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Analytical method development and validation of Tenofovir Alafenamide by using RP-HPLC of bulk drug

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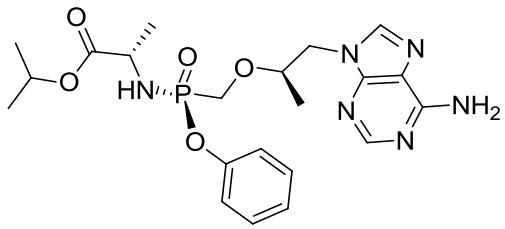
ABSTRACT

A fast, simple, sensitive, precise and reproducible (liquid chromatography) RP-HPLC method was developed and validated for the analysis of Tenofovir alafenamide bulk dosages form. The separation was conducted by using C-18 HPLC column. Which was maintained at ambient temperature. The mobile phase consist of methanol (100 v/v) was delivered at a rate of 1mL/min. The analysis was detected by using UV detector at the wavelength 259 nm. The method was validated for its precision, limit of quantitiation (LOQ) linearity and robustness. The method was found to be linear over the concentration range 10-100 µg/mL (r^2 =0.999). The retention time for Tenofovir alafenamide was found to be 4.107± 25 min. limit of quantitation of method was 6.3139 µg/mL and limit of detection 2.0836µg/mL. Thus, the developed method is found to be robust and rugged which can be applied as a rapid tool for routine analysis of tenofovir alafenamide in the bulk and in the pharmaceutical dosage form.

Keywords: C18, HPLC, Methanol, Tenofovir alafenamide,

INTRODUCTION

Antiretroviral drugs which are used for manage of HIV \AIDS normally includes the use of anti retro viral drugs in an attempt to control HIV infection. several classes of anti retro viral agents that act on different stages of HIV life cycle [1] .The use of multiple drugs that act on different viral targets is known as (HAART) highly active antiretroviral therapy [2]. Tenofovir Alafenamide is a nucleotide reverse transcriptase inhibitor and a novel prodrug of tenofovir. It is closely related to tenofovirdisoproxil fumarate but has greater antiviral activity. Mainly used in the treatment of HIV infection and chronic hepatitis B [3]. Tenofovir alafenamide (TA) Fig.1 is chemically (S)-isopropyl 2-(((R)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl)oxy)methyl)phoshoryl)amino)propanoate.



TA available in market in combination with Emtricitabine in tablet dosage form. The literature survey shows that TA one method of LC [4] and one spectroscopic method [5]. The main purpose of this work to develop a HPLC method for the determination of TA in its bulk form so as to provide better scope for further research on the drug and pharmaceutical industry.

MATERIALS AND METHOD

Chemicals

TA was received as a gift sample from Mylan Laboratories Limited, Hyderabad, India. HPLC-grade Methanol obtained from Molychem, Mumbai, India. Double distilled water as solvent was used for the other purpose.

Instrumentation

The chromatographic technique was performed on a Shimadzu LC-2010C_{HT} Liquid chromatography with UV-visible detector and LC-Solution software, reversed phase C18 column (Inertsil ODS-3V 5 um 250×4.6 mm), Shimadzu analytical balance AY-220, Vacuum micro filtration unit with 0.45µ membrane filter was used in the study. Double beam UV-visible spectrophotometer (UV-probe 2.32 software).

Determination of Working Wavelength (λmax)

10 mg of Tenofovir Alafenamide was weighed and transferred in to 100 mL volumetric flask and dissolved in methanol and then made up to the mark with methanol and diluted to produce 10 µg /mL of solution by diluting 0.1 mL to 10 mL with methanol. The wavelength of maximum absorption (λmax) for 10 µg/mL solution of the drug in methanol was scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The absorption curve shows characteristic absorption maxima at 259 nm for Tenofovir Alafenamide.

Chromatographic conditions

The mobile phase for the proposed method methanol 100% was filtered through a 0.45-µm membrane filter degassed with a helium spurge for 20 min and pumped from the respective solvent reservoir to the column inerstil C 18 column (250×4.6 mm) at flow rate 1.0 mL/min. The run time was set at 10 min the column temperature was maintained at room temperature. Prior to injecting the drug solution in to the column, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluent was monitored at 259 nm. The data was stored and analyzed with the software "LC-Solution".

Selection of mobile phase

The solution of Tenofovir Alafenamide was injected into the HPLC system and run in different solvent systems. Different mobile phases containing methanol, water, acetonitrile and phosphate buffer in different proportions were tried and finally methanol (100v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for Tenofovir Alafenamide.

EVALUATION OF ANALYTICAL METHODS

Linearity

Aliquots ranging from 10-100 µg /mL were prepared by suitable dilution of standard stock solution using mobile phase. Though linear response was obtained at lower concentrations for Tenofovir Alafenamide, the higher concentration range was used to improve signal to noise ratio. Linearity was determined by analyzing five working standard solutions over the concentration range of 10-100 µg /mL for Tenofovir Alafenamide

Limit of detection LOD

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD is calculated from the formula,

$LOD = 3.3\sigma/s$

Where, σ = standard deviation of the response

S = slope of the calibration curve.

Limit of Quantification (LOQ)

The limit quantification is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. LOQ is calculated from formula,

$LOQ = 10\sigma/s$

Where, σ = standard deviation of the response

S = slope of calibration curve

Accuracy

The accuracy of the method was carried out using one set of different standard addition methods at different concentration levels, 80%, 100% and 120%, and then comparing the difference between the spiked value (theoretical value) and actual found value.

Precision

Five sets of aliquots with same concentration (50 µg /mL) were prepared and these solutions were analyzed to record any intra and inter day variations in the results. The results obtained for Intra and inter day variations.

Robustness

Robustness of the proposed method for Tenofovir Alafenamide sulfate was carried out by the slight variation in flow rate, temperature and mobile phase ratio. The percentage recovery and RSD were noted for Tenofovir Alafenamide.

RESULT AND DISCUSSION

Checking of resolution of drug and materrials

The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Standard solution of Tenofovir Alafenamide was injected to get the chromatogram. The retention time for Tenofovir Alafenamide was found to be 4.107 min. It is shown in the Table 1.

Table 1 Resolution of drug material

Drug	Ret. Time	Area	Height	Theoretical plate	Tailing factor
Tenofovir Alafenamide	4.107	3956744	330511	3306.216	1.884

Linearity

The data of the peak area vs drug concentration were evaluated by linear regression analysis as shown in the Table 2 and calibration curve obtained after plotting drug concentration vs area shown in the fig. 1. Linear regression analysis demonstrated that chromatograph response for the drug was highly linear (r^2=0.999) in the studied concentration range of 10-100 µg/mL. A typical chromatogram of Tenofovir Alafenamide (100 µg/mL) shown in fig. 1.

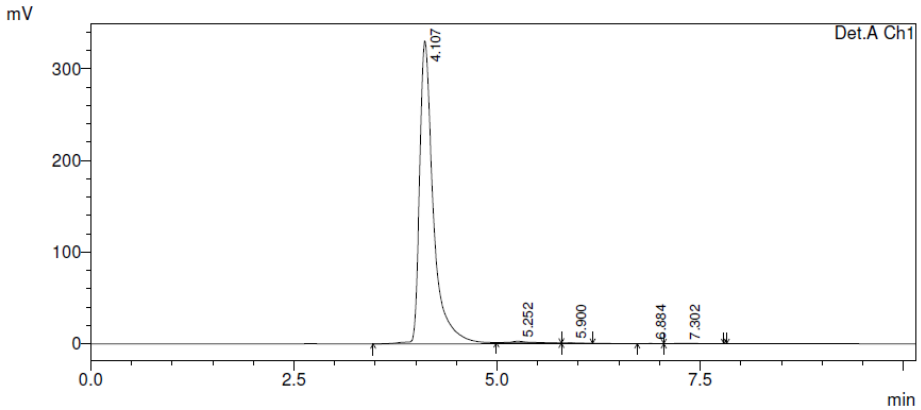


Fig.1. A typical chromatogram for Tenofovir Alafenamide (100µg /mL)

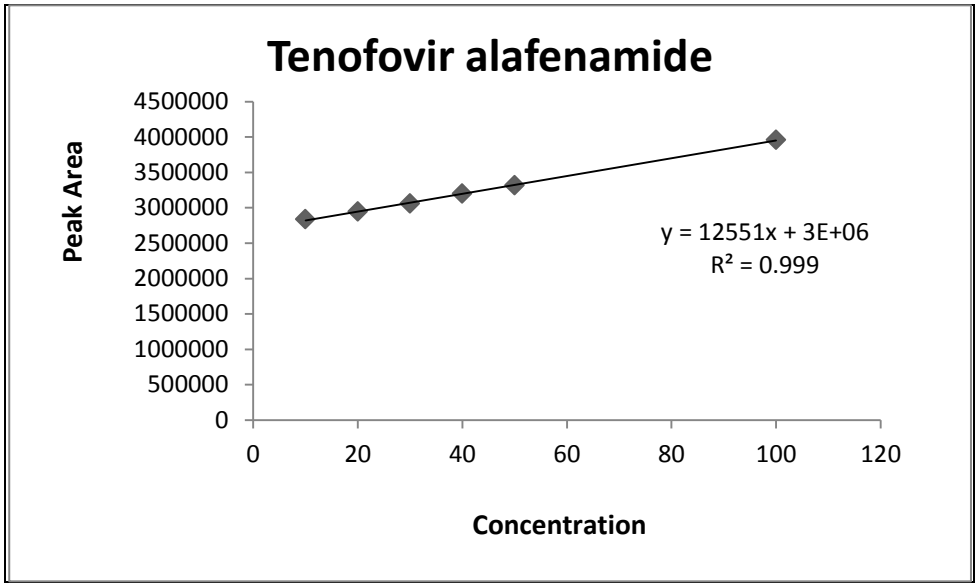


Fig. 2 calibration curve of Tenofovir Alafenamide

Table 2. calibration of Tenofovir Alafenamide

Sr no.	Concentration(µg /mL)	Peak area
1	10	2835678
2	20	2945454
3	30	3058971
4	40	3198265
5	50	3314724
6	100	3956744

Precision

The result depicted in the table3a,3b indicated that the given method has sufficient precision as indicated by the corresponding values of %RSD ranging 0.12 for inter day studies respectively. The values of %RSD for both the studies are well below 1.0% constructing adequate precision.

Table. 3a.Intra-day Precision for Tenofovir Alafenamide

Concentration((µg /mL)	Peak area	Mean (n=5)	S.D.	%RSD
50	3756489			
50	3812456			
50	3756237	3776730	12622.96	0.12
50	3802345			
50	3756123			

Table. 3b.Inter-day Precision for T enofovir Alafenamide

Concentration((µg /mL)	Peak area	Mean (n=5)	S.D.	%RSD
50	3716489			
50	3712456			
50	3856231	3788928.8	38217.53	0.15
50	3901345			
50	3758123			

Limit of detection and quantification

Standard error and slope of linear data is used to predict LOD and LOQ of rivastigmine and precision was established at the predict concentration. The result was shown in the table.4

Table 4 Limit of detection and Limit of quantification

Limit of detection	Limit of quantification
2.0836µg/mL	6.3139µg/mL

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