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● *RESEARCH NOTE* ●

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Method development and validation for simulataneous estimation of emtricitabine and tenofovir disoproxil fumerate in pharmaceutical dosage form

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ABSTRACT

A rapid, sensitive and specific RP-HPLC method involving UV detection was developed and validated for determination and quantification of Emtricitabine and Tenofovir disoproxil fumerate in tablet dosage form. Chromatography was carried out on a pre-packed zorbax SB - phenyl, 250 x 4.6 mm, 5 m column using filtered and degassed Buffer:Methanol as mobile phase at a flow rate of 1.0 ml/min in gradient method and effluent was monitored at 265 nm. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of quantification and limit of detection. The assay was linear over the concentration range of Emtricitabine and Tenofovir disoproxil fumerate was 20 mcg-60 mcg/ml and 30 mcg/ml to 90 mcg/ml, respectively. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the pre analyzed test solution and was found to be 98.49 % - 98.9 % and 98.6 % - 99.6 % within precision RSD of 0.54 and 0.55 for Emtricitabine and Tenofovir disoproxil fumerate, respectively. The system suitability parameters such as retention time, theoretical plates and tailing factors were found to be 4.86, 12081, 1.08 and 7.84, 31182, 0.99, respectively for Emtricitabine and Tenofovir disoproxil fumerate. The method required only 12 mins as run time for analysis which prove the adoptability of the method for the routine quality control of the drug.

Key words : Emtricitabine, Tenofovir disoproxil fumerate, Gradient, Method development, Validation

Emtricitabine[1-3] is chemically 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl] cytosine and used for treating HIV infection in adults in combination with other antiretroviral agents. Emtricitabine enters cells by passive diffusion and is phosphorylated by deoxycytidine kinase and cellular kinases to its active metabolite, emtricitabine triphosphate. The intracellular triphosphate acts as a competitive inhibitor of reverse transcriptase and is incorporated into HIV DNA to cause chain termination. Tenofovir disoproxil fumerate[1-3] is chemically 9 [(R) 2 [[bis[[(isopropoxycarbonyl)oxy]methoxy]phoshinyl]-Methoxy] propyl] adenine fumarate (1:1) and used for treating HIV infection in adults in combination with other antiretroviral agents. Tenofovir disoproxil fumarate is hydrolyzed rapidly to tenofovir and then is phosphorylated by cellular kinases to its active metabolite, tenofovir diphosphate. The active moiety is, in fact, a triphosphate compound because the parent drug starts out as the monophosphate. The intracellular diphosphate is a competitive inhibitor of viral reverse transcriptases and is incorporated into HIV DNA to cause chain termination because it has an incomplete ribose ring. There is a plethora of analysis of such formulations without prior separation. For the estimation of multi-component formulation, the instrumental techniques, which are commonly employed, are spectrophotometry, GLC, high performance thin layer chromatography (HPTLC), HPLC etc. These methods are based upon the measurement of specific and nonspecific physical properties of the substances. The literature survey reveals that there is no methods have been reported. This present study is to develop[4] an accurate and reliable HPLC method for simultaneous estimation of Emtricitabine and Tenofovir disoproxil fumerate in solid dosage form.

In this paper we describe a simple, inexpensive, sensitive and validated HPLC method[5-9] for the simultaneous determination of Emtricitabine and Tenofovir disoproxil fumerate in pharmaceutical formulation.

Working standards of Emtricitabine and Tenofovir disoproxil fumerate were obtained from well reputed

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research laboratories. HPLC grade Methanol, AR grade O-Phosphoric acid and Milli-Q water were procured from the market. The separation was carried out on Gradient HPLC system (AGILENT1100) with pre-packed zorbax SB - phenyl, 250 x 4.6 mm, 5 m column using filtered and degassed mixture of Buffer:Methanol (20:80) as mobile phase.

Transferred about 1 ml of ortho phosphoric acid to 1000ml volumetric flask containg water, made up to volume with water and mixed, filter through 0.45µm nylon membrane filtered and degassed.

Accurately weighed about 60.0 mg of Tenofovir disoproxil fumarate working standard into a 100 ml clean dry volumetric flask, added about 60 ml of diluent, sonicated for 5 mins, and diluted to volume with diluent and mixed.

Accurately weighed about 40.0 mg of Emtricitabine working standard into a 100 ml clean dry volumetric flask, added about 60 ml of diluent, sonicated for 5 mins, and *i.e.* was diluted to volume with diluent.

Pipetted out 5ml of Emtricitabine standard stock solution and 5 ml Tenofovir DF standard stock solution and diluted to 50 ml with diluent and mixed.

Flow rate 1.0 ml/min; detection wavelength 265nm; injection volume 20 µl; column used zorbax SB - phenyl, 250 x 4.6 mm, 5 µ; column temperature: ambient, mobile phase: Buffer: methanol with gradient program showed in the Table 1.

Table 1 : Gradient programme

Time(min)	Flow (ml/min)	%A	%B
0.00	1.0	95	5
8.00	1.0	50	50
13.00	1.0	95	5
20.00	1.0	95	5

Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

Weighed five tablets and transferred into a 500 ml volumetric flask. Added about 100 ml of buffer and shake the volumetric flask on a rotary shaker and allow rotating until complete disintegration for 20 min, 200 ml of methanol was added and sonicated for 20min with intermittent shaking and diluted to volume with methanol and mixed. From this pipetted out 2 ml of sample solution into a 100 ml volumetric flask, made up the volume with diluent, mixed and filtered the solution through 0.45 µ nylon membrane filter to obtain clear solution.

20 µl of the standard preparation and assay preparation were separately injected and chromatographed which was shown in Fig. 1.

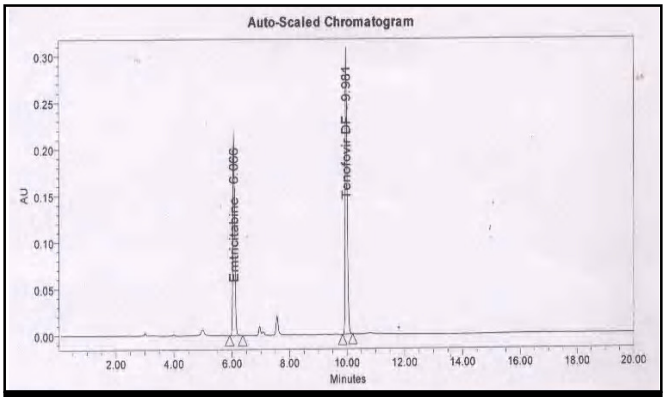

Fig. 1 : Chromatogram of Emtricitabine and Tenofovir disoproxil fumerate

The standard, sample and placebo were degraded by acid, base, peroxide, thermal, phootolytic and accelerated degradation. There was no interference of main peak with degraded products peak which is shown in the Table 2.

Linearity[10-12] was demonstrated by analysing six different concentrations of active compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs concentrations of Emtricitabine and Tenofovir disoproxil fumerate which were found to be linear in the range of 20 mcg/ml – 60 mcg/ml and 30 mcg to 90 mcg/ml, respectively. Coefficient of correlation was 0.9999 and 0.9998.

Accuracy[10-12] was done by recovery study using standard addition method, known amount of standard Emtricitabine and Tenofovir disoproxil fumerate in to pre-analysed samples and subjected to proposed HPLC method. The results of recovery studies are shown in Table 2.

To demonstrate agreement among results, a series of measurements are done with Emtricitabine and Tenofovir disoproxil fumerate six replicate injections of

Table 2 : Analysis of tablet containing Emtricitabine and Tenofovir disoproxil fumerate

Drug	Injected sample (mcg/ml)	Amount found (mcg/ml)	Found (%)	Amount std. added	Amount recovered (mcg/ml)	Recovery (%)
Emtricitabine	40	39.76	99.42	40	39.44	98.60
Tenofovir DF	60	59.52	99.20	60	58.96	98.26

the specific standard at various time intervals on the same day were injected into the chromatograph and the value of % RSD was found to be 0.79 and 0.74 for Emtricitabine and Tenofovir disoproxil fumerate, respectively. In inter-day precision same standard was injected on different days and the found % RSD were 0.36 and 0.38 for Emtricitabine and Tenofovir disoproxil fumerate, respectively. The results are shown in the Table 3.

Table 3 : Precision

Amount found on	Intra-day		Inter-day	
	Mean %	RSD (%)	Mean %	RSD (%)
Emtricitabine	98.5	0.54	98.9	0.79
Tenofovir DF	99.6	0.55	98.6	0.74

The regression value was found to be 0.9998 and 0.9999 for Emtricitabine and Tenofovir disoproxil fumerate, respectively, which shows the response, is linear from 20 mcg/ml - 60 mcg/ml and 30 mcg/ml to 90 mcg/ml, respectively. Specificity experiment showed that there was no interference or overlapping of the peaks either due to excipients or diluents and degradation products with the main peak of Emtricitabine and Tenofovir disoproxil fumerate. The percentage RSD for precision is < 2 which confirms that method is sufficiently precise and the total run time required for the method is only 12mins for eluting both Emtricitabine and Tenofovir disoproxil fumerate. The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control of Emtricitabine and Tenofovir disoproxil fumerate.

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