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(RESEARCH ARTICLE)

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# Analytical method development and validation of RP-HPLC method for estimation of Tenofovir Alafenamide

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## Abstract

Analytical monitoring of the pharmaceutical products, or of specific ingredients within the product, its necessary to insure it's safely and efficacy throughout all phases of its shelf-life, including storage, distribution and use. Essentially, the techniques of chromatography is based on the differences in the rate at which the components of the mixture move through a porous medium (called stationary phase) under the influence of same solvent or gas (called mobile phase). Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The mobile phase of ACN: aqueous buffer (phosphate buffer pH 4.0) was selected after several permutations and combination. The flow rate was kept at 0.7 mL/min and 285 nm was selected as detection wavelength. Tenofovir Alafenamide was degraded in alkaline (0.1 N NaOH), acidic (0.1 N HCl), oxidative (3% $H_2O_2$), and thermal conditions. Tenofovir Alafenamide drug were almost stable in neutral conditions

**Keywords:** Tenofovir Alafenamide; RP-HPLC; Stability Indicating Study; Forced Degradation; Analytical Chemistry

## 1. Introduction

Analytical chemistry is mainly concerned with determining the qualitative and quantitative composition of material under study[2]. Analytical monitoring of the pharmaceutical products, or of specific ingredients within the product, its necessary to insure it's safely and efficacy throughout all phases of its shelf-life, including storage, distribution and use. Analysis is important in every product, but it is especially vital in medicine as it deals with life. In the manufacture of drug, it is necessary to follow certain recommended practices for overall control to ensure that consumer receives drug of high quality.

Qualitative analysis: it deals with the identification of substances. It is concerned with what elements or compounds are present in the sample.

Quantitative analysis: it provides numerical information concerning the quantity of the same species (the analyte) in a measured amount of matter (the sample). Various techniques employed for analysis and method development are as follows:-

Spectrophotometric method : - UV

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Chromatographic method : - HPLC, HPTLC C] Other methods: - LC-MS, GC-MS

Essentially, the techniques of chromatography is based on the differences in the rate at which the components of the mixture move through a porous medium (called stationary phase) under the influence of same solvent or gas (called mobile phase).

The chromatographic method of separation, in general, involves the following steps

- Retention of a substance or substances on the stationary phase
- Separation of the adsorbed substances by the mobile phase
- Recovery of the separated substances by a continuous flow of the mobile phase
- Qualitative and quantitative analysis of the eluted substance

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirement for the intended analytical applications.

Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. It, is mainly an investigation of the intrinsic stability of the molecule providing the foundation for developing and validating the analytical methods and for developing the stable formulations. Forced degradation is carried out to produce representative samples for developing stability-indicating methods for drug substances and drug product

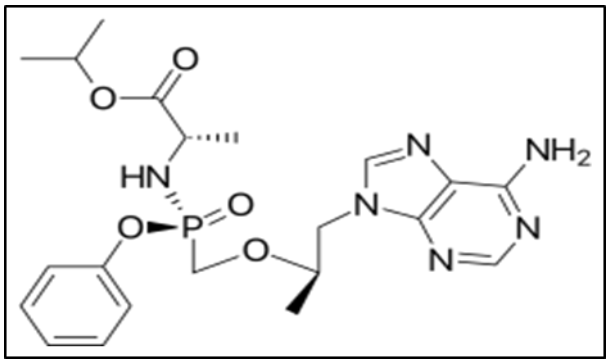
### 1.1. Drug profile

Drug profiles are scientifically sound descriptions of drugs in the form of 'drug profiles'. Presented in a standardized way.

Tenofovir Alafenamide Structure:

It is official in IP[28a].

**IUPAC Name:** [(2R)-1-(6-Amino-9H-purin-9-yl) propan-2 yl] oxymethylphosphoryl] oxy-methyl-N-phenylalaninamide




**Molecular Formula:** C21H29N6O5P **Molecular Weight:** 476.46 g/mol **Appearance:** White to off-white powder

**Description:** Tenofovir alafenamide is a nucleotide reverse transcriptase inhibitor (NRTI) and a prodrug of tenofovir. It is used in the treatment of HIV-1 and chronic hepatitis B infection.

**Melting Point:** 279–282°C

**Mode of action:** TAF is converted intracellularly to tenofovir, which is then

phosphorylated to the active metabolite tenofovir diphosphate. This active form inhibits HIV reverse transcriptase by competing with natural substrates and causing DNA chain termination.

## 2. Experimental work and results

### 2.1. Chemicals and Reagents

HPLC grade Acetonitrile, Methanol and Milli-Q water was used for dilution of stock solution.

**Table 1** Reagents/ Materials

| Sr. No. | Reagents/ Materials | Grade |
|---|---|---|
| 1 | Milli-Q water | HPLC grade |
| 2 | Methanol | HPLC grade |
| 3 | Acetonitrile | HPLC grade |
| 4 | phosphate buffer | HPLC grade |

### 2.2. Instrument

Agilent- 1100 series HPLC system comprising of Quaternary gradient pumps G1311A with on line Degasser G1322A, Variable wavelength UV detector G1314A, Autosampler G1313A, Zodiac 100 C18 column (250mm x 4.6 mm i.d, 5um).

- UV Spectrophotometer (PROLAB)
- Digital Balance (RADWAG)
- Ultra-Sonicator
- Digital pH meter (Prolab India).
- Digital Hot air oven
- Preparation of Standard Solutions

Preparation of Stock standard solution of Tenofovir Alafenamide:

Accurately weighed quantity of 10.0 mg of Tenofovir Alafenamide transferred to 10.0 ml volumetric flask. & the volume was made up to 10 ml using methanol.

Working standard solution of Tenofovir Alafenamide

Dilute 5 ml of standard stock solution to 10 ml volumetric flask and make up the volume with mobile phase to get a concentration of 500 µg/ml.

Fixation of suitable parameters for the drugs Location of λmax

An aliquot portion of standard stock solution A, were appropriately diluted with mobile phase to get conc. 100µg/mL. The solutions were scanned in the range of 400nm to 200nm against solvent blank. Tenofovir Alafenamide shows maximum absorbance at 285 nm.

### 2.3. Selection of mobile phase

The chromatographic conditions were set as per the given parameters. Various mobile phases were tried by permutation and combination shown in Table. The mobile phase containing mixture of ACN: aqueous buffer (phosphate buffer pH 3.0) (60:40) was found to be most satisfactory as it gave good resolution and sharp peaks. The detection wavelength selected was 285 nm and flow rate of 0.7 ml/min

### 2.4. Final chromatographic condition

**Sample Preparation:** 10mg Tenofovir Alafenamide taken in 10ml volumetric flask and make up the volume with Methanol.

**Table 2** Final Chromatographic Condition

| **Column** | **Zodiac 100 C18 Column** | | |
|---|---|---|---|
| Mobile phase | ACN: aqueous buffer (phosphate buffer pH 4.0):(60:40) | Injection volume | 10µL |
| Wavelength | 285nm | Temperature | 25°C |
| Flow rate | 0.7 ml/min | Run time | 15min |
| Retention Time | 4.408 | Diluent | ACN: aqueous buffer (phosphate buffer pH 4.0) |
| pH | 4.0 | | |

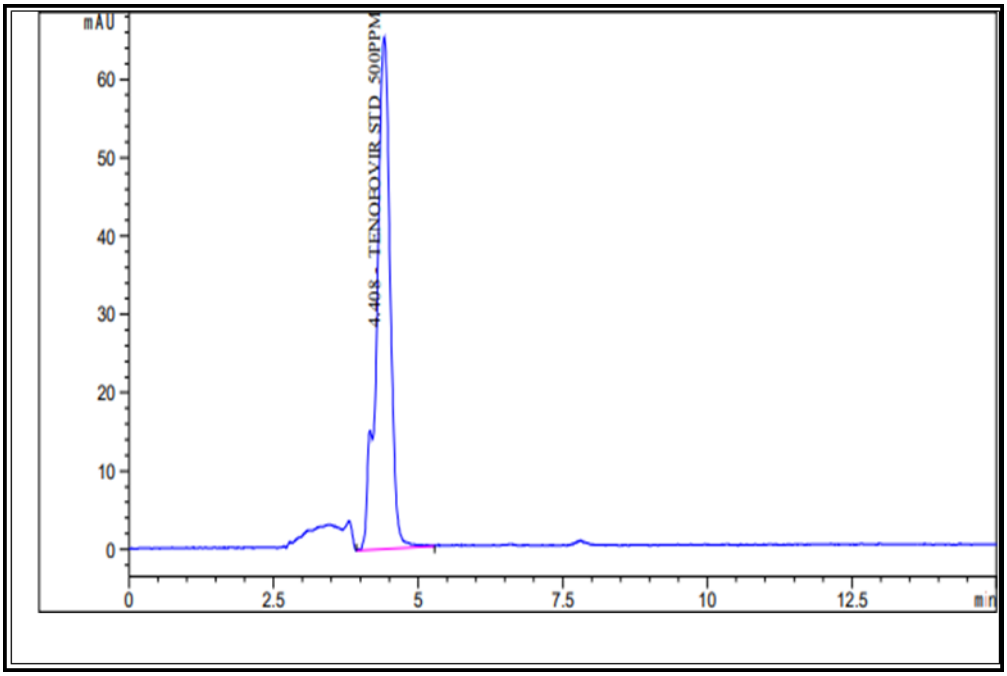



**Figure 1** Chromatogram of Tenofovir Alafenamide

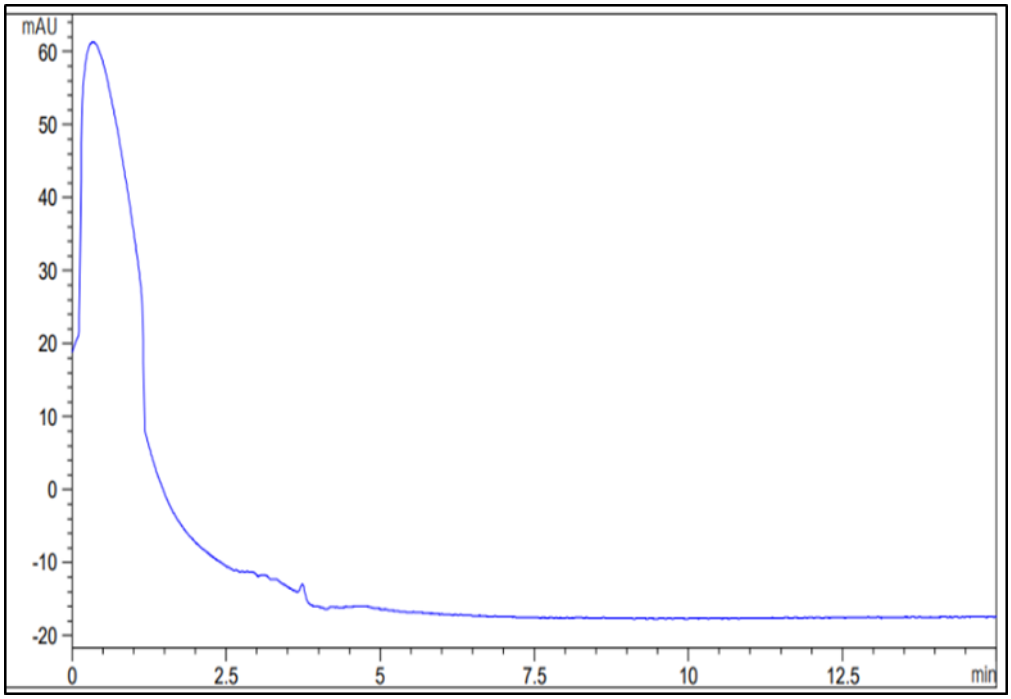



**Figure 2** HPLC Chromatogram of Blank

### 2.5. Validation of proposed method.

According to the guidelines of ICH Q2 (R1) all the parameters as discussed below were analyzed and validated accurately following the procedure of the proposed method.

### 2.6. System suitability test parameters of Tenofovir Alafenamide

The system suitability is the pharmacopeial requirement and is used to verify the resolution and reproducibility of the chromatographic system. The test was performed by collecting the data from five replicate injection of standard solution.

**Table 3** System suitability test parameters of Tenofovir Alafenamide

| Sr.No | Retention time | Peak area | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| 1 | 4.41 | 1050 | 2155 | 0.88 |
| 2 | 4.42 | 1053 | 2162 | 0.84 |
| 3 | 4.41 | 1050 | 2158 | 0.87 |
| 4 | 4.42 | 1052 | 2160 | 0.88 |
| 5 | 4.41 | 1053 | 2158 | 0.88 |

**Table 4** Statistical Parameters

| Statistics | | | | |
|---|---|---|---|---|
| Mean | 4.41 | 1051 | 2159 | 0.87 |
| ±SD | 0.00 | 1.36 | 2.33 | 0.02 |
| %RSD | 0.11 | 0.13 | 0.11 | 1.78 |

**Accuracy:** It was determined on the basis of recovery study performed by standard addition method.

**Table 5** Accuracy parameters

| **Drug Name: Tenofovir Alafenamide** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Std. conc. (%) | Std. (ppm) | Peak area | Drug (%) | Drug (ppm) | Peak area | Avg. peak area | Drug Rec. (%) |
| 100% | 500 ppm | 1050 | 50 | 250 | 526 | 523 | 99.43 |
| | | | | 250 | 520 | | |
| | | | 100 | 500 | 1050 | 1052 | 99.81 |
| | | | | 500 | 1054 | | |
| | | | 150 | 750 | 1570 | 1569 | 99.40 |
| | | | | 750 | 1567 | | |
| Drug recovery Range (%) as per ICH = 100±10% | | | | | | | 99.46%-99.81 % |

### 2.7. Preparation of Calibration Curves for Tenofovir Alafenamide

Each of the standard solution was injected separately. The aliquot portion of stock solution A mobile phase to get concentration of 20, 40, 60, 100, 120 µg/ml for Tenofovir Alafenamide. The chromatogram was recorded.

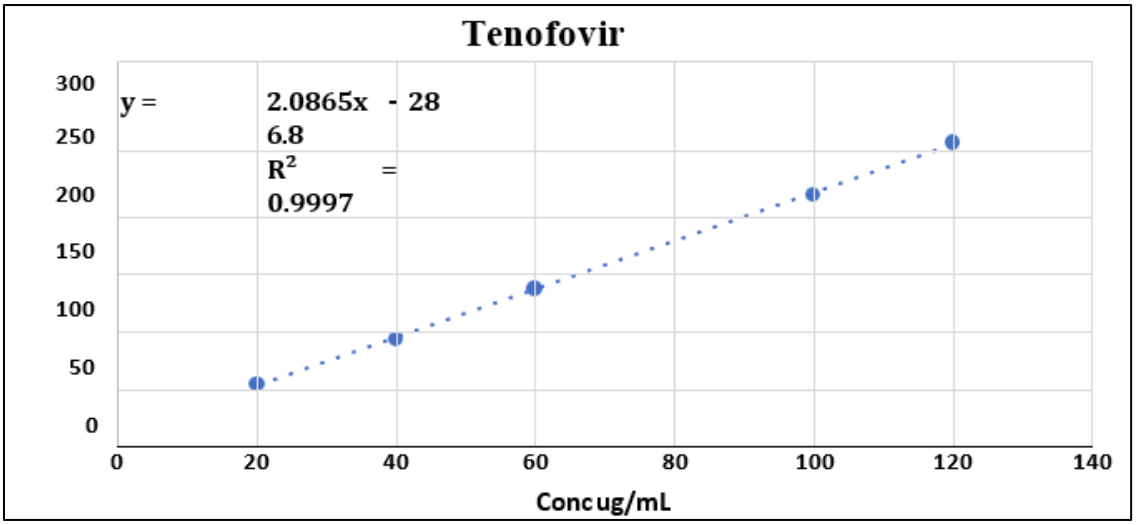



**Figure 3** Calibration curve for Tenofovir Alafenamide

**Linearity:** The series of solution of curve were analyzed in 10 to 120 µg/ml, results are shown in Fig. 10

**Table 6** Linearity parameters

| **Sr. No.** | **Parameters** | **Tenofovir Alafenamide** |
|---|---|---|
| 1 | Linear dynamic range (µg/ml) | 10-120 |
| 2 | Slope | 2.0865 |
| 3 | Y-intercept | 6.8628 |
| 4 | Correlation coefficient ($R^2$) | 0.9997 |

### 2.8. Specificity study

Forced degradation study of Tenofovir Alafenamide was carried out under acidic, alkaline, neutral and thermal stress conditions. All chromatographic parameters were kept constant as in method development.

*2.8.1. Acidic hydrolysis study*

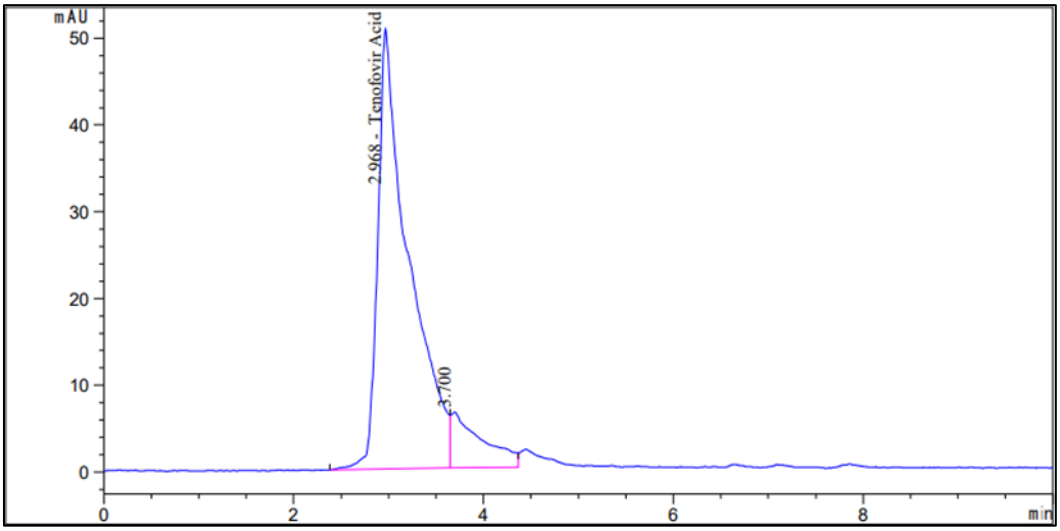



**Figure 4** HPLC Chromatogram of specificity study of Tenofovir Alafenamide of 0.1 M HCL (At room temp. for 24hrs.)

### 2.9. Neutral degradation study

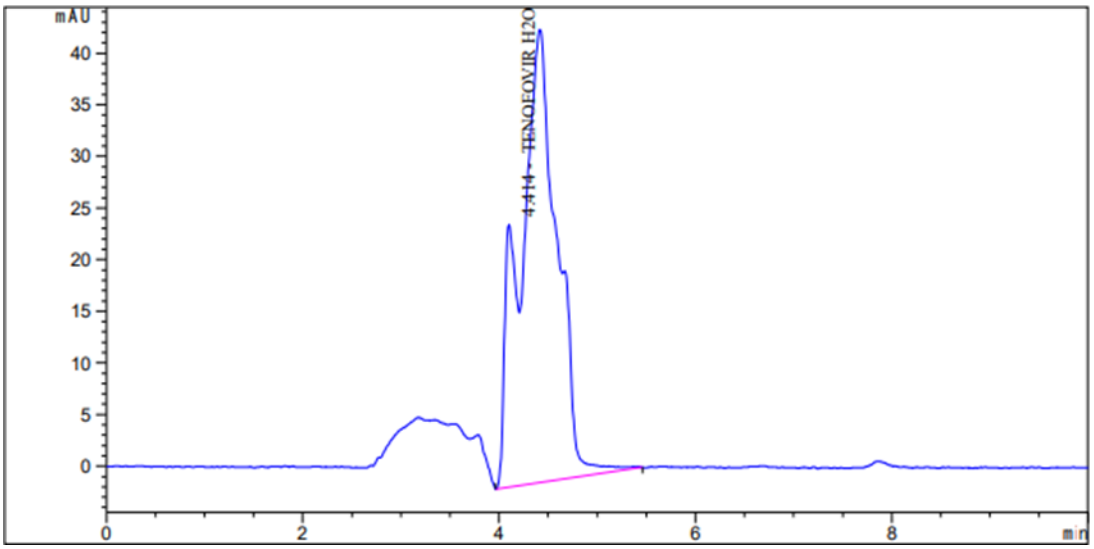



**Figure 5 HPLC** Chromatogram of specificity study of Tenofovir Alafenamide of water (neutral solvent at room temperature for 24hrs.)

### 2.10. Alkaline hydrolysis study

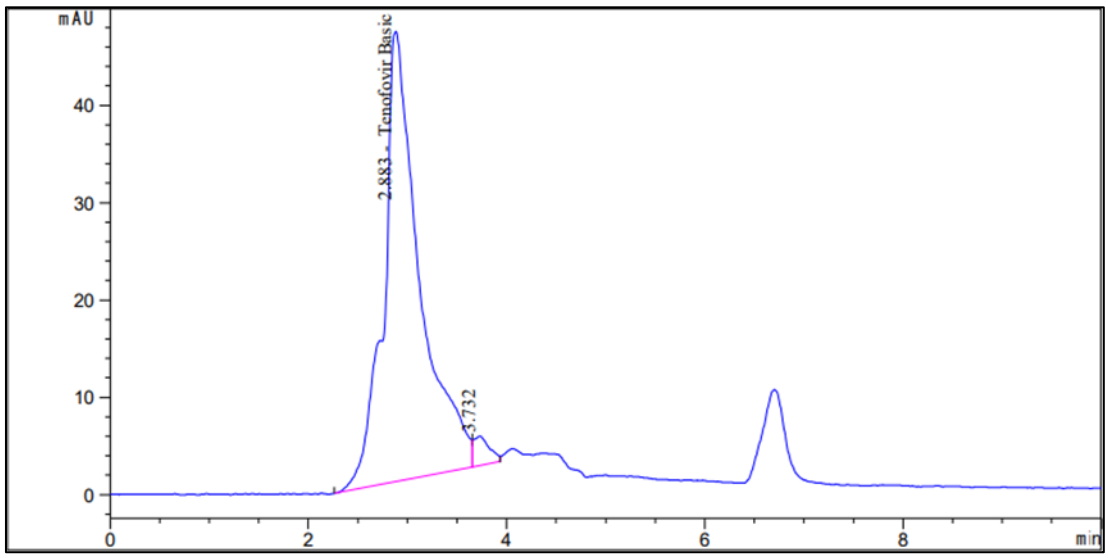



**Figure 6** HPLC Chromatogram of specificity study of Tenofovir Alafenamide of 1 M NaOH (At room temp. for 24hrs.)

### 2.11. Thermal Degradation study

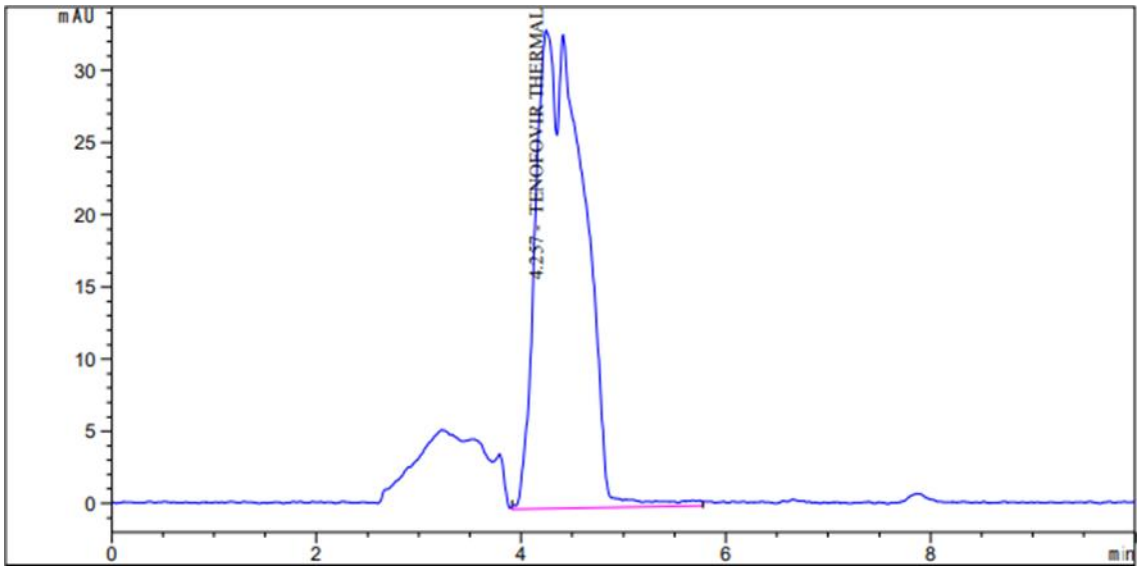



**Figure 7** HPLC Chromatogram of specificity study of Tenofovir Alafenamide of Thermolysis

**Table 7** Specificity study of Tenofovir Alafenamide by HPLC

| Medium | AREA | % AREA | PERCENT RECOVERY (%) |
|---|---|---|---|
| Standard | 1050 | NA | NA |
| Heat (at 60ºC)) | 1032 | 98.3 | 1.7 |
| Alkaline Hydrolysis (0.1 M NaOH) | 994 | 94.7 | 5.3 |
| Acid Hydrolysis (0.1 M HCl | 889 | 84.7 | 15.3 |
| Neutral | 1034 | 98.5 | 1.5 |

### 2.12. Precision study

**Table 8 A)** Data of intraday study

| **Conc. of Standard (µg/ml)** | **Weight of tablet powder taken (mg)** | **AUC of standard solution** | **AUC of Sample** | **% Drug estimated** |
|---|---|---|---|---|
| Drug Name: Tenofovir Alafenamide | | | | |
| 500 | 242.3 | 1050 | 1054 | 100.38 |
| | | | 1046 | 99.62 |
| | | | 1041 | 99.14 |

**Table 9 B**) Data of Interday

| **Conc. of Standard(µg/ml)** | **Weight of tablet powder taken (mg)** | **AUC of standard solution** | **AUC of Sample** | **% Drug estimated** |
|---|---|---|---|---|
| Drug Name: Tenofovir Alafenamide | | | | |
| 500 | | 1050 | 1041 | 99.14 |
| | | | 1012 | 96.38 |
| | | | 1049 | 99.90 |

**Table 10 C**) Data of different analyst

| **Conc. of Standard (µg/ml)** | **Weight of tablet powder taken (mg)** | **AUC of standard solution** | **AUC of Sample** | **% Drug estimate d** |
|---|---|---|---|---|
| Drug Name: Tenofovir Alafenamide | | | | |
| 500 | 242.3 | 28678 | 1038 | 98.86 |
| | | | 1032 | 98.29 |
| | | | 1029 | 98.00 |

**Table 11** D) Statistical parameters

| **Sr. No.** | **Parameter** | **% of labeled claim** | | |
|---|---|---|---|---|
| | | Intermediate precision | | |
| | | Intraday | Interday | Different |
| | | Drug Name: Tenofovir Alafenamide | | |
| 1 | Mean | 99.71 | 98.48 | 98.38 |
| 2 | ± S.D | 0.51 | 1.51 | 0.36 |
| 3 | %RSD | 0.51 | 1.54 | 0.36 |

## 3. Summary and discussion

These methods are optimized for accuracy, precision, and cost-effectiveness, aiming to ensure reliable quantification in complex matrices. The simple, precise and accurate HPLC method was developed for estimation of Tenofovir Alafenamide using $C_{18}$ column. The mobile phase of ACN: aqueous buffer (phosphate buffer pH 4.0) was selected after several permutations and combination. The flow rate was kept at 0.7 mL/min and 285 nm was selected as detection wavelength. The stress degradation study of Tenofovir Alafenamide was carried out according to ICH guidelines by HPLC. The stability of both the drug was evaluated under six type of conditions. Tenofovir Alafenamide was degraded in alkaline (0.1 N NaOH), acidic (0.1 N HCl), oxidative (3% $H_2O_2$), and thermal conditions. Tenofovir Alafenamide drug were almost stable in neutral conditions

**Table 12** Summarized results of proposed HPLC method

| Parameters | ROS |
|---|---|
| Linearity range (µg/mL) | 20-120 |
| Correlation coefficient | 0.9997 |
| Retention time (min) | 4.41 |
| Tailing factor | 1.78 |
| Theoretical plates | 2159 |
| % Recovery | 99.46% - 99.81 % |
| Specificity | 84.07-98.5 |
| Intraday Precision (%RSD) | 0.51 |
| Interday Precision (%RSD) | 1.54 |
| Different Analyst (%RSD) | 0.36 |

## 4. Conclusion

The proposed HPLC methods were developed for the determination of Tenofovir Alafenamide in pharmaceutical formulation were simple, accurate, sensitive and reproducible. Statistical analysis proves that the methods were repeatable and selective for the analysis of Tenofovir Alafenamide. The methods were completely validated as per ICH Q2 (R1) guidelines showing satisfactory data for all the parameter tested. The HPLC method presented are direct, simple, selective, reproducible, sensitive and linear. The procedure was successfully applied to the simultaneous estimation of studied compound in pharmaceutical formulations without any interference from the additives and endogenous substances.

Stress degradation study were carried out on all the three developed methods as per ICH Q1A (R2) guidelines. The degradation study shows that the drugs were almost stable in neutral condition. Both the drugs were degraded in alkaline, oxidative, photolytic and thermal condition,

## Compliance with ethical standards

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*Disclosure of conflict of interest*

The authors declare no conflicts of interest, financial or otherwise.

*Availability of data and materials*

Supplementary material that is available on the publisher's website along with the published article contains spectroscopic data of all compounds.

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