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Der Pharmacia Sinica, 2011, 2 (5):163-168

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Simultaneous RP-HPLC method for the estimation of Emitricitabine and Tenofovir Disoproxil Fumarate in Pharmaceutical dosage forms

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ABSTRACT

An accurate, sensitive high performance liquid chromatography method for the simultaneous determination of Tenofovir disoproxil Fumarate (TNF) and Emitricitabine (EMT) in dosage form was developed and validated as per ICH Guidelines. The separation and quantification were achieved on Agilent C_{18} column using Methanol – Phosphate buffer7.0 (65:35 v/v) as mobile phase at a flow rate of 1 ml / min with UV detection at 260nm for both analytes. Emitricitabine and Tenofovir disoproxil Fumarate were eluted at 4.7 min and 5.8 min with asymmetry of 1.27 and 1.22 respectively. Theoretical plates were found to be more than 9000 and the linearity ranges were 5- 70 µg / ml and 5 -70 µg/ml, respectively for TNF and EMT. The intra and inter – day RSD's were in the range of 0.6 – 0.9% and 1.2 – 1.6% for TNF and EMT. The LOD and LOQ values for EMT and TNF were found to be 0.054 µg/ml, 0.165 µg/ml and 0.063µg/ml,0.192µg/ml respectively. The recovery studies were found to be 98.7±0.6 %, 100.5±1.1, 100.3±0.5 and 99.8±1.1, 101.2±0.8, 101.2 ±0.3 for LQC, MQC, HQC samples of TNF and EMT, respectively. The assay values were in the range of 98 – 102 %.

Keywords: Emitricitabine, Tenofovir disoproxil Fumarate, Simultaneous, RP-HPLC.

INTRODUCTION

Emtricitabine (EMT) is a nucleoside reverse transcriptase inhibitor (NRTIs), chemically named as 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3–oxathiolan–5-yl]cytosine. The (-) enantiomer of thio analog of cytidine which differs from other cytidine analogs, in which it has fluorine atom at 5th position. EMT is an antiviral agent used for the prevention of perinatal HIV-1 reverse transcriptase [1] and also active against Hepatitis B virus [2, 3]. Emtricitabine, Tenofovir disoproxil Fumarate combinations represents the first once-daily, antiretroviral regimen in the present remedy. Tenofovir is also a NRTIs, anti-retroviral drug and chemically, called as 1-(6-aminopurin-9-yl) propan-2-yloxymethylphosphoric acid. Literature survey reveals few Chromatographic methods were reported for Emitricitabine along with other antiretroviral dugs.

Some HPLC methods has been reported for the estimation of Emtricitabine, along with Tenofovir disoproxil fumerate and Efavirenz in pharmaceutical dosage form [4, 5, 6].

Recently, Estimation of EMT by HPLC with Fluorometric detection [7] in human plasma and Stability indicating liquid chromatographic [8] methods were reported. LC-MS/MS [9] method was also been reported for simultaneous estimation of EMT and TEN in human plasma. HPTLC [10, 11] method was reported for simultaneous estimation of EMT and TNF in pharmaceutical formulation. Hence, the present study was to develop simple, rapid, precise and accurate RP – HPLC method for the simultaneous estimation of both EMT and TNF in tablet dosage form.

MATERIALS AND METHODS

2.1 Apparatus

RP-HPLC determination of both TNF and EMT was performed with a Agilent LC with VWD UV 2075 – visible detector, and a Rheodyne injection capacity 20 µL. The monitoring software was EZ – Chrom Elite. The equipment was controlled by a PC workstation. Compounds were separated on Agilent (4.6 x 250 mm, 5 microns) C18 column under reversed-phase adsorption chromatographic conditions. The work was carried out in an air-conditioned room maintained at temperature 25 ± 2°C. The flow rate was 1 ml/ min and the analytes were monitored at 260 nm.

2.2. Chemicals and Reagents

Working Standards of pharmaceutical grade TNF and *EMT* were obtained as gift samples from Hetero drugs Pvt. Ltd, Hyderabad, India. The tablet dosage forms were procured from the local community Pharmacy. All the chemicals and reagents used were of HPLC grade and purchased from Merck Ltd, Mumbai, India.

2.3. Mobile phase

The mobile phase consisting of Methonol : Phosphate buffer pH 7.0 (65:35 v/v) prepared and degassed with Ultrasonicator and filtered through 0.2 micron membrane filter, whenever necessary.

2.4. Standard stock solution and Construction of Calibration curve

Standard stock solution of TNF and EMT were prepared separately in mobile phase with suitable dilution to get the concentration of 100 µg / ml. From the standard stock solution of drugs, different dilutions were prepared, injected and their peak area was measured. Calibration curves were drawn between concentration against their respective peak area for TNF (5- 70µg / ml) and EMT (5 - 70µg / ml) respectively. Unknown samples were determined by using these regression equations of (Y= mx + c) calibration curves.

2.5. Standard mixture solution

Mixed standard analysis was performed to validate the procedure with different mixed standard solutions of 2 : 12, 4:10, 6:8, 8:6, 10:4, 12:2 of TNF and *EMT*, respectively and were analyzed using statistical results (Acceptance % RSD < 2.0 and S.D < 1.0).

2.6. Sample preparation

Twenty tablets were weighed and their average weight was determined. The tablets were then crushed to a fine powder and the tablet powder equivalent to 300 mg of TNF and 200 mg of EMT was transferred into a volumetric flask and extracted with HPLC grade methanol. The solution was shaken for 5 min and sonicated for 15-20 min. The solution was filtered through Whatman filter paper 41. This filtrate was further diluted with mobile phase to get the final

concentration of 20 µg / ml for TNF and 30 µg / ml for EMT theoretically. 20 µL of the sample solution was injected for quantitative analysis. Identification is done by comparing retention times of the sample solution with those of standard solution. The amount of TNF and EMT per tablet was calculated from Regression Plot. The results are reported in Table 5.

RESULTS AND DISCUSSION

3.1. HPLC method development and optimization

The mobile phase conditions were optimized so that the components were not interfered from the solvent and excipients. Mobile phase and flow rate selection was done based on peak parameters such as height, area, tailing, theoretical plates, capacity factor, resolution and run time. The best result was obtained by the use of methanol – Phosphate buffer 7.0 (65:35 v/v) at a flow rate of 1 ml/ min. Based on the overlain UV spectra (Systronics), suitable wavelength considered for monitoring the drugs and it was 260 nm . Solutions of TNF and *EMT* in diluents were also injected (n = 5) directly for HPLC analysis and the responses (peak area) were recorded. It was observed that there was no interference from the mobile phase or baseline disturbances and the analytes were detected well with more than 9000 theoretical plates at 260 nm. The chromatogram of standard mixture is shown in Fig 2. Under the optimum chromatographic conditions, the retention time obtained for EMT and TNF were 4.7 and 5.8 min, respectively. The results of capacity factor, tailing factor, Number of theoretical plates and resolution are reported in Table 1

3.2. Validation of the developed method

The method was validated for linearity, accuracy, precision, repeatability, selectivity and specificity study as per ICH norms [12, 13]. All the validation studies were carried out by replicate injection of the sample and standard solutions.

3.3. Linearity

Linearity was found to be 5 – 70 µg / ml for EMT and 5- 70 µg / ml for TNF. The linear regression equations for EMT and TNF were y = 91934x + 6555.8 (r2 = 0.9996) and y = 82413 x + 6667.3 (n=3, r2 = 0.9994), respectively. The regression data were shown in table 2.

3.4. Accuracy

Accuracy of developed method was confirmed by doing recovery study as per ICH norms at three different concentration levels by replicate analysis (n=3). The result of accuracy study was reported in Table 3.

3.5. Precision, Limit of Detection, and Limit of Quantitation

The concentrations of both the drugs were measured three times on the same day at intervals of 1 hr and on three different days for intra and interday study respectively. LOD and LOQ were calculated by use of the equations LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation and S is the slope of the calibration curve. The results are reported in Table 4.

3.6. Selectivity and Specificity

The selectivity of the method was performed by injecting drugs solutions into the optimized system and it was observed that two sharp peaks of TNF and EMT having resolution of 2.9 were obtained at retention time of 4.7 and 5.8 min, respectively with reference to placebo solution. Comparing the chromatograms obtained from standard drugs, with the chromatogram obtained from tablet solutions, the specificity of the method was assessed. As the retention time of standard drugs and the retention time of the drugs in sample solution was same, so the method

was specific. The developed method was found specific and selective, as there was no interference of excipients found.

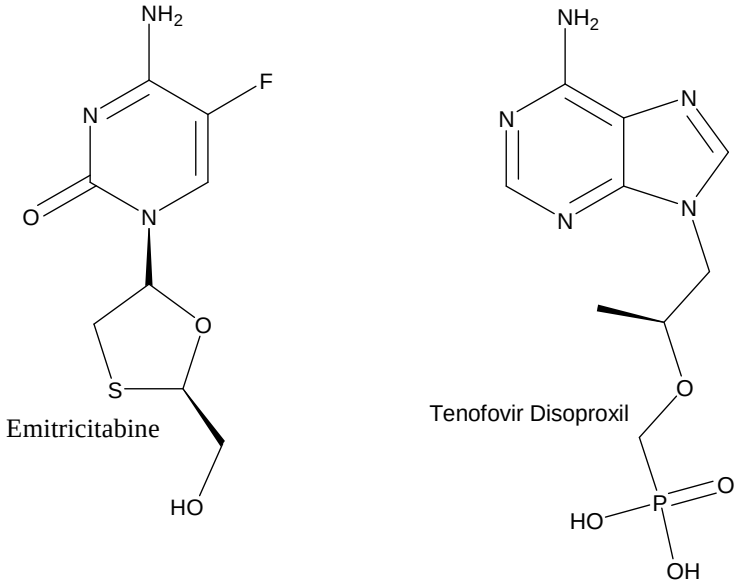

Fig. 1. Structures of Tenofovir disoproxil Fumarate and Emitricitabine

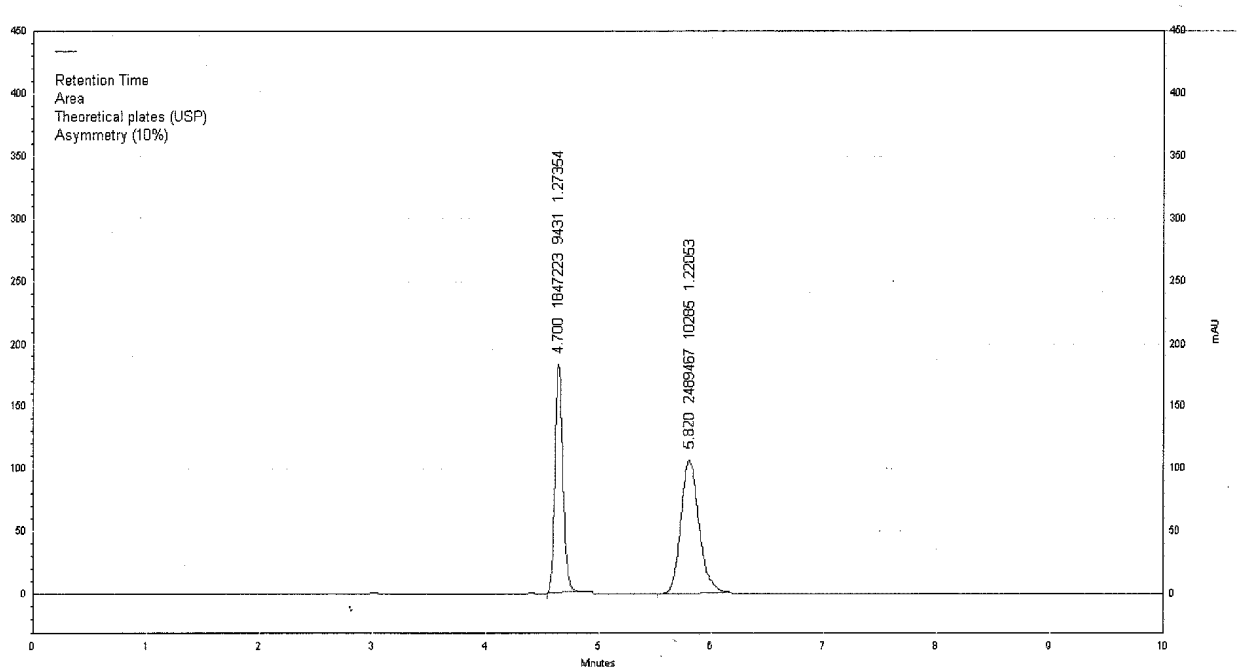

Fig. 2. Chromatogram of Tenofovir disoproxil Fumarate and Emitricitabine

Table 1. Validation Summary ; System Suitability

Parameter	Emitricitabine(EMT)	Tenofovir disoproxil Fumarate (TNF)
Regression equation	y = 91934x + 6555.8	y = 82413x + 6667.3
Retention time	4.7 min	5.8 min
Peak Asymmetry	1.27	1.22
Capacity factor	0.904	1.344
Resolution	-	2.9
Theoretical plates	9431	10285

Table 2. Regression data for the developed RP – HPLC method

Parameter	Emitricitabine(EMT)	Tenofovir disoproxil Fumarate (TNF)
Regression equation	y = 91934x + 6555.8	y = 82413x + 6667.3
Slope	91934	82413
Intercept	6555.8	6667.3
Correlation coefficient (R^2)	0.9996	0.9994
% RSD	0.12	0.14
Concentration Range	5- 70µg/ml	5- 70 µg/ml

Table 3. Accuracy

Analyte	Amount Taken	Amount Added	% Recovery*	% RSD
EMT	20 µg/ml	16 µg/ml	*99.8±1.1*	1.102
		20µg/ml	*101.2±0.8*	0.790
		24µg/ml	*101.2±0.3*	0.296
TNF	30 µg/ml	24 µg/ml	*98.7±0.6*	0.607
		30 µg/ml	*100.5±1.1*	1.094
		36 µg/ml	*100.3±0.5*	0.498

** values are presented as Mean±SD*

Table 4. Precision, LOD and LOQ

Analyte	Intra-day % RSD	Inter-day %RSD	LOD µg/ml	LOQ µg/ml
EMT	1.2334	1.6124	0.054	0.165
TNF	0.6644	0.9421	0.063	0.192

Table 5. Assay

S.NO	Brand Name	Label claim	Amount Found	Recovery (Mean±S.D)	%RSD
1	Forstavir - EM	EMT - 200mg	196.78	98.39±0.8	0.81
		TNF - 300mg	304.25	101.41±0.5	0.49

CONCLUSION

The developed RP – HPLC method for the simultaneous analysis of TNF and EMT in a tablet formulation, was shown linear, accurate, reproducible, repeatable, precise, selective and specific with significant LOD and LOQ.

Acknowledgement
The authors are thankful to Centre for Pharmaceutical Research, Pharmaceutical Analysis Division, Raghavendra Institute of Pharmaceutical Education and Research, for providing necessary facilities to carry out this work. Also the authors are thankful to Hetero Drugs Pvt. Ltd, Hyderabad, for providing the gift samples of EMT and TNF.

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