RESEARCH ARTICLE

VALIDATED FIRST-ORDER DERIVATIVE SPECTROPHOTOMETRY FOR SIMULTANEOUS DETERMINATION OF EMTRICITABINE AND TENOFOVIR DISOPROXIL FUMARATE IN PHARMACEUTICAL DOSAGE FORM

*Sunitha. P. G[1], Kaliappan.Ilango[2],

[1]Department of Pharmaceutical Chemistry,College of Pharmacy, Madras Medical College, Chennai-600 003

[2]Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRM University, Kattankulathur-603 203

Corresponding Author's E-mail: sunitha.srm@gmail.com

ABSTRACT

A simple, precise, accurate, and economical spectrophotometric method has been developed for simultaneous estimation of emtricitabine(EMB) and tenofovir(TEN) by employing first-order derivative spectrophotometric method. The first derivative spectrum is a plot of the rate of change of absorbance with wavelength against wavelength (dA /dλ versus λ). It is characterized by a maximum, minimum and a cross- over point at the λmax of the absorption band. The linearity was established over the concentration range of 6-16 μg/mL and 9-24 μg/mL for EMB and TEN with correlation coefficients 0.9994and 0.9992, respectively. The mean percentage recoveries were found to be 99.99% for EMB and 100.43%for TEN. The proposed method has been validated as per ICH guidelines and successfully applied for the simultaneous estimation of EMB and TEN in combined tablet dosage form.

Keywords: emtricitabine, tenofovir, spectrophotometry

INTRODUCTION:

Emtricitabine and Tenofovir are antiretroviral drugs used for the treatment of HIV[1]. Forstavir - EM is the combination of the two drugs containing 200mg of EMB and 300mg of TEN. Emtricitabine is chemically 4-Amino-5-fluoro-1-[2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-pyrimidin-2-one[2,3].It is a nucleoside reverse transcriptase inhibitor. The structure of EMB is given in Figure 1.
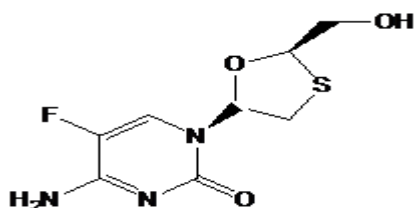

Figure 1: Chemical structure of EMB

Chemically Tenofovir is 1-(6-Aminopurin-9-yl)-propan-2-yl-oxymethylphosphonic acid[4]. It is a nucleotide analogue reverse transcriptase inhibitor. The structure of TEN is given in Figure 2.
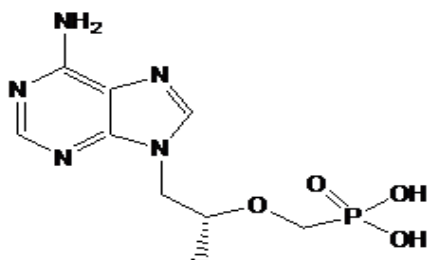

Figure 2: Chemical structure of TEN

Extensive literature survey revealed that only LC-MS/MS[5]and RP-HPLC[6] methods for the determination of EMB and TEN in human plasma, RP-HPLC[7] for determination of TEN in plasma, LC/MS/MS for determination of plasma TEN concentrations[8], LC-MS method for determination of plasma TEN concentrations[9] and HPLC with fluorimetric detection for determination of EMB in human plasma[10] have been reported so far.However, to the best of our knowledge, no first derivative spectrophotometric method is published for the simultaneous determination of EMB and TEN in tablet dosage form. The present work describes the development of a simple, precise, accurate, and reproducible spectrophotometric method for the simultaneous estimation of EMB and TEN in combined dosage form. The developed method was validated in accordance with ICH guideline and successfully employed for the assay of EMB and TEN in combined tablet dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents

Working standards of EMB and TEN were provided as gift samples by Ranbaxy Laboratories, India. Forstavir - EM tablets were purchased from a local pharmacy.

Instruments

Shimadzu UV-Vis Spectrophotometer-1650, 1 cm matched quartz cells, and UV probe 2.34 software was used. Calibrated analytical balance Shimadzu BP211D (Sartorius Gottingen AG, Germany) was used for weighing purpose. All statistical calculations were carried out using Microsoft Excel 2007 analytical tool.

Preparation of Standard Stock Solutions

Accurately weighed 25 mg of EMB and TEN standards was transferred to a separate 25 mL volumetric flask and dissolved in 10 mL distilled water. The flasks were shaken and volume was made up to the mark with distilled water. These solutions are 1000 µg/mL EMB and 1000 µg/mL TEN respectively.

Selection of Analytical Wavelength

Working standard solutions of 6-16 µg/mL of EMB and 9-24 µg/mL of TEN were prepared in distilled water by appropriate dilution, and the spectrum was recorded between 200 and 400 nm, and all zero-order spectrums (D^0) were converted to first derivative spectrums (D^1). The overlain first derivative spectrums of LCT and PHE at different concentrations were recorded.

Method Validation[11]

The proposed method was validated in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and reproducibility.

Linearity

Appropriate aiquots from EMB and TEN standard stock solution were transferred to volumetric flasks of 10 mL capacity. The volume was adjusted to the mark with distilled water to give working standard solutions containing 6-16 µg/mL for EMB and 9-24 µg/mL for TEN respectively. All D^1 spectra were recorded using the above spectrophotometric condition. D^1 absorbance at 257 nm and 282 nm was recorded for EMB and TEN, respectively. Calibration curves were constructed by plotting average absorbance versus concentrations for both drugs. Straight line equations were obtained from these calibration curves.

Accuracy

Accuracy was assessed by the determination of the recovery of the method by addition of standard drug to the prequantified sample preparation. Each concentration was analyzed 3 times, and average recoveries were measured.

Precision

The repeatability was evaluated by assaying 6 times the sample solution prepared for assay determination. The results are reported in terms of % RSD.

Detection Limit and Quantitation Limit

In the present study, the LOD and LOQ were calculated by the use of standard deviation of the response and the slope of the calibration curve.

Reproducibility

The absorbance readings were measured in a different laboratory, using another spectrophotometer by another analyst, and the values obtained were evaluated to verify their reproducibility.

Determination of EMB and TEN in Their Combined Tablet Dosage Form

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed powder equivalent to 300mg of TEN and 200mg of EMB was transferred to a 100mL volumetric flask and dissolved in about 75mL of methanol. The volume was then made upto the mark with methanol. The solution was sonicated for about 15 minutes and filtered through Whatmann filter paper(0.45 µ) and diluted to prepare the concentration of 9-24µg/mL TEN and 6-16µg/mL of EMB. The resulting solution was analyzed by the proposed method. The quantitation was carried out by keeping these values to the straight line equation of the calibration curve.

RESULTS AND DISCUSSION:

First-order derivative spectrophotometric method was developed for determination of EMB and TEN. Figure 3 shows overlain D^1 spectra of EMB and TEN at different concentrations.
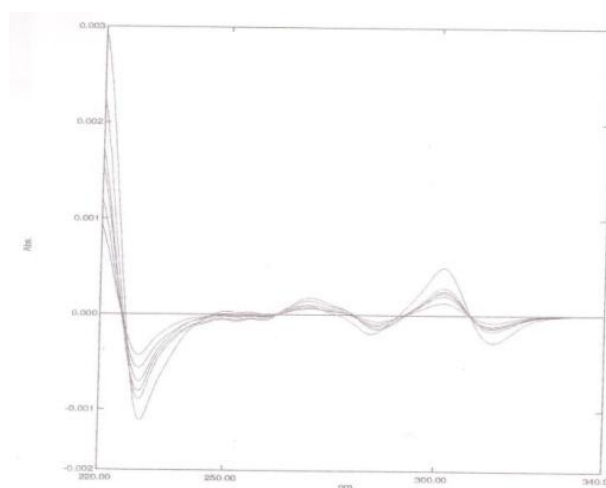

Figure 3: Overlain D^1 spectra of EMB and TEN

The optical characteristics such as absorption maxima, Beer's law limits and the regression characteristics like slope(b),intercept(c), correlation co-efficient(r), percent relative standard deviation(% RSD) and standard error(SE) were calculated and the results are summarized in Table-1. The results of sample analysis showed that the drug determined by the proposed methods was in good agreement with the label claim proving the accuracy of the proposed methods.

To study the accuracy and reproducibility of the proposed methods, recovery experiments were carried out by adding a known amount of drug to preanalysed sample and the percentage recovery was calculated. The results are furnished in Table-2. The results indicate that there is no interference of other ingredients present in the formulations. Thus, the proposed method is simple, sensitive, economical, accurate and reproducible and useful for the simultaneous estimation of EMB and TEN in combined tablet dosage form.

Table 1: Optical and Statistical parameters

Parameters	EMB	TEN
Absorption maximum/ Wavelength range(µg/mL)	200-400	200-400
Linearity Range(µg/mL)	6-16	9-24
Correlation coefficient	0.9994	0.9992
% RSD	0.0460	0.0307
Standard Error(SE)	0.0424	0.0122
Regression Equation y=mx+c	0.9089x+0.1414	0.5065x+0.1223
Intercept (c)	0.1414	0.1223
Slope (m)	0.9089	0.5065
LOD(µg/mL)	0.1180	0.1212
LOQ(µg/mL)	0.3601	0.3672

Table 2: Assay and recovery of EMB and TEN

Method	Labelled amount(mg)	Amount obtained(mg)*	Percentage recovery**
EMB	200	199.75	99.99%
TEN	300	299.45	100.43%

Average of six determinations   **Average of three determinations*

CONCLUSIONS:

The proposed first-order derivative method provides simple, specific, precise, and accurate quantitative analysis for simultaneous determination of EMB and TEN in combined tablet dosage form. The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and reproducibility. The proposed method can be used for routine analysis of EMB and TEN in combined dosage form.

REFERENCES:

1. Martindale. The Complete Drug Reference, 35th Edition, **2007**, Pharmaceutical Press London, 811.
2. Indian Pharmacopoeia 2010. Volume II. Government of India, Ministry of Health and Family Welfare. The Indian Pharmacopoeia Commission.Ghaziabad: 1272-1273.
3. The Merck Index. An Encyclopedia of Chemicals, Drugs and Biological, 13th Edition. Merck Research Laboratories: 630, 1631-1632.
4. Indian Pharmacopoeia 2010.Volume III. Government of India, Ministry of Health and Family Welfare.The Indian Pharmacopoeia Commission. Ghaziabad: 2188-2193.
5. Gomes N.A, Vaidya V.V, Pudage A, Joshi S.S &Parakh S.A. 2008. *J. Pharm. Biomed. Anal*, 48(3): 918-926.
6. Baietto L, D'Avolio A, Sciandra M &Siccardi M. 2008. *J. Chromatogr. Sci* 46 (6): 524-528
7. Sentenac S, Fernandez C, Thrillers A, Lachat P &Aymard G. 2003. *J. Chromatogr. B*, 793(2): 317-324.
8. Delahunty Tom, Bushman Lane, et.al.2006. *J. Chromatogr. B*830 : 6-12
9. Takahashi Masaaki, Kudaka Yuichi et.al.2007. *Biol. Pharm. Bul*30 : 1784-1786.
10. Droste A. H, Aarnoutse R. E et.al. 2007. *J. Liq. Chromatogr. Rel. Tech* 30: 2769 – 2778.
11. ICH Harmonized Tripartite Guidelines, Validation of Analytical Procedures: Text and Methodology, Q2(R1), Geneva, Switzerland, 2005.