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NEW STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TENOFOVIR ALAFENAMIDE AND EMTRICITABINE IN BULK AND COMBINED TABLET DOSAGE FORMS

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ABSTRACT

The present study accurate and precise RP-HPLC method has been developed for the validated of Tenofovir Alafenamide and Emtricitabine, in its bulk and Combined Tablet dosage forms. Chromatography was carried out system C_{18} (4.6 x 150mm, 5µm) column using a mixture of Acetonitrile: ortho phosphoric acid bufferadjusted pH 2.5 (55:45v/v) as the mobile phase at a flow rate of 1.2 mL/min, the detection was carried out at 266 nm. The retention time of the Tenofovir alafenamide and Emtricitabine were found 2.1 and 3.4 minand respectively. The method produce linear responses in the concentration range of 5-30µg/mL of Tenofovir alafenamide and40-240µg/ml of Emtricitabine. The method precision for the determination of assay was below 2.0%RSD. The Proposed RP-HPLC method is useful in the quality control of bulk drug and Tablet dosage forms.

KEYWORDS

Emtricitabine, Tenofoviralafenamide, RP-HPLC, ICH validation and Tablet dosage forms.

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INTRODUCTION

Tenofovir alafenamide

It is chemically isopropyl(2S)-2-[[[(1R)-2-(6-aminopurin-9-yl)-1- methyl-ethhoxy] methyl-phenoxy-phoshoryl] amino]propanoate. TA is a nucleotide reverse transcriptase inhibitor and a prodrug of tenofovir. It is used in the treatment of HIV infection and chronic hepatitis B. It is distantly associated to the normally used reverse-transcriptase inhibitor tenofovirdisproxil, TA has greater antiviral activity and better distribution into

lymphoid tissues than that tenofovirdisproxil Figure No.1.

Drug category : Antiviral Agents, Antiviral for systemic use.

Molecular Formula: $C_{21}H_{29}N_6O_5P$

Molecular Weight : Average 476.474 g/mole.

Emtricitabine (EMT)

Figure No.2 is chemically 4-amino-5-fluoro-1-[(2R, 5S)-2 (hydroxyl -methyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydro- pyrimidin-2-one. EMT is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. EMT is an analogue of cytidine.

TA and EMT in combination are available in tablet dosage forms containing 25 mg TA and 200 mg EMT. Literature survey shows that five UV spectroscopic[1-5] and five HPLC[6-10] analytical methods are reported for the estimation of EMT. For TA one method ofLC[11] and one spectroscopic method[12] reported in its bulk form. No method is reported for the simultaneous estimation of TA and EMT in bulk and pharmaceutical dosage forms. The purpose of this work was to develop a simple, basic, rapid and economic simultaneous RP-HPLC method for the determination of TA and EMT in bulk and pharmaceutical dosage forms so as to provide better scope for further research on the drugs. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelinesQ2 (R1)[14].

Structure

IUPAC Name **:** 4-amino-5-amino-1-[(2R, 5S)-2 (hydroxyl-methyl)-1, 3- oxathiolan-5-yl]-1, 2-dihydro-pyrimidin-2-one.

Molecular Formula **:** C8H10FN3O3S

Molecular Weight **:** Average: 247gm/mole

Momoisotopic **:** 247.042690096

MATERIAL AND METHODS

Instrumentation

To develop a high pressure liquid chromatographic method for simultaneous estimation of EMT and TA by using HPLC. C_{18} column (250-4.6 mn) was used. The instrument is equipped with an UV 730 D detector. Shimadzu SPD 10 Abinary pump, an auto sampler and a 2996 photo diode array detector was employed for the study. The integration processed with the Empower-2 software.

Preparation of Mobile Phase

Accurately measured prepared by mixing 55ml Acetonitrile, 45mL buffer was adjusted (PH-2.5) a mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of standard solution

Accurately weighed and transferred 10 mg of Tenofovir alafenamide and Emtricitabine working standard into a 10mL of clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Mobile phase. Further pipette 0.15 and 0.6mL of the above Tenofovir alafenamide and Emtricitabine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Optimized Chromatogram

Mobile phase : Acetonitrile: Phosphate Buffer pH 2.5 (55:45v/v)
Column : Primesil C18 (4.6×150mm, 5.0 µm)
Flow rate : 1.2 ml/min
Wavelength : 266nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 5 minutes

METHOD VALIDATION

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Tenofovir Alafenamide and Emtricitabine in drug product.

Linearity

The linearity was observed for both the drugs 1-5µg/mL and 100-500 µg/mL for Tenofovir Alafenamide and Emtricitabine respectively. The result ants are shown in Table No.1 and 2 and Figure No.3 and 4.

LINEARITY PLOT

Precision

Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of Tenofovir (180µg/mL) and Emitricitabine (75µg/mL), have been analyzed by injecting them into a HPLC column on the same day. The intermediate precision was estimated by injecting samples prepared at the same concentrations on three different days by different operators. The peak area ratios of all injections were taken and standard deviation, % relative standard deviation (RSD), was calculated.

REPEATABILITY

Obtained Five (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Accuracy

At each concentration, sample was injected thrice to check repeatability and from the %RSD values it was analyzed that the method was accurate as % recovery values found to be in the range of 99.25-100.90% for the Emtricitabine and 99.59-101.05% for Tenofovir Alafenamide at three different concentrations 50%, 100%, 150%. The results are given in Table No.7,8.

Limit of detection

The LOD and LOQ values were found to be for Emtricitabine and Tenofovir were mentioned below.

Emtricitabine: LOD =3.3 × 1760.8/78322=0.07µg/ml

Tenofovir Alafenamide : LOD =3.3 × 61155/11150=18.0µg/ml

Limit of quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

Emtricitabine : LOQ =10×1760.8/78322 = 0.2µg/ml

Tenofovir Alafenamide : LOQ**=** 10 × 61155/11150= 54.8µg/ml

ROBUSTNESS

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Tenofovir Alafenamide and Emtricitabine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Tenofovir Alafenamide and Emtricitabine were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 255nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Primesil C_{18} because it was giving good peak. 35 ° C temperatures was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0 mL/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Phosphate Buffer pH 3.9 (55:45v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 8min because analyze gave peak around 2.061, 2.462 ±0.02min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range 25-125µg/ml of Emtricitabine and 60-360µg/ml of Tenofovir Alafenamide of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

Table No.1: Linearity data of Tenofovir Alafenamide

S.No	Concentration µg/ml	Average Peak Area
1	25	88442
2	50	165724
3	75	242754
4	100	315906
5	125	396371

Table No.2: Linearity data of Tenofovir Alafenamide

S.No	Concentration µg/ml	Average Peak Area
1	60	1131032
2	120	2345302
3	180	3355282
4	240	4429382
5	300	5623754
6	360	63664809

Table No.3: Data of Emtricitabine

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Emtricitabine	2.065	249684	12079	5343	1.0
2	Emtricitabine	2.064	249696	12068	5473	1.2
3	Emtricitabine	2.064	246325	11949	5473	1.1
4	Emtricitabine	2.065	249816	11811	5389	1.1
5	Emtricitabine	2.067	249892	11735	5180	1.0
Mean			3237814			
Std. Dev			10060.62			
% RSD			0.310722			

Table No.4: Precession Data of Tenofovir alafenamide

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Tenofovir	2.486	3233700	59095	6654	1.2
2	Tenofovir	2.484	3241323	57552	6524	1.3
3	Tenofovir	2.482	3245927	57213	6440	1.3
4	Tenofovir	2.483	3245927	57096	6411	1.4
5	Tenofovir	2.483	3222194	54363	6260	1.4
Mean			3237814			
Std. Dev			10060.62			
% RSD			0.310722			

Table No.5: The accuracy results for Emtricitabine

S.No	%Concentration (at specification Level)	Peak Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
1	50%	124675.7	15	15.1	101%	100.4%
2	100%	242006.3	30	30.1	100.5%	
3	150%	357449	45	44.9	99.7%	

Table No.6: The accuracy results for Tenofovir Alafenamide

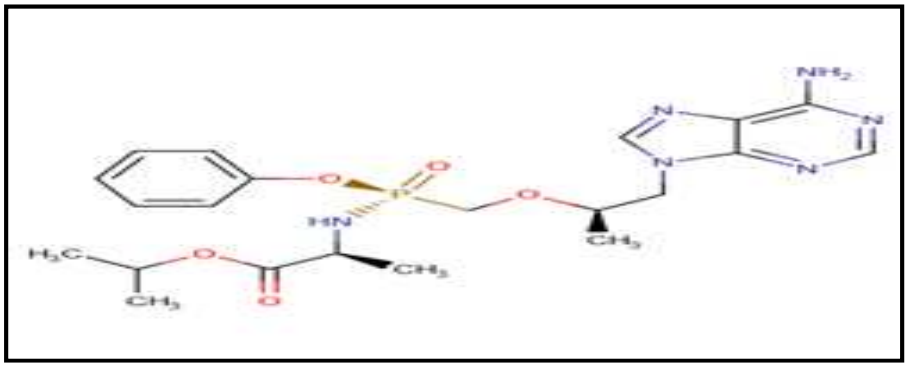
S.No	%Concentration(at specification Level)	Peak Area	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean Recovery
1	50%	1696259	18.75	18.71	99.8%	99.2%
2	100%	3351661	37.5	37.2	99.4%	
3	150%	4975094	56.25	55.47	98.6%	

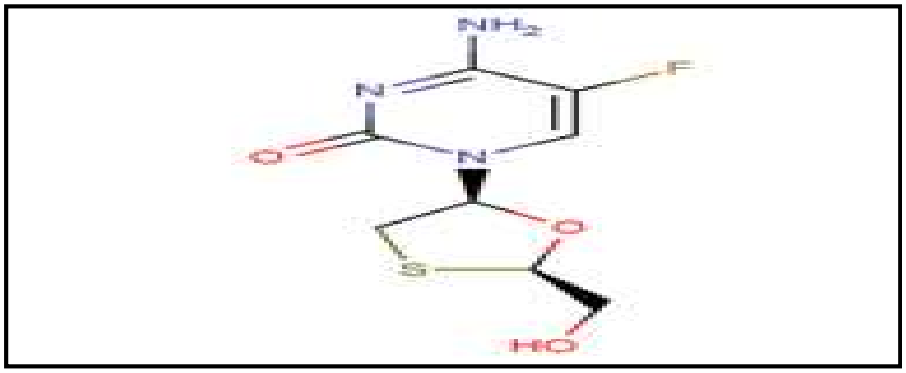
Table No.7: Robustness data of Emtricitabine

S.No	Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
1	Actual Flow rate of 1.0 mL/min	247392	2.061	7243	1.2
2	Less Flow rate of 0.9 mL/min	69214	2.267	4713	1.3
3	More Flow rate of 1.1 mL/min	388838	1.864	4740	1.2
4	Less organic phase	445628	2.165	4709	1.2
5	More organic phase	69404	1.967	5590	1.4

Table No.8: Robustness data of Tenofovir alafenamide

S.No	Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
1	Actual Flow rate of 1.0 mL/min	3530866	2.462	3389	1.1
2	Less Flow rate of 0.9 mL/min	527373	2.690	5275	1.0
3	More Flow rate of 1.1 mL/min	4363129	2.284	5611	1.0
4	Less organic phase	3965572	2.590	5550	1.0
5	More organic phase	527708	2.390	6273	1.0





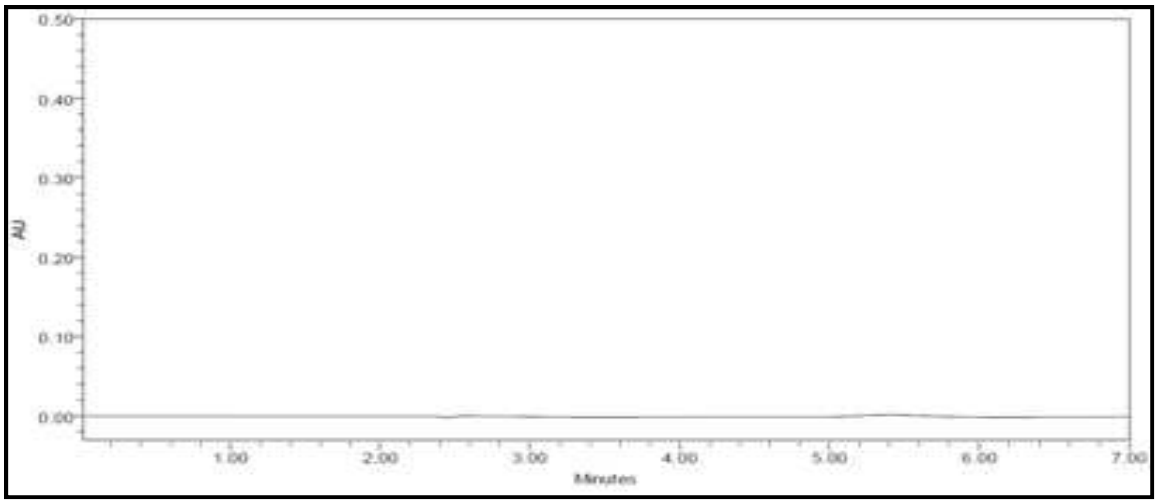


Figure No.1: Chromatogram of blank

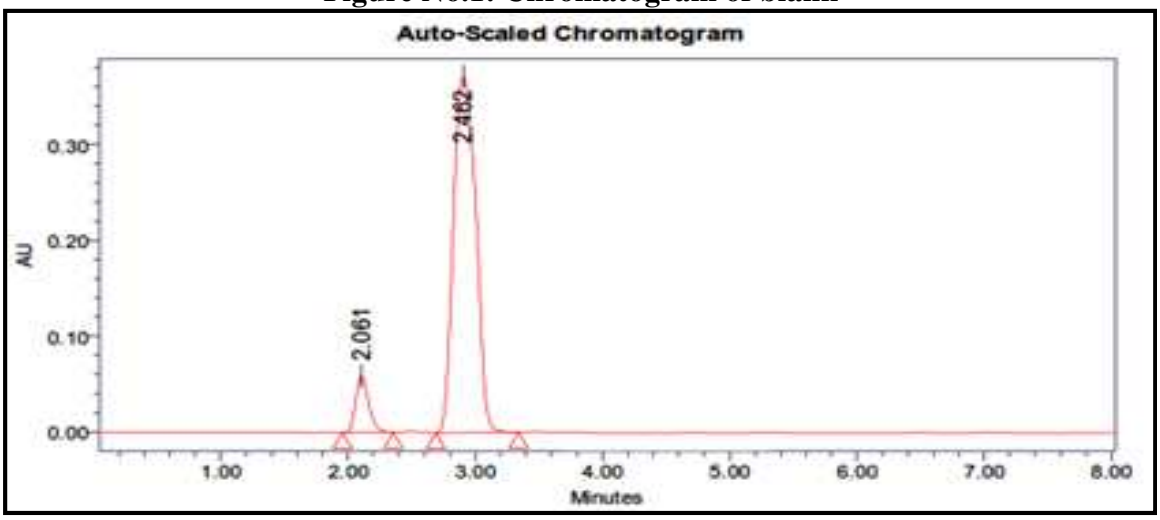


Figure No.2: Typical Chromatogram of Mixed working standard solution

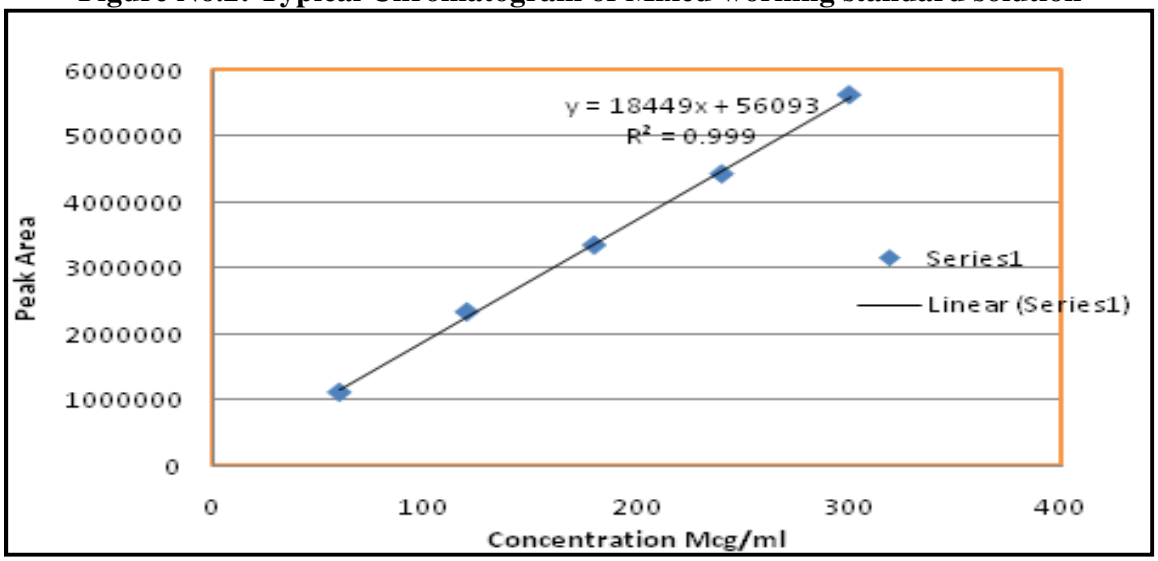


Figure No.3: Calibration graph for Emtricitabine

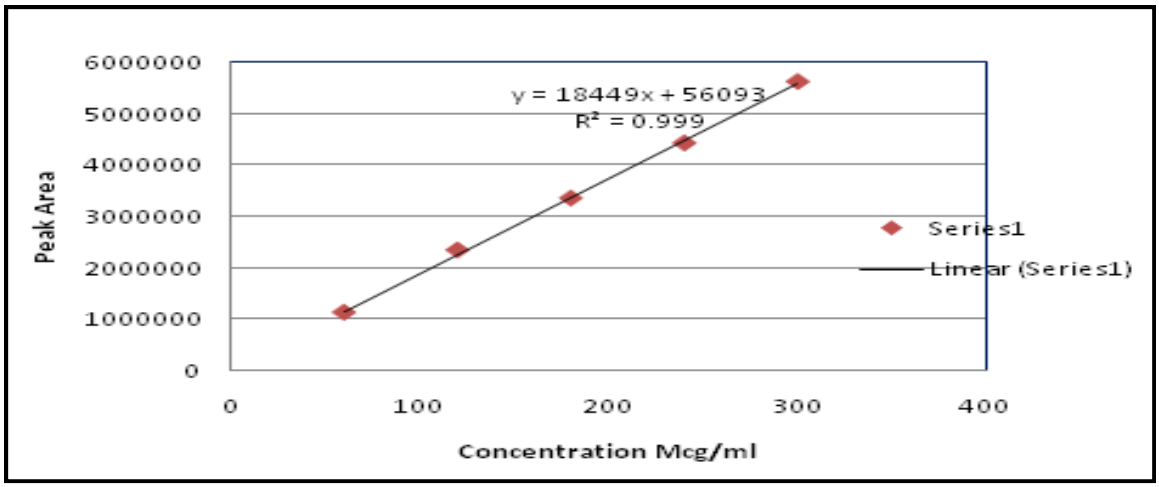


Figure No.4: Calibration graph for Emtricitabine

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Tenofovir Alafenamide and Emtricitabine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Tenofovir Alafenamide and Emtricitabine was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Phosphate Buffer pH 3.9 (55:45v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Tenofovir Alafenamide and Emtricitabine in bulk drug and in Pharmaceutical dosage forms.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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