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New Validated Rp-Hplc Analytical Method for Simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate

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ABSTRACT:
A new method was established for simultaneous estimation of Emtricitabine and Tenofovir Disoproxil Fumarate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Emtricitabine and Tenofovir Disoproxil Fumarate by using Inspire C18 column (150×4.6mm) 5.0µm, flow rate was 1.0ml/min, mobile phase ratio was (30:70 v/v) Ortho-phosphoric acid Buffer (adjust the pH 2.5 with NaOH solution): Methanol. The detection of wavelength was 272nm.
The developed and validated method was successfully used for the quantitative analysis of commercially available dosage forms. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, UV detector 2487, Empower-software version-2. The retention times of Emtricitabine 2.802mins and Tenofovir Disoproxil Fumarate were found to be 3.677 mins. The % purity of Emtricitabine and Tenofovir Disoproxil Fumarate and was found to be 99.77% and 99.04 % respectively. The system suitability parameters for Emtricitabine and Tenofovir Disoproxil Fumarate such as theoretical plates and tailing factor were found to be 2744.20 and 1.56; 3375and 1.19 respectively, while the resolution was found to be 6.0.
The analytical method was validated according to ICH guidelines [ICH, Q2 (R1)]. The linearity study Emtricitabine and Tenofovir Disoproxil Fumarate was found in concentration range of 20µg-100 µg and 30µg-150µg and correlation coefficient (R2) was found to be 0.999 and 0.999 while % recovery was found to be 100.35% and 100.24% respectively. %RSD for repeatability was 0.22 and 0.5, % RSD for intermediate precision was 0.6 and 0.69 respectively. The study was precise, robust, and repeatable. LOD value was 2.98 and 2.96 , and LOQ value was 9.98 and 9.96 respectively.
Keywords: Emtricitabine, Tenofovir Disoproxil Fumarate and RP-HPLC.

I. INTRODUCTION

Emtricitabine[1] is a nucleoside reverse transcriptase inhibitor (NRTI) indicated for the treatment of HIV infection in adults or combined with Emtricitabine alafenamide for the prevention of HIV-1 infection in high risk adolescents and adults. Emtricitabine is a cytidine analogue. The drug works by inhibiting HIV reverse transcriptase, preventingtranscription of HIV RNA to DNA.
Emtricitabine is described chemically 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3oxathiolan-5-yl]cytosine.It has a molecular formula$C_8H_{10}FN_3O_3S$ and has the following structural formula:

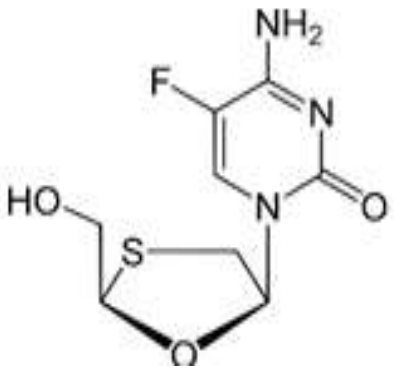

Fig.1:Chemical structure of Emtricitabine

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Tenofovir belongs to a class of antiretroviral drugs known as nucleotide4 analogue reverse transcriptase inhibitors (NtRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. Tenofovir is currently in late stage clinical trials for the treatment of hepatitis B.

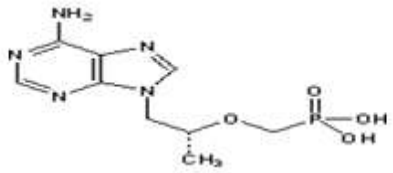

Fig.3: Chemical structure of Tenofovir

Tenofovir disoproxil fumarate is an acyclic nucleoside phosphonate di-ester analog of adenosine monophosphate.Tenofovir inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination. Secifically, the drugs are analogues of the naturally occurring deoxynucleotides needed to synthesize the viral DNA and they compete with the natural deoxynucleotides 5 for incorporation into the growing viral DNA chain. Hence in the present communication we would like to report a simple ,economic, feasible, rapid,sensitive and validated 6-12 specific RP-HPLC method for the simultaneous estimation of Emtricitabine and Tenofovir disoproxil fumarate in Bulk and formulation.

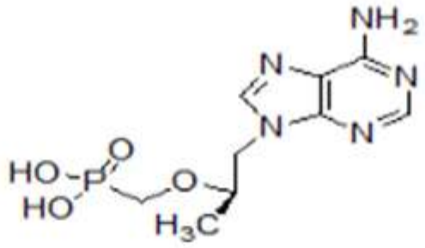

Fig.4: Chemical structure of Tenofovir disoproxil fumarateMATERIALS AND METHODS

Instruments used:

Chromatographic isocratic pump; Rheodyne injector , fixed volume loop, EZICHROME ELITE, Double beam UV-Visible spectrophotometer (Labindia-3120) ,UVWIN-5 software, Ultrasonicator (1.5 L), shimadzu electronic analytical balance (AX-220), Systronics digital pH meter.

Chemicals and Reagents :

Emtricitabine and Tenofovir DF were supplied from Hetero Laboratories, Hyderabad and Potassium dihydrogen o-phosphate and Methanol (MOLY CHEM,HPLC GRADE),Double distilled water and o-phosphoric acid(MERCK) were employed in the present work.

Selection Of Wavelength: 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.

Preparation of buffer and mobile phase: Preparation of 0.1% Ortho phosphoric acid buffer: Pipetted 1 ml of ortho phosphoric acid in 1000 ml HPLC water and adjust the pH 2.5 with NaOH solution. Preparation of mobile phase: Mix a mixture of above buffer 300 ml (30%) and 700 ml MethonolHPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through045 µ filter under vacuum filtration.

Diluent Preparation: Use the Mobile phase as Diluents.

Optimized chromatographic conditions:

Instrument used : High performance liquid chromatography equipped withAuto Sampler and DAD or UV detector

Temperature : Ambient

Buffer : Ortho phosphoric acid pH 2.5Mobile phase : 30%

buffer: 70% Methanol Flow rate : 1.0 ml per min Wavelength : 272 nm Injection volume : 20 µl Run time : 7min.

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.

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

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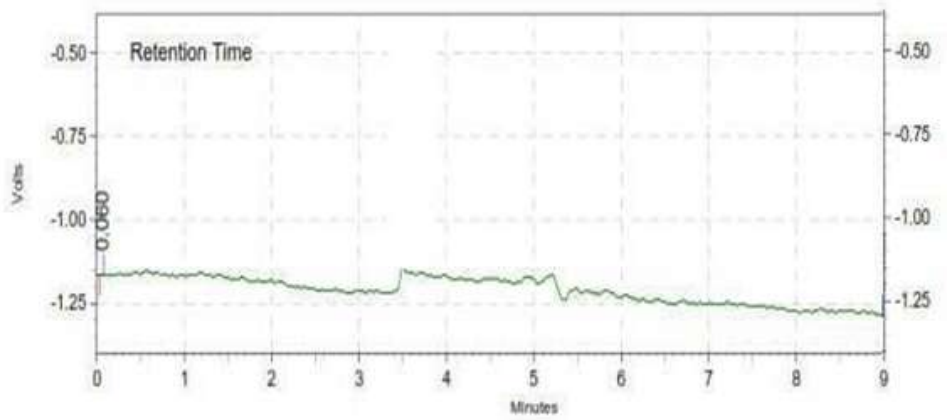
and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

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: Injected 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.



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METHOD VALIDATION

The parameters studied for validation were system suitability, specificity, linearity, precision, accuracy (recovery), ruggedness and robustness, limit of detection and limit of quantification, filter validation and solution stability.

A. Specificity:

If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. It has been observed that there were no peaks of diluents and placebo at main peaks. Hence, the chromatographic systemused for the estimation of Emtricitabine and Tenofovir disoproxil fumarate was very selective and specific. Specificity studies indicating that the excipients did not interfere with the analysis. The standard solution has shown symmetric peak with retention times of 2.86 min for Emtricitabine and 7.40 min for Tenofovir disoproxil fumarate.

B. System suitability:

Standard solution was prepared as per the proposed method and injected into the HPLC system in six replicates and the results were depicted in Table. 5.5.2

C. Linearity & Range:

A series of standard concentrations were prepared from 50 % to 150 % of the target concentration of ETB and TDF. Linearity was assessed by performing single measurement at several analyte concentration varying quantities of stock standard solution diluted with the mobile phase to get final concentrations of 40, 80, 120, 160, 200, 240 µg/mL of ETB and 60,120, 180, 240, 300, 360 µg/mL of TDF. Injection was made at intervals of 10.0 min. Linearity of ETB was found to be exist between 40-240 µg/mL and for TDB was 60 - 360 µg/mL. The chromatograms were recorded and linearity graph was plotted by using peak area of drug against respective concentrations to obtain the linearity range.

D. Precision :

The intra-day and inter-day precision studies were carried out using a test sample assay method with six replicates on the same day and different days.

E. Accuracy (Recovery)

The accuracy of the method was determined by calculating recoveries of ETB and TDF by method of standard additions. Known amount of ETB and TDF were added to a pre quantified sample solution (containing ETB and TDF in 80 and 120 µg/ mL proportion, respectively), and the amount of ETB and TDF were estimated by measuring the peak areas and by fitting these values to the straight-line equation of Method Validation calibration curve.

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F. Ruggedness

This is to prove the lack of influence of operational and environmental variables of the test results by using the method. Ruggedness is a measure of reproducibility of test results underthe variation in conditions normally expected from system to system and from analyst to analyst. It was carried out by using a test sample assay method with six replicates using different analyst.

G. Robustness

Robustness was performed by change in mobile phase ratio, mobile phase flow rate and wavelength of the detector. The test was carried out by small variation in the chromatographic conditions at a concentration equal to standard concentrations 200 µg/mL for ETB and 300 µg/mL for TDF and % change was calculated. % Change in the results was calculated.

H. Limit of detection and Limit of quantification

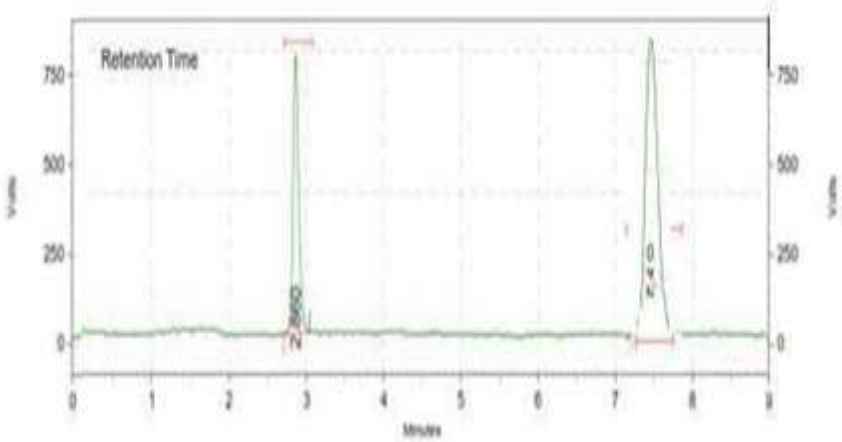
The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy.

I. Solution Stability

Solution stability was assed using standard and test stock solutions. These stocks were prepared and stored at room temperature and refrigerated conditions (2-8°C) for 36 h and % differences were calculated

J. Filter validation

A study was conducted to determine the effect of filter on the assay, dissolution and impurities. Test solution was prepared as per the test method. Some portion of the above solution was filtered through three different filters namely 0.45µ PVDF filter, 0.45µ PTFE and 0.45µ Nylon filter and some portion was centrifuged and injected into the HPLC system. The Chapter-5 Method Validation Page 61 % difference values between centrifuged and filtered sample were calculated. Preparation test solution A total of 20 tablets were accurately weighed and powdered in a mortar. An amount equivalent to 66.5 mg tablet (Containing 20 mg of Emtricitabine and 30 mg of Tenofovir disoproxil fumarate) was transferred to 50 mL volumetric flask, 10 mL of mobile phase was added and content of the flask was sonicated for 10 min and make up to 50 mL with mobile phase. The solution was filtered through whatmann filter paper No. 41. The filtered sample solution 5mL was diluted to 10mL with mobile phaseto get the solution containing ETB and TDF in 200 and 300 µg/mL proportion, respectively. The test solution was injected in to HPLC and % assay was calculated



Standard chromatogram of Emtricitabine and Tenofovir disoproxil fumarate

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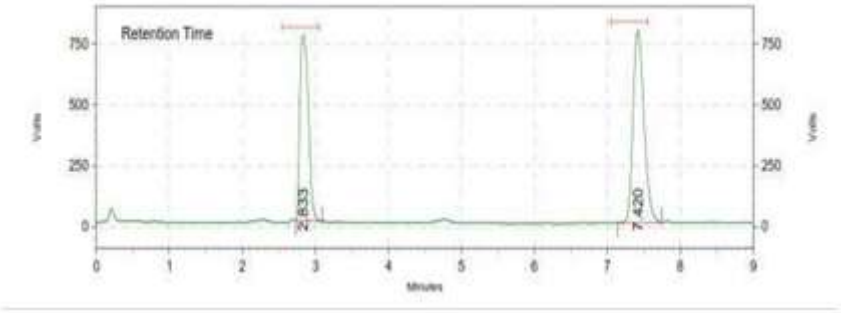
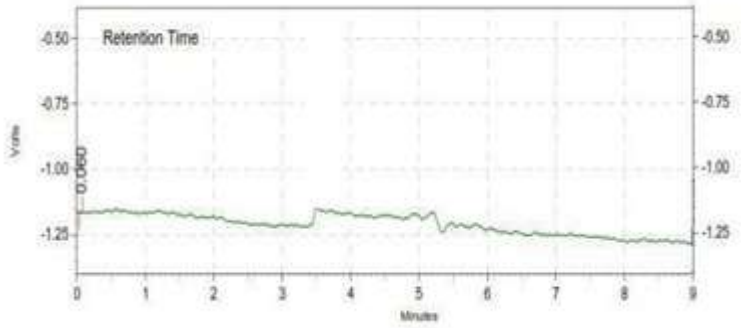


Test formulation chromatogram Emtricitabine and Tenofovir disoproxil fumarate

II. RESULTS & DISCUSSION

In this RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate analytes. The mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The system with 10 mM phosphate buffer (pH 6.8): acetonitrile (40: 60) (v/v) at flow rate of 1.0 mL/min was found to be robust method. Thus the method developed in the present investigation is simple, sensitive, accurate, rugged, robust, rapid and precise. The absence of additional peaks in the chromatogram indicated that there is no interference of the common excipients used in the tablets. Hence, the developed method can be successfully applied for the estimation of Emtricitabine and Tenofovir disoproxil fumarate in tablet dosage forms by RP-HPLC



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III. SUMMARY AND CONCLUSION

It was concluded that there were few methods reported for the simultaneous estimation of the selected multi component dosage forms, which promote to pursue the present work. The present work aimed to assess the applicability of High performance liquid chromatography coupled with UV detector (RP-HPLC with PDA) for analysis of drugs in pharmaceutical formulations.

Precision of the developed methods was studied under intra and inter day precision. The % RSD values for precision was found to be within the acceptable limit, which revealed that the developed method was precise. The developed method was found to be robust. The result indicates satisfactory accuracy of method for simultaneous estimation of the selected drugs The present work compiled with initial research objectives and successfully demonstrated the applicability of RP-HPLC for pharmaceutical analysis of different classes of drugs namely Emtricitabine and Tenofovir disoproxil fumarate, in pharmaceutical formulations by RP-HPLC with UV detection. The developed and validated methods shown high degree of sensitivity, selectivity, reproducibility and good recovery, stability with negligible matrix effects when compared with previously reported methods. This research work has contributions in four important scientific fields.

From an analytical research and

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development (AR□ &D) point of view, useful for analytical research scientists, particularly developing new analytical methods for these selected drugs.

From a formulation research and development (FR□ &D) point of view, useful for formulation research scientists, particularly working on these selected drugs in developing new formulations and pharmacokinetic parameter calculations.

From a drug regulatory point of view, generated data meeting regulatory standards□ and it is acceptable for regulatory submission.

From GLP (Good laboratory practices) point of view, all analytical lab instruments□ and methods were calibrated and validated before performing analysis for acquiring of precise and accurate results. The tremendous potential of RP-HPLC for pharmaceutical analysis is evident and will unquestionably expand future research capabilities in terms of shorter runtime, high rugged and reproducible analytical methods with high precision and accuracy.

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