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Research Article

**APPLICATIONS OF SIMULTANEOUS EQUATION METHOD
FOR THE DETERMINATION OF EMTRICITABINE AND
TENOFIVIR DISOPROXIL FUMARATE TO DISSOLUTION
STUDIES IN MARKETED TABLETS****Rajendra Mogal***, Madhavi shinde¹, Ashwini Shelke¹, Rahul Sable², Anil Jadhav³

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¹ Department of Pharmaceutical Chemistry, Sandip Institute of Pharmaceutical Sciences, Nashik, India-422213² Department of Pharmacology, Sandip Institute of Pharmaceutical Sciences, Nashik, India-422213³ Department of Pharmacognosy, Sandip Institute of Pharmaceutical Sciences, Nashik, India-422213**Abstract:**

The main objective of present work was to develop simple spectrophotometric multicomponent method of analysis i. e. simultaneous equation method. Tenofovir disoproxil fumarate & Emtricitabine was first approved in US in July 2003 and is indicated for adults aged > or =18 years. This method is used for dissolution study of Tenofovir disoproxil fumarate and Emtricitabine in combined tablet dosage form. As per USFDA guidelines, dissolution study was carried out in 900 mL 0.01 N HCl. Dissolution study was carried out using a USP Type 2 (Paddle) apparatus at a stirring rate of 50 rpm for 45 minutes as per USFDA guidelines. Tenofovir disoproxil fumarate and Emtricitabine showed 258 and 286 nm as λ_{max} in 0.01N HCl respectively. The drug release from tablet was evaluated by developed spectroscopic methods i. e. simultaneous equation method. At the end of 45 minutes, % Cumulative drug release of Emtricitabine & Tenofovir disoproxil fumarate was found to be 104.9 & 98.8 % based on developed method. The developed method was found to be simple, rapid, less tedious & economical compare to other reported methods in literature. The developed method can be a good alternative method for quality control testing of Emtricitabine & Tenofovir disoproxil fumarate in combined dosage form.

Keywords: Tenofovir disoproxil fumarate, Emtricitabine, Simultaneous Equation method, Dissolution, USFDA.**Corresponding author:****Rajendra Tryambak Mogal,**

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INTRODUCTION:

Emtricitabine (EMT) (4-amino-5-fluoro-1-[(2*S*,5*R*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one), an analogue of cytidine, Fig. No. 1 and Tenofovir disoproxil fumarate (TDF) (([[(2*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-yl]oxy)methyl]phosphonic acid), are nucleoside reverse transcriptase inhibitor (NRTI) that are used for the treatment of HIV infection fig. no. 2 [1] TDF is a bioavailable prodrug of Tenofovir, a potent nucleotide analogue reverse-transcriptase inhibitor with activity against human immunodeficiency virus (HIV) and hepatitis B virus [2].

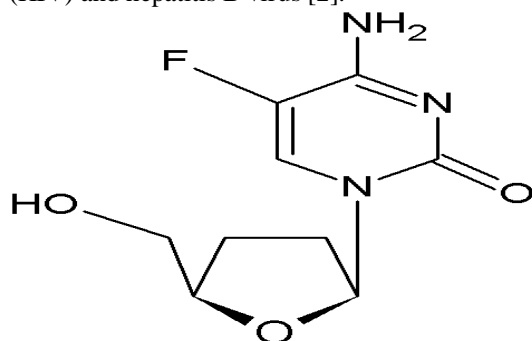


Fig.1: Chemical Structure of Emtricitabine

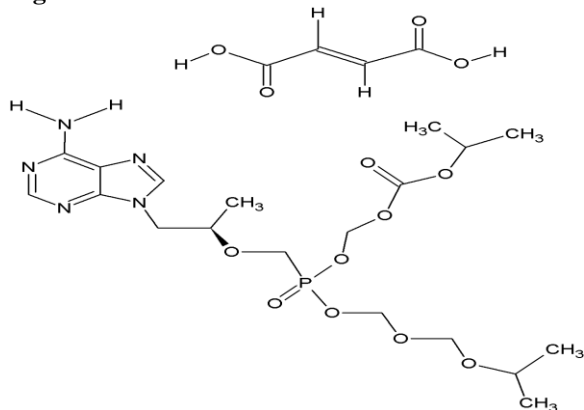


Fig.2: Chemical Structure of Tenofovir disoproxil fumarate

Drug dissolution (or release) testing is an analytical technique used to assess release profiles of drugs in pharmaceutical products, generally for solid oral products such as tablets and capsules[3]. Dissolution test gains its significance from the fact that if a drug from a product is to produce its effect; it must be released from the product and should generally be dissolved in the fluids of the gastrointestinal (GI) tract from which it will subsequently get absorbed easily. Thus, a drug dissolution test may be considered as an indicator of potential drug release and absorption characteristics of a product in humans as well as in animals. Therefore, a dissolution test is

often considered a surrogate for the assessment of availability of drugs in the body, generally termed bioavailability[4].

The TDF and EMT combined tablet is official in Indian pharmacopoeia 2010. The method given for dissolution is by High Performance Liquid Chromatography (HPLC) which is tedious, costly and time consuming [5]. A literature survey revealed that HPLC[6], spectrophotometric techniques by simultaneous[7]& Area Under Curve [8]& High Performance Thin Layer Chromatography (HPTLC)[9] have been reported for the simultaneous determination of EMT, and TDF in pure drug, pharmaceutical dosage forms and biological samples. The reported methods are quite tedious, time consuming & complicated. Hence, the authors have attempted to develop a simple method for the simultaneous estimation of these drugs in tablet dosage forms and its application for dissolution study.

MATERIAL AND METHODS:**Chemicals and Reagents:**

Pharmaceutical grade of Emtricitabine and Tenofovir disoproxil fumarate were kindly supplied as gift sample by Lupin Pharmaceuticals Ltd. All the chemicals were used of analytical grades. The marketed formulations were purchased from local market were obtained from retail pharmacist.

Instruments:

UV-Visible Spectrophotometer (Agilent-Cary 60) with cuvette cells of 1 cm light path was used for the measurement of absorbance. The USP dissolution apparatus (Electrolab ETC-11L) was used for dissolution study. Electronic Balance (Schimadzu BL-220H) was used for weighing the samples. Class 'A' volumetric glassware's were used. Ultra-Sonicator (Wensar-WUC-2L) was used for sonication purpose.

Procedure:**Selection of Dissolution Medium:**

The solvent i.e. 0.01N HCl was selected as Dissolution medium for estimation of Emtricitabine and Tenofovir Disoproxil fumarate in tablet dosage form as per US FDA guidelines (USFDA 2007).

Preparation of standard stock solution:

An accurately weighed quantity of about 20 mg Emtricitabine and Tenofovir Disoproxil fumarate was taken in a 100 mL volumetric flask and was dissolved in 0.01N HCl with sonication. The volume was made up to mark with Solvent to get the concentration of 200 ppm.

Preparation of Working Standard Solution of Emtricitabine and Tenofovir Disoproxil fumarate:

The aliquot portion of standard stock solution of Emtricitabine and Tenofovir Disoproxil fumarate was diluted appropriately with 0.01N HCl obtaining

concentration 10 µg/mL. Solutions were taken in 1 cm cell and scanned in the range 200 nm to 400 nm and spectrum were recorded as showed in Fig. 3 & Fig. 4 for EMT & TDF respectively.

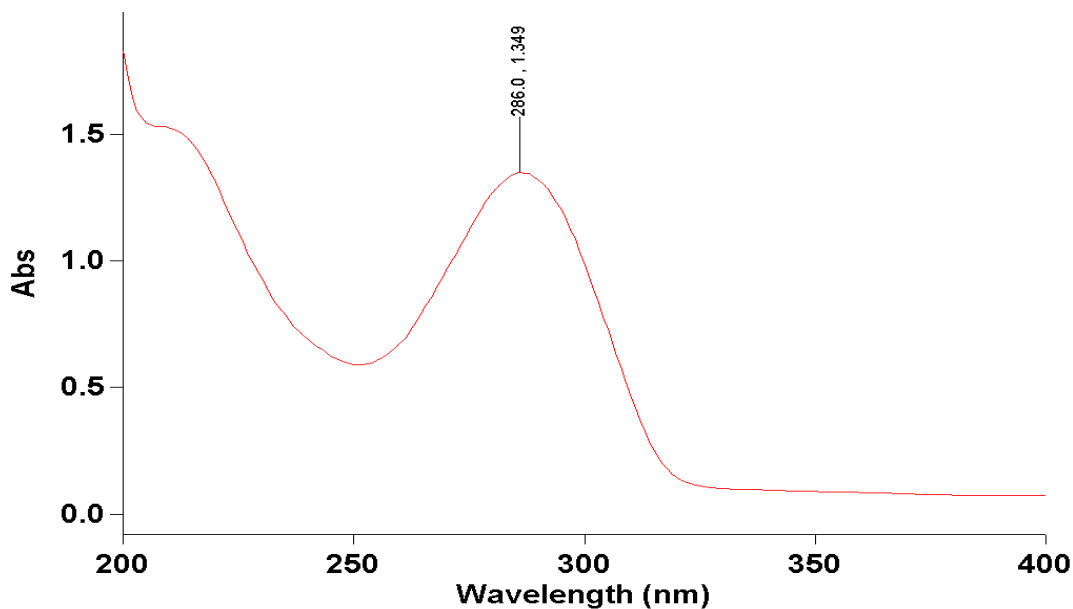


Fig. 3: UV spectra of Emtricitabine in 0.01 N HCl

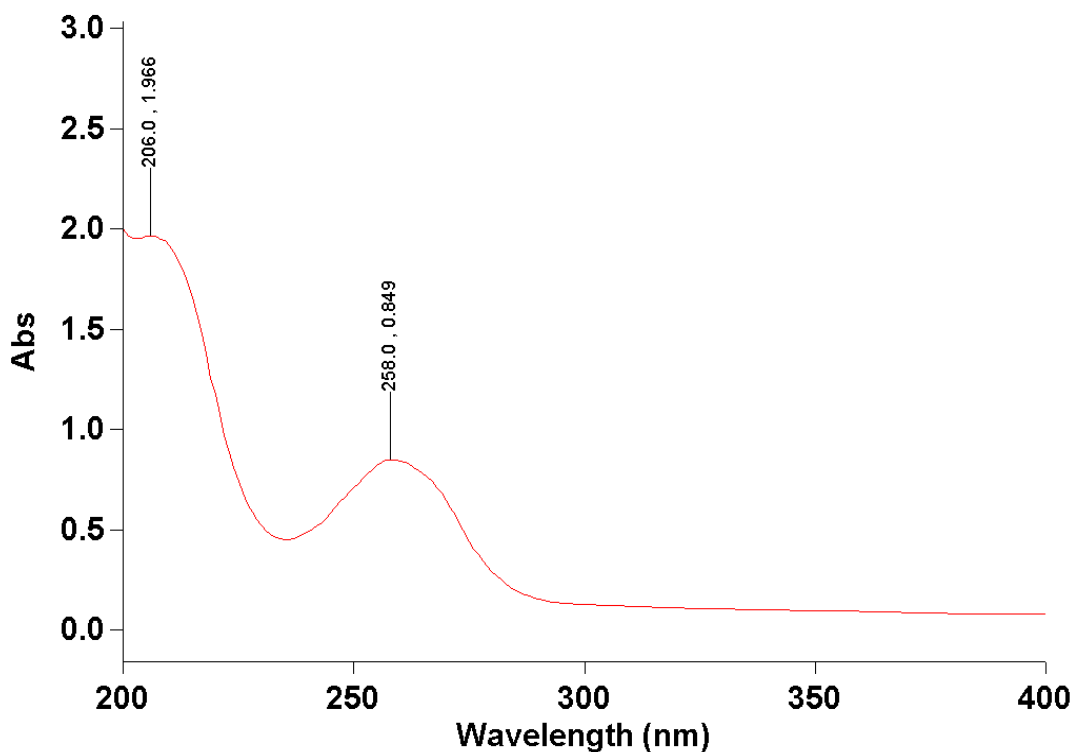


Fig. 4: UV spectra of Tenofovir disoproxil fumarate in 0.01 N HCl

Method I: Simultaneous equation method

From the spectra of pure drugs, two wavelengths, 258 nm (λ_{\max} of TDF) and 286 nm (λ_{\max} of EMT) were selected for the formation of simultaneous equation. The A (1%, 1 cm) was determined at both the wavelengths selected for each drug. Samples containing two absorbing species EMT and TDF (X & Y) & both absorb at the λ_{\max} of the other. So the absorbance of each drug at both wavelengths λ_1 & λ_2 were measured respectively. Both the drugs were determined by simultaneous method (Vierodt's method). The absorptivity of EMT (X) at λ_1 (286) and λ_2 (258) is ax_1 and ax_2 , respectively. The absorptivity of TDF (Y) at λ_1 (286) and λ_2 (258) is ay_1 and ay_2 , respectively [10].

The absorptivity of each solution was calculated by using the following formula:

Absorptivity = Absorbance / concentration (g/100 mL)

The absorbance of the sample (formulation) at λ_1 (286) and λ_2 (258) is A_1 and A_2 respectively.

The total absorbance of the mixture is equal to the sum of individual absorbance of X and Y.

$$A_1 = ax_1bCx + ay_1bCy \text{ ----- (1)}$$

$$A_2 = ax_2bCx + ay_2bCy \text{ ----- (2)}$$

$$Cx = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1ay_2} \text{ ----- (3)}$$

$$Cy = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2} \text{ ----- (4)}$$

Cx: Concentration of EMT

Cy: Concentration of TDF

Dissolution study:

Dissolution testing was carried out using paddle (USP Apparatus 2) at 50 rpm using 900 mL of 0.01 N HCl as dissolution medium for 45 min at 37 ± 0.5 °C. 5.0 mL sample aliquots were withdrawn at intervals of 5, 10, 15, 30, and 45 min and replaced with an equal volume of the fresh medium to maintain sink condition. After the end of each test time, sample aliquots were filtered, diluted with 0.01N HCl and quantified. The analysis of dissolution samples was

performed using simultaneous equation method. The proposed method was employed to calculate the percentage release at respective time interval of dissolution profile. The dissolution parameters set is as per table no. 1.[11,12]

Table No. 1: Dissolution parameters for Emtricitabine and Tenofovir disoproxil fumarate

Dissolution parameters	
Dissolution apparatus	USP apparatus II (Paddle)
Dissolution medium	0.01N HCl
Volume of dissolution medium (mL)	900
Speed of paddle rotation (rpm)	50
Temperature (°C)	37 ± 0.5
Sampling time (minutes)	5, 10, 15, 20, 30, & 45

RESULT AND DISCUSSION:

Simultaneous equation:

Two wavelengths, 258 nm (λ_{\max} of TDF) and 286nm (λ_{\max} of EMT) were found in 0.01N HCl as per spectral analysis. The absorptivity of EMT (X) at λ_1 and λ_2 is ax_1 (130.85) and ax_2 (361.70) respectively. The absorptivity of TDF (Y) at λ_1 and λ_2 is ay_1 (230.25) and ay_2 (18.25) respectively. After solving equation no. 3 & 4, the concentrations of EMT & TDF were calculated.

Dissolution study

The dissolution study was carried out using 0.01N HCl as dissolution medium at stated conditions. The absorbance at different time points were measured & respective concentrations of EMT and TDF were calculated by simultaneous equation method. After that % CDR for both drugs were calculated & obtained results shown in Table no. 2.

Table 2: % CDR at different time points

Time Points (Min)	% CDR of Emtricitabine	% CDR of Tenofovir disoproxil Fumarate
5	56.16	51.92
10	88.82	82.49
15	96.05	92.3
30	102.05	92.83
45	104.9	98.8

The graph of % CDR against Time point was plotted as shown in Fig. no. 5. At the end of 45 min, 104.9 EMT & 98.8 TDF percent drug release was found.

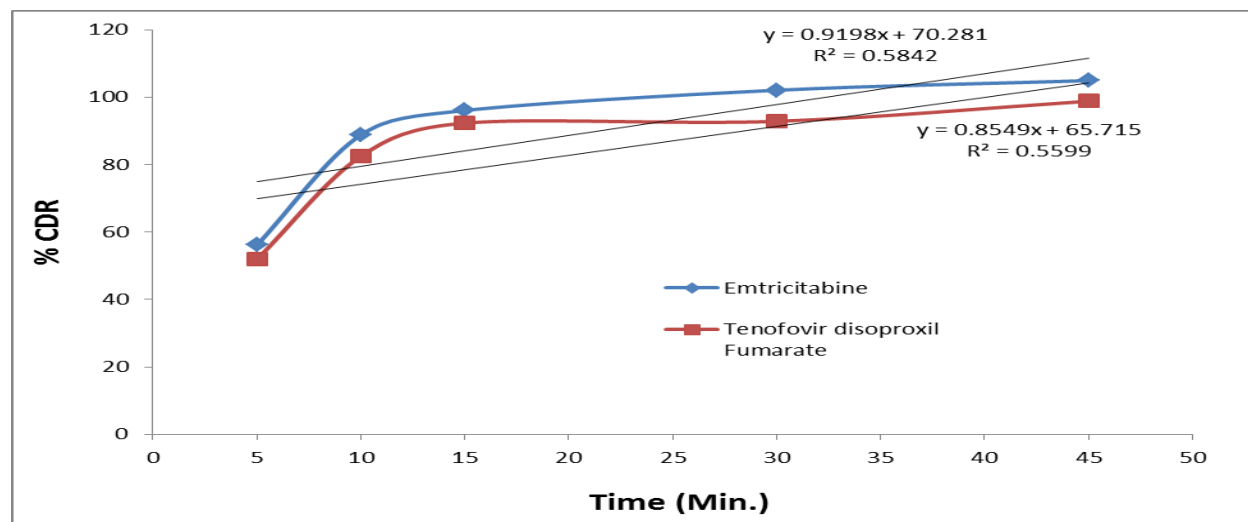


Fig.5: A plot of % CDR against time points (Dissolution Profile) for Emtricitabine & Tenofovir Disoproxil Fumarate

CONCLUSION:

The developed method offers numerous advantages over chromatographic (HPLC) analysis like comparatively simple, rapid, reproducible, less time consuming, & economical method for simultaneous estimation of Emtricitabine and Tenofovir disoproxil fumarate. Hence the proposed method can be used for routine analysis of both the drugs in combined dosage form. The method could be satisfactorily employed in dissolution studies to determine the percentage drug release.

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