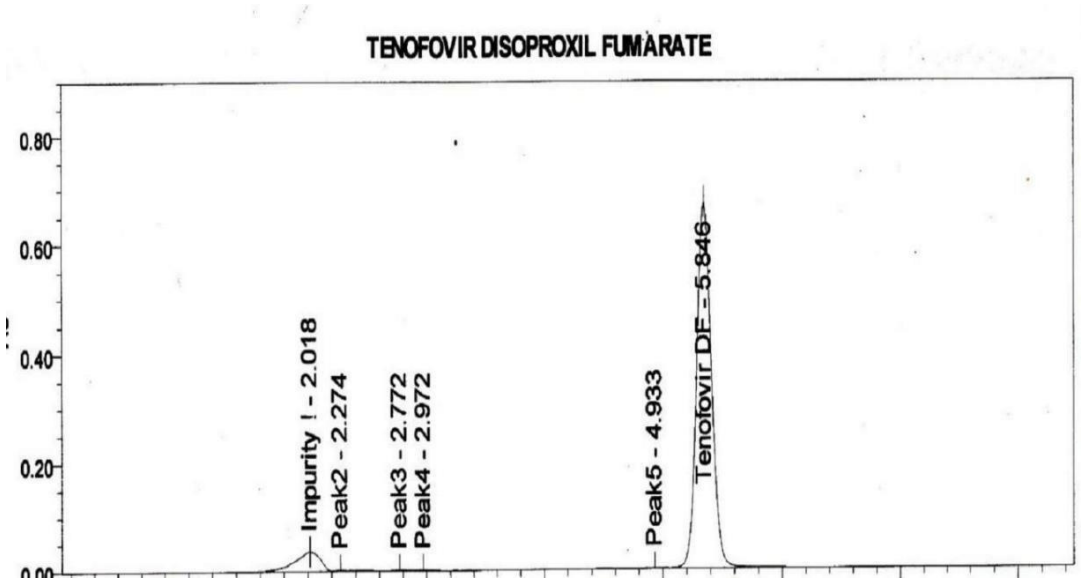
Several flow rates were tested. Flow rates of 0.6 mL/min and 0.8 mL/min resulted in fronting and tailing. A final flow rate of 1.0 mL/min was selected as it provided well-defined peak shapes and optimum resolution.

RESULTS AND DISCUSSION:

Method Development Trials for TDF by RP-HPLC

Parameter	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5 (Optimized Method)
Column	ODS C18 (250×4.6 mm, 5 µm)	ODS C18 (250×4.6 mm, 5 µm)	ODS C18 (250×4.6 mm, 5 µm)	ODS C18 (250×4.6 mm, 5 µm)	ODS C18 (250×4.6 mm, 5 µm)
Mobile Phase	Methanol : Water (35:65)	Methanol : Water (50:50) + 1 mL OPA	Methanol : Water + 0.1% OPA	ACN : MeOH : Water (5:50:45) + 0.1% OPA (Gradient)	ACN : MeOH : Water (5:50:45) + 0.1% OPA
Detection Wavelength	260 nm	260 nm	260 nm	260 nm	260 nm
Flow Rate	1 mL/min	1 mL/min	1 mL/min	1 mL/min	1 mL/min
Injection Volume	10 µL	10 µL	10 µL	10 µL	10 µL
Run Time	15 min	15 min	15 min	15 min	15 min
TDF Retention Time	11.405 min	12.06 min	11.97 min	8.084 min	5.346 min
TDF % Area	96.39%	68.86%	67.27%	96.48%	91.08%
TDF Tailing Factor	1.86	1.51	1.18	1.33	1.07
TDF Plate Count	5041	5242	30525	8286	9069
Observations	High tailing; impurities not resolved	Tailing + merging of impurity peaks	Long retention time	Better resolution; shorter Rt but minor impurity merging	Best peak shape, reduced Rt, good resolution – optimized

Trial 5 (Optimized Method):



QUANTITATIVE DETERMINATION OF TENOFOVIR DISOPROXIL FUMARATE USING DEVELOPED RP-HPLC METHOD

Sample Details

- Sample Name: Tenofovir Disoproxil Fumarate Tablets
- Label Claim: 300 mg
- Diluent: Methanol:Water:Acetonitrile in 50:30:20 (v/v/v), sonicated and filtered through 0.45 µm

Preparation of Mobile Phase

- Composition: Acetonitrile : Methanol : Water in the ratio 5:50:45 (v/v/v)
- Additive: 1 mL of 0.1% Ortho Phosphoric Acid (OPA) to improve peak resolution
- Filtering: Filtered through 0.45 µm membrane

Preparation of Standard Solution

- Accurately weigh 50 mg of TDF
- Dissolve in small volume of diluent
- Sonicate and make up volume to 100 mL → 500 µg/mL (0.5 mg/mL)
- Filtered through 0.45 µm membrane

Preparation of Sample Solution

1. Weigh 20 tablets, calculate average tablet weight.
2. Transfer powder equivalent to 50 mg TDF into a 50 mL volumetric flask.
3. Add 10 mL diluent, sonicate, and make up to the mark with diluent.
4. Filter through 0.45 µm filter.
5. Dilute 5 mL of filtrate to 10 mL → Final conc. 0.5 mg/mL.

Chromatographic Conditions

Parameter	Value
Column	ODS C18 (250 × 4.6 mm, 5 µm)
Mobile Phase	ACN:MeOH:Water (5:50:45) + 1 mL OPA
Flow Rate	1.0 mL/min
Detection	260 nm (UV)
Injection Volume	20 µL
Run Time	15 min
Needle Wash	HPLC Grade Water

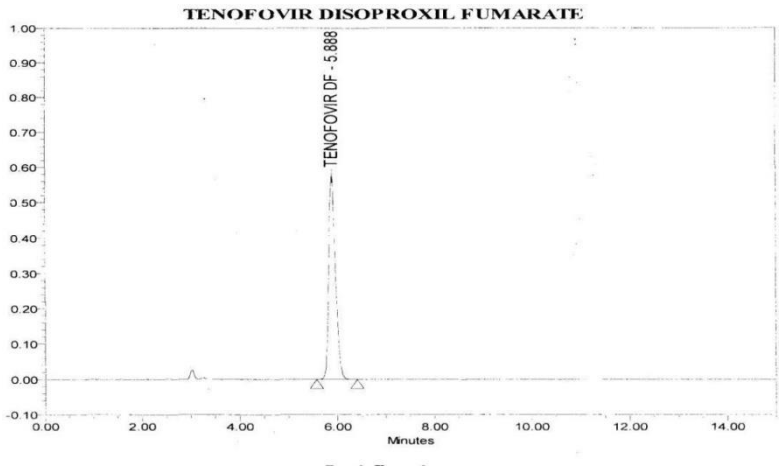
Procedure

- Inject 20 µL each of standard and sample solution into the HPLC system.
- Record chromatograms and peak areas.
- Calculate % Assay using the formula:

Assay Results

Data for Assay

S.No	Content	Label Claim (mg)	Peak Area (Standard)	Peak Area (Sample)	Amount Present (mg)	Percent Content%
1	TDF	300	5,575,121.8	5,532,089.7	294.74	99.12%



Acceptance Criteria

- As per ICH/USP: 97.0% – 102.0%
- Result: Meets the acceptance criteria ✅

Chromatogram

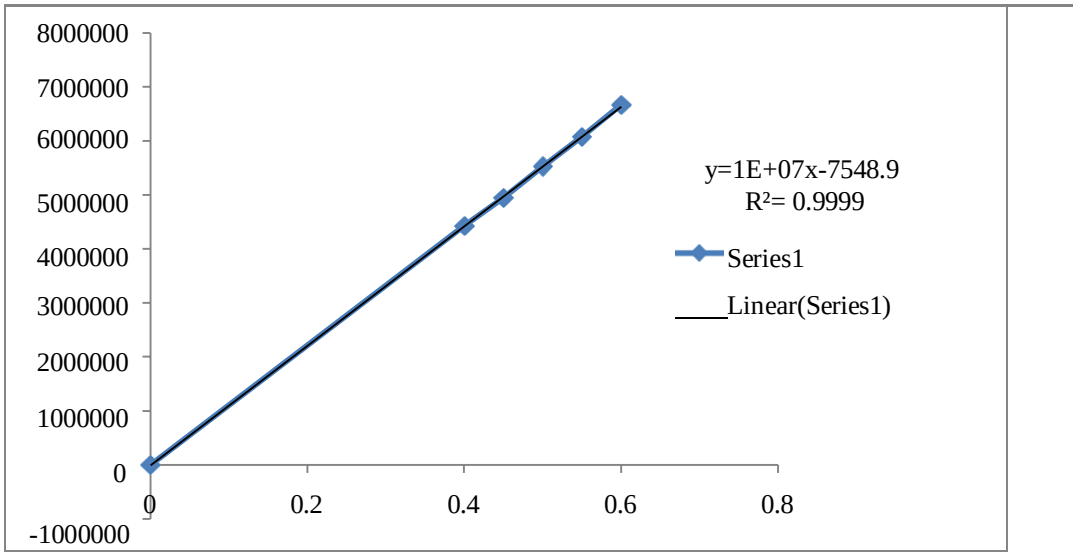
- Chromatogram of Blank
- Chromatogram of Standard Preparation
- Chromatogram of Sample Assay Preparation

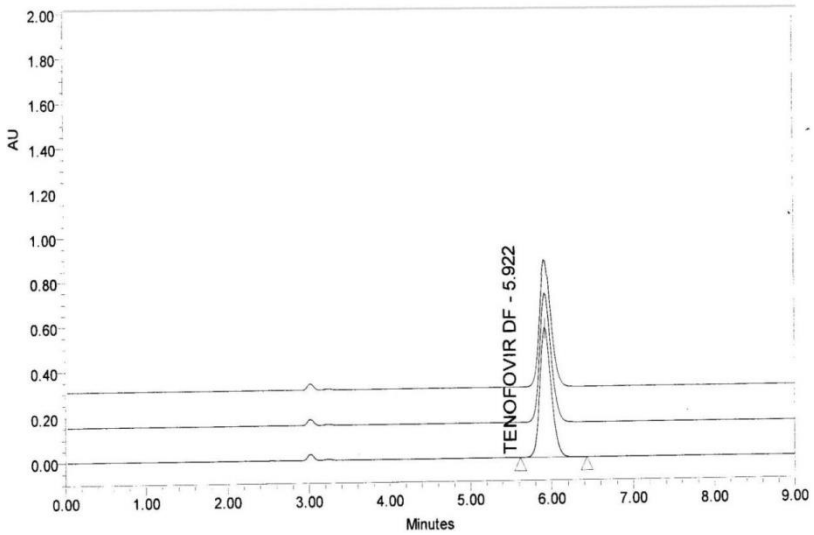
Linearity Validation Table for Tenofovir Disoproxil Fumarate (TDF)

Linearity Level (%)	Concentration (mg/mL)	Mean Retention Time (min)	Mean Area	USP Plate Count	USP Tailing	% RSD (Area)
80%	0.4	5.948	4,425,374.66	8760–8815	1.15	0.15
90%	0.45	5.933	4,947,196.03	8631–8674	1.16–1.17	0.31
100%	0.5	5.923	5,525,741.66	8535–8550	1.19	0.23
110%	0.55	5.922	6,174,048.66	8451–8459	1.20	0.15
120%	0.6	5.927	6,668,287.33	8446–8486	1.20–1.21	0.23

Linearity Curve

- Regression Equation: y = 1E+07x – 7548.9
- Correlation Coefficient (R^2): 0.9999
- Acceptance Criteria: $R^2 \geq 0.999$ ✅
- Observation: The method exhibits excellent linearity across the range of 80%–120%.





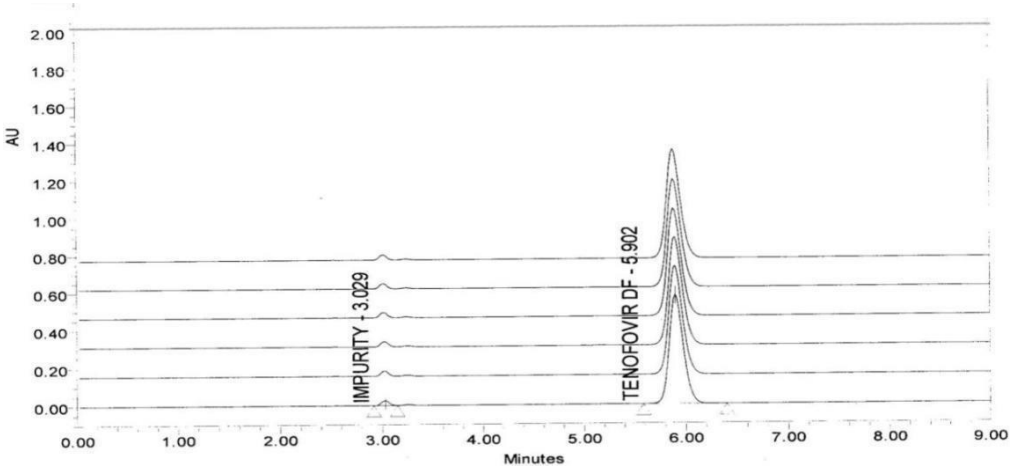
System Suitability Table for Tenofovir Disoproxil Fumarate

Injection No.	Retention Time (min)	Area	USP Resolution	USP Plate Count (N)	USP Tailing Factor (T)
1	5.902	5,530,064	14.18	8683.90	1.17
2	5.897	5,540,327	14.13	8728.94	1.17
3	5.897	5,532,090	14.18	8751.37	1.17
4	5.888	5,524,221	14.30	8615.91	1.18
5	5.887	5,527,200	14.29	8619.38	1.17
6	5.884	5,569,517	14.24	8677.53	1.17
Mean	5.892	5,537,236.46	—	—	—
Standard Deviation	0.007	16,731.15	—	—	—
% RSD	0.12%	0.30%	—	—	—

Acceptance Criteria & Observations

Parameter	Acceptance Criteria	Observed	Status
% RSD of Retention Time	NMT 2.0%	0.12%	✅ Pass
% RSD of Area	NMT 2.0%	0.30%	✅ Pass
USP Plate Count (N)	NLT 2000	All ≥ 8615	✅ Pass
USP Tailing Factor (T)	NMT 2.0	1.17–1.18	✅ Pass
Resolution	NLT 2.0	~14.13–14.30	✅ Pass

System suitability parameters of Tenofovir Disoproxil Fumarate met all ICH criteria, confirming the reliability



and performance of the HPLC system for routine analysis.

SPECIFICITY

Specificity is defined as the ability of an analytical method to unequivocally assess the analyte in the presence of expected components such as:

- Placebo excipients
- Degradants
- Impurities
- Matrix components

This test ensures that Tenofovir Disoproxil Fumarate (TDF) is accurately identified without interference.

A) Standard Solution

S.No	Name	RT (min)	Area	% Area	Purity Angle	Purity Threshold	USP Tailing	USP Plate Count
1	TDF	5.888	5,524,221	100.00%	0.082	0.283	1.17	8751
Sum			5524221					

B) Placebo Interference

- A placebo solution (excipients without active drug) was injected to evaluate interference.
- No peaks were observed at or near the RT of 5.888–5.902 min (TDF RT).

AcceptanceCriteria:
No peak should appear at the retention time of the TDF analyte in the placebo chromatogram.

Result: No interference observed. Placebo is considered non-interfering.

C) Blank Interference

- A blank solution (mobile phase) was injected to confirm the absence of interfering peaks.

Injection	Interference Observed	RT (min)
Blank	Nil	No interference at 5.902
Placebo	Nil	No interference at 5.902
TDF (Sample)	Analyte at 5.902	Peak confirmed

AcceptanceCriteria:
No peak should appear at the retention time of the analyte (TDF) in the blank chromatogram.

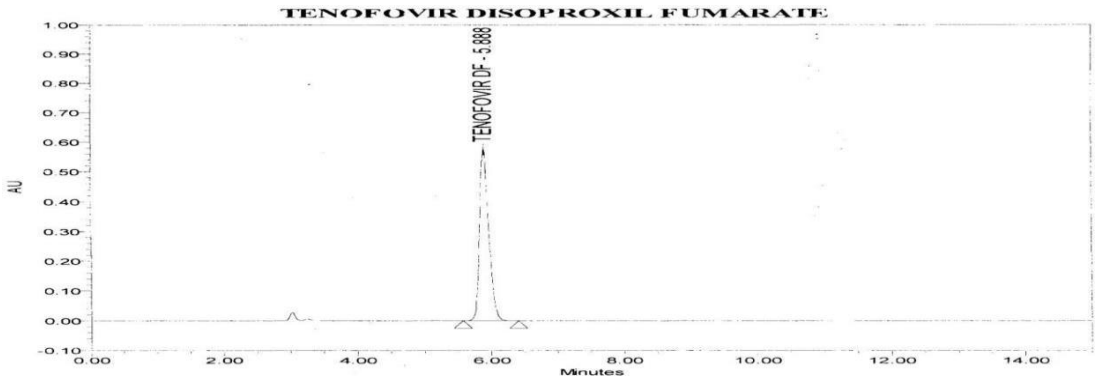
Result: No interference observed. Blank confirmed as non-interfering.

Observation & Conclusion:

The chromatograms confirm that there was:

- No interference from blank or placebo solutions.
- The TDF peak was pure with satisfactory purity angle and threshold.

Therefore, the developed RP-HPLC method is specific for Tenofovir Disoproxil Fumarate.



Comparison of Precision Studies (Repeatability & Reproducibility)

S. No	Parameter	Type of Study	Sample Details	No. of Injections	%RSD	Acceptance Criteria	Result
1	Repeatability	Method Precision	Tablet powder (0.5 mg/mL)	6	0.30	NMT 2.0%	Complies
2	System Precision	Instrumental Precision	Working standard solution (0.5 mg/mL)	6	0.12	NMT 2.0%	Complies
3	Intra-day Precision	Solution Stability	TDF Tablet (0.5 mg/mL) @ 0–12 hrs	6	0.30	NMT 2.0%	Stable up to 12 h
4	Inter-day Precision	Solution Stability	TDF Tablet (0.5 mg/mL) Day 1 vs Day 2	6 (3 per day)	0.46 (Day 1), 0.10 (Day 2)	NMT 2.0%	Stable across days

Observation:

- All %RSD values were well below 2%, confirming the precision, reliability, and stability of the method for TDF assay under various conditions.

Accuracy Results of Tenofovir Disoproxil Fumarate

% Level	Amount Added (mg/mL)	Recovered Conc. (mg/mL)	Mean % Recovery	% RSD	Acceptance Criteria	Result
80%	0.40	0.399	99.7%	0.15	95–105%	Complies
100%	0.50	0.499–0.501	99.8–100.2%	0.23	95–105%	Complies
120%	0.60	0.58–0.59	98%	0.24	95–105%	Complies

Observation:

- The % recovery values across all three concentration levels (80%, 100%, 120%) fall within the acceptable range.
- % RSD values are well below 2%, indicating excellent method accuracy and consistency.

LOD and LOQ for Tenofovir Disoproxil Fumarate

Parameter	Definition	Acceptance Criteria	Observed Value	Result
LOD (Limit of Detection)	Lowest concentration detectable but not quantifiable	S/N ratio ≥ 3	S/N = 2.74	*Below limit*
LOQ (Limit of Quantification)	Lowest concentration quantifiable with acceptable accuracy and precision	S/N ratio ≥ 10	S/N = 11.67	Complies

Observations:

- LOD is slightly below the acceptable S/N threshold (2.74 vs required ≥ 3). May require optimization or reevaluation.
- LOQ meets acceptance criteria with S/N of 11.67, showing that the method can accurately quantify low concentrations of TDF.

ROBUSTNESS STUDY

The robustness of the method was evaluated by introducing deliberate variations in:

1. Flow Rate
2. Column Temperature
3. Mobile Phase Composition

Table: Effect of Method Parameter Variations on Tenofovir Disoproxil Fumarate

Parameter Changed	Condition	RT (min)	Peak Area	USP Tailing	%RSD	Observation
Flow Rate	0.9 mL/min	6.522	6161521.55	1.19	0.85%	Slight increase in RT; within acceptable limit
	1.0 mL/min	5.8886	5524221	1.18		—

Parameter Changed	Condition	RT (min)	Peak Area	USP Tailing	%RSD	Observation
	(normal)					
	1.1 mL/min	5.349	5039639.13	1.16		Slight decrease in RT; acceptable
Temperature	38°C	5.972	5506498.40	1.19	0.83%	Lower temp delayed elution; acceptable
	40°C (normal)	5.800	5524221.04	1.18		—
	42°C	5.795	5438375.04	1.41		Slight early elution, increased tailing
Mobile Phase Composition	40% Water	5.9	5506498	1.19	1.42%	Retention within limit
	45% Water (normal)	5.8	5524221	1.18		—
	50% Water	6.5	6161522	1.19		Delayed elution; acceptable

Conclusion:

- All deliberate variations produced %RSD < 2.0, confirming the robustness of the developed RP-HPLC method for Tenofovir Disoproxil Fumarate.
- The method is reliable under small, deliberate variations in flow rate, temperature, and mobile phase composition.

Ruggedness Study Table for TDF Assay

Parameter	Analyst 1	Analyst 2	Lab 1	Lab 2	Instrument 1 (Waters)	Instrument 2 (Agilent)
Retention Time (min)	5.899	5.879	5.899	5.879	5.887	5.886
Area	5,531,076.96	5,536,208.27	5,531,076.96	5,536,208.27	5,525,710.45	5,546,869.09
USP Plate Count	8717.5	8740	8717.5	8740	8617.5	8647
USP Tailing Factor	1.17	1.17	1.17	1.17	1.175	1.175
Standard Deviation (Area)	1432.28	5824.49	1432.28	4131.73	2106.34	32029.18
% RSD (Area)	0.03%	0.11%	0.03%	0.07%	0.04%	0.58%
Acceptance Criteria	NMT 2.0% RSD	NMT 2.0% RSD	NMT 2.0% RSD	NMT 2.0% RSD	NMT 2.0% RSD	NMT 2.0% RSD
Within Limits?	Yes	Yes	Yes	Yes	Yes	Yes

Observations:

- All ruggedness tests (across analysts, labs, and instruments) had %RSD well below the 2.0% acceptance limit, confirming the method's robustness.
- Instrument 2 (Agilent) showed the highest %RSD (0.58%), still within acceptable limits.
- All tests showed excellent reproducibility, supporting the method's suitability for consistent analytical results under varying conditions

FORCED DEGRADATION STUDIES (TDF)

Purpose:

To study how Tenofovir Disoproxil Fumarate (TDF) breaks down under different stress conditions like acid, base, water, heat, and light.

Conditions Used:

- Acid Hydrolysis: 0.1 M HCl
- Base Hydrolysis: 0.1 M NaOH
- Neutral Hydrolysis: Water
- Thermal Degradation: 60°C
- Photodegradation: UV light at 220 nm

Acid, base, and neutral hydrolysis were done at 100°C.

Types of Degradation:

- Acid degradation
- Base degradation
- Neutral degradation
- Thermal degradation
- ☑ Photodegradation

Degradation Study Table:

S. No	Type of Degradation	Conditions	Agent Used	Sample Taken (ml)	Total Volume (ml)	Exposure Time	After Treatment
1	Acid Hydrolysis	2N HCl	1 ml	5 ml	10 ml	24 hrs	Kept aside for 1 hr
2	Base Hydrolysis	2N NaOH	1 ml	5 ml	10 ml	24 hrs	Kept aside for 1 hr
3	Neutral Hydrolysis	Water at 105°C	5 ml	5 ml	10 ml	24 hrs	Kept aside for 1 hr
4	Thermal Degradation	60°C	None	10 ml	10 ml	24 hrs	Cooled for 1 hr
5	Photodegradation	UV light at 220 nm	None	10 ml	10 ml	24 hrs	Kept aside

Sample Preparation:

1. 20 TDF tablets were weighed to get the average weight.
2. A portion containing 50 mg of TDF was taken.
3. It was added to a 50 ml volumetric flask with 10 ml of diluent, sonicated to dissolve.
4. Volume made up to 50 ml with diluent and filtered (0.45 µm filter).
5. From this, 5 ml was taken and diluted to 10 ml → Final conc.: 0.5 mg/ml

Acid Degradation – Trial 1

Condition	1 mL 1N HCl at room temperature for 24 hrs
Observation	No degradation was found
TDF Retention Time (Rt)	Not applicable

Acid Degradation – Trial 2

Condition			1 mL 1N HCl, reflux at 100°C for 24 hrs						
TDF Rt			6.085 min						
Degradation Products (DP)			4						
S.No	Name	Rt (min)	Area	% Area	USP Resolution	USP Tailing	USP Plate Count	Purity Angle	Purity Threshold
1	DP1	2.550	1937	0.03	—	0.80	4718	40.575	47.575
2	DP2	3.032	167857	2.55	3.29	1.06	7311	0.131	0.323
3	DP3	3.266	102564	1.56	1.47	1.15	5862	13.387	1.328
4	DP4	3.946	5288	0.08	3.34	1.07	4602	13.778	16.240
5	TDF	6.085	6309064	95.69	2.83	1.18	8537	0.069	0.290

Acid Degradation – Trial 3

Condition	1 mL 2N HCl, reflux at 100°C for 24 hrs
TDF Rt	6.053 min
Degradation Products (DP)	6
Observation	More extensive degradation observed

Base Degradation – Trial 1

Condition	1 mL 1N NaOH at room temperature for 24 hrs
Observation	No degradation was found
TDF Retention Time (Rt)	Not applicable

Base Degradation – Trial 2

Condition	1 mL 1N NaOH, reflux at 100°C for 24 hrs
Observation	No degradation was found
TDF Retention Time (Rt)	Not applicable

Base Degradation – Trial 3

Condition	1 mL 2N NaOH, reflux at 100°C for 24 hrs
TDF Rt	6.126 min
Degradation Products (DP)	4
Observation	Significant degradation observed

S.No	Name	Rt (min)	Area	% Area	USP Resolution	USP Tailing	USP Plate Count	Purity Angle	Purity Threshold
1	DP1	2.771	377,798	7.54	—	0.91	5439	0.511	0.415
2	DP2	3.011	236,430	4.72	1.52	1.00	5519	1.634	0.284
3	DP3	3.262	1,602,426	31.97	1.49	1.12	5749	2.989	0.280
4	DP4	3.939	1,515,065	30.23	3.55	1.07	6036	0.171	0.296
5	TDF	6.126	1,280,236	25.54	9.44	1.05	9443	0.149	0.331
	Total Area	—	5,011,955.2	100%					

Thermal Degradation – Trial 1

Condition	TDF kept at 60°C for 3 hours
TDF Rt	6.085 min
Degradation Products (DP)	4
Observation	Moderate degradation observed

S.No	Name	Rt (min)	Area	% Area	USP Resolution	USP Tailing	USP Plate Count	Purity Angle	Purity Threshold
1	DP1	2.550	1937	0.03	—	0.802	4715	40.575	47.575
2	DP2	3.302	167857	2.55	3.29	1.06	7311	0.131	0.323
3	DP3	3.266	102564	1.56	1.47	1.15	5862	13.387	1.328
4	DP4	3.946	5288	1.08	3.34	1.07	4602	13.778	16.240
5	TDF	6.085	6309064	95.69	2.83	1.18	8537	0.069	0.290

Thermal Degradation – Trial 3

Condition	TDF kept at 60°C for 6 hours
TDF Rt	6.053 min
Degradation Products (DP)	6
Observation	Significant degradation observed

S.No	Name	Rt (min)	Area	% Area	USP Resolution	USP Tailing	USP Plate Count	Purity Angle	Purity Threshold
1	DP1	2.770	41140	0.34	—	1.12	5534	6.552	1.771
2	DP2	3.020	313176	2.58	1.68	1.05	7328	0.580	1.417
3	DP3	3.250	407881	3.37	1.48	1.15	5609	6.988	0.459
4	DP4	3.930	268371	2.21	3.55	1.06	5686	0.603	0.666
5	DP5	4.490	3445	0.03	2.42	1.71	6059	55.406	53.406
6	DP6	5.300	12537	0.10	2.92	0.99	5152	21.573	21.573
7	TDF	6.050	1107311	91.36	2.64	1.29	8057	0.148	0.148

Thermal Degradation – Trial 4

Condition	TDF kept at 100°C for 24 hours
TDF Rt	6.066 min
Degradation Products (DP)	5
Observation	Extensive degradation observed

S.No	Name	Rt (min)	Area	% Area	USP Resolution	USP Tailing	USP Plate Count	Purity Angle	Purity Threshold
1	DP1	2.780	39592	0.33	—	1.12	5534	5.615	1.477
2	DP2	3.029	305972	2.58	1.71	1.03	7542	0.625	1.102
3	DP3	3.263	355695	2.99	1.46	1.13	5629	15.339	0.476
4	DP4	3.942	261140	2.20	3.48	1.06	5821	0.671	0.633
5	DP5	5.305	11843	0.10	5.20	1.02	5922	17.929	12.731
6	TDF	6.066	10903727	91.80	2.09	1.28	8156	0.199	0.460

Neutral Degradation Studies:

5 mL of the standard stock solution (0.5 mg/mL) was mixed with 5 mL of distilled water at room temperature. The solution was then heated at 100°C.

Chromatogram for Neutral Degradation

Observation: No degradation was observed.

Photodegradation Studies:

10 mL of the previously prepared test stock solution (0.5 mg/mL) was taken at room temperature. The solution was exposed to UV light for 24 hours.

ExperimentalConditions:
TDF was exposed to 220 W·h/m² of UV light for 2 days.

Chromatogram for Photodegradation

Data for Photodegradation

S. No.	Name	Rt (min)	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
1	DP1	2.785	2,744	0.05	—	1.16	7,087
2	DP2	3.024	128,729	2.52	1.75	1.08	7,881
3	DP3	3.254	166,018	3.25	1.48	1.15	5,847
4	DP4	3.929	133,652	2.62	3.56	1.05	5,981
5	TDF	6.075	4,676,148	91.56	9.19	1.15	9,075

Observation:
TDF was degraded into four degradation products (DP1–DP4). The retention time (Rt) of TDF was found to be 6.075 minutes.

Validation Summary for RP-HPLC Method (TDF)

S. No.	Parameter	Result / Observation	Acceptance Criteria	Status
1	Specificity	Passes (No interference from blank/placebo)	No interference	Accepted
2	Linearity	Correlation Coefficient = 0.999	NLT 0.999	Accepted
3	Accuracy (Mean Recovery)	98.62% (Range: 98%–99.86%)	97–103%	Accepted
4	Precision	RSD ≤ 0.30% for all levels	NMT 2% RSD	Accepted
5	Robustness	RSD ≤ 1.42%, Tailing Factor ≤ 1.26	RSD ≤ 2%, Tailing ≤ 2.0	Accepted
6	Ruggedness	RSD ≤ 0.58% across analysts, instruments, labs	NMT 2% RSD	Accepted
7	System Suitability	Plates: 8777.3, Tailing: 1.17, RSD: 0.30%	NLT 2000, NMT 2.0	Accepted
8	LOD	S/N = 2.74	NMT 3	Accepted
9	LOQ	S/N = 11.64	NMT 13	Accepted

Degradation Studies :

S. No.	Degradation Type	Conditions Applied	Results
1	Acid Degradation	1N/2N HCl, RT & Reflux at 100°C for 24 hrs	0 to 6 degradation products observed
2	Base Degradation	1N/2N NaOH, RT & Reflux at 100°C for 24 hrs	0 to 4 degradation products observed
3	Thermal Degradation	60°C for 1, 3, 6 hrs	0 to 6 degradation products observed
4	Neutral Degradation	Water, heated at 100°C	No degradation observed
5	Photo Degradation	UV light exposure for 24 hrs	4 degradation products observed

DISCUSSION:

- Method Suitability: The RP-HPLC method was found to be specific, linear, accurate, precise, robust, and rugged per ICH guidelines.
- System Performance: High theoretical plate count (8777), low tailing (1.17), and low %RSD indicate excellent chromatographic performance.
- Sensitivity: LOD and LOQ values indicate the method is suitable for detecting low concentrations of TDF.
- Degradation Behavior: TDF is stable under neutral and some thermal/basic conditions but shows notable degradation under acidic and photolytic conditions, producing up to 6 degradation products.

- A RP-HPLC method was developed and validated for the estimation of Tenofovir Disoproxil Fumarate (TDF) in tablet form, as per ICH guidelines.
- The method was tested for the following parameters:
  System suitability, Linearity, Precision, Intermediate precision, Specificity, Accuracy, Ruggedness, Robustness, LOD, and LOQ.
- All validation results were within the acceptance criteria, showing that the method is:
  - Simple
  - Accurate
  - Precise
  - Specific
  - Robust
  - Rugged
- The method was also reliable and worked well across different systems, analysts, and lab conditions.
- TDF is an antiretroviral drug used to treat HIV and hepatitis B. Based on literature, several methods like HPLC, HPTLC, UV, and LC-MS are used to estimate TDF.

- This method was designed to be easy, fast, and suitable for routine quality control.
- The robustness was confirmed by testing small changes in flow rate, mobile phase, and wavelength — results remained consistent.

CONCLUSION:

A simple, accurate, fast, and repeatable RP-HPLC method was developed for estimating TDF in tablets.

- It was validated for key parameters such as system suitability, linearity, precision, accuracy, LOD, LOQ, and robustness, following ICH guidelines.
- Important system values like retention time, peak area, tailing factor, and theoretical plates were all within acceptable limits.
- The mobile phase used was easy to prepare and cost-effective.
- The method remained stable even with impurities and during robustness testing.
- Therefore, this RP-HPLC method can be easily used for routine analysis of TDF in pharmaceutical formulations.

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