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RP-HPLC SIMULTANEOUS ESTIMATION AND VALIDATION OF EMTRICITABINE AND TENOFOVIR

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ABSTRACT

The estimation of Emtricitabine and Tenofovir DF was done by RP-HPLC. The assay of Emtricitabine and Tenofovir DF was performed with tablets and the % assay was found to be 99.77 and 99.04 which shows that the method is useful for routine analysis. The linearity of Emtricitabine and Tenofovir DF was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.22 and 0.5 for Emtricitabine and Tenofovir DF which shows that the method is precise.

Key words: Emtricitabine, Tenofovir, RP-HPLC

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INTRODUCTION

During the last four decades, very fast development has been going on in what is now being described as High Performance Liquid Chromatography (HPLC) in columns. With the availability of pumps capable of producing pressures of a few thousand psi, a typical HPLC analysis takes only a few minutes, compared to Several hours required by its "classical" form to achieve a similar result. This situation is essentially the culmination of the efforts put forward by instrument manufacturers. As a result, HPL chromatographs have taken their rightful place beside the gas chromatographs in almost every type of analytical laboratory. HPLC today is the product of quarter century of refinement, driven by technical advances and economic competition in a USD 2 Billon plus equipment market. Recently, manufactures have improved HPLC'S performance easier. The proteomics researchers need high resolution and small sample capabilities and the pharmaceutical industries demand for high throughput screening for drug discovery. The HPLC instrumentation took on the basis of these demands. To increase the throughput, the systems with multi columns and more efficient stationary phases are being developed. It appears that

the advances in column technology have significantly overtaken the advances in instrumental and hardware aspects of HPLC. The temperature programming of columns in HPLC is a new trend emerging. The temperature programming adds a third dimension (in addition to mobile phase and stationary phase) to HPLC. This increases the speed, selectivity and efficiency of HPLC. Lowering limits of detection and increasing method sensitivity have the goals of analysts since the advent of liquid chromatography. It was soon recognized that using smaller bore columns would improve sensitivity, but a lack of affordable and suitable HPLC instrumentation meant that micro HPLC was the province of research departments. Only recently has micro HPLC become popular, due mainly to the increasing popularity of LCMS and to the improvements in instrument technology. This demand was largely driven by chromatographers working with small complex biological samples. It requires highly sensitive separation techniques (1). The choice of multi detectors and diode array and mass spectrometers to analyze the stream from the chromatography column means the results with more data per sample and hence data handling and interpretation tools are required (2-5).

Emtricitabine (4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one) is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. Emtricitabine is an analogue of cytidine. The drug works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA.

Tenofovir disoproxil fumarate (({[(2R)-1-(6-amino-9H-purin-9-yl)propan-2-yl]oxy}methyl)phosphonic acid) (a prodrug of tenofovir), marketed by Gilead Sciences under the trade name Viread®, belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (nRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. [Wikipedia] In vivo tenofovir disoproxil fumarate is converted to tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate.

Literature reveals different methods for their analysis in their formulations. But our present plan is to develop a new, simple, precise& accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validated (6-9).

MATERIALS AND METHOD (10)

Wave length selection

UV spectrum of 10 µg / ml Emtricitabine and Tenofovir DF in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 272. At this wavelength both the drugs show good absorbance.

Assay

Standard Solution Preparation

Accurately weigh and transfer 20mg of Emtricitabine & 30mg of Tenofovir DF working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1ml of Emtricitabine & Tenofovir DF of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of Emtricitabine & Tenofovir DF of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Sample Solution Preparation

Accurately weigh and transfer equivalent to 20mg of Emtricitabine & 30mg Tenofovir DF equivalent weight of the sample into a 10ml clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1ml of Emtricitabine & Tenofovir DF of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of Emtricitabine & Tenofovir DF of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Emtricitabine & Tenofovir DF peaks and calculate the %Assay by using the formulae.

Accuracy

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation of Standard stock solution

Accurately weigh and transfer 20mg of Emtricitabine & 30mg of Tenofovir DF working standard into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1ml of Emtricitabine & Tenofovir DF the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.Further pipette 3ml of Emtricitabine & Tenofovir DF the above stock solution into a10ml volumetric flask and dilute up to the mark with Diluents.

Preparation Sample solutions

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10mg of Emtricitabine and 15mg of Tenofovir DF working standard into a 10ml clean dry volumetric flask add about 7 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1ml of Emtricitabine & Tenofovir DF the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of Emtricitabine & Tenofovir DF the above stock solution into a10ml volumetric flask and dilute up to the mark with Diluents

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 20mg of Emtricitabine and 30mg of Tenofovir DF working standard into a into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1ml of Emtricitabine & Tenofovir DF the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of Emtricitabine & Tenofovir DF the above stock solution into a10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 30mg of Emtricitabine and 45mg of Tenofovir DF equivalent weight of tablet powder into a 10ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of Emtricitabine & Tenofovir DF the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of Emtricitabine & Tenofovir DF the above stock solution into a10ml volumetric flask and dilute up to the mark with Diluents

Procedure

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Emtricitabine & Tenofovir DF and calculate the individual recovery and mean recovery values.

RESULTS AND DISCUSSION

Standard and sample solution injected as described under experimental work. The corresponding chromatograms (Fig-1 and 2) and results are shown in Table-1.

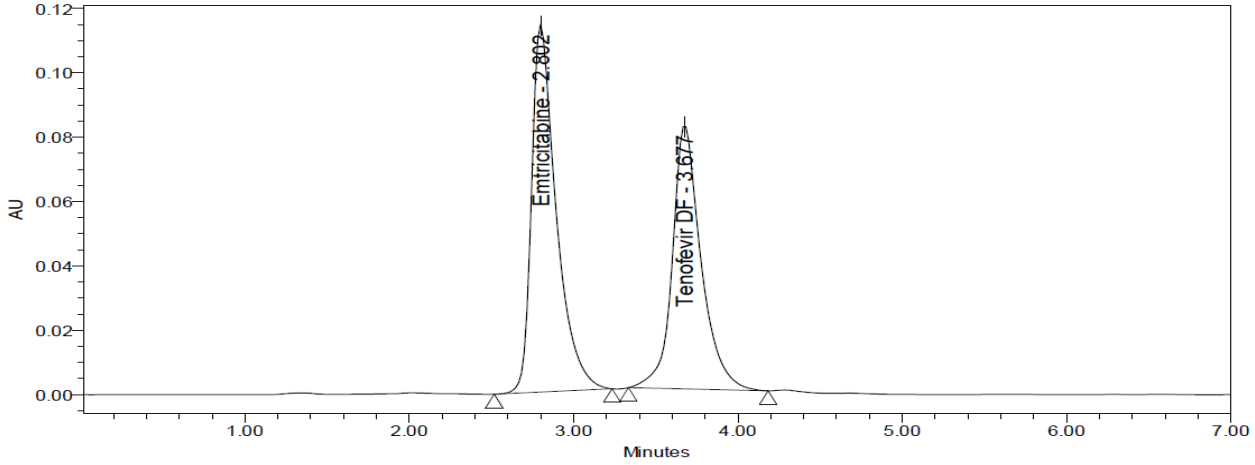

Figure-1 Chromatogram for Standard

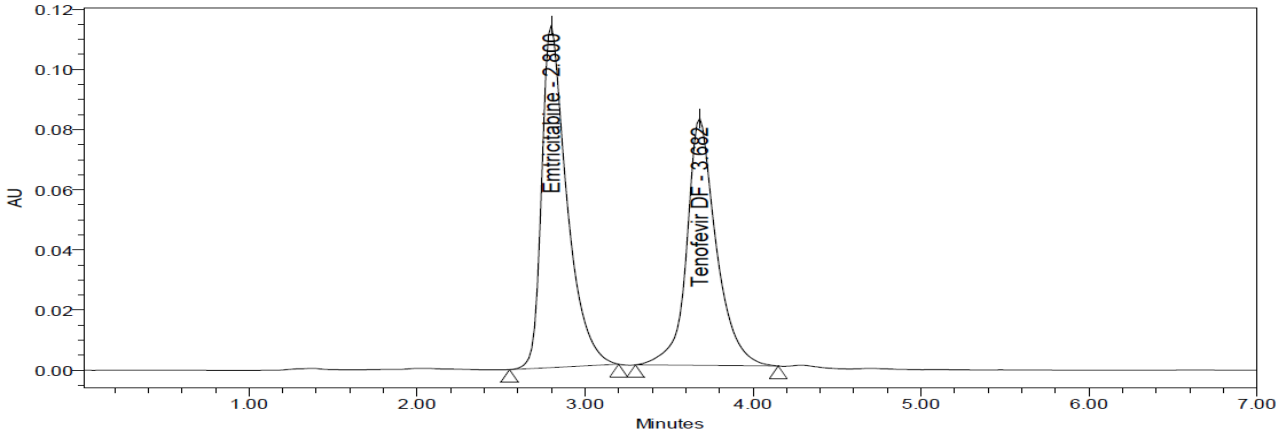

Figure-2 Chromatogram for Sample

Table-1 Results of Assay for Emtricitabine and Tenofovir DF

	Label Claim (mg)	**% Assay**
Emtricitabine	200	99.77
Tenofovir DF	300	99.04

The linearity range was found to lie from 20µg/ml to 100µg/ml of Emtricitabine, 30µg/ml to 150µg/ml 0f Tenofovir DF and linearity graph are given in fig-3 and 4.

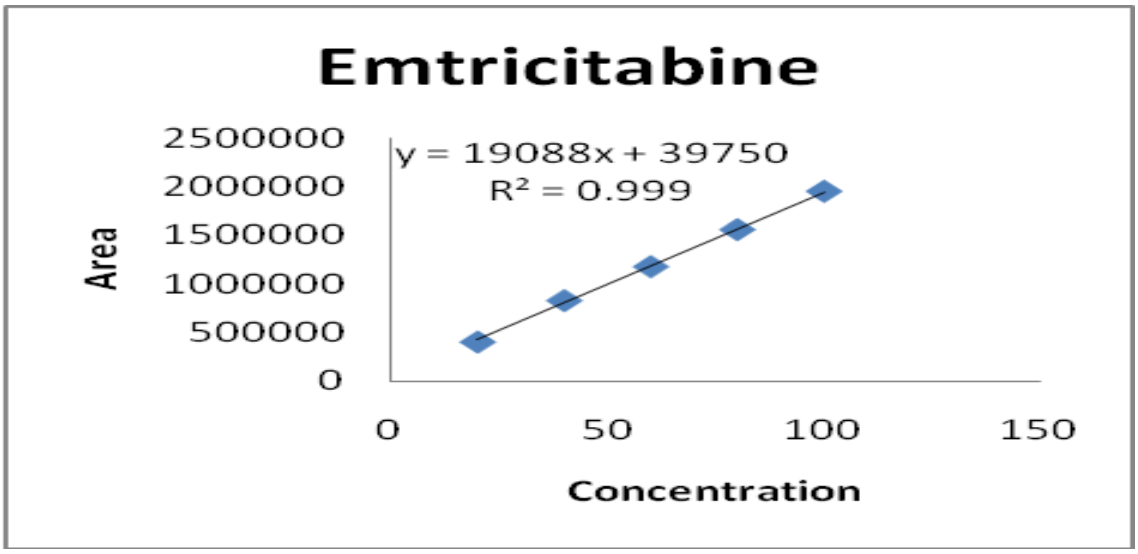

Figure-3 Calibration graph for Emtricitabine

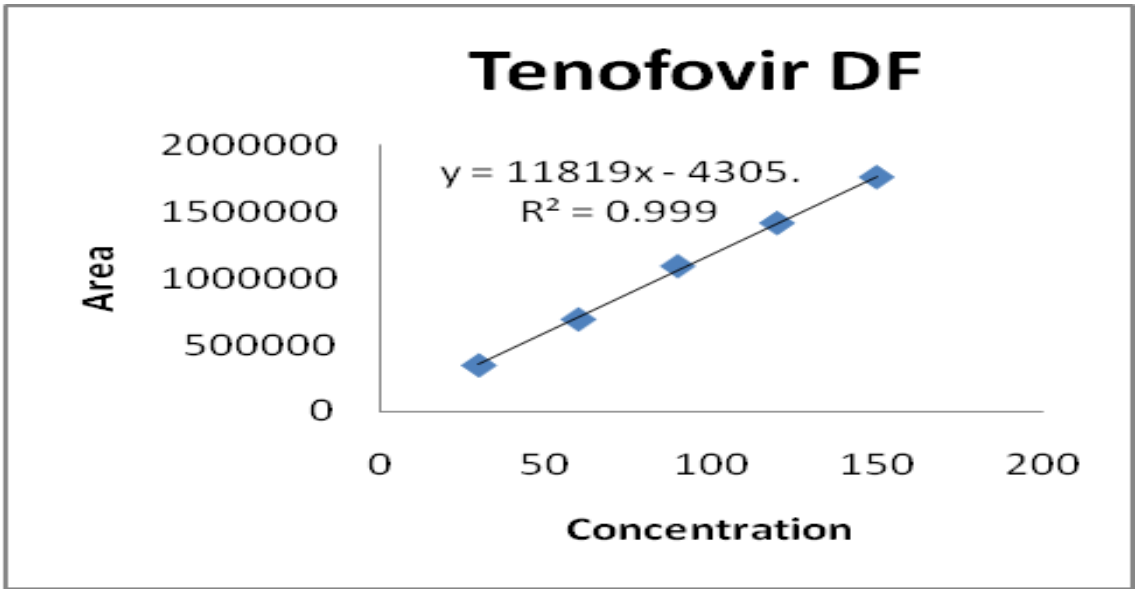

Figure-4 Calibration graph for Tenofovir DF

The correlation coefficient obtained was 0.999 which is in the acceptance limit.

The %RSD for the standard solution is below 1, which is within the limits hence method is precise. There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated and results are given in table-2 and 3.

Table-2 Accuracy (recovery) data for Emtricitabine

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	598249	10	10.06	100.41	100.35
100%	1186057	20	19.95	99.77	
150%	1798511	30	30.26	100.86	

*Average of three determinations

Table-3 Accuracy (recovery) data for Tenofovir DF

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	501791	15	15.05	100.36	100.24
100%	992126	30	29.76	99.21	
150%	1517427	45	45.52	101.16	

*Average of three determinations

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.
The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio (Table-4 and 5).

Table-4 Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Emtricitabine	48	143	2.98
Tenofovir DF	48	142	2.96

Table-5 Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Emtricitabine	48	479	9.98
Tenofovir DF	48	478	9.96

The standard and samples of Emtricitabine and Tenofovir DF 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. Hence the method is robust.

CONCLUSION

The estimation of Emtricitabine and Tenofovir DF was done by RP-HPLC. The assay of Emtricitabine and Tenofovir DF was performed with tablets and the % assay was found to be 99.77 and 99.04 which shows that the method is useful for routine analysis. The linearity of Emtricitabine and Tenofovir DF was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.22 and 0.5 for Emtricitabine and Tenofovir DF which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.69 for Emtricitabine and Tenofovir DF which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.35% and 100.24% for Emtricitabine and Tenofovir DF. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Tenofovir DF was found to be 2.98 and 9.98 and LOD and LOQ for Emtricitabine was found to be 2.96 and 9.96. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

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