*Journal of Pharmaceutical Research International*

***33(44A): 189-196, 2021; Article no.JPRI.73941***
***ISSN: 2456-9119***
*(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)*

# RP-HPLC Method for Quantification of Emtricitabine and Tenofovir Alafenamide Fumarate Drug Substance and Tablet Dosage Form

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/JPRI/2021/v33i44A32606
Editor(s):
(1) Dr. Thomas F. George, University of Missouri- St. Louis, USA.
Reviewers:
(1) Jinal N. Tandel, Charutar Vidyamandal University, India.
(2) Hind Hadi Abdullah, University of Baghdad, Iraq.
Complete Peer review History: https://www.sdiarticle4.com/review-history/73941

***Original Research Article***

***Received 03 July 2021***
***Accepted 13 September 2021***
***Published 17 September 2021***

## ABSTRACT

**Aim:** The primary objective of the research work is to develop a effective, sensitive, economical and simple reverse phase HPLC method to estimate Emtricitabine and tenofovir alafenamide fumarate in its pure and binary mixture of tablets.
**Study Design:** HPLC based Quantification Studies.
**Place and Duration of Study:** 1Department of Chemistry, Acharya Nagarjuna University,Guntur, Andhra Pradesh between April 2019 and August 2020.
**Methodology:** Separation of the analytes were done by using Eclipse XDB-Phenyl (250 x 4.6mm, 5µ,100 A0) column and a mobile phase ratio of 30:10:70 percentage of 0.1% trifluoro acetic acid: acetonitile: methanol at a flow rate of 1 ml/min. The injected standard and sample solutions were detected 260nm wavelength.
**Results:** The retention time of Emtricitabine and tenofovir alafenamide fumarate were found at 2.3min and 2.8 min respectively. The method has good linearity range about 50 to 150µg/ml of Emtricitabine and 6.5 to 19.5 µg/ml of tenofovir alafenamide fumarate. The method has validated as per ICH guidelines and all the validation parameterwere satisfy the ICH Q2 specification acceptance limits

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**Conclusion:** The developed method said to be highly sensitive, accurate, specific and robust, therefore this method has high probability to adopt in pharmaceutical industry for regular analysis of Emtricitabine and tenofovir alafenamide.

*Keywords: Tenofovir alafenamide fumarate; Emtricitabine, HPLC based Quantification Studies; Eclipse XDB-Phenyl column; Sensitive.*

## ABBREVIATIONS

*FDA: Food and Drug Administration*
*HIV: Human Immune Virus*
*TAF: Tenofovir alafenamide fumarate*
*HPLC: High-Performance Liquid Chromatography*
*RT: Retention Time*
*LOD: Limit of Detection*
*LOQ: Limit of Quantification*
*ICH: International Council on Harmonization*
*SD: Standard Deviation*
*RSD: Relative Standard Deviation*

## 1. INTRODUCTION

Research scientists and health care experts have been doing research against effective treatment of human immune virus (HIV) infection for 20 years. To treat this infection, till now around 20 anti viral agents have been approved by food and drug administration (FDA). Significant changes have been occurring in the treatment due to drug resistance, pill burden, and drug tolerability. To overcome this problem, fixed dose combinations of anti viral agents have been developed. Among those a fixed dose combination Emtricitabine and Tenofovir alafenamide fumarate (TAF) tablet administered once a day is effect in the treatment of HIV infection.

Chemically Emtricitabine is 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one with chemical formula $C_8H_{10}FN_3O_3S$. The antiviral activity of Emtricitabine is due to its metabolite Emtricitabine 5'-triphosphate, which effectively inhibits the reverse transcriptase enzyme blocks replication of the HIV [1,2]. Tenofovir alafenamide fumarate is a prodrug of Tenofovir prepared by reacting one mole of fumaric acid with two moles of tenofovir alafenamide. The IUPAC name of TAF is (2E)-but-2-enedioic acid, propan-2-yl(2S)-2-[[[(2R)-1-(6-aminopurin-9-yl)propan-2-yl]oxymethyl phenoxyphosphoryl] amino]propanoate with chemical formula $C_{46}H_{62}N_{12}O_{14}P_2$ . It is also reverse transcriptase inhibitor most commonly used in antiviral combination therapy. Based on literature as of now different analytical method were reported for both Emtricitabine and TAF individually [2-8]. Along with the individual methods, analytical and bio analytical methods were available in simultaneous estimation in combination with other antiviral drugs [9,10]. Only few RP-HPLC method were reported for simultaneous estimation of Emtricitabine and TAF in fixed combination regimen which are not economical due to longer runtime, less sensitivity and composition of complicated mobile phase [11-12]. Hence a new method development was attempted to make a good method with cost effective and industrial use for routine analysis of fixed dose combination of Emtricitabine and TAF. The Chemical structures of Emtricitabine and TAF were mentioned in Fig. 1.

## 2. MATERIALS AND METHODS

Pure drug substance of Emtricitabine and TAF were collected as gift sample from Fortune Pharma private limited, Hyderabad. HPLC grade solvents were obtained from Merck India, Mumbai, India

### 2.1 Chromatographic Conditions

To carry out the present reverse phase liquid chromatography method WATERS HPLC, Model: 2695 with 2487 PDA detector having an automated sample injecting system was used. The output signal was processed and computed using Empower 2 software. Chromatographic separation was done by using Eclipse XDB-Phenyl (250 x 4.6mm, 5µ,100 $A^0$) column and a mobile phase ratio of 30:10:70 percentage of 0.1% trifluoro acetic acid : acetonitile: methanol at a flow rate of 1ml/min. The injected standard and sample solutions were detected 260nm wavelength. All the solutions have been filtered through the 0.45µm nylon filters. To prepare standard and sample solution water: methanol (50:50) selected as diluent based on the solubility of Emtricitabine and TAF. Optimized chromatogram was shown in Fig. 2.

### 2.2 Preparation of Standard Solution

10mg of Emtricitabine and 1.3 mg of TAF pure API's were weighed accurately and dissolved

with to diluent (Methanol and water(50:50)) to made a solution having 100µg/ml and 13µg/ml concentration of Emtricitabine and TAF respectively, which is expressed as 100% level solution.

## 2.3 Preparation of Standard Solution

Tablet (Taficita™) powder equivalent to 10mg of Emtricitabine and 1.3 mg of TAF were weighed accurately and dissolved with to diluent (Methanol and water(50:50)) to made a solution having 100µg/ml and 13µg/ml concentration of Emtricitabine and TAF respectively.

## 2.4 Method Validation

The adopted method has been validated with respective to Q2 guidelines of ICH.

### 2.4.1 System SUITABILITY TEST

Five replicate injections of Emtricitabine and TAF standard solution used to carry out the system suitability test. Parameters such as USP plate count (N), USP tailing (T) and percentage relative standard deviation (%RSD) values have been computed.

### 2.4.2 Linearity

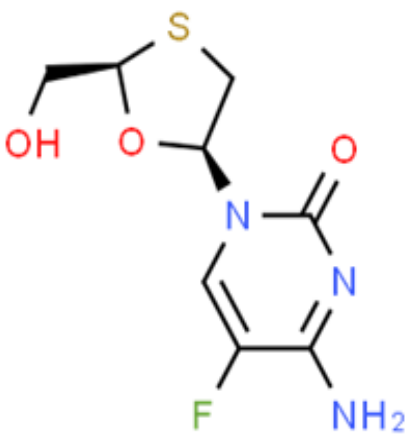
The linearity of the method reflects that the peaks areas are directly proportional to concentrations. Linearity of the method was done by plotting a graph between concentration Vs peak area for both drugs in standard solutions concentrations of 50µg/ml to 150µg/ml of Emtricitabine and 6.5µg/ml to 19.5µg/ml of TAF into HPLC instrument. Regression coefficient ($r^2$) values were determined from the linearity graphs of the both drugs.

### 2.4.3 Precision

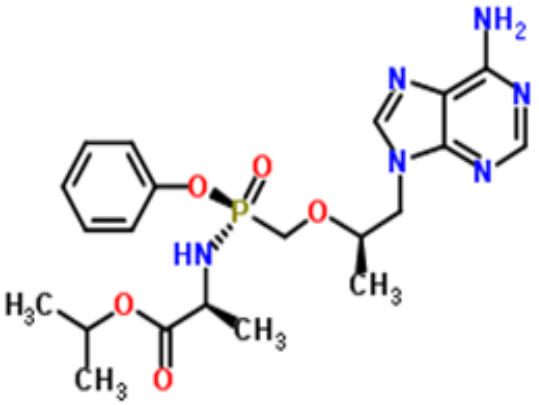
The closeness relationship among the peak areas of homogenous solution on repeated injections termed as precision. it has performed by injecting 100% level solution for 5 replicates in a day for three days, % RSD value was computed for obtained responses.

### 2.4.4 Accuracy

The accuracy represents the closeness relationship between standard and observed responses. It was done by performing percentage recovery studies, where spiking of sample solution in to standard solution at three levels like 50, 100, and 150%. The each spiked level solutions introduced in to HPLC system in triplicate. The mean percentage recovery at three different levels of the drug solution was calculated.

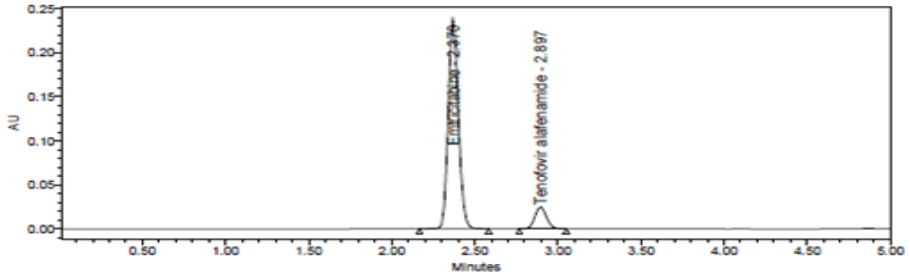



Emtricitabine




Tenofovir alafenamide fumarate

**Fig. 1.Chemical structures of Emtricitabine and Tenofovir alafenamide fumarate**




Chromatogram

| Peak Name | RT (Min) | Area | USP Plate Count | USP Tailing | USP Resolution |
|---|---|---|---|---|---|
| Emtricitabine | 2.370 | 1003973 | 7210 | 1.11 | - |
| Tenofovir alafenamide | 2.897 | 120631 | 8048 | 1.08 | 4.1 |

Results of obtained chromatogram

**Fig. 2. Optimized chromatogram of Emtricitabine and TAF**

### 2.4.5 Specificity

The capacity of the method to determine the substance to be analyzed in the presence impurities and other substances without intrusion represents methods specificity. It has been performed by injecting blank, standard solution and standard solution with placebo. Chromatograms were observed for interference with the RT of Emtricitabine and TAF.

### 2.4.6 Sensitivity

The LOD and LOQ of the method were calculated by using Standard deviation method.

**LOD=3σ/S**

**LOQ = 10 σ/S**

Where, σ - Standard deviation of the responses
*S* - Slope of the standard curve

### 2.4.7 Robustness

To check the robustness of the adopted method small changes were made in the flow rate (± 0.1 ml/min) and maximum absorption wave length (± 2nm).

## 2.5 Assay

The percentage purity of marketed tablets were estimated by injecting 100 % level concentration of standard solution and sample solution.

## 3. RESULTS AND DISCUSSION

## 3.1 Method Validation

### 3.1.1System suitability

All the system suitability parameters values were within the showed acceptance limits, which are mentioned by ICH. Results and acceptance limits were shown in Table 1 and Table 2 respectively.

**Table 1. System Suitability test of Emtricitabine and TAF**

| | Emtricitabine (100µg/ml) | | | | TAF(13µg/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| **Injection no** | **RT** | **Peak area** | **USP plate count** | **USP tailing** | **RT** | **Peak area** | **USP plate count** | **USP tailing** |
| 1 | 2.37 | 1003973 | 7210 | 1.11 | 2.897 | 120631 | 8048 | 1.06 |
| 2 | 2.369 | 1004573 | 7277 | 1.1 | 2.898 | 120664 | 7936 | 1.06 |
| 3 | 2.37 | 1003068 | 7102 | 1.11 | 2.899 | 120644 | 7876 | 1.07 |
| 4 | 2.368 | 1003764 | 7293 | 1.11 | 2.895 | 120518 | 8107 | 1.07 |
| 5 | 2.369 | 1000930 | 7253 | 1.11 | 2.897 | 120077 | 7933 | 1.06 |
| **Mean** | | **1003262** | **7227** | **1.108** | | **120506.8** | **7980** | **1.064** |
| **STDEV** | | **1409.9** | **76.56** | **0.0044** | | **246.8** | **94.4** | **0.0054** |
| **%RSD** | | **0.14** | **1.0** | **0.40** | | **0.20** | **1.18** | **0.51** |

**Table 2. Acceptance limits of system suitability parameters**

| Parameter | Acceptance limit |
|---|---|
| USP Plate count | >2000 |
| USP tailing | ≤2 |
| %RSD | ≤2 |
| Resolution | >2 |

### 3.1.2 Linearity

The $r^2$ values for the linearity curves of 50 to 150µg/ml concentration of Emtricitabine and 6.5 to 19.5 µg/ml of TAF were found as 0.999 and 0.999 respectively. Those values states that the proposed method has acceptable linearity over the specified concentration ranges. The results were stated in Table 3 and Fig. 3.

### 3.1.3 Accuracy

The Percentage mean recovery At the mentioned three different levels mean recovery percentages for Emtricitabine and TAF were in the range of 99.2 to 99.8% which are within the Table 4.

### 3.1.4 Precision

The % RSD of the injected 100% level standard solution of Emtricitabine and TAF and were not more than 2 and results were represented in Table 5 states the methods precision.

### 3.1.5 Sensitivity

The LOD and LOQ of Emtricitabine **was** 1.3µg/ml and 4µg/ml and **TAF** was 1µg/ml and 2µg/ml states high level sensitivity of the developed method.

### 3.1.6 Robustness

Intentionally made small changes in flow rate and maximum absorption wavelength in the proposed method has produced system suitability parameter observed values were in the acceptance limit (Table 6) depicts the robustness of the developed method.

## 3.2 Assay

The percentage purity of the marketed tablet was found to be 100.02% and 99.3% mentioned in Table 7.

**Table 3. Linearity curve of Emtricitabine and TAF**

| | Emtricitabine | | TAF | |
|---|---|---|---|---|
| **% level** | **Concentration(µg/ml)** | **Peak area** | **Concentration(µg/ml)** | **Peak area** |
| 50 | 50 | 504197 | 6.5 | 60649 |
| 75 | 75 | 728128 | 9.75 | 88129 |
| 100 | 100 | 995636 | 13 | 116620 |
| 125 | 125 | 1253650 | 16.25 | 149852 |
| 150 | 150 | 1514911 | 19.5 | 181028 |
| **Correlation coefficient($r^2$)** | | **0.9996** | | **0.9993** |

**Table 4. Results of accuracy studies**

| % | Emtricitabine (µg/ml) | | | | TAF (µg/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| **Level** | **Amount added** | **Amount recovered** | **% Recovery** | **% Mean recovery** | **Amount added** | **Amount recovered** | **% Recovery** | **% Mean recovery** |
| | 50 | 49.7 | 99.4 | 99.3 | 6.5 | 6.46 | 99.5 | |
| 50% | 50 | 49.8 | 99.7 | | 6.5 | 6.48 | 99.8 | 99.8 |
| | 50 | 49.4 | 98.9 | | 6.5 | 6.51 | 100.3 | |
| 100% | 100 | 99.8 | 99.8 | 99.4 | 13 | 12.96 | 99.7 | |
| | 100 | 99.5 | 99.5 | | 13 | 12.83 | 98.7 | 99.2 |
| | 100 | 98.9 | 98.9 | | 13 | 12.92 | 99.4 | |
| | 150 | 148.6 | 99.1 | 98.7 | 19.5 | 19.4 | 99.5 | |
| 150% | 150 | 147.7 | 98.5 | | 19.5 | 19.46 | 99.8 | 99.7 |
| | 150 | 148.0 | 98.7 | | 19.5 | 19.48 | 99.9 | |
| Acceptance limit: 100±2 | | | | | | | | |

**Table 5. Results of precision of 100% level solution**

| Precision | | Emtricitabine | | TAF | |
|---|---|---|---|---|---|
| Repeatability | **S.NO** | **RT** | **Area** | **RT** | **Area** |
| Or | Mean | 2.31 | 1003262 | 2.89 | 120506.8 |
| Intra day | Std. Dev. | 0.001 | 1409.9 | 0.011 | 246.8 |
| | % RSD | 0.04 | 0.14 | 0.38 | 0.20 |
| | Mean | 2.369 | 999966 | 2.901 | 118414 |
| Intra day | Std. Dev. | 0.001 | 2450 | 0.001 | 528 |
| | % RSD | 0.02 | 0.25 | 0.03 | 0.45 |

**Table 6. Robustness results of Emtricitabine and TAF**

| Parameter | | RT (Min) | Peak area | USP plate count | RT (Min) | Peak area | USP Plate count |
|---|---|---|---|---|---|---|---|
| **Flow rate (±0.1ml)** | 0.9 | 2.584 | 1084907 | 7193 | 3.17 | 115306 | 8127 |
| | 1 | 2.37 | 1003973 | 7210 | 2.897 | 120631 | 8048 |
| | 1.1 | 2.19 | 926656 | 7283 | 2.685 | 98598 | 8201 |
| **Maximum wavelength (± 2nm)** | 258 | 2.37 | 1003973 | 7365 | 2.897 | 120631 | 8195 |
| | 260 | 2.37 | 1003973 | 7210 | 2.897 | 120631 | 8048 |
| | 262 | 2.369 | 1004573 | 7156 | 2.898 | 120664 | 8056 |

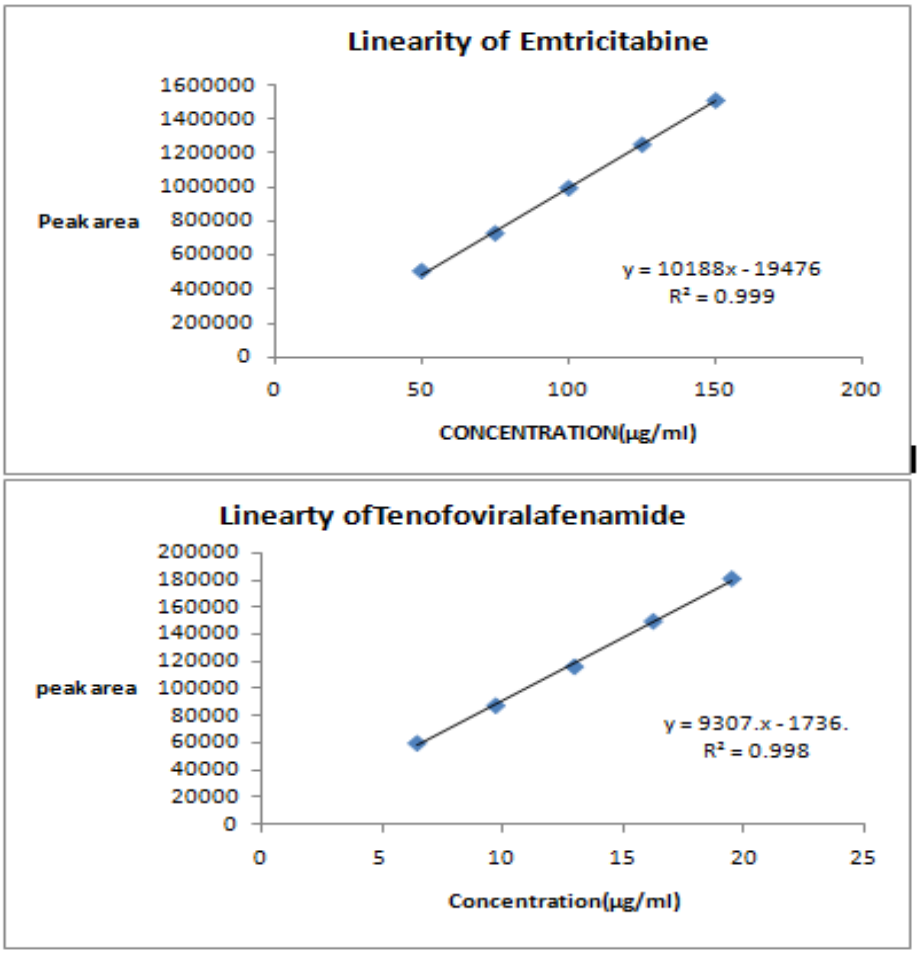



**Fig. 3. Calibration curve of Emtricitabine and TAF**

**Table 7. Results of % assay of the tablet dosage form**

| Drug | Peak name | Retention time | Peak Area | USP Tailing | USP Plate count | %Assay |
|---|---|---|---|---|---|---|
| Emtricitabine | Standard | 2.369 | 1000291 | 1.12 | 7365 | **100.02%** |
| | Test | 3.369 | 1002599 | 1.11 | 7059 | |
| TAF | Standard | 2.901 | 119223 | 1.07 | 8265 | **99.3%** |
| | Test | 2.9 | 118428 | 1.04 | 8726 | |
| **Acceptance limit** | | | | **≤2** | **>2000** | **100±2** |

The RP-HPLC method has key role in both qualitative and quantitative analysis of drug. As of now, few RP-HPLC methods were available for analysis of Emtricitabine and TAF. But the retention times in the previously reported studies were more, and a method with high retention time cannot be said as economical because it requires huge volume of mobile phase and takes longer run time. To overcome these problem developed a new method, in this the retention time was reduced hence with in the short period of time more number of drug samples can be analyzed. In this RP-HPLC method the retention time of Emtricitabine and TAF were 2.3 and 2.8 minutes respectively which was attained by a simple mobile phase composition of 30:10:70 percentage of 0.1% trifluoro acetic acid: acetonitile: methanol at a flow rate of 1 ml/min). The lowest linearity range and sensitivity was gained by this method.

## 4. CONCLUSION

An effective, unique simple and specific RP HPLC method with isocratic elution method was developed to estimate Emtricitabine and TAF simultaneously in API and its combined marketd tabletsdosage form. Hence, the proposed approach is predicted as revival to normal evaluation of mixed dosage in pharmaceutical enterprise**.**

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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