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Original Article

SIMULTANIOUS DETERMINATION OF ANTIRETROVIRAL DRUGS EMTRICITABINE AND TENOFOVIR DESOPROXIL FUMERATE BY A STABILITY INDICATING RP-HPLC METHOD

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ABSTRACT

Objective: The objective of the present investigation is to develop a stability indicating RP-HPLC method for the simultaneous estimation of Emtricitabine (EMT) and Tenofovir Desoproxil Fumerate (TDF) in pure and tablet dosage forms.

Methods: Waters (Alliance) HPLC 2695 series systems equipped with UV detector was adopted for the chromatographic analysis. The separation was achieved by injecting 20 µl of working standard solution of concentration 60 µg/ml of EMT and 90 µg/ml of TDF into the Inertsil ODS C-18 (250 mm x 4.6 mm, 5 µm) column maintained at ambient temperature, elution was carried out by 0.1% triflouro acetic acid (TFA) buffer and methanol in the ratio 39:61 (v/v) as mobile phase at a flow rate of 1.2 ml/min, and the detection at a wavelength at 261 nm.

Results: The system suitability parameters such as tailing factor (1.41&1.43), theoretical plates (2470&3059), resolution (4.21) and chromatographic parameters like retention time (2.387&3.293 min), peak area (147228&250057), peak height (20731&26892) of EMT and TDF were evaluated. The developed method was validated in terms precision (for system precision and method precision, the %RSD was found to be 0.4637&0.3176 and 0.3502&0.2789 for EMT and TDF respectively), accuracy (percent of recovery was found to be 99.21-100.16&100.78-101.16), linearity (20-100µg/ml&30-150 µg/ml), limit of detection (0.48&0.56 µg/ml), limit of quantitation (1.64&1.89 µg/ml) for EMT and TDF respectively. The developed method was applied for the determination of assay of Truvada and the percent of assay was found to be in the range of 100.74-101.56.

Conclusion: A simple, rapid and economic RP-HPLC method for simultaneous determination of EMT and TDF in bulk and formulations was developed and validated. The developed method was found to be precise, accurate, linear, robust and rugged. This method was successfully applied for the assay of Truvada; hence it can be adopted for the determination of quality in any quality control laboratory.

Keywords: RP-HPLC method, Truvada, Validation, Stability, Emtricitabine, Tenofovir Desoproxil Fumerate.

INTRODUCTION

The development and use of antiviral drugs for the treatment of viral infections such as acquired immune deficiency syndrome (AIDS), hepatitis, and avian and swine flu epidemics has become a very active area for the last few years. Recently, the combination of EMT and TDF has demonstrated significantly greater human immunodeficiency virus (HIV) ribonucleic acid (RNA) suppression compared to the combination of zidovudine and lamivudine. TDF is formulated in binary mixture of the reverse transcriptase inhibitor emtricitabine to prevent HIV from altering the genetic material of healthy cells. Combining the two drugs in one tablet (Truvada) helps in reduction of the pill burden and increases the compliance with antiretroviral therapy. These antiviral drugs work by preventing HIV cells from multiplying in the body.

Truvada consists of 200 mg of EMT and 300 mg of TDF (equivalent to 245 mg of tenofovir). Emtricitabine, a nucleoside reverse transcriptase inhibitor (NRTI) is chemically known as 4-amino-5-fluoro-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydro pyrimidin-2-one, has molecular formula $C_8H_{10}FN_3O_3S$ and molecular weight 247.248 g/mol. EMT is indicated in combination with other antiretroviral agents for the treatment of HIV infection in adults. Tenofovir disoproxil Fumerate belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NRTIs), which block reverse transcriptase, a crucial viral enzyme in human immunodeficiency virus 1 (HIV-1) and hepatitis B virus infections. It is chemically known as ({[(2*R*)-1-(6-amino-9*H*-purin-9-yl) propan-2-yl]oxy}methyl)phosphoric acid with molecular formula $C_9H_{14}N_5O_4P$ and molecular weight 287.213 g/mol. Tenofovir is indicated in combination with other anti retroviral agents for the treatment of HIV-1 infection in adults. The molecular structures of EMT and TDF were presented in fig. 1 and fig. 2 respectively.

An extensive literature survey was carried out and found some simultaneous spectrophotometric methods [1-8] for the determination of emtricitabine and tenofovir disoproxil Fumerate in pure and pharmaceutical formulations. Several authors developed reversed phase liquid chromatographic methods for the simultaneous estimation of EMT and TDF in tablet dosage forms [9-13] and biological fluids [14]. Several liquid chromatography-tandem mass spectrometric methods [15-19] were present in the literature for the determination of low concentrations of these drugs in especially in human plasma. In addition, two HPTLC methods [20] and one RP-UPLC method [21] were reported. Different experimental methods such as spectrophotometry [22], reverse phase HPLC [23-26] and LC/MS/MS [27] methods were reported for the individual determination of emtricitabine in tablet dosage form or human plasma and for the study of related impurities in the drug substance. Several spectrophorometric methods [28-30], RP-HPLC methods [31-36], LC/MS/MS methods [37] were found in the literature for the estimation of tenofovir disoproxil Fumerate in dosage forms and human plasma. Since spectrophotometric methods are the lack of sensitivity and though LC/MS/MS technique is highly sensitive but costly and lot of care should be taken during analysis. Therefore HPLC methods have wide applications for the analysis of pharmaceutical analysis especially in quality assessment. Though there were a few HPLC methods, there is a scope to develop a new simple, rapid and economic method. Hence the author made some investigations to decrease retention time of the analytes and hence run time of the analysis which makes the method simple, rapid and economic. The other objectives are to make the peaks symmetrical (decrease tailing and fronting factor), and to improve its sensitivity. The foremost goal of the study is to ascertain the percent of degradation when the drugs are exposed to some degradation conditions such as acidic, basic, oxidative, thermal and photolytic exposure and to find the stability under the study forced degradation.

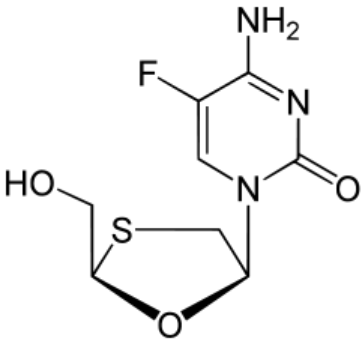

Fig. 1: Molecular structure of Emtricitabine

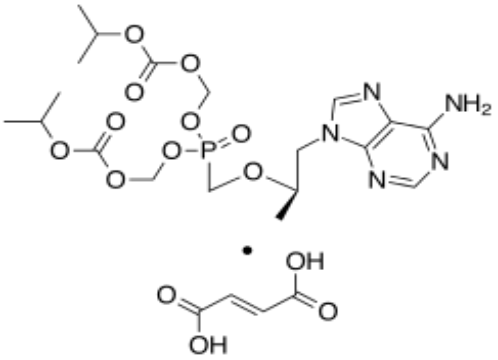

Fig. 2: Molecular structure of Tenofovir disoproxil Fumerate

MATERIALS AND METHODS

Instrumentation

Waters (Alliance) HPLC 2695 series consisting of four auto sampler racks and DAD or UV detector and Shimadzu HPLC model (VP series) containing LC-10AT pump, variable wave length programmable UV/Visible detector SPD-10AVP and rheodyne injector (7725i) with 20 µl fixed loop were used for the present investigation.

Chemicals and reagents

Emtricitabine and Tenofovir Desoproxil Fumerate reference samples (99.8% pure) were obtained from Finoso Pharma Pvt. Ltd., Hyderabad, Telangana, India. HPLC grade water and AR grade methanol (MeOH), Triflouro acitic acid (TFA), hydrochloric acid (HCl), sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) were procured from Merck India.

Preparation of solutions

Preparation of 0.1% TFA buffer

1.0 ml of triflouro acetic acid was accurately transferred into a 1000 ml volumetric flask and made up to the mark with HPLC grade water, and sonicated for 5 min using an ultrasonic water bath.

Preparation of mobile phase

Mobile phase or diluent of composition buffer and methanol 39:61 (v/v) was prepared by mixing exactly 390 ml of 0.1% TFA buffer and 610 ml of methanol. The solution was degassed by using an ultrasonic water bath and filtered through 4.5 µm filter under vacuum filtration and used for further dilutions.

Preparation of working standard solution

An amount of 20 mg of EMT and 30 mg and TDF were weighed accurately and transferred into a clean 100 ml dry volumetric flask, dissolved in diluent, sonicated and made up to the mark, the concentration of resulting solution was found to be 200 µg/ml of EMT and 300 µg/ml TDF. Then 3.0 ml of an above stock solution was transferred into a 10 ml volumetric flasks and diluted up to the mark with diluents, and the concentration of the resulting working standard solution was 60 µg/ml of EMT and 90 µg/ml TDF.

Preparation of sample solution

Average weight of five Truvada tablets (200 mg of EMT and 300 mg of TDF) was determined, grinded well and an amount of the powder (74.5 mg) equivalent to 20 mg of EMT & 30 mg TDF was accurately weighed and transferred into a clean 100 ml volumetric flask, dissolved in diluent, sonicated and used as sample stock solution. Further 3.0 ml of the above solution was diluted to 10.0 ml and used for the analysis.

Method development and optimization

The RP-HPLC method was developed based on physico-chemical information of selected drugs like chemical structure, molecular weight, solubility, pKa value, UV absorption maxima, inactive ingredients and impurities present in the sample. The selected drugs were completely soluble in moderately polar or polar solvents such as water, methanol and acetonotrile, hence a reversed phase liquid chromatographic technique was the best method. In this mode, non polar stationary phase (a nonpolar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel) and a polar mobile phase were chosen. The optimum chromatographic conditions were obtained from different trials by changing one of the chromatographic conditions such as stationary phase (column), mobile phase and its composition, flow rate of the mobile phase, injection volume, run time, column temperature and detection wavelength keeping other constant, and finally good separation was achieved by injecting 20 µl of working standard solution into the Inertsil-ODS, C18, 250 mmx4.6 mm, 5µ column maintained at ambient temperature, elution was carried out by using mobile phase of 0.1% TFA buffer and methanol in the ratio 39:61 at a flow rate of 1.2 ml/min, and the detection at wavelength of 261 nm.

Method validation

Validation is establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specification and quality characteristics. The developed RP-HPLC method was validated for specificity, precision, accuracy, linearity, robustness and ruggedness as per ICH guidelines.

Specificity

Specificity is the ability to asses unequivocally the analyte in the presence of impurities, degradants and matrix. To determine the specificity of the proposed method, 20 µl of blank, standard and sample solutions were injected separately into the column and triplicate chromatograms were recorded under the optimized chromatographic conditions.

System precision and method precision

Precision refers to the reproducibility of measurement within a set, one of the most common statistical terms employed is the standard deviation of a population of observation, and percent relative standard deviation (%RSD) is the most convenient property to express precision. To find out system precision, 20 µl of the working standard solution was injected into HPLC column six times and chromatograms were recorded and chromatographic parameters were evaluated under the optimized conditions. In the study of method precision, six replicate working standard solutions of the same concentration were prepared from the standard stock solution, injected into the HPLC system, chromatograms were recorded and chromatographic parameters were obtained under similar conditions.

Accuracy

The study accuracy was carried out at three different levels (50%, 100% and 150% with respect to target concentration by standard addition method in which known amounts of standards were added to pre-analysed sample solution in triplicate. Chromatograms were obtained and the percent of recovery was evaluated from the peak area.

Linearity

The linearity between peak area and concentration of EMT and TDF was demonstrated by preparing a series of standards by taking different aliquots of standard stock solution into 10 ml standard flasks, made up to the mark. The concentrations of EMT and TDF in resulting solutions were 20-100µg/ml and 30-120µg/ml respectively. Then 20 µl of each solution was injected into the column twice and chromatograms were obtained under the identical chromatographic conditions and the average peak area was calculated for each concentration level.

Limit of detection and Limit of quantitation

Sensitivity of an analytical technique is expressed in terms of limit of detection (LOD) and limit of quantitation (LOQ). Exactly 1.0 ml of standard stock solution (200µg/ml EMT and 300µg/ml of TDF) was accurately transferred into a 10 ml volumetric flask and diluted up to the mark with diluents, further 3.0 ml of the above solution was transferred accurately into a 10 ml volumetric flask and diluted up to the mark with diluent. Then 0.8 ml of the above stock solution was pipette and introduced into a 10 ml volumetric flask and dilute up to the mark with diluents and the final concentration of the solution was 0.48µg/ml and 0.56µg/ml of EMT and TDF respectively. Similarly LOD stock solution of concentration 1.64µg/ml EMT and 1.89 µg/ml TDF was prepared by a series of dilutions (diluting 1.4 ml of working standard to 10 ml and then 2 ml of this solution to 10 ml). Base line noise for blank and area of the peak for LOD/lOQ standards was experimentally determined by injecting 20 µl of blank and LOD/lOQ standard in triplicate under the optimized chromatographic conditions.

Robustness

The study of robustness in the present investigation was demonstrated by carrying out deliberate variations in flow rate 1.2±0.2 ml and mobile phase compositions (Organic composition in the mobile phase was varied from 51% to 71%). The chromatograms were recorded in triplicate for each variation and the system suitable parameters were evaluated. In the study of flow rate variation(1.0 ml/min to 1.4 ml/min.), standard solution of concentration 60 & 90 µg/ml of EMT and TDF was prepared and analysed using the varied flow rates along with method flow rate. In case of variation in organic solvent composition in the mobile phase (51%to 71%), working standard solution of concentration 60 µg/ml of EMT and TDF was prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

Ruggedness

Ruggedness is a study of repeatability of results between two analysts, laboratories, different days and different instruments. In the present investigation, the author made investigations to find the repeatability of the results between two different days and different instruments by obtaining chromatograms for same working standard solution under the identical conditions.

Assay of pharmaceutical formulations

The percent of assay was calculated using peak area of standard and sample, average weight of standard, sample, and their concentrations by using the following equitation. %Assay= (AT/AS)*(WS/DS)*(DT/WT)*(P/100)*(Average weight/label claimed)*100. Where AT = Average area counts of sample preparation, AS = Average area counts of standard preparation, WS = Weight of working standard taken in mg, P = Percentage purity of the working standard, LC **=** Label claim mg/ml. Exactly 20 mg of EMT and 30 mg of TDF were weighed accurately and transferred into two clean 100 ml dry volumetric flask, dissolved in diluent, sonicated and made up to the mark. Then 3.0 ml of the above solution was accurately measured by using micro burette and transferred into 10 ml volumetric flasks and diluted up to the mark with diluents, and the concentration of the resulting solution was 60µg/ml EMT and 90µg/ml TDP respectively. After that 20 µl of these solutions were injected into the column, chromatograms were obtained under the optimized chromatographic conditions.

Stability studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies of EMT and TDF in Truvada samples. Average weight of ten Truvada tablets (200 mg of EMT and 300 mg of TDF) was determined, and made them into a fine powder, and 74.5 mg of this powder was found to be equivalent to 20 mg of EMT and 30 mg TDF. In this study, the drug sample was exposed to different chemical and physical degradation conditions such as 0.1N HCl (acid hydrolysis), 0.1N NaOH (base hydrolysis), 3% H_2O_2 (oxidation), heat (thermal decomposition) and UV-light (radiation decomposition) for specified time, and then diluted as similar as standard dilution, and then duplicate chromatograms were obtained under the similar chromatographic conditions, the percent of degradation was calculated from the peak area of the chromatograms.

Acid or base hydrolysis

An amount of sample powder equivalent to 20 mg of EMT and 30 mg of TDF was transferred into 100 ml of round bottom flask and added 50 ml of freshly prepared 0.1 N HCl or 0.1N NaOH. Allowed for 24 hours for hydrolysis, then filtered the solution through 0.45µfilter into a 100 ml standard flask and neutralized the unreacted acid or base with 0.1N NaOH or 0.1 N HCl and made up to the mark. Then 3.0 ml of this filtrate was diluted to 10 ml with the mobile phase. Now 20 µl of freshly prepared working standard solution and acid/base hydrolyzed solution was injected into chromatographic column, duplicate chromatograms were recorded and percent of degradation was calculated from the area of the peaks.

Hydrogen peroxide degradation

An amount of sample powder equivalent to 20 mg of EMT and 30 mg of TDF was accurately transferred into 100 ml of round bottom flask and refluxed for 24 hours by adding 50 ml of freshly prepared 3%H_2O_2, then filtered the solution through 0.45µfilter into a 100 ml standard flask and made up to the mark, then 3.0 ml of this filtrate was diluted to 10 ml with mobile phase. Then 20 µl of freshly prepared working standard solution and oxidized solution was injected into the chromatographic column separately, chromatograms were recorded and percent of degradation was calculated from the mean peak area of two chromatograms.

Thermal degradation

In the study of thermal decomposition, an amount of sample powder equivalent to 20 mg of EMT and 30 mg of TDF was accurately transferred into a clean and dry watch glass, placed in an oven which was maintained at 80^{o}C for 24 hrs. Then removed from the oven and allowed to cool to room temperature. The substance was accurately transferred into 100 ml volumetric flask and dissolved in diluents and made up to the mark, filtered and 3.0 ml of this filtrate was diluted to 10 ml with the mobile phase. Then 20 µl of freshly prepared working standard solution and solution of decomposed drug was injected into chromatographic column separately, chromatograms were recorded twice and percent of degradation was calculated from the area of the peaks.

Degradation by UV exposure

In the study of decomposition of the drugs in the presence of UV radiation, an amount of sample powder equivalent to 20 mg of EMT and 30 mg of TDF was accurately transferred into a clean and dry Petridish, placed the dish in a UV cabinet for 24 hours, then compounds were kept at room temperature for a few min, accurately transferred into a 100 ml volumetric flask containing 50 ml mobile phase, sonicated for 10 min and diluted up to the volume by the mobile phase and filtered the solution. Then 3.0 ml of this filtrate was diluted to 10 ml with mobile phase. Then 20 µl of this solution and freshly prepared working standard solution were injected into chromatographic column two times separately, chromatograms were recorded and percent of degradation was calculated from the area of the peaks.

RESULTS AND DISCUSSION

System suitability parameters

20 µl of the working standard solution was injected into the column, and buffer solution and methanol in the ration 39:61 were allowed to flow through the column at a rate of 1.2 ml per min from two separate channels, and the system response (absorbance in mV) was recorded automatically at a detection wavelength 261 nm as a function of time for 8.0 min of run time. Chromatograms were recorded and the system suitability parameters such as tailing factor (1.41&1.43), theoretical plates (2470&3059), resolution (4.21) and chromatographic parameters like retention time (2.387&3.293 min), peak area (147228&250057), peak height (20731&26892) of EMT and TDF were obtained. A representative chromatogram for standard was presented in fig. 3.

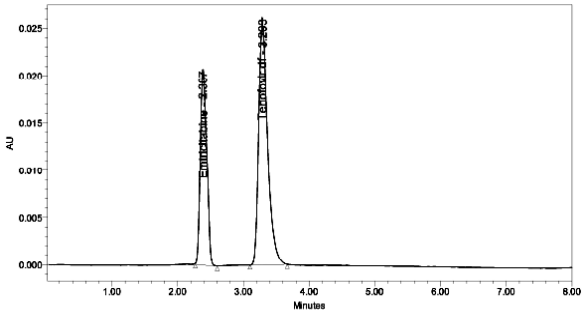

Fig. 3: Chromatogram of working standard solution (n=3)

Specificity

The chromatograms for blank and sample solution were recorded under optimized chromatographic conditions, compared with the chromatogram of the standard solution, and found no additional peaks.

The two peaks were completely separated and resolution was found to be 4.15 min even in the presence of inactive excipients of the sample. The overlain chromatogram for blank and sample was given in fig. 4

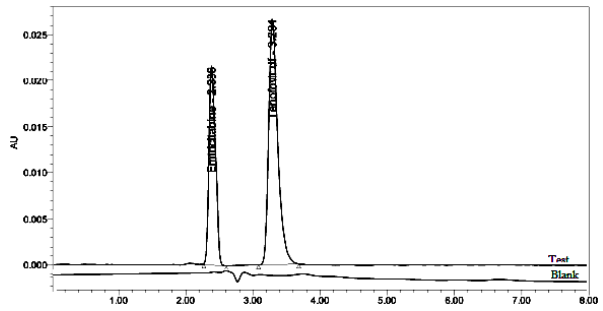

Fig. 4: A typical chromatogram of blank and sample solution (n=3)

Precision

The precision of the developed method was expressed as percent of standard deviation (%RSD) of six replicates and calculated from mean and standard deviation (SD) of six replicate measurements (peak area) by the formula % RSD=(SD/X)*100, and found to be less than 2.0. The experimental data and statistical parameters of system precision and method precision were presented in table 1.

Accuracy

Triplicate chromatograms of the three different concentrated solutions 50%, 100% and 150% with respect to target concentration were obtained and the amount of drug recovered and percent of recovery was obtained from the peak area at each level, and the results of percent of recovery were formulated in table 2.

Table 1: A study of system precision and method precision

System precision			Method precision		
Injection	**Area of EMT**	**Area of TDF**	**Injection**	**Area of EMT**	**Area of TDF**
Injection-1	149568	255421	Injection-1	149724	255384
Injection-2	150452	255899	Injection-2	149452	255768
Injection-3	150547	255837	Injection-3	150715	255737
Injection-4	148894	253797	Injection-4	149547	253915
Injection-5	150624	255096	Injection-5	150497	255296
Injection-6	149642	255874	Injection-6	149768	255749
Maximum	150624	255899		150715	255768
Minimum	148894	253797		149497	253915
Spread	1730	2102		1218	1853
Mean	149954.2	255320.7		149950.5	255308.2
Median	150047	255629		149746	255560
SD	695.484	811.09		525.17	712.248
%RSD	0.4637	0.3176		0.3502	0.2789
Variance	483698	657869		275809	507298

Sample size (n) is six, SD: Standard deviation, %RSD: Percent of relative standard deviation

Table 2: Accuracy study of proposed method

%Concentration	Mean peak area*	Amount Added (mg)	Amount found (mg)*	% Recovery	Mean recovery
		EMT			
50%	73875	10	10.10	100.16%	99.68%
100%	145200	20	19.84	99.21%	
150%	218773	30	29.90	99.66%	
		TDF			
50%	126799	15	15.27	101.83%	100.96%
100%	250977	30	30.23	100.78%	
150%	374627	45	45.13	100.29%	

- Indicates average of three determinations

Linearity

Linearity limits, slope, intercept and correlation coefficient of the two array data (concentration of the analyte and peak area) were determined by linear regression analysis. It was found that the range of linearity 20-100 µg/ml and 30-150µg/ml for EMT and TDF respectively. The correlation value more than 0.999 indicate that the peak area was linearly increases with concentration. The experimental data and results of linearity study were shown in table 3.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The chromatograms for LOD and LOQ concentration levels were recorded (fig. 5) under optimized conditions, the average baseline noise for blank and average peak area for LOD concentration with was determined and calculated signal to noise ration and found to be more than 3.0. The average baseline noise was obtained for the blank and average peak area of LOQ concentration was also determined and calculated signal to noise ration and found to be more than 10.0. The results of LOD and LOQ were presented in table 4.

Table 3: Study of linearity between mean peak area and concentration

S. No.	EMT		TDF	
	Concentration µg/ml	Mean peak Area*	Concentration µg/ml	Mean peak Area*
1	20	61320	30	91371
2	40	106380	60	170219
3	60	147992	90	250626
4	80	190764	120	326001
5	100	237000	150	404952
Linearity µg/ml		20-100		30-150
Slope		2178.7		2609.8
Intercept		17968		13751
Correlation coefficient		0.9997		0.9999

*Mean of two determinations

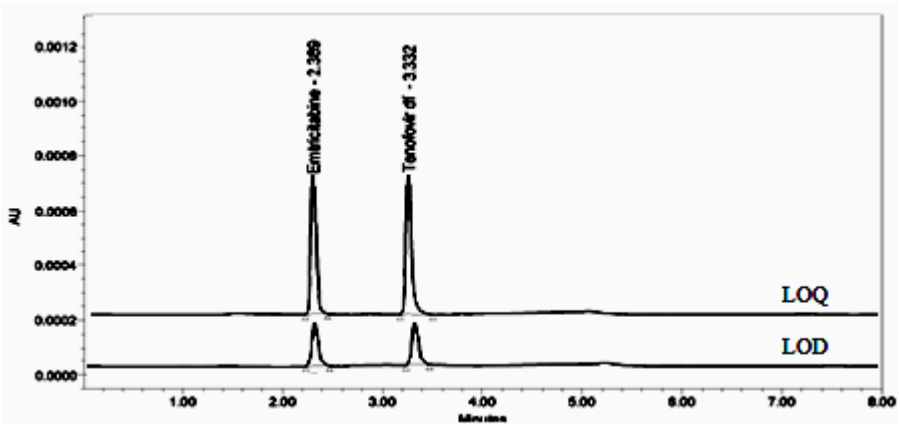

Fig. 5: A typical LOD &LOQ chromatograms (n=3)

Table 4: Results of limit of detection and quantitation

	EMT*	TDF*
Baseline noise	N=56 µV	N=56
Peak area of LOD standard	S=165 µV	S=166
Peak area of LOQ standard	S=559 µV	S=558
LOD=S/N	2.95	2.96
LOQ=S/N	9.98	9.96
LOD Concentration	0.48 µg/ml	0.56 µg/ml
LOQ Concentration	1.64 µg/ml	1.89 µg/ml

* Average of three determinations, S: Signal, N: Noise, S/N: Signal-to-noise ratio, LOD: Limit of detection, LOQ: Limit of quantitation

Robustness

The robustness, a measure of its capacity to remain unchanged by small but deliberate variations in method parameters like flow rate, polarity of the solvent, pH of the buffer solution, temperature and wave length. On evaluation of the above results, it can be concluded that the variation in flow rate and polarity of the solvent did not affect the method significantly. Hence developed the method was found to be robust even by change in the flow rate±10% and mobile phase±10%. The results of ruggedness were shown in table 5.

Ruggedness

In the study of ruggedness, the reproducible results were obtained by the analysis of the same samples under a variety of conditions, such as different days and different instruments. The results of study of ruggedness were shown in table 6.

Assay

The percent of assay was calculated using peak area of standard and sample, average weight of standard, sample, and their concentrations. The results of percent of assay were given in table 7.

Table 5: Study of robustness

Parameter		Flow rate				Mobile phase composition			
		EMT		TDF		EMT		TDF	
Variation		Plate count	Tailing	Plate count	Tailing	Plate count	Tailing	Plate count	Tailing
Actual	1	2465	1.42	3056	1.44	2465	1.42	3056	1.44
	2	2472	1.40	3063	1.42	2472	1.40	3063	1.42
	3	2473	1.41	3058	1.43	2473	1.41	3058	1.43
	AVG	2470	1.41	3059	1.43	2470	1.41	3059	1.43
	SD	4.3589	0.01	3.6055	0.01	4.3589	0.01	3.6055	0.01
	%RSD	0.1765	0.7092	0.1178	0.6993	0.1765	0.7092	0.1178	0.6993
Less	1	2507	1.40	3063	1.51	2595	1.41	3334	1.34
	2	2503	1.41	3065	1.49	2593	1.39	3331	1.33
	3	2506	1.38	3062	1.5	2594	1.40	3332	1.32
	AVG	2505	1.3967	3063	1.5	2594	1.40	3332	1.33
	SD	2.0816	0.0153	1.5275	0.01	1.0	0.01	1.5275	0.01
	%RSD	0.0831	1.0937	0.0498	0.6667	0.0385	0.7143	0.0458	0.7518
More	1	2283	1.26	2804	1.37	2345	1.38	2805	1.33
	2	2280	1.27	2801	1.37	2341	1.39	2801	1.36
	3	2281	1.25	2803	1.36	2342	1.37	2800	1.42
	AVG	2281	1.26	2802	1.3667	2342	1.38	2802	1.37
	SD	1.5275	0.01	1.5275	0.0057	2.0816	0.01	2.6457	0.0458
	%RSD	0.0669	0.7936	0.0545	0.4224	0.0888	0.7246	0.0944	3.3449

AVG: Average, SD: Standard deviation, RSD: Relative standard deviation

Table 6: Study of ruggedness

Injection	Day-1	Day-2	Instrument-1	Instrument-2
	Peak area EMT	Peak area TDF	Peak area EMT	Peak area TDF
Injection-1	145634	250866	145758	249687
Injection-2	147426	249318	146824	249572
Injection-3	144695	244872	145695	245172
Injection-4	149570	253526	149070	245226
Injection-5	147072	249319	147372	248319
Injection-6	144997	245337	145818	246337
Mean	146565.7	248873	146756.2	247385.5
SD	1835.793	3302.444	1322.138	2079.013
%RSD	1.25254	1.32696	0.900908	0.840394

At each variety of conditions, averages of three determinations were taken, SD: Standard deviation, %RSD: Percent of relative standard deviation

Table 7: Results of assay studies

EMT	LC	P	AS*+SD	AT*+SD	%A+SD
	200	99.8	146059+3.60	148629+2.08	101.56+1.56
TDF	LC	P	AS*	AT*	%A
	300	99.8	248542+2.14	250890+1.47	100.74+1.16

* Average of three determinations, LC: Label claimed, P: Purity, AS: Mean peak area for standard, AT: Mean peak area for test, %A: Percent of assay, SD: Standard deviation

Stability studies

A study of forced degradation was carried out to evaluate the stability of the drugs either in pure form (API) or formulations. In the present investigation acid, base and peroxide degradation studies and degradation in the presence of thermal energy or photo light were carried out, and the percent of degradation was calculated from the peak area of degradation standard and degraded test solution.

The chromatograms in the degradation study were presented from fig. 6 to fig. 10, and results of degradation and stability of drugs were presented in table 8.

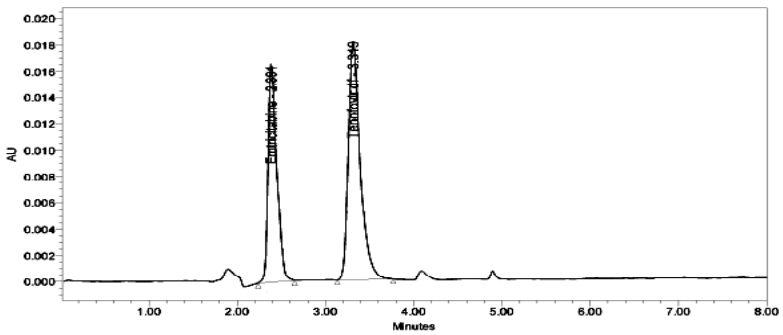

Fig. 6: Chromatogram of acid degradation studies (n=2)

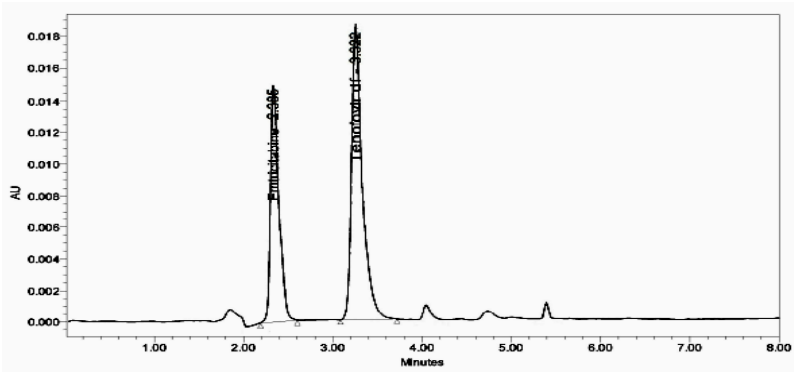

Fig. 7: Chromatogram of base degradation studies (n=2)

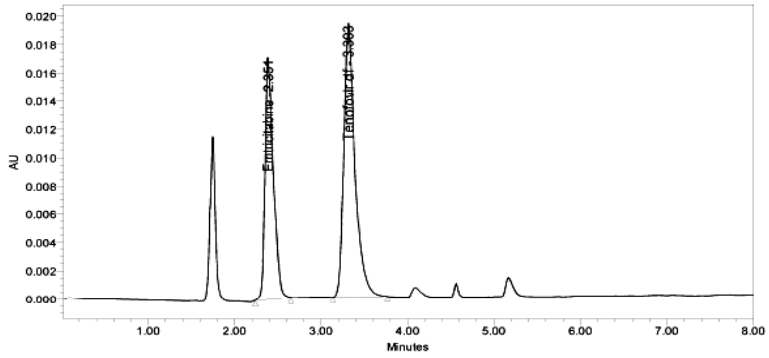

Fig. 8: Chromatogram of peroxide degradation studies (n=2)

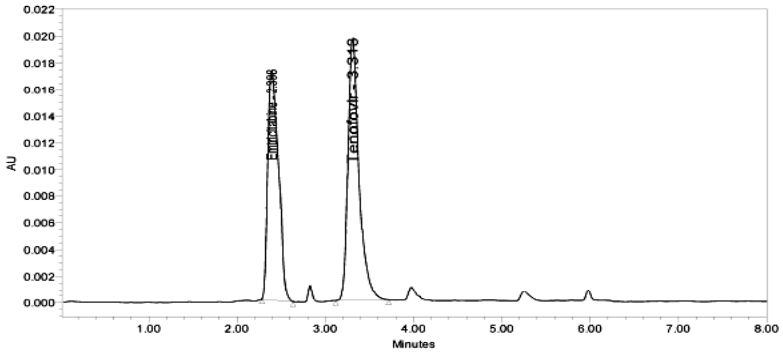

Fig. 9: Chromatogram of thermal degradation studies (n=2)

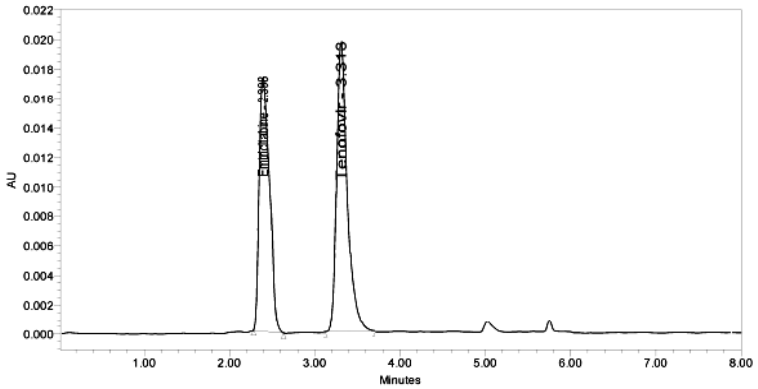

Fig. 10: Chromatogram of photo light degradation (n=2)

Table 8: Results of degradation studies

	EMT		TDF	
	Area*+SD	**%DEG+SD**	**Area*+SD**	**%DEG+SD**
Standard	155211+1.43	--	211002+1.52	--
Acid	138548+2.51	10.73+2.49	184305+1.49	12.65+1.50
Base	133216+1.47	14.17+1.45	181173+2.07	14.13+2.06
Thermal	139435+2.04	10.16+2.06	184316+1.96	12.64+1.95
Peroxide	142373+1.94	8.27+1.97	191668+2.14	9.16+2.13

* Average of two determinations, DEG: Degradation, SD: Standard deviation

A novel reversed phase liquid chromatographic method was developed for the estimation of EMT and TDF in bulk drugs and pharmaceutical formulations. The separation and quantitation of each drug was achieved by injecting 20 µl of standard or sample solution of concentration 60 µg/ml of EMT and 90 µg/ml of TDF into the Inertsil ODS C_{18} (250 mm x 4.6 mm, 5 µm) column, and the components were eluted by passing 0.1% triflouro acetic acid (TFA) buffer and methanol in the ratio 39:61 (v/v) as mobile phase at a flow rate of 1.2 ml/min through the column. The components were detected at a wavelength at 261 nm by using UV detector. The developed method was validated as per ICH guidelines, precision, accuracy, linearity of the proposed method were determined. Limit of detection and limit of quantitation was determined, and the developed method was applied for determination of assay of Truvada. The stability of the drug was examined under different forced degradation conditions such as hydrochloric acid, sodium hydroxide, hydrogen peroxide, thermal and photo light. The developed method was found to be simple, sensitive and selective. The proposed method was applied for the determination of quality of the pharmaceutical formulations and bulk drugs.

CONCLUSION

The developed isocratic RP-HPLC method was found to be simple, rapid, accurate and specific for the determination of Emtricitabine, and Tenofovir Desoproxil Fumerate in tablet dosages. Hence the proposed method can be adopted for the analysis for quality control in any quality control and testing laboratory.

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CONFLICT OF INTERESTS

Declared None

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