Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (17):185-195
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Stability indicating UV-Spectrophotometric method for simultaneous determination of emtricitabine and tenofovir desoproxil fumerate in truvada

Valli Purnima B.[1,2], Santha Kumari M.[2], Ramu G.[3], Vijaya Bhaskara Reddy T.[1] and Ramachandran D.[1*]

[1]Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India
[2]Sir C. R. Reddy College for Women, Eluru, West Godavari, Andhra Pradesh, India
[3]Sir C. R. Reddy College, P. G. Courses, Eluru, West Godavari, Andhra Pradesh, India

---

ABSTRACT

The objective of the present investigation is to develop a stability indicating UV-Spectrophotometric method for the simultaneous estimation of Emtricitabine (EMT) and Tenofovir Desoproxil Fumerate (TDF) in pure and tablet dosage forms. From the observation of the absorption spectra of EMT and TDF it was found that, TDF does not interfere with the measurement of EMT at λ_1=282.4 nm, but EMT absorb appreciably along with TDF at λ_2=258.7 nm. In this case the concentration of EMT is determined directly from the absorbance of the solution at 282.4 nm. The molar extinction coefficient of EMT at λ_1=282.4 nm and λ_2=258.7 nm is determined and found to be $9.7182x10^3$ and $4.5672x10^3$ lt/mol/cm and respectively. Then the absorbance contributed at 258.7 nm by this concentration of EMT is calculated from the previously known molar absorptivity of EMT at 258.7 nm, and this contribution is subtracted from the measured absorbance of the solution at 258.7, yielding the absorbance due to TDF, whose concentration is then calculated in the usual manner. The developed method was validated in terms precision, accuracy, linearity, LOD, LOQ. The system precision and method precision expressed as %RSD were found to be 1.6813&1.747 and 1.762&1.739 for EMT and TDF respectively. The mean percent of recovery in accuracy at 50,100 and 150% spiked levels were found to be 101.81, 100.00 and 100.48 for EMT and 100.74, 100.43 and 100.58 for TDF respectively. The developed method was found to be linear in the limits of 4-24 µg/ml & 6-30 µg/mL for EMT and TDF respectively. Limit of detection and limit of quantitation were found to be 0.755&2.518 and 0.332&1.108 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 99.75±0.291 and 99.8±0.282 f or EMT and TDF respectively. A simple, rapid and economic stability indicating UV-Spectrophotometric method for simultaneous determination of EMT and TDF in bulk and formulations was developed validated. The developed method was found to be precise, accurate, linear, robust and 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: UV-spectrophotometric method, Validation, Stability, Emtricitabine, Tenofovir Desoproxil Fumerate, and Truvada.

INTRODUCTION

Profile of the drugs

The development and usage 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 Emtricitabine (EMT) and Tenofovir disoproxil Fumerate (TDF) has demonstrated significantly greater human immunodeficiency virus (HIV) ribonucleic acid (RNA) suppression compared to the combination of zidovudine and lamivudine. Combining the two drugs in one tablet (Truvada

consists of 200 mg of EMT and 300 mg of TDF equivalent to 245 mg of tenofovir) 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. Emtricitabine, a nucleoside reverse transcriptase inhibitor (NRTI) is chemically known as 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-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. The molecular structures of EMT and TDF were presented in Figure.1 and Figure.2 respectively.

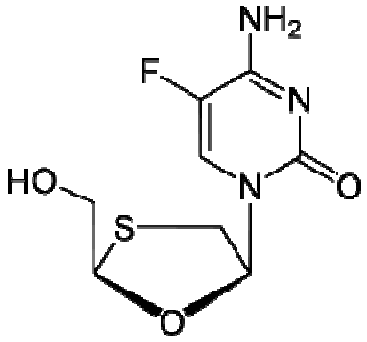

Figure.1: Molecular structure of Emtricitabine (EMT)

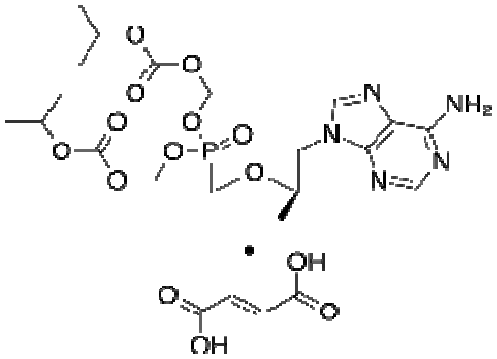

Figure.2: Molecular structure of Tenofovir disoproxil Fumerate (TDF)

Literature review

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. These methods were found to be lack of stability studies. 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 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.

Objective of the investigation

The aim of the present investigation is to determine potency and to test the stability of drug sample under different degradation conditions. The main objectives of the present work are , to develop UV spectrophotometric method by choosing different solvents, validate the developed method as per the ICH guidelines to determine precision, accuracy, sensitivity, linearity, robustness and ruggedness of the developed method, and finally to determine the assay of EMT and TDF simultaneously in pharmaceutical formulations. The developed method was also extended to study the stability of EMT and TDF under different degradation conditions such as acid, base, peroxide, thermal and photo light, and to find out the percent of degradation of the drug moiety present in the sample.

Spectrophotometry

Spectrophotometry is the most accurate method for determining the concentration of substance in solution among other things. The term spectrophotometry means measuring the extent of radiation absorbed by a chemical substance as a function of the wavelength (λ) or frequency of the radiation and the measurements of absorption at a fixed wavelength i.e. wavelength of maximum absorbance ((λ_{max}). In spectrophotometric analysis a source of radiation is used that extends into the ultraviolet region of the spectrum. Spectrophotometry mainly concerned with the following regions of the electromagnetic spectrum: ultraviolet 185 to 400nm; visible 400 to 760 nm and infrared 0.76 to 15µm. When light falls upon a homogeneous medium, a portion of the incident light is reflected, a portion is absorbed within the medium, and the rest is transmitted. Two separate laws governing absorption are usually known as Lambert's law and Beer's law, in combined form they are known as Beer-Lambert law. On combining Lambert's law and Beer's law, we get $I_t=I_0 10^{-acl}$ or $A=\log_{10}(I_0/I_t)=acl$. If c is expressed in mol/L and l in cm then 'a' is given the symbol € and is called the molar absorption coefficient or molar extinction coefficient. Finally the relationship

between absorbance, transmittance and molar extinction coefficient is given by A=€cl=log (1/T) =-log (T). When the value of € is known, the concentration of an unknown solution can be calculated from the formula $c_{unknown}$=A/€l. In double beam spectrophotometers, monochromatic beam of radiation from tungsten or deuterium lamps is divided into two identical beams, one of which passes through the reference cell and the other through the sample cell. The absorption signal produced by the reference cell is automatically subtracted from the absorption signal of sample solution. In these instruments, once the operator set up the conditions, the spectral scan was automatically performed and the absorption spectrum displayed as a pen-and–ink plot on graph paper. A schematic representation of working of double beam spectrophotometer is presented in Figure-3.

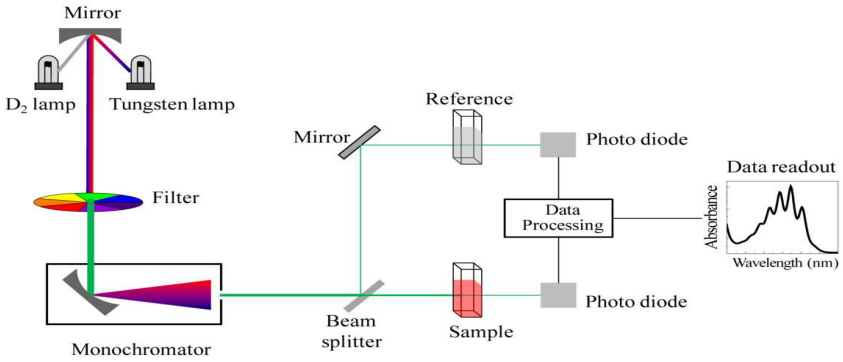

Figure.-3: Schematic diagram of a double beam UV-Visible spectrophotometer

Multicomponent analysis - Theory

Since absorbances are additive, it is possible to determine the concentration of two absorbing constituents X and Y by spectrophotometry if provided there is no chemical reaction between the two solutes. The complexity of the situation depends upon the absorption spectra of X and Y.

No overlap of spectra: The spectra do not overlap, or at least it is possible to find a suitable wavelength (λ_1) where X absorbs and Y does not, and a similar wavelength (λ_2) for measuring Y. The constituents X and Y are simply measured at wavelength (λ_1) and (λ_2)

One-way Overlap spectra: In this case, Y does not interfere with the measurement of X at λ_1, but X does absorb appreciably along with Y at λ_2. In this case the concentration of X is determined directly from the absorbance of the solution at λ_1. Then the absorbance contributed at λ_2 by this concentration of X is calculated from the previously known molar absorptivity of X at λ_2. This contribution is subtracted from the measured absorbance of the solution at λ_2, yielding the absorbance due to Y, whose concentration is then calculated in the usual manner.

Two-way Overlap spectra: When no wavelength can be found where either X or Y absorbs exclusively, it is necessary to solve simultaneous equations in two unknowns. Since the total absorbance is the sum of the contributions of the individual absorbing constituents of the solution, then by measuring the absorbance of the mixture at λ_1 and λ_2, the concentrations of the two components can be calculated by solving these simultaneous equations. $A_1= €_{x1}\ C_x+€_{y1}\ C_y$ and $A_2= €_{x2}\ C_x+€_{y2}\ C_y$. The solution of these simultaneous equations gives $C_x= (€_{y2}A_1-€_{y1}A_2) / (€_{x1}\ €_{y2}\ -€_{y1}\ €_{x2})$ and $C_y= (€_{x1}A_2-€_{x2}A_1) / (€_{x1}\ €_{y2}\ -€_{y1}\ €_{x2})$. The values of $€_x$ and $€_y$ can be deduced from the measurements of pure solutions of X and Y respectively. Where A_1= Measured absorbance at λ_1, A_2= Measured absorbance at λ_2, $€_{x1}$= Molar absorptivity of X at λ_1, $€_{x2}$= Molar absorptivity of X at λ_2, $€_{y1}$= Molar absorptivity of Y at λ_1, $€_{y2}$= Molar absorptivity of Y at λ_2, C_x= Concentration of X and C_y= Concentration of Y

MATERIALS AND METHODS

Instrumentation

Elico-SL159 model, 2nm high resolution, double beam, 1cm length quartz coated optics and wavelength range190-1100nm is used for all the spectral measurements.

Chemicals and Reagents

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

Preparation of solutions

Preparation of working standard solution: An amount of 20 mg of EMT and 30mg and TDF were weighed accurately and transferred into a clean 100 mL dry volumetric flask, dissolved in ethanol, 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.

Preparation of sample solution: Average weight of ten Truvada tablets (200 mg of EMT and 300mg of TDF) was determined, grinded well and an amount of the powder (74.5mg) equivalent to 20 mg of EMT & 30mg TDF was accurately weighed and transferred into a clean 100 mL volumetric flask, dissolved in ethanol, sonicated and used as sample stock solution.

Method Development and Optimization

Choice of solvent

Solvent used for the spectrophotometric determination of a substance must be a good and pure solvent which should not interact with the solute, and must not show significant absorption at the wavelength employed in the determination. For the majority of the analysis of organic compounds and drugs it is necessary to use polar solvents such as water, methanol, ethanol etc. Cut-off wavelengths of some commonly used solvents are 190 nm for water, 207 nm for ethanol and 210 nm for methanol.

Determination of absorption spectra

The development of UV-spectrophotometric method was based on selection of wavelength of maximum absorbance of the selected drugs in different solvents. Since the selected drugs were completely soluble in water, methanol, diethyl ether and ethanol, wavelength of maximum absorbance and stability of the absorbance was determined in these solvents and found that methanol was a good choice for the method development. Exactly 3.0 ml of the standard stock solutions of EMT and TDF were introduced into two separate 50 ml volumetric flasks and diluted up to the mark with water, and final individual concentrations of EMT and TDF were found to be 12 and 18 µg/ml respectively. Reagent blank is also prepared in similar manner without EMT / TDF. Into two cuvettes, reagent blank and standard solutions EMT or TDF were taken; absorption spectra of these solutions were recorded as a function of wavelength against reagent blank. The absorption spectra of EMT and TDF were represented in Figure-4 and Figure-5 respectively.

The wavelength of maximum absorbance of EMT and DTF were found to be 282.4 3 and 258.7 nm respectively. From the observation of the absorption spectrum of EMT, two peaks were found, peak-1 at 239.9 nm and peak-2 at 282.4 nm, but the peak-2 at 282.4 nm has maximum absorbance relative to peak-1 at 239.9 nm, whereas only one peak was found at wavelength 258.7 nm in case of absorption spectrum of TDF. From the observation of two absorption spectra it was found that absorption of TDF at 282.4 nm was significantly zero, therefore TDF did not interact the measurements of EMT at 282.4 nm, and hence measurements of absorbance at wavelength 282.4 nm were used for the method validation for EMT.

EMT has significant absorbance at 258.7 nm; therefore molar extinction coefficient was determined at 258.7 nm for EMT and found to be 4.5672×10^3 lt/mol/cm. Then the absorbance contributed at 258.7 nm by EMT is calculated from the previously known molar absorptivity of EMT at 258.7 nm. This contribution is subtracted from the measured absorbance of the combined solution at 258.7 nm, yielding the absorbance due to TDF, whose concentration is then calculated in the usual manner. The molar extinction coefficient of EMT at λ_1=282.4 nm and $\lambda_{2=}$258.7 nm is determined and found to be 9.7182×10^3 and 4.5672×10^3 lt/mol/cm and respectively. The molar extinction coefficient of TDF at $\lambda_{2=}$258.7 nm is determined and found to be 1.0449×10^4 lt/mol/cm.

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 UV method was validated as per the ICH guidelines.

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. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

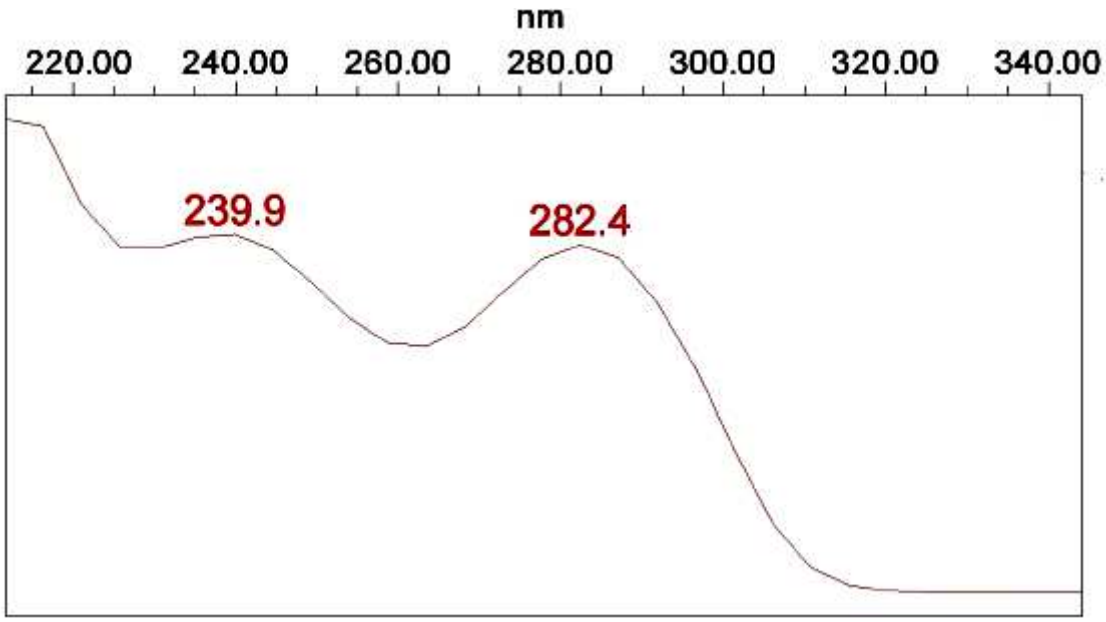

Figure-4: Absorption spectrum of EMT in ethanol

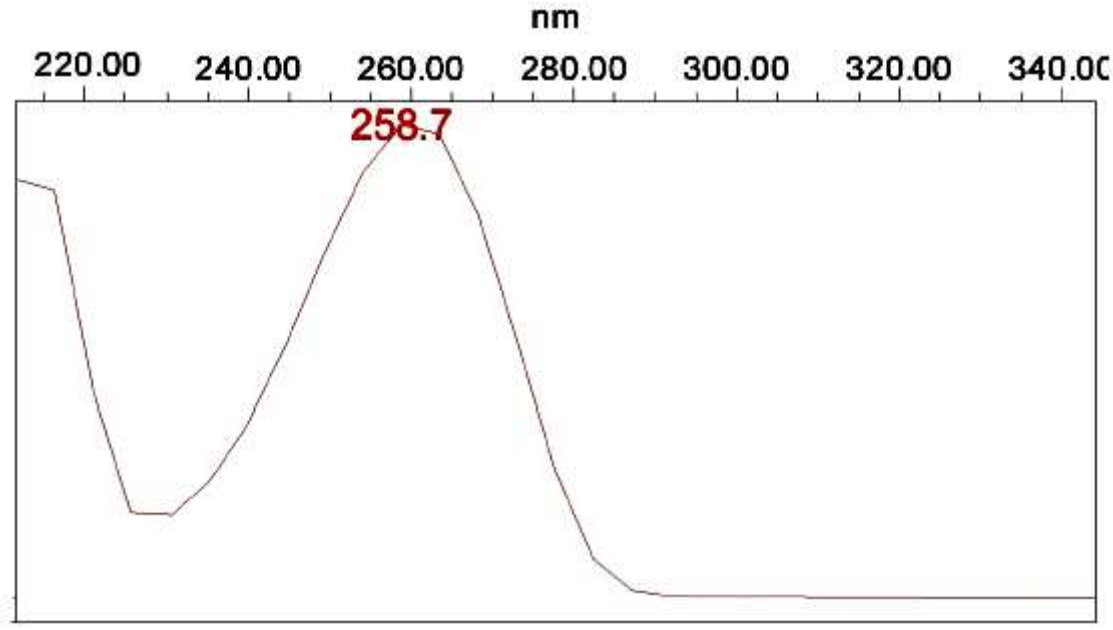

Figure-5: Absorption spectrum of TDF in ethanol

Repeatability: In order to demonstrate repeatability (system precision) of the system, exactly 3.0 mL of standard stock solution was accurately transferred into a 50 mL volumetric flask and diluted up to the mark with diluents, and the concentration of the resulting working standard solution was 12 µg/mL of EMT and 18 µg/mL TDF. In similar manner reagent blank was also prepared omitting drug standards. The standard solution and reagent blank were taken in two clean and transparent cuvettes, and then absorbance of the solution was measured against reagent blank six times at two wavelengths 258.7 and 282.2 nm. Since TDF did not absorb significantly at 282.2 nm, therefore measured absorbance values at this wavelength were considered to evaluate the standard deviation (SD) and percent of relative standard deviation (%RSD) for EMT and found to be 0.0079 and 1.6813. Since EMT has significant absorbance at 258.7 nm, the absorbance contributed by EMT at 258.7 nm was calculated from the previously known molar absorptivity of EMT at 258.7 nm, and this contribution was subtracted from the measured absorbance of the combined solution at 258.7 nm, yielding the absorbance due to TDF. Now standard deviation (SD) and percent of relative standard deviation (%RSD) for TDF were determined and found to be 0.011 and 1.747. In the determination of repeatability (method precision) of the method, working standard mixture solution of EMT and TDF were prepared six times as prescribed procedure; absorbance of these solutions were measured against reagent blank at

258.7 and 282.2 nm. Standard deviation (SD) and percent of relative standard deviation (%RSD) were calculated and found to be 0.0083&0.010 and 1.762&1.739 respectively. The results of system and method precision were presented in Table-1

Table-1: System precision and method precision of the developed method

S.No.	System precision		Method precision	
	Absorbance of EMT at 282.2 nm	Absorbance of TDF at 258.7 nm	Absorbance of EMT at 282.2 nm	Absorbance of TDF at 258.7 nm
1	0.477	0.612	0.472	0.602
2	0.472	0.618	0.471	0.612
3	0.479	0.604	0.474	0.591
4	0.457	0.612	0.456	0.582
5	0.476	0.592	0.479	0.602
6	0.473	0.594	0.478	0.601
Mean	0.4723	0.606	0.4717	0.598
SD	0.0079	0.011	0.0083	0.010
%RSD	1.6813	1.747	1.762	1.739

Intermediate precision: Precision determined within same laboratories but different days, different analysts, different equipment, etc. is known as intermediate precision. In the developed method, intermediate precision was determined on two different days i.e. on the same day (Intraday precision) and on different days (Inter day precision). %RSD values of intraday precision and inter day precision were found to be 1.1558&1.593 and 1.0472&1.854 respectively. The results of intermediate precision were given in Table-2.

Table-2: Intraday precision and inter day precision of the developed method

S.No.	Intraday precision		Inter day precision	
	Absorbance of EMT at 282.2 nm	Absorbance of TDF at 258.7 nm	Absorbance of EMT at 282.2 nm	Absorbance of TDF at 258.7 nm
1	0.479	0.597	0.481	0.577
2	0.468	0.602	0.476	0.602
3	0.469	0.601	0.483	0.577
4	0.472	0.577	0.479	0.579
5	0.467	0.591	0.475	0.587
6	0.463	0.600	0.469	0.572
Mean	0.469	0.595	0.477	0.583
SD	0.0054	0.009	0.0050	0.011
%RSD	1.1558	1.593	1.0472	1.854

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found or it may be defined as the concordance between found value and the true or most probable value. Accuracy should be assessed using a minimum of nine determinations over a minimum of 3 concentration levels covering the specified range (e.g., three concentrations / three replicates each of the total analytical procedure). Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals. In the present investigation, study of accuracy was carried out at 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. Mean percent of recovery was found to in between 100.0-100.81 and the results were presented in Table-3 and Table-4.

Table-3: Results of accuracy (percent recovery) studies of EMT at three spiked levels

Spiked level	Amount added	Amount found	%Recovery	Mean % Recovery
50%	6.0	6.21	103.50	
	6.0	5.986	99.77	101.81
	6.0	6.13	102.17	
100%	12.0	11.97	99.75	
	12.0	11.89	99.08	100.00
	12.0	12.14	101.17	
150%	18.0	18.24	101.33	
	18.0	18.05	100.28	100.48
	18.0	17.97	99.83	
Mean				100.76

Table-4: Results of accuracy (percent recovery) studies of TDF at three spiked levels

Spiked level	Amount added	Amount found	%Recovery	Mean % Recovery
50%	9.0	8.95	99.44	
	9.0	9.14	101.56	100.74
	9.0	9.11	101.22	
100%	18.0	17.89	99.39	
	18.0	18.21	101.17	100.43
	18.0	18.13	100.72	
150%	27.0	27.51	101.89	
	27.0	26.97	99.89	100.58
	27.0	26.99	99.96	
Mean				100.58

Linearity

The linearity between response of the instrument (absorbance) and concentration of EMT and TDF was demonstrated by preparing a series of standards by taking different aliquots (1.0-6.0ml) of standard stock solution into 50 mL standard flasks, made up to the mark. Absorbance values at wavelengths 258.7 and 282.2 nm were measured. Linearity plots were plotted by taking mean response on y-axis against concentration of the EMT and TDF and were represented by Figure-6 and Figure-7 respectively.

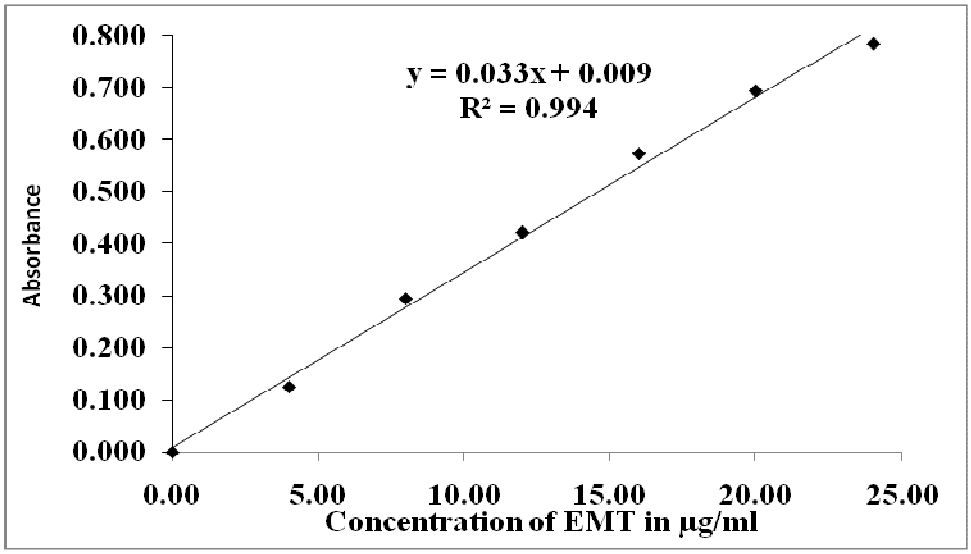

Figure-6: Linearity plot of absorbance against concentration of EMT

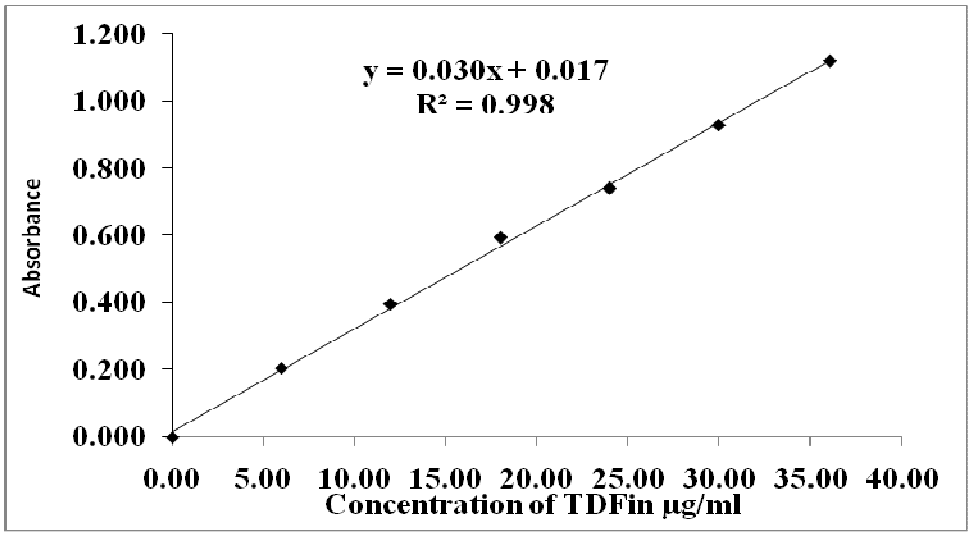

Figure-7: Linearity plot of absorbance against concentration of TDF

Form these plots it was found that EMT and TDF were found to be linear in the range of concentration 4-24 µg/ml and 6-30µg/ml respectively. Slope, intercept and correlation coefficient were determined and correlation coefficient was found to be 0.9940 and 0.9980 for EMT and TDF respectively. The results of linearity were presented in Table-5.

Table-5: Results of linearity studies of EMT and TDF

S.No	Volume in ml	EMT		TDF	
		Concentration µg/ml	Absorbance	Concentration µg/ml	Absorbance
1	1.00	4.00	0.125	6.00	0.206
2	2.00	8.00	0.295	12.00	0.396
3	3.00	12.00	0.421	18.00	0.594
4	4.00	16.00	0.574	24.00	0.74
5	5.00	20.00	0.693	30.00	0.93
6	6.00	24.00	0.784	36.00	1.12
Cor.Coefficient		0.9990		0.998	
Slope		0.016		0.010	
Intercept		0.003		0.014	

Limit of detection and Limit of quantitation

Limit of detection and limit of quantitation were evaluated from standard deviation of the response (σ) and the slope (s). Limit of detection and limit of quantitation may be expressed as LOD=3σ/s and LOD=10 σ/s. LOD and LOQ values were calculated and found to be 0.755&0.332 and 2.518&1.108 µg/ml, and the results were presented in Table-6.

Table-6: Results of LOD and LOQ of EMT and TDF

Constituent	Property	Concentration
EMT	LOD	0.755 µg/ml
	LOQ	2.518 µg/ml
TDF	LOD	0.332 µg/ml
	LOQ	1.108 µg/ml

Assay of pharmaceutical formulations

Standard solution was prepared by weighing precisely 20 mg of EMT and 30 mg of TDF and transferred into two clean 100 mL dry volumetric flask, dissolved in diluent, sonicated and made up to the mark. To prepare sample solution, average weight of ten Truvada tablets (200 mg of EMT and 300mg of TDF) was determined, grinded well and an amount of the powder equivalent to 20 mg of EMT & 30mg TDF was accurately weighed and transferred into a clean 100 mL volumetric flask, dissolved in diluent, sonicated and used as sample stock solution. Then 3.0 mL of the standard / sample solution was accurately measured by using micro burette and transferred into 50 mL volumetric flasks and diluted up to the mark with diluents, and measured absorbance values twice against reagent blank.

The percent of assay was calculated from the measurements of absorbance 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 absorbance of sample, AS = Average absorbance of standard, WS = Weight of working standard taken in mg, WT = Weight of working sample taken in mg, P = Percentage purity of working standard, DT and DS were dilutions of sample and standard respectively. LC = Label claim mg. The percent of assay of EMT and TDF was found to be 99.75 and 98.0 %, and the results were given in Table-7.

Table-7: Assay of EMT and TDF in Truvada formulation

Brand name	Name of the drug	Label claimed	Amount found ± SD	% of assay±%RSD
Truvada	EMT	200	199.5±0.581	99.75±0.291
	TDF	300	299.4±0.845	99.80±0.282

**Average of three determinations*

Stability studies

The aim of this work was to perform the stress degradation studies of EMT and TDF in Truvada samples. 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.

Degradation standard: An amount of 20 mg of EMT and 30mg and TDF were weighed accurately and transferred into a clean 100 mL dry volumetric flask, dissolved in ethanol, 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.

Acid or base hydrolysis: An amount of sample powder equivalent to 20mg 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 0.45µ filter 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.

Hydrogen Peroxide Degradation: An amount of sample powder equivalent to 20mg 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 0.45µ filter into a 100 mL standard flask and made up to the mark.

Thermal Degradation: In the study of thermal decomposition, an amount of sample powder equivalent to 20mg 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 24hrs. 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.

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 20mg 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 ethanol, sonicated for 10min and diluted up to the volume by water and filtered the solution.

Procedure: After that 3.0 mL of standard solution or acid/base/peroxide/thermal and photo light degradation solution was accurately transferred into a 50 mL volumetric flask and diluted up to the mark with water. In similar manner reagent blank was also prepared omitting drug standard or sample. The standard or test solution and reagent blank were taken in two clean and transparent cuvettes, and then absorbance of the solution was measured against reagent blank twice at two wavelengths 258.7 and 282.2 nm. The results of stability were presented in Table-8

Table-8: Results of study of degradation

Degradation condition	% Degradation of EMT	% Degradation of TDF
Acid	11.2	12.4
Base	14.81	14.92
Peroxide	9.97	10.15
Thermal	10.48	13.24
Photo light	9.51	11.54

RESULTS AND DISCUSSION

UV-spectrophotometric method was developed for the estimation of EMT and TDF in bulk drugs and pharmaceutical formulations. 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 were determined, and the developed method was applied for the determination of assay of Truvada.

Absorption spectra of EMT and TDF were recorded by auto scan mode from wavelength 200-400 nm range and were presented in Figure-3 and Figure-4 respectively. From the observation of the absorption spectrum of EMT, two peaks were found, peak-1 at 239.9 nm and peak-2 at 282.4 nm. The molar extinction coefficient values of EMT at λ_1=282.4 nm and λ_2=258.7 nm were determined and found to be 9.7182x10^3 and 4.5672x10^3 lt/mol/cm and respectively. But only one peak was found at wavelength 258.7 nm in case of absorption spectrum of TDF and the molar extinction coefficient of TDF at λ_2=258.7 nm is determined and found to be 1.0449x10^4 lt/mol/cm. From these spectra it was evident that TDF does not interfere with the measurement of EMT at λ_1=282.4 nm, therefore absorption measurements at 282.2 nm were directly used for the method validation and assay analysis. Whereas EMT absorb appreciably along with TDF at λ_2=258.7 nm, then the absorbance contributed at 258.7 nm by EMT is calculated from the previously known molar absorptivity of EMT at 258.7 nm, and this contribution is subtracted from the measured absorbance of the solution at 258.7, yielding the absorbance due to TDF, whose concentration is then calculated in the usual manner.

The developed method was validated as per the guidelines. System precision, method precision and intermediate precision were determined and %RSD was found to be less than 2.0. Table-2 and Table-3 represents the results of precision. Accuracy of the method was determined at three different concentrations was determined and the percent of recovery was found to be within the limits 98-102, and the results were presented in Table-4 and Table-5. The proposed method was found to be linear in the range of concentration 4-24 µg/ml and 6-30µg/ml respectively. Slope,

intercept and correlation coefficient were determined and correlation coefficient was found to be 0.9940 and 0.9980 for EMT and TDF respectively. Figure-6 and Figure-7 represents the linearity plots of EMT and TDF respectively. The results of linearity were presented in Table-5.

The method was found to be sensitive, and LOD and LOQ values were calculated and found to be 0.755&0.332 and 2.518&1.108 µg/ml, and the results were presented in Table-6. The percent of assay of EMT and TDF was found to be 99.75 and 98.0 %, and the results were given in Table-7. A study of forced degradation was carried out to evaluate the stability of the drugs sample. In the present investigation acid, base and peroxide degradation studies and degradation in presence of thermal energy or photo light was carried out, and the percent of degradation was calculated from the peak area of degradation standard and degraded test solution. The percent of degradation of EMT and TDF was found to be in the range of 9.51-14.81and 10.15-14.92 respectively. The results of degradation and stability of drugs were presented in Table-8.

CONCLUSION

The developed UV –spectrophotometric 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.

Acknowledgments
The authors are thankful to Pharma Train, Pharmaceutical training and testing laboratory, Hyderabad, Telangana state, India for providing laboratory facilities and for technical support throughout the analysis.

REFERENCES

[1] Anandakumar K, Kannan K, Vetrichelvan T, *Digest Journal of Nanomaterial and Biostructures*, **2011**, 6(3),1085.
[2] Patel S, Baghel US, Rajesh P, Prabhakar D, Engla G, Nagar PN, *Int J PharmClini Res*, **2009**, 1(1), 28.
[3] Choudhari VP, Ingale KD, Barhate A, Kale AN, Bobade CD, Kuchekar BS, *J Pharm Res* ,**2010**, 9, 11.
[4] Ghorpade SA, Sali MS, Kategaonkar AH, Patel DM, Choudhari VP, Kuchekar BS, *J.Chil Chem Soc*, **2010**, 54, 331.
[5] Vishnu PC, Snehal Ingale, Sacchidanand RG, Dipali DT, Vikram GM, Archana Ambekar, *Pharm Methods*, **2011**, 2(1), 47.
[6] Ahindita P, Aurobinda KM, Amit GS, Dannana K, Swapna M, Sudam CSI, *Int J PharmTech Res* ,**2010**, 3(3),1874.
[7] Ghorpade SA, Sali, Kategaonkar AH, Patel DM, Choudhari VP, Kuchekar BS, *J Chil Chem Soc*, **2010**, 55(1), 115.
[8] Choudhari VP, Ingale S, Gite SR, Tajane DD, Modak VG, Ambekar A, *Pharm Methods*, **2011**,1(2), 47.
[9] Karunakaran A, Kamarajan K, Thangarasu V, *Pharm Sin*, **2010**, 1(2),52.
[10] Sharma R, Gupta P, *Eurasian J Anal Chem* ,**2009**, 4(3), 276.
[11] Pranitha D, Vanitha C, Francies P,*J Pharm Res*, **2010**, 5(8), 4600.
[12] Raju NA, Begum S, *Res J Pharm Technol*, **2008**, 1(1), 522.
[13] Devrukhakar PS, Borkar R, Shastri N, Surendranath KV, *ISRN Chromatogr*, **2013**, 8 pages.
[14] Rezk NL, Crutchley RD, Kashuba AD, *J Chromatogr B* ,**2005**,822, 201.
[15] Nirogi R, Bhyrapuneni G, Kandikere V, Mudigonda K, Komarneni P, Aleti R, et al.,*J Biomed Chromatogr* ,**2009**, 23, 371.
[16] Gomes NA, Vaidya VV, Pudage A, Joshi SS, Parekh SA, *J Pharm Biomed Anal*,**2008**,48(3), 918.
[17] Delahunty T, Bushman L, Robbins B, Fletcher CV, *J Chromatogr* B, **2009**, 877(20-21), 1907.
[18] Avolio AD, Sciandra M, Siccardi M et al., *J Chromatogr Sci*, **2008**, 46(6), 524.
[19] Yadav M, Singhal P, Goswami S, Pande UC, Sanyal M, and Shrivastav PS, *J Chromatogr Sci* ,**2010**, 48(9), 704.
[20] Joshi M, Nikalje AP, Shahed M, Dehghan M,*Indian J Pharm Sci* **2009**, 71(1), 95.
[21] Kavitha KY, Geetha G, Hariprasad R, Venkatnarayanan R, Kaviarasu M, *Int Res J Pharm*, **2013**, 4(1),150.
[22] Nagaraju PT, Channabasavaraj KP, Shantha Kumar PT, *Int J ChemTech Res*,**2011**, 3(1), 23.
[23] Droste JAH, Aarnoutse RE, Burger DM, *J Liq Chromatogr Relat Technol* ,**2007**, 30(10),2769.
[24] Ashenafi D, Verbeek A, Hoogmartens J, Adams E, *J Sep Sci*, **2009**, 32 (11), 1823.
[25] Seshachalam U, Haribabu B, Chandrasekhar KB, *J Sep Sci* **2007**, 30(7), 999.
[26] Pendela M, Mamade DA, Hoogmartens J, Van Schepdael A, Adams E, *Talanta* **2010**, 82(1),125.
[27] Atul HB Charashila Sajai JS, *Pak J Pharm Sci*, **2009**, 22 (1), 27.
[28] Pratap Reddy J and Chakravarthy IE, *IOSR J Appl Chem* ,**2012**, 1(2), 29.

[29] Rani TS, Sujatha K, Chitra K et al., *Research & Review: J Pharm Anal*, **2012**, 1(1), 9.
[30] Onah JO, Ajima U, *Int J Drug Dev Res* ,**2011**, 3(1), 199.
[31] Kandagal PB, Manjunatha DH, Seetharamappa J, Kalanur SS, *Anal Lett* **2008**, 41 (4), 561.
[32] Jullien V, Tréluyer JM, Pons G, Rey E. *J Chromatogr B* **2003**,785(2), 377.
[33] Sparidans RW, Crommentuyn KML, Schellens JHM, Beijnen J, *J Chromatogr B: Anal Technol Biomed Life* Sci ,**2003**, 791(1-2), 227.
[34] Bhavsar S, Patel BN, Patel CN, *Pharm Methods* ,**2012**, 3(2), 73.
[35] Barkil ME, Gagnieu M, Guitton J, *J Chromatogr B* ,**2007**, 54,192.
[36] Sentenac S, Fernandez C, Thuillier A, Lechat P, Aymard G, *J Chromatogr B* ,**2003**, 793,317.
[37] Delahunty T, Bushman L, Fletcher CV, *J Chromatogr B*, **2006**, 830 (1), 6.