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Method development and validation of tenofovir disoproxil fumerate and emtricitabine in combined tablet dosage form by UV-spectrophotometry and RP-HPLC

Dr.R.Srinivasan*[1], K.Lurdhu Mary, G.Lakshmana, D.Rajesh Kumar, B.Rajini
Siddhartha Institute of pharmaceutical sciences, Jonnalagadda, Narsaraopet, Guntur (DT), India.
*** Corresponding author: Dr.R.Srinivasan**
E-mail id: rangusha75@gmail.com

ABSTRACT

A simple, accurate, rapid, precise and novel UV-Spectrophotometry and Reverse phase High Pressure liquid chromatographic method (RP-HPLC) has been developed and validated for simultaneous determination of Tenofovir Disoproxil Fumerate(TDF) And Emtricitabine(EMT) in combined tablet dosage form. At wavelength 260 nm both drugs have considerable absorbance. The UV-Spectrophotometry method was found to be linear between the range of 5-25 µg/ml for TDF and 7-35 µg/ml for EMT.The selected and optimized mobile phase was Acetonitrile : Phosphate pH 3.5 buffers in the ratio of 60:40 was fixed due to good symmetrical peak. and conditions were flow rate (1.0 ml/minute), wavelength (270 nm), Run time was 5 min. The retention time were found to be 2.85 min and 3.55 min for Tenofovir Disoproxil Fumerate And Emtricitabine respectively. Linearity and range was found to be 3-15 µg/ml for Tenofovir Disoproxil Fumerate and 2-10 µg/ml for Emtricitabine. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drugs.The method was validated for accuracy, precision, specificity, linearity, robustness, sensitivity, LOD and LOQ. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible.

Keywords: UV-Spectrophotometry, Reverse phase High Pressure liquid chromatography, Tenofovir Disoproxil Fumerate(TDF), Emtricitabine(EMT) and Acetonitrile.

INTRODUCTION

Analytical chemistry[1] is the science to analyze morphologies, compositions, and quantities of analytical targets.

CHROMATOGRAPHY[2]

Chromatography **(**from Greek: chroma, colour and: "grafein" to write) is the collective term for a family of laboratory techniques for the separation of mixtures

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

NORMAL PHASE CHROMATOGRAPHY

Normal phase HPLC (NP-HPLC) was the first kind of HPLC chemistry used, and separates analytes based on polarity. This method uses a polar stationary phase and a non-polar mobile phase, and is used when the analyte of interest is fairly polar in nature.

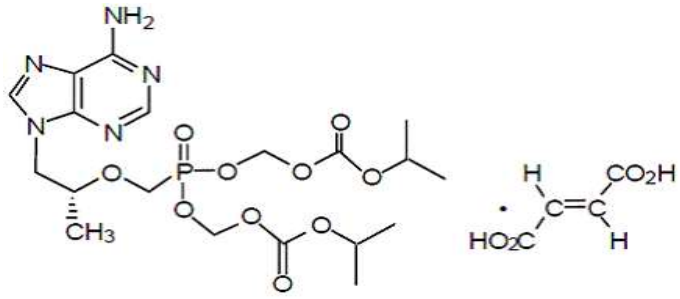
REVERSED PHASE CHROMATOGRAPHY

Reversed phase HPLC (RP-HPLC) consists of a non-polar stationary phase and an aqueous, moderately polar mobile phase.

DRUG PROFILE [3-7]

TENOFOVIR DISOPROXIL FUMERATE

Structure



Chemical name: salt of bis(isopropyloxycarbonyloxymethyl ester of (R)-9-(2-phosphonomethoxypropyl)adenine with fumaric acid.

Empirical formula: $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$

Molecular weight: 635.5

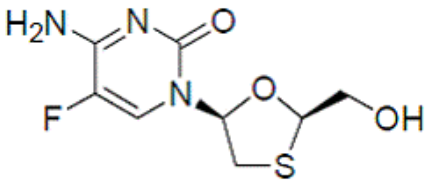
Description: A white to off white crystalline powder

Solubility: soluble in water : methanol (1:1)

Category: Anti-HIV Agents
Nucleoside and Nucleotide Reverse Transcriptase Inhibitors
Reverse Transcriptase Inhibitors

EMTRICITABINE

Structure



Chemical name: 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl) -1,3-oxathiolan-5-yl]-2 (1H)-pyrimidone

Empirical formula: $C_8H_{10}FN_3O_3S$

Molecular weight: 247.3

Description: A white to off-white crystalline powder

Solubility: Soluble in water and sparingly soluble in methanol

Category**:** Antiviral Agent, Anti-HIV Agent

MATERIALS & METHODS

UV-METHOD

Materials

i. TDF
ii. EMT
iii. Methanol
iv. Distilled water

Instruments used

i. Digital balance – shimadzu
ii. UV-Visible spectrophotometer – UV-1700 shimadzu

Method

1. Solvent : methanol (50% v/v)
2. Identification of spectrum :

Accurately weighed quantities (100 mg) of TDF and EMT were taken in 100ml standard flasks, dissolved separately by adding 50 ml methanol and volumes were made up with distilled water (1000 µg/ml). These solutions were used as working standards. Aliquot portions of stock solutions of TDF and EMT were diluted appropriately with distilled water to obtain concentration 30 µg/ml of TDF and 20 µg/ml of EMT. The working standard solutions were scanned from 200 to 400 nm to select the wavelengths for estimation. the wavelength selected for estimation of TDF was 260 nm, where EMT has no significant absorbance and for EMT it was 290 nm, where absorbance of EMT is corrected. Different binary mixture solutions of TNF and EMT were then run in entire range from 200 to 400 nm. The drugs obey Beer's law in the concentration range of 5 to 30 µg/ml and 7 to 35 µg/ml for TDF & EMT respectively.

RP-HPLC METHOD

ANALYSIS OF FORMULATION

PREPARATION OF MOBILE PHASE

PREPARATION OF BUFFER SOLUTION

4.08g of potassium dihydrogen phosphate is dissolved in 1000 ml of volumetric flask and make up with water and adjust the pH to 3.5. Filtered through a finer porosity membrane filter and degassed.

MOBILE PHASE

Mixed ACN and Buffer in the ratio of 600:400v/v respectively and degassed by sonication.

PREPARATION OF DILUENT

Taking into consideration the solubility of the drugs in different solvents, the common diluent was selected for all the two drugs which is nothing but the Water.

PREPARATION OF STANDARD: (TDF & EMT STANDARDS)

Standard stock solution of TDF 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 (3- 15µg / ml) and EMT (2 - 10µg / ml) respectively. Unknown samples were determined by using these regression equations of (Y= mx + c) calibration curves.

SAMPLE PREPARATION: (TENVIR-EM TABLETS)

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 30 mg of TNF and 20 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 15 µg / ml for TNF and 10 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.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

The following parameters were used for RP-HPLC analysis of assay of pharmaceutical dosage form.

Mode of operation : Isocratic
Stationary phase : PHENOMENEX Luna C18 A,250*4.6,5µ
Mobile phase : Acetonitrile : 0.03M Potassium dihydrogen phosphate pH(3.5)

Ratio : 60:40
Diluent : Water
Detection wavelength : 270nm
Flow rate : 1 ml/min
Temperature : 25°C
Sample volume : 20 µl

PROCEDURE

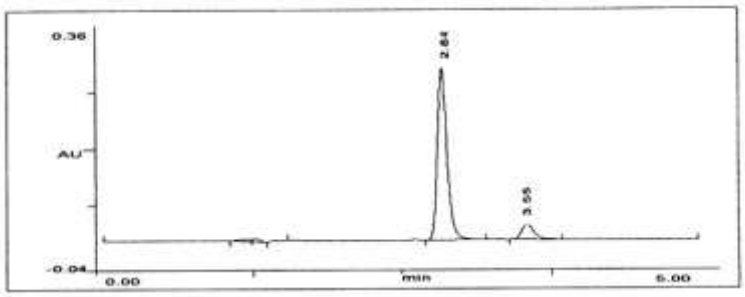
The chromatographic conditions were set as per the established parameter and mobile phase allowed to equilibrate with the stationary phase. Working standard solution was injected separately and chromatograms have been reproduced.

STUDY OF SYSTEM SUITABILITY PARAMETERS

PROCEDURE

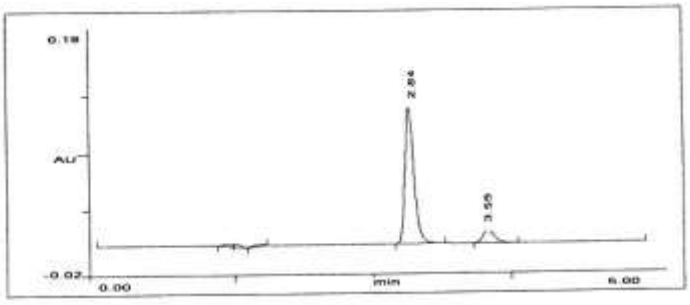
The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline.

RESULTS & DISCUSSION



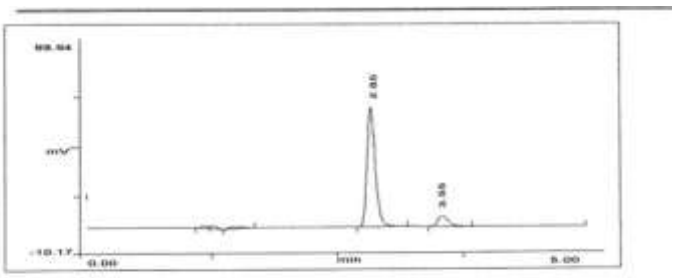
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.84	66797	9060931	91.8867	89.8669	BB	0.093
2	3.55	5898	1021681	8.1133	10.1331	BB	0.118
		7e+04	10082612				

OPTIMIZED CHROMATOGRAM OF TDF & EMT USING ACETONITRILE : PHOSPHATE BUFFER (pH-3.5) (60:40)



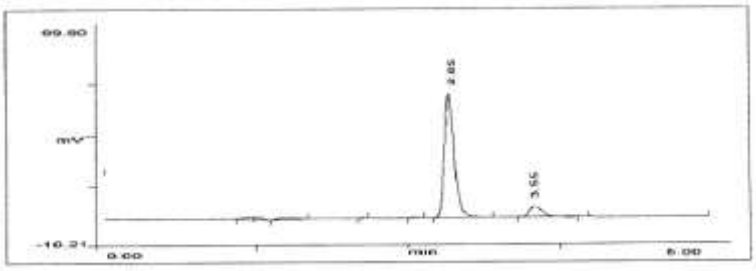
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.84	26353	3698325	91.9440	90.0617	BB	0.094
2	3.55	2309	402594	8.0560	9.9383	BB	0.119
		3e+04	4050919				

QUANTIFICATION OF TDF & EMT IN FORMULATION sample-1



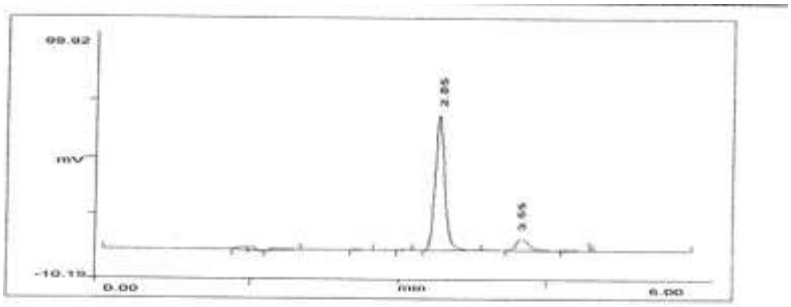
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.85	26388	3678130	91.9090	90.0346	BB	0.095
2	3.55	2323	407109	8.0910	9.9654	BB	0.120
		3e+04	4085239				

QUANTIFICATION OF TDF & EMT IN FORMULATION sample-2



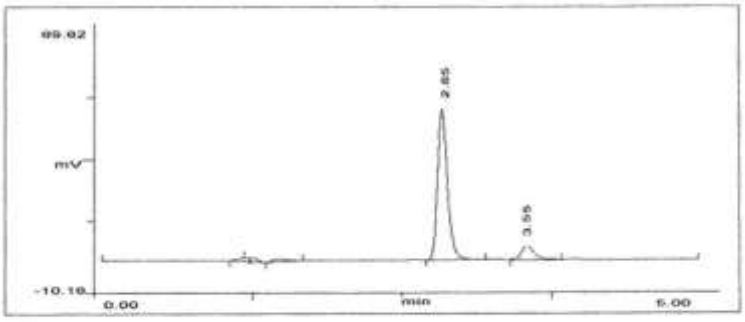
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.85	26541	3687131	91.9870	90.2653	BB	0.095
2	3.55	2312	397640	8.0130	9.7347	BP	0.117
		3e+04	4084771				

QUANTIFICATION OF TDF & EMT IN FORMULATION – sample-3



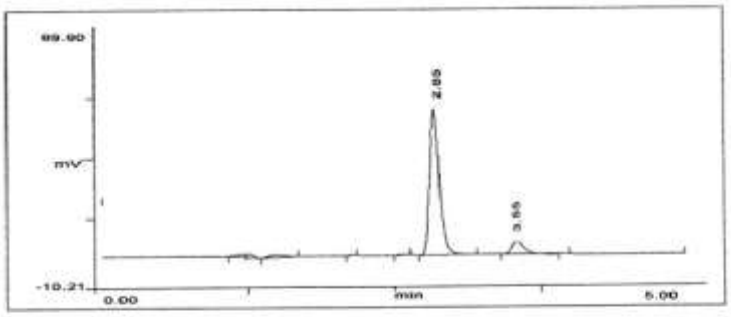
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.85	26064	3695945	91.9430	89.9022	BB	0.095
2	3.55	2284	407264	8.0570	10.0978	BB	0.122
		3e+04	4033209				

QUANTIFICATION OF TDF & EMT IN FORMULATION – sample-4



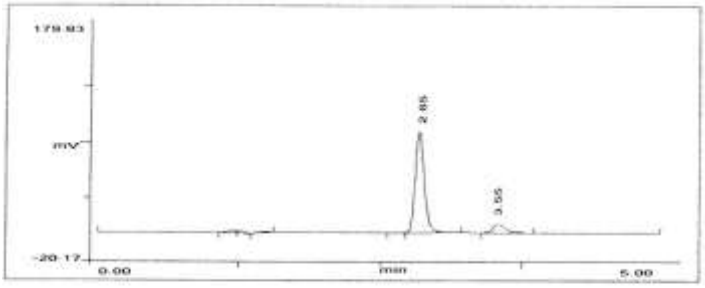
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.85	26739	3716866	91.9783	90.1010	BB	0.095
2	3.55	2332	408354	8.0217	9.8990	BB	0.120
		3e+04	4125220				

QUANTIFICATION OF TDF & EMT IN FORMULATION-sample-5



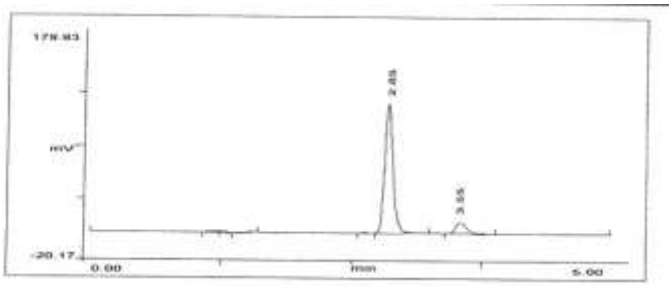
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.85	26541	3687231	91.9870	90.2653	BB	0.095
2	3.55	2312	397640	8.0130	9.7347	BP	0.117
		3e+04	4084771				

QUANTIFICATION OF TDF & EMT HCL BY RP-HPLC- sample-6



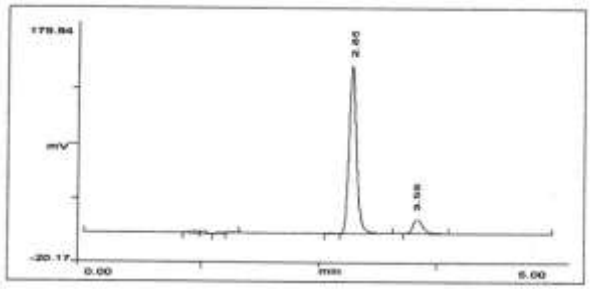
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.85	40229	5545540	91.9499	89.9762	PB	0.094
2	3.55	3522	617802	8.0501	10.0238	BB	0.120
		4e+04	6163342				

RECOVERY STUDIES OF TDF & EMT BY RP-HPLC



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.85	53564	7355009	91.9633	89.9935	PB	0.094
2	3.55	4681	817810	8.0367	10.0065	BB	0.119
		6e+04	8172819				

RECOVERY STUDIES OF TDF & EMT HCL BY RP-HPLC



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.85	66734	9140587	91.9581	89.9876	PB	0.093
2	3.55	5836	1017017	8.0419	10.0124	BB	0.119
		7e+04	10157604				

RECOVERY STUDIES OF TDF & EMT HCL BY RP-HPLC

Two new methods have been developed and validated for simultaneous estimation of TDF & EMT in a tablet dosage form (TENVIR-EM). The first method, the absorbance correction method was based on the measurement of the absorbance at two wavelengths, namely, 290 nm at which TDF has no absorbance while at wavelength 260 nm both drugs have considerable absorbance. The method was found to be linear between the range of 5-25 µg/ml for TDF and 7-35 µg/ml for EMT. The mean percentage recovery was found in the range of 99.24%-100.42% and 100.03-101.04% for TDF and EMT at three different levels of standard additions. The precision (intra-day, inter-day) of method was found within limits (RSD <2%).

The second method, RP-HPLC method for the simultaneous estimation of TDF & EMT was developed by studying different parameters. First of all, maximum absorbance was found to be at 270nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was PHENOMENEX LUNA C18 chosen. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase of Acetonitrile : Phosphate pH 3.5 buffers in the ratio of 60:40 was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Acetonitrile was selected because of maximum extraction and all the drug particles were completely soluble and showed good recovery. Run time was selected to be 5min because analyze gave peaks for TDF & EMT around 2.84 min

and 3.55. After the development of the method, it was validated for accuracy, linearity, precision, robustness, LOD and LOQ studies. The precision of the System and Method were checked and found to be within limits. This indicates that the method is precise. Linearity study, correlation coefficient and curve fitting was found to be 0.999. From the results shown in the accuracy table, it was found that recovery value of pure drug was between 99.4 % to 101.7 %.

CONCLUSION

It could be concluded from the results obtained in the present investigation that the two methods for the simultaneous estimation of TDF & EMT in tablet dosage form are simple, rapid, accurate, precise and economical and can be used, successfully in the quality control of pharmaceutical formulations and other routine laboratory analysis.

REFERENCES

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[2] K. Anand kumar et. al., "Development and validation of emtricitabine and tenofovir disoproxil fumerate in pure and in fixed dose combination by uv Spectrophotometry", Digest Journal of Nanomaterials and Biostructures, Vol. 6, No 3, July-September 2011, pages 1085-1090.

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