IJBPAS, August, 2020, 9(8): 1832-1840

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

RP-HPLC DEVELOPMENT AND VALIDATION OF METHOD FOR THE DETERMINATION OF TENOFOVIR ALAFENAMIDE IN BULK AND TABLET DOSAGE FORM

ANSARI YA[1*], SHAIKH MT[2], KHAN RA[3], INAMDAR ZE[4], QAZI SS[5], SHAIKH NS[6], ANSARI MR[7]

[1, 2, 3, 4, 6]Jamia College of Pharmacy, Akkalkuwa, Dist- Nandurbar, Maharashtra, India

[5]Ali-Allana College of Pharmacy, Akkalkuwa, Dist- Nandurbar, Maharashtra, India

[7]School of Pharmacy and Medical Sciences, Singhania University, Rajasthan, India

***Corresponding Author: E Mail: yasirpharma222@gmail.com; Mob.No: +91-9370211222**

Received 7th Dec. 2019; Revised 8th Jan. 2020; Accepted 9th Feb. 2020; Available online 1st Aug. 2020

https://doi.org/10.31032/IJBPAS/2020/9.8.5129

ABSTRACT

A new RP-HPLC method was developed for estimation of Tenofovir Alafenamide. In RP-HPLC method, good resolution and separation of drug was achieved. Buffer, pH 5.0: Methanol (20:80) was used as mobile phase. Retention time of Tenofovir Alafenamide was found to be 5.050 min with a flow rate of 1.0 ml/min. The drug was found to be linear in concentration of 5-15 µg/ml. The developed method was validated for specificity, Linearity, Precision, LOD, LOQ and robustness of the system. The assay of marketed formulation was also performed, % purity of drug was found to be 96.007%. The proposed method was robust, accurate and precise. Therefore proposed method can be used for routine analysis of Tenofovir Alafenamide in tablets.

Keywords: HPLC, Method development, Method validation, Tenofovir Alafenamide, Anti-viral

1. INTRODUCTION

1.1 HIV Disorder

HIV is a virus that attack on immune system. It increases the risk of other infections and diseases. HIV attack on immune cells called CD4 cells (type of T cells). These cells move around the body detecting fault and

anomalies in cells as well as infections. The HIV spreads through bodily fluids that include: blood, semen, breast milk, vaginal and rectal fluids **[1-3]**.

The first few weeks after infection is called the acute infection stage. During this time the virus rapidly reproduces. Your immune system responds by producing HIV antibodies. Many people experience temporary flu-like symptoms during this stage. Even without symptoms, HIV is highly contagious during this time **[4-6]**.

Symptoms:

Some people infected with HIV are asymptomatic at first. Most people experience symptoms in the first month or two after becoming infected. That's because your immune system is reacting to the virus as it rapidly reproduces. This early stage is called acute stage. Symptoms are similar to those of the flu and may last anywhere from a few days to several weeks. These include: fever, swollen lymph glands, general aches and pains. During the first few months of infection, an HIV test may provide a false-negative result. This is because it takes time for the immune system to build up enough antibodies to be detected in a blood test. But the virus is active and highly contagious during this time. The clinical latent infection, or chronic stage of HIV, can last from a few years to a few decades. During this time the virus is still reproducing, but at lower levels. Some people have few, if any, symptoms. Others may have many symptoms **[5-8]**.

Diagnosis

Antibody test

Between 21 and 84 days after infection, about 97% of people will develop detectable HIV antibodies, which can be found in the blood or saliva.

There's no preparation necessary for blood tests or mouth swabs. Some tests provide results in 30 minutes or less and can be performed in a doctor's office or clinic. There are also home test kits available:

- **OraQuick HIV Test**: An oral swab provides results in as little as 20 minutes.
- **Home Access HIV-1 Test System**: After pricking your finger, you send a blood sample to a licensed laboratory. You can remain anonymous and call for results the next business day.

If you think you've recently been exposed to HIV, but tested negative, repeat the test in three months. If you have a positive result, follow up with your doctor to confirm.

Antibody/antigen test

An antigen is part of the virus that activates your immune system. It takes from 13 to 42

days for antibodies and antigens to be detectable.

Nucleic acid test (NAT)

- ✓ This expensive test isn't used for general screening. It's for people who have early symptoms of HIV or recently had a high-risk exposure. This test doesn't look for antibodies, but for the virus itself. It takes from seven to 28 days for HIV to be detectable in the blood. This test is usually accompanied by an antibody test **[9-13]**.

2. EXPERIMENTAL AND RESULTS

2.1 Standards and Reagents:

Tables 1-3, show the standards and reagents used in the study.

2.2 Instrumentation (Table 4)

2.3 Development of RP-HPLC Method

2.3.1 Preparation of Solutions

(A) Tenofovir Alafenamide standard stock solution: (100µg/mL)

A 10 mg of Tenofovir Alafenamide was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with Diluent.

(B) Preparation of working standard solution of Tenofovir Alafenamide (10µg/mL)

Take 1 mL from the Tenofovir Alafenamide stock solution and transferred to 10 mL volumetric flask and volume made up to the mark with Diluent.

(C) Diluent: Water: Methanol (10:90)

2.3.2 Selection of Mobile phase for Tenofovir Alafenamide

Trial contains various mobile phase which are considered of Methanol and Water in different proportions and different volumes at different flow rate were tried. On the basis of various trial the mixture of Buffer, pH 5.0: Methanol (20:80) at 1 mL/min flow rate, proved to be better than the other mixture in terms of peak shape, theoretical plate and asymmetry.

Trials are summarizes in following **Table 5**.

2.4 Validation of RP-HPLC method:

2.4.1 Specificity:

Shown in **Figure 1-3**.

2.4.2 Linearity: (Table 8, Figure 4)

Correlation co-efficient for calibration curve Tenofovir Alafenamide was found to be 0.999

The regression line equation for Tenofovir Alafenamide is as following:

For Tenofovir Alafenamide **y = 190.31x - 8.6338**

2.4.3 Precision

I. Repeatability

The data for repeatability of peak area measurement for Tenofovir Alafenamide (10 µg/ml) The % RSD for Tenofovir

Alafenamide (10 µg/ml) was found to be 0.376 **(Table 9)**.

II. Intraday precision

% RSD in Intraday precision for Tenofovir Alafenamide was found in range of 0.178-0.490 **(Table 10)**.

III. Interday precision

% RSD in Interday precision for Tenofovir Alafenamide was found in range of 0.435-1.254 **(Table 11)**.

2.4.4 Accuracy:

For Tenofovir Alafenamide

5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 50% , 100%, 150% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 285 nm. The amount of Tenofovir Alafenamide was calculated at each level and % recoveries were computed **(Table 12)**.

2.4.5 LOD and LOQ:

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated **(Table 13)**. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts

2.4.6 Robustness:

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation **(Table 14)**.

2.4.7 Assay of Marketed formulation: (Table 15)

Table 1: Standard API Procurement

Standard	Source
Tenofovir Alafenamide	Yash Pharmaceuticals

Table 2: Sample Procurement

Sample	Source
Hepbest Tablet 25mg	Mylan

Table 3: Reagents Used In Experiment:

Chemical/ Reagent	Grade	Manufacturer
Methanol	HPLC Grade	Finar
Potassium Dihydrogen Phosphate	AR	Finar
Water	HPLC Grade	Finar
Acetic Acid	AR	Spectrochem pvt Ltd.

Table 4: Instrumentation for HPLC

Component	Brand / Model / Software
HPLC	1200 series/ Agilent,
HPLC Column	Inertsil ODS- 3v (250*4.6mm)
Detector	UV detector
Ultrasonicator	Frontline machinery
Digital pH meter	Analab
Analytical Balance	Shimadzu

Table 5: List of Mobile Phase trials for Tenofovir Alafenamide

Trial	Mobile Phase	Retention time (minute)	Remark
1	Water: Methanol (50:50)	12.163	Irregular peak observed
2	Water: Methanol (40:60)	9.920	Retention time decreased
3	Water: Acetonitrile (40:60)	6.850	Peak shape is irregular
4	Buffer, pH 5.0: Methanol (40:60)	9.850	Peak is sharp, but retention time is high
5	Buffer, pH 5.0: Methanol (20:80)	5.05	Retention time decreased

Table 6: System suitability parameter

Parameters	Tenofovir Alafenamide
Retention Time	5.050
Theoretical Plates	8833
Asymmetry	1.375

Table 7: RP-HPLC optimized chromatographic conditions

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	Buffer, pH 5.0: Methanol (40:60)
Column	Inertsil ODS 3V (250× 4.6mn)
Flow rate	1.0 ml/min
Runtime	10 min
Injection volume	20 µL
Detection wavelength	237 nm

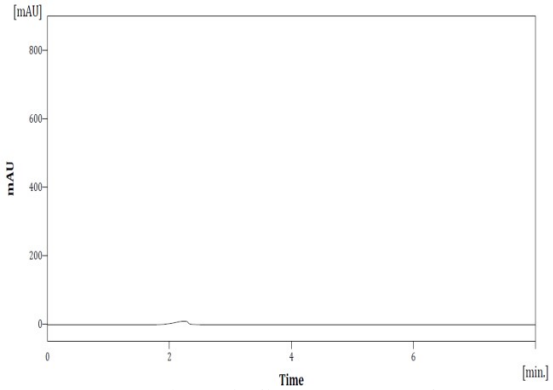

Figure 1: Chromatogram of Blank

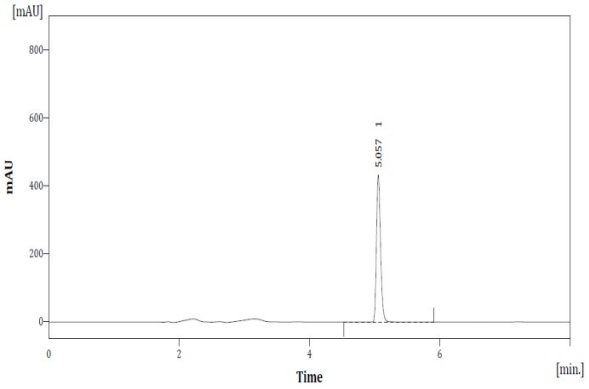

Figure 2: Chromatogram of Standard

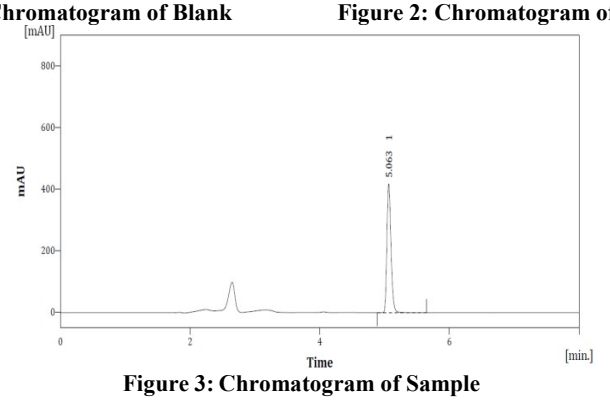

Figure 3: Chromatogram of Sample

Table 8: Linearity data for Tenofovir Alafenamide

Sr. No	Concentration (µg/ml)	Area
1	5	957.125
2	7.5	1395.775
3	10	1911.274
4	12.5	2348.839
5	15	2859.518

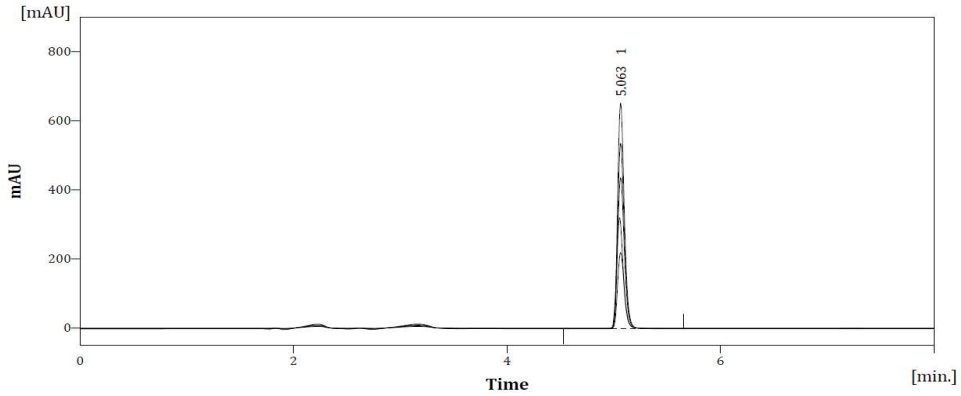

Figure 4: Overlay chromatogram of different concentrations of Tenofovir Alafenamide

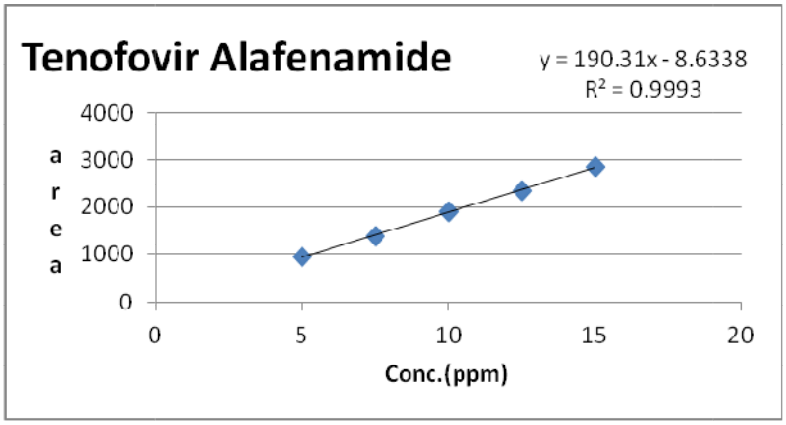

Figure 5: Calibration Curve of Tenofovir Alafenamide (5-15 µg/ml)

Table 9: Repeatability data for Tenofovir Alafenamide

Tenofovir Alafenamide (10 µg/ml)				
Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	10	1898.016	1890.137±7.109	0.376
		1886.558		
		1882.782		
		1897.839		
		1882.638		
		1892.992		

Table 10: Intraday precision data for Tenofovir Alafenamide

Sr. No.	Conc. (µg/ml)	Mean ± S.D (n=6)	% R.S.D
1	5	958.395±2.215	0.231
2	10	1898.688±9.307	0.490
3	15	2874.191±5.109	0.178

Table 11: Interday data for Tenofovir Alafenamide

Sr. No.	Conc. (µg/ml)	Mean ± S.D (n=6)	% R.S.D
1	5	947.144±11.874	1.254
2	10	1909.368±8.318	0.435
3	15	2845.380±13.112	0.461

Table 12: Recovery data for Tenofovir Alafenamide

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Average	% RSD
1	50 %	5	4	4.014	100.354	98.509	1.754
2		5	4	3.877	96.927		
3		5	4	3.930	98.246		
4	100 %	5	5	5.035	100.698	101.427	1.576
5		5	5	5.134	102.689		
6		5	5	5.195	103.894		
7	150 %	5	6	6.036	100.606	99.638	0.843
8		5	6	5.951	99.181		
9		5	6	5.948	99.126		

Table 13: LOD and LOQ data for Tenofovir Alafenamide

LOD	LOQ
LOD = 3.3 x (SD / Slope) = 3.3 x (23.451/190.31) = 0.407 µg/ml	LOQ = 10 x (SD / Slope) = 10x (23.451/190.31) = 1.232 µg/ml

Table 14: Robustness data for Tenofovir Alafenamide

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	1905.366	1891.976	1924.327	1848.856
2	1897.745	1903.750	1935.875	1859.972
3	1912.918	1898.630	1922.318	1846.951
% R.S.D	0.398	0.311	0.380	0.380

Table 15: Assay of Marketed formulation

Sr. No.	Label claim (mg)	Result (mg)	% Assay	average % Assay	SD	%RSD
1	25	24.078	96.314	96.007	0.409	0.426
2	25	23.886	95.542			
3	25	24.041	96.165			

3. CONCLUSION

RP-HPLC method was developed for estimation of Tenofovir Alafenamide. In RP-HPLC method, good resolution and separation of two drugs was achieved. Buffer, pH 5.0: Methanol (20:80) was used as mobile phase. Retention time of Tenofovir Alafenamide was found to be 5.050 min with a flow rate of 1.0 ml/min. Validation was performed on developed method and method observed specific, linear, precise, accurate and robust. Therefore proposed method can

be used for routine analysis of Tenofovir Alafenamide in tablets.

4. ACKNOWLEDGEMENT

Authors are thankful to the entire Management, Principals and Teaching & Non-Teaching Staff of Jamia & Ali-Allana College of Pharmacy, Akkalkuwa Dist-Nandurbar (M.S) for their kind support and motivations during research work.

5. REFERENCES

[1] "Drug profile for Tenofovir Alafenamide", May-2019 https://www.medkoo.com/products/4516

[2] Kasture AV., Mahadik KR., Wododkar SG and More HN "A Text Book of Pharmaceutical Analysis" 1^{7th}Edn; Nirali Prakashan, Pune, 2002, pp.48-57.

[3] Shethi PD,"HPLC-Quantitative Analysis of Pharmaceutical Formulations" CBS Publishers & Distributers, New Delhi, 1996, pp. 3-46.

[4] Chatwal GR, "Instrumental Method of Chemical Analysis",Part-1, Himalaya Publishing House,5th Edition,2002, pp. 2.624-2.631.

[5] Robinson JW, Skelly Frame EM and Frame GM, "Undergraduate Instrymental Analysis", 6th Edition, Marcel Dekker, 2005, pp. 806.

[6] Ahuja S and Scypinski S, "Handbook of Modern Pharmaceutical Analysis", Academic Press 3, 2001, pp.356-367.

[7] FDA, "Guidance for Industry; Analytical Procedures and Methods Validation (Draft guidance), Food & Drug Administration," Rockville, US Department of Health and Human Services, 2000.

[8] ICH, Validation of Analytical Procedures; Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva 1996.

[9] Aggarwal NN, Bhat KI, Jacob JT, "Stability Indicating Assay Method Development and Validation for Tenofovir Alafenamide Fumarate by RP-HPLC" *Pharm. Anal. Act.,* **2018,** 9(12), pp.1-6.

[10] Mastanamma SK, Jyothi JA, Saidulu P, "Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Lamivudine, Tenofovir Alafenamide and Dolutegravir Bulk and their Combined Dosage Form" *Pharm. methods,* **2018,** 9(2), pp.49-55.

[11] Mastanamma SK, Reddy DV, Saidulu PP, "Development and Validation of Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Emtricitabine Tenofovir Alafenamide

Bulk and their Combined Dosage Form" *J. Chem. and Pharm. Res.,* **2017,** 9(9), pp.70-80.

[12] Akram NM, Umamahesh M, "A New Validated RP-HPLC Method for the Determination of Emtricitabine and Tenofovir AF in its Bulk and Pharmaceutical Dosage Forms" *J. Chem. and Pharm. Sci.,* **2017,** 10(4), pp.54-59.

[13] Tejaswi JD, Rajan G, "RP-HPLC Method development and validation for simultaneous estimation and forced degradation study of Tenofovir Alafenamide and Emtricitabine and Rilpivirine in solid dosage form", *Asi. J. of Pharm. and Cli. Res.,* **2019,** 12(1).