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Research

Validation Parameters Using Rp – Hplc Method For The Simultaneous Determination Of *Emtricitabine* And *Tenofovir Disoproxil Fumarate*

Dr. D. Jeevan Mani Babu[1], M. Sireesha[2], J. Madhavi Latha[3], T. Aswini[2]

[1]Principal,Department of Pharmacology, St.Xavier Institute of Pharmacy, Deenapur, Phirangipuram, Andhra Pradesh, Pin code:522529.
[2]Department of Pharmaceutical Analysis, St.Xavier Institute of Pharmacy, Deenapur, Phirangipuram, Andhra Pradesh, Pin code:522529.
[3]Associate Professor, Department of Pharmaceutical Analysis, St.Xavier Institute of Pharmacy, Deenapur, Phirangipuram, Andhra Pradesh, Pin code:522529.

Author correspondence: Dr. D. Jeevan Mani Babu
Email id: drjeevanbabu@gmail.com

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Abstract

Separation of Emtricitabine was successfully achieved Dona:YMC PACK PRO 150X4.6mm, 5µm, C18 or equivalenting an isocratic mode utilizing KH2PO4: Methanol(65:35) at a flowrate of 1.0mL/mins and eluate was monitored at 265nm, with a retention time of 3.150 minutes for Emtricitabine respectively. Assay Results 98.82%The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Assay.

Keywords: Emtricitabine, RP-HPLC, Method development, Validation

INTRODUCTION

Instrumental analysis

The instrument is only one component of the total analysis. Often, it is necessary to use several instrumental techniques to obtain the information required to solve an analytical problem. Instrumental method may be used by analytical chemists to save time, to avoid chemical separation or to obtain increased accuracy.

Based on Principle Types of Chemical Instrumentation

A) Spectrometric Techniques

□□□□□□□□□□□□□□□□□□Atomic Spectrometry (Emission and Absorption)
□□□□□□□□□□□□□□□□□□Electron Spin Resonance Spectroscopy
□□□□□Fluorescence and phosphorescence Spectrophotometry
□□□□□Infrared Spectrophotometry
□□□□□Nuclear Magnetic Resonance Spectroscopy
□□□□□Radiochemical Techniques including activation analysis
□□□□□Raman Spectroscopy
□□□□□Ultraviolet and visible Spectrophotometry
□□□□□X-Ray Spectroscopy

B) Electrochemical techniques

- Potentiometry
- Voltametry
- Stripping techniques
- Amperometric techniques
- Coulometry
- Electrogravimetry
- Conductance techniques.

C) Chromatographic Techniques:

□□□□□Gas Chromatography
□□□□□High performance Liquid Chromatography
□□□□□Thin Layer Chromatography

D) Miscellaneous Techniques:

□□□□Kinetic Techniques
□□□□Mass Spectrometry
□□□□Thermal Analysis

D) Hyphenated Techniques:

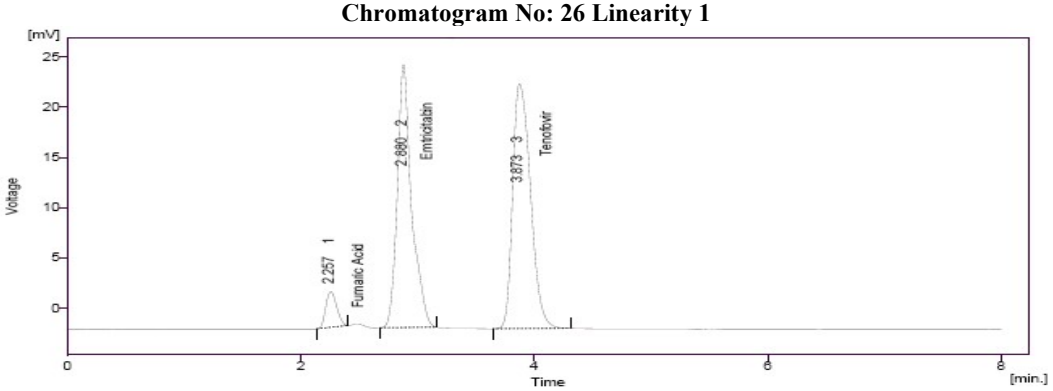
□□□□GC-MS (Gas Chromatography - Mass Spectrometry)
□□□□ICP-MS (Inductivity Coupled Plasma - Mass Spectrometry)
□□□□GC-IR (Gas Chromatography - Infrared Spectroscopy)

□□□□MS-MS (Mass Spectrometry - Mass Spectrometry **(Willard H.H. *et al 1986)***		Thin layer Chromatography
		Adsorption Column Chromatography
		High Performance Liquid Chromatography
SOLID (Ion exchange resin)	LIQUID	Ion exchange Chromatography
SOLID	GAS	Gas-Solid Chromatography
SOLID MATRIX	LIQUID	Gel permeation Chromatography (Exclusion Chromatography)
LIQUID	GAS	Gas-Liquid Chromatography
LIQUID	LIQUID	Liquid-Liquid Chromatoraphy

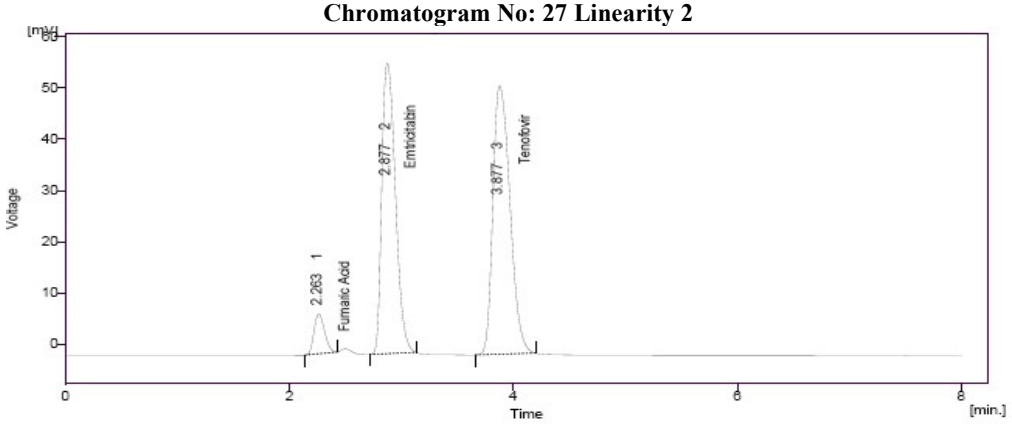
Validation of rp-hplc method

After development of HPLC method for the estimation of the multi component dosage forms validation of the method was carried out. This section describes the procedure followed for the validation of the developed method.

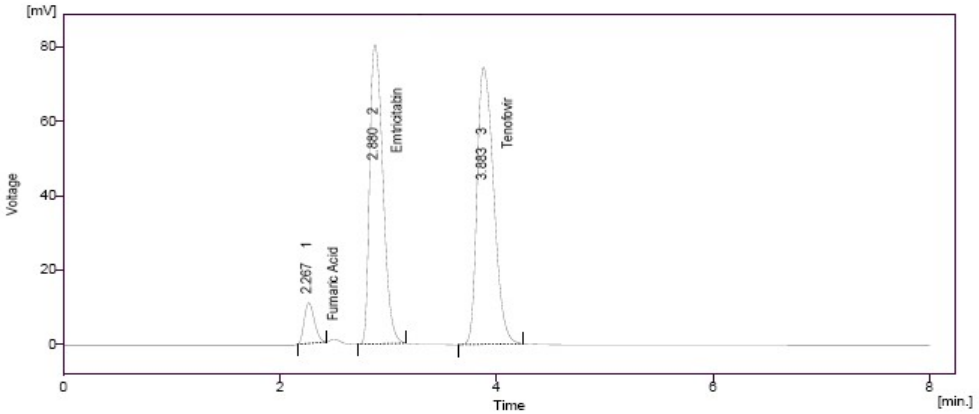
Chromatogram No: 26 Linearity 1



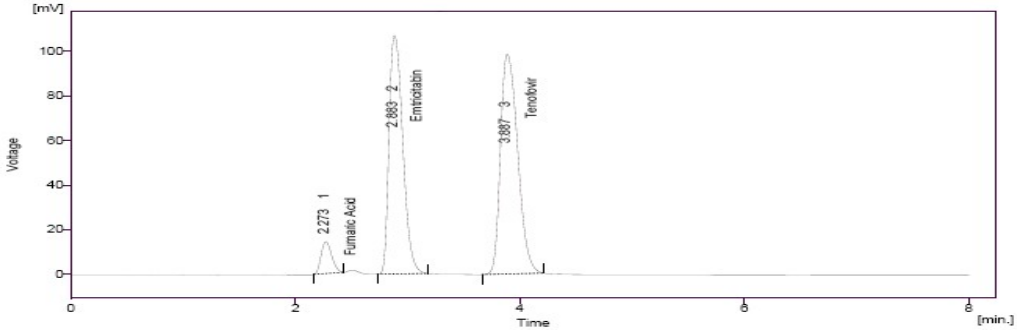
Chromatogram No: 27 Linearity 2



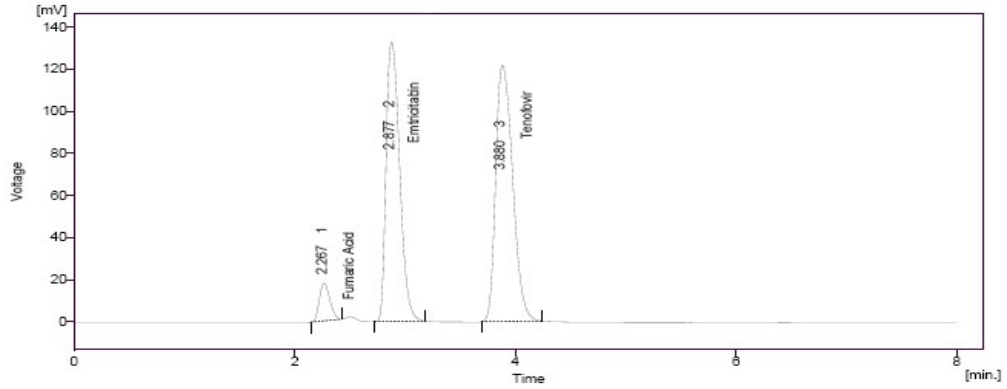
Chromatogram No: 28
Linearity 3



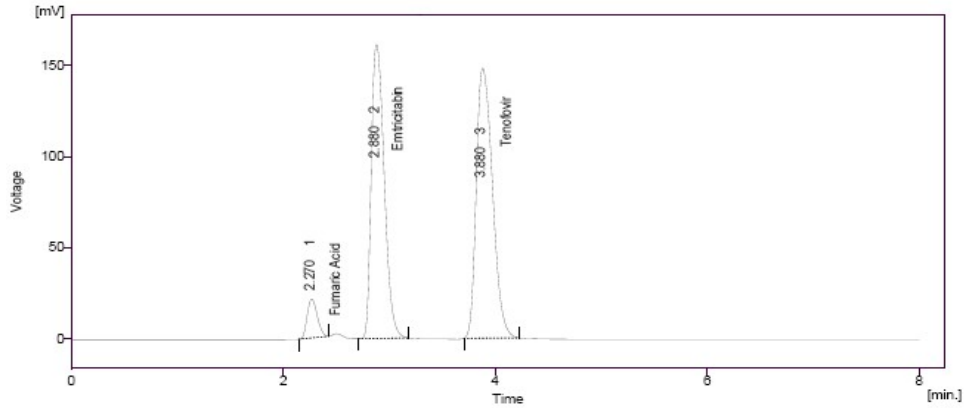
Chromatogram No: 29
Linearity 4



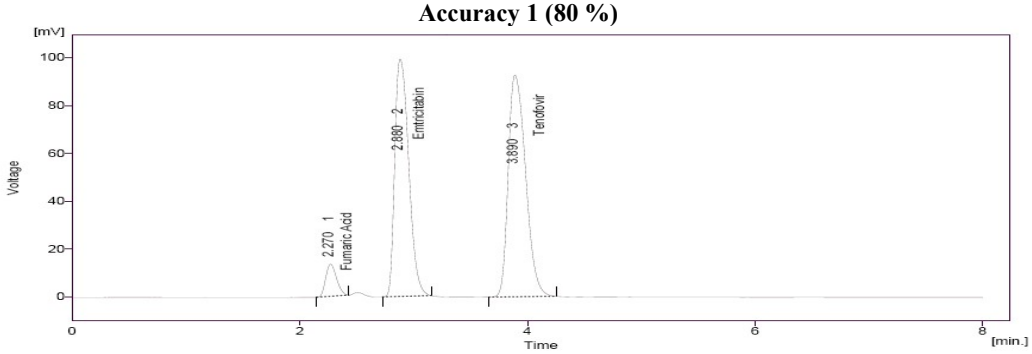
Chromatogram No: 30 Linearity 5



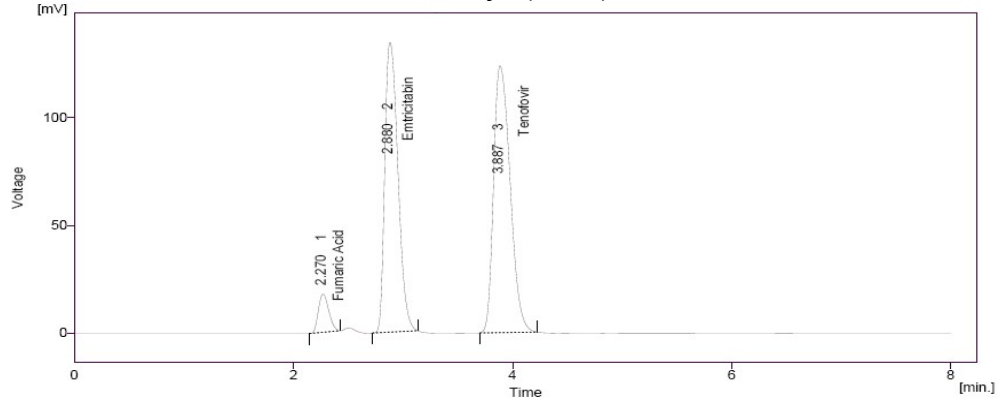
Chromatogram No: 31
Linearity 6



Chromatogram No: 32
Accuracy 1 (80 %)

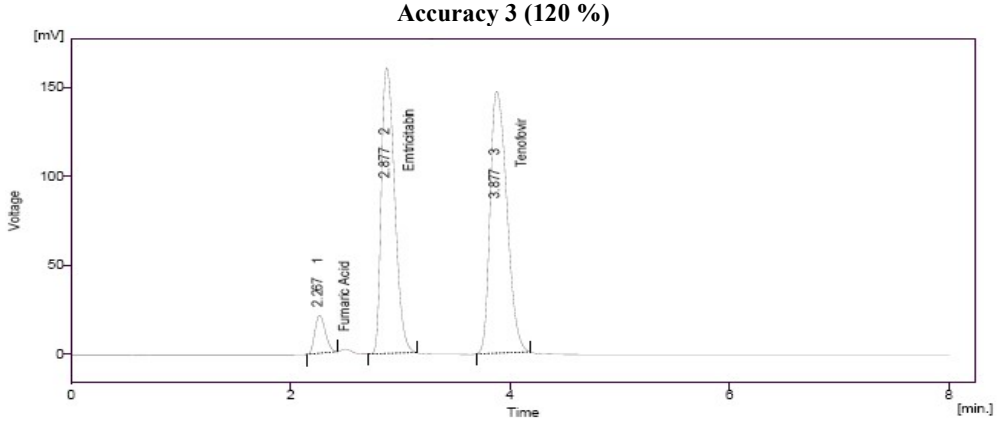


Chromatogram No: 33
Accuracy 2 (100 %)



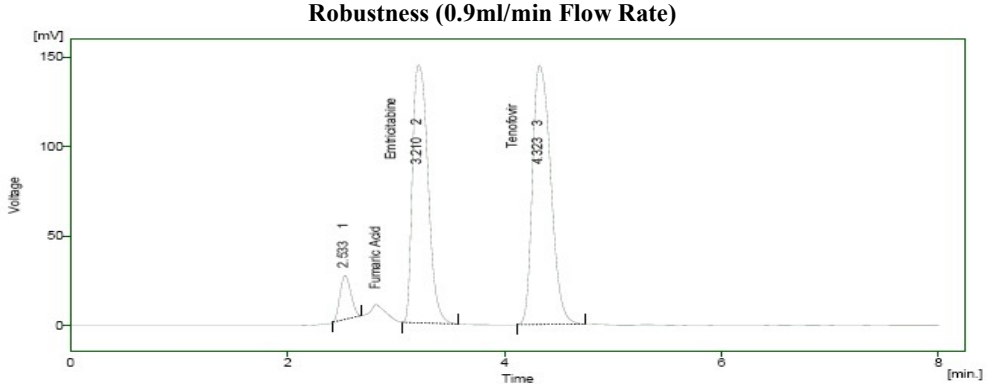
Chromatogram No: 34

Accuracy 3 (120 %)



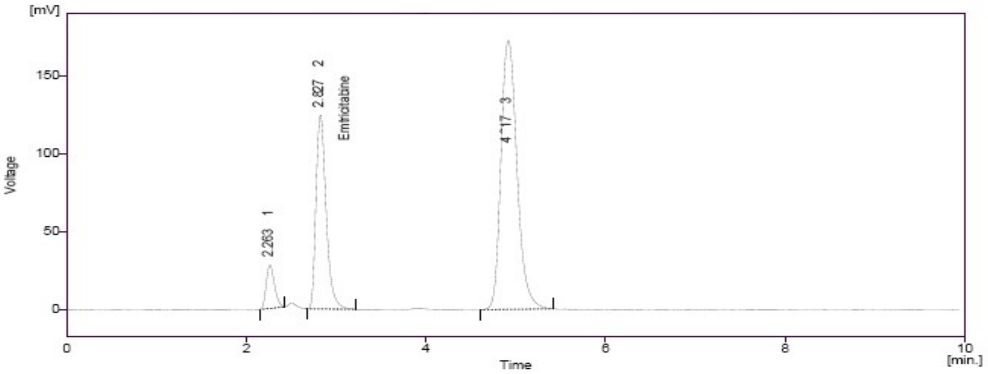
Chromatogram No: 35

Robustness (0.9ml/min Flow Rate)



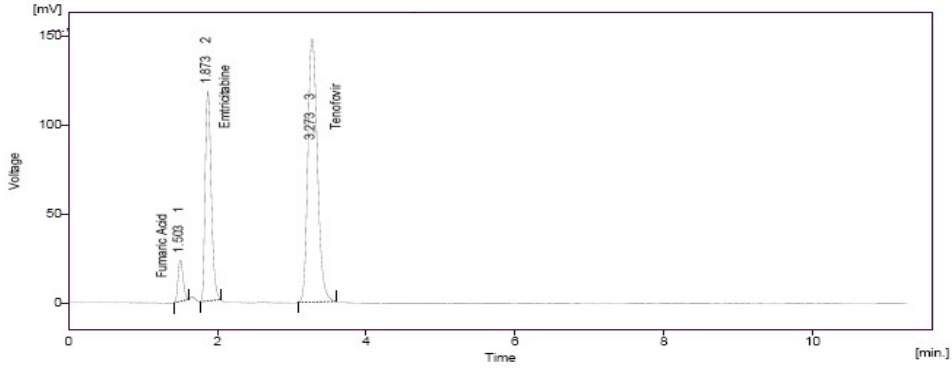
Chromatogram No: 36

Robustness (1.2 ml/min flow rate)



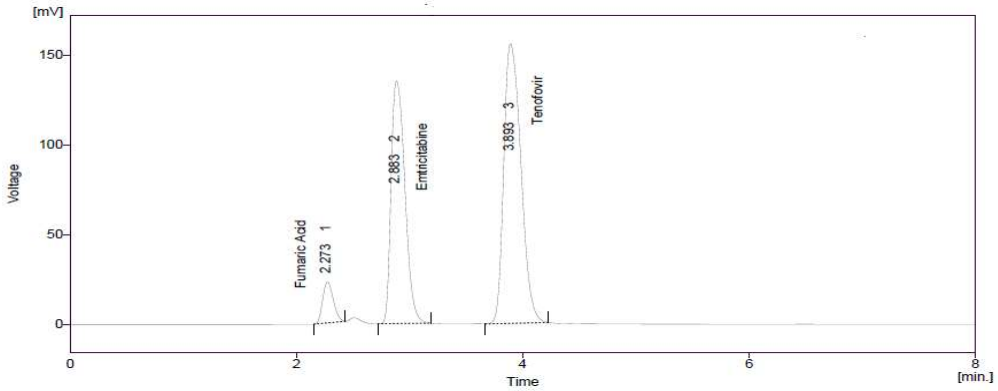
Chromatogram No: 37

Robustness (1.5 ml/min Flow rate)



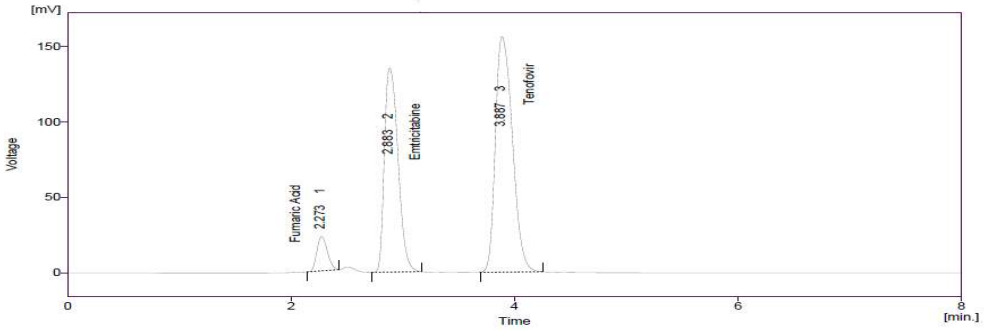
Chromatogram No: 38

Robustness (265 nm)



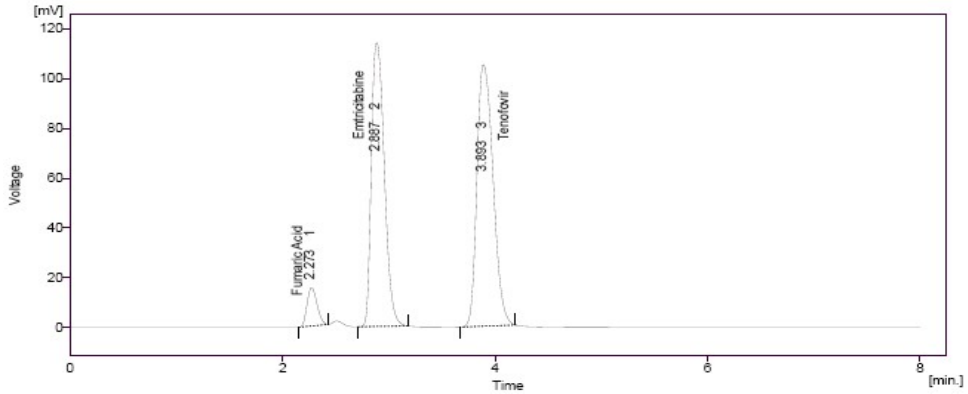
Chromatogram No: 39

Robustness (270 nm)



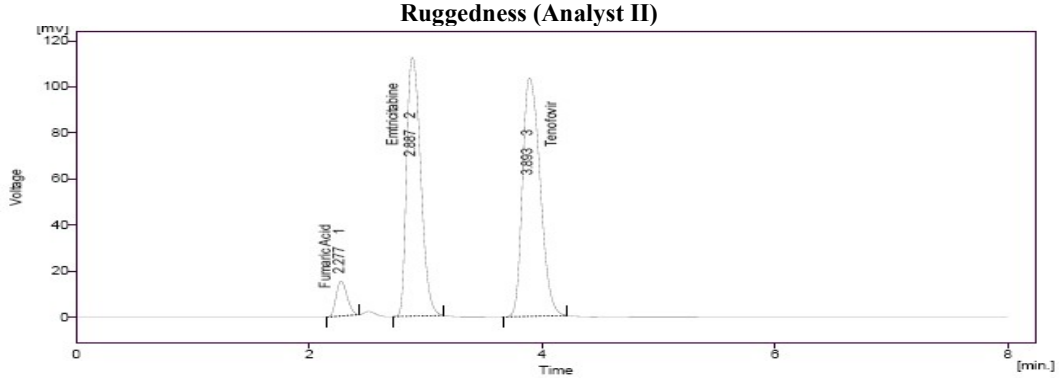
Chromatogram No: 40

Ruggedness (Analyst I)



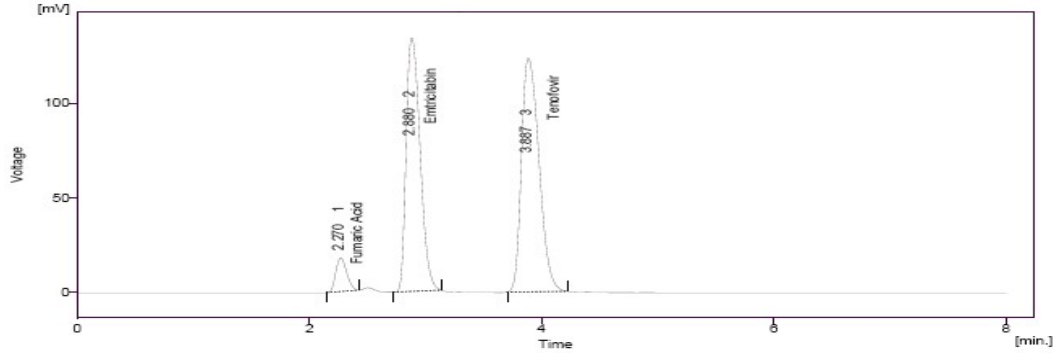
Chromatogram No: 41

Ruggedness (Analyst II)



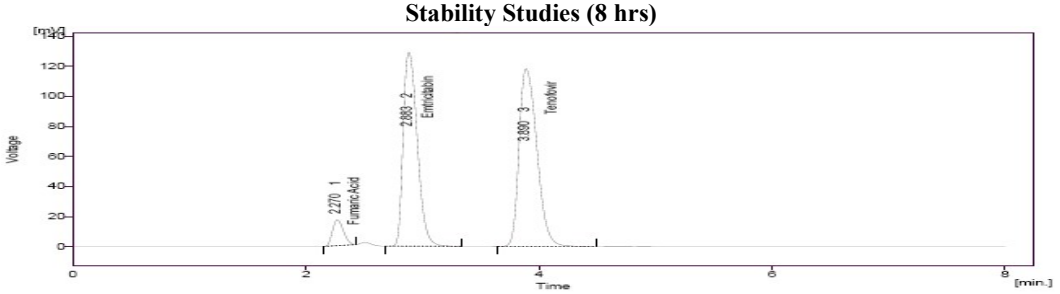
Chromatography No: 42

Stability studies (0 hrs)



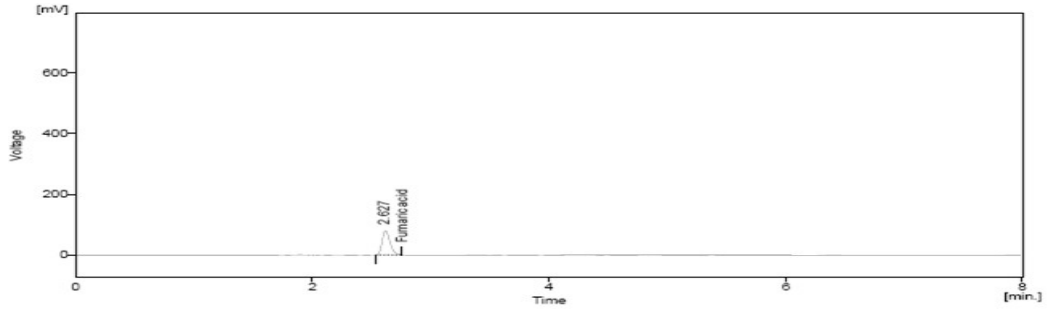
Chromatogram No: 43

Stability Studies (8 hrs)



Chromatogram No: 44

Fumaric acid Standard



RESULT AND DISCUSSION

VALIDATION OF THE METHOD

The precision of the method was demonstrated by system and method precision. All solutions were injected into the chromatographic system. The peak area and percentage relative standard deviation were calculated and presented in tables (5) & (6). The linearity of proposed method were performedby using the concentration range of 20% to 120% of standard concentration i.e 4 µg/ml to 24 µg/ml of *Emtricitabine* and 6 µg/ml and 36 µg/ml of *Tenofovir disoproxil fumarate* and was presented in Table 7, 8 & 9 .The response factor, slope, intercept and correlation co-efficient were calculated. The slope, intercept, correlation co-efficient were found to be 56.47, 3.867, 0.999respectively for *Emtricitabine* and 47.21, 8.114, 0.999 for *Tenofovir disoproxil fumarate*. The calibration curves were plotted using response factor Vs concentration of standard solutions(fig: 6 &7). The calibration graph shows that linear response was obtained over the range of concentration used in the assay procedure. These data demonstrates that the method have adequate sensitivity to the analytes. The range demonstrate that the method is linear outside the limits of expected use.

The robustness of the method was studied by carrying out experiments by changing conditions discussed earlier. The response factors for these changed chromatographic parameters were almost same as that of the fixed chromatographic parameters (table11&12) and hence developed method is said to be robust and ruggedness performed by analyst 1 and analyst 2 (Table 10). The LOD and LOQ were calculated for *Emtricitabine* and *Tenofovir* and it was presented in Table 13. The limit of detection for *Emtricitabine* was found to be 0.05318 µg/ml and for *Tenofovir disoproxil fumarate* was found to be 0.06782µg/ml. The Limit of Quantitation for for *Emtricitabine* was found to be 0.16115 µg/ml and for *Tenofovir disoproxil fumarate* was found to be

0.2553µg/ml. The stability studies were carried out at zero hour and after 8 hour (table 14). The assay procedure was performed and the assay percentage was calculated and presented in Table (15).

SUMMARY AND CONCLUSION

From the reported literature, there were few methods established for the determination of *Emtricitabine* and *Tenofovir disoproxil fumarate* in individual and in combination with other drug. It was concluded that there was two method reported for the simultaneous estimation of the above selected multi component dosage form, which promote to pursue the present work. The scope and object of the present work is to develop and validate a new simple HPLC method for simultaneous estimation of *Emtricitabine* and *Tenofovir disoproxil fumarate* in combined dosage form.

The *Emtricitabine* and *Tenofovir disoproxil fumarate* showed in the range of 4-24µg/ml and 6 - 36 µg/ml respectively. The slope intercept and correlation coefficient(s) were found to be, 56.47, 3.867, 0.999 respectively for *Emtricitabine* and 47.21, 8.114, 0.99 respectively for *Tenofovir disoproxil fumarate* which indicates excellent correlation factor Vs concentration of standard solutions. Precision of the developed methods was studied under system precision, method precision. The %RSD values for precision was found to be within the acceptable limit, which revealed that the developed method was precise. The developed method was found to be robust. The %RSD values for recovery percentage of *Emtricitabine* and *Tenofovir disoproxil fumarate* was found to be within the acceptable criteria. The result indicates satisfactory accuracy of method for simultaneous estimation of the above mentioned drugs.

Hence, the chromatographic method developed for *Emtricitabine* and *Tenofovir disoproxil fumarate* are rapid, simple, specific, sensitive, precise, Accurate. The RP-HPLC was simple and does not suffer from common excipients in pharmaceutical preparation and highly useful in the analysis of drugs in pharmaceutical formulation.

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