



Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (1):303-314
(<http://scholarsresearchlibrary.com/archive.html>)



A novel validated RP-HPLC method for the simultaneous estimation of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine in bulk and pharmaceutical tablet dosage forms

Uttam Prasad Panigrahy^{1*} and A. Sunil Kumar Reddy²

¹Department of Pharmaceutical Analysis and Quality Assurance, Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad, India

²Department of Pharmaceutical Chemistry, Bharat Institute of Technology-Pharmacy, Ibrahimpatnam, Hyderabad, India

ABSTRACT

An accurate, precise, simple, efficient and reproducible, isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine in bulk and pharmaceutical tablet dosage forms. Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine were separated using an Inertsil ODS 3V C₁₈ column (250mm×4.6 mm, 5µm particle size), Waters Alliance e2695 HPLC system with 2998 PDA detector and the mobile phase contained a mixture of 0.01M Potassium dihydrogen phosphate (pH adjusted to 4 with orthophosphoric acid) and Acetonitrile (30:70, v/v). The flow rate was set to 1ml/min with the responses measured at 265nm. The retention time of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine was found to be 1.976min, 2.661min and 4.316min respectively with resolution of 3.1 and 6.8. Linearity was established for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine in the range of 50-300µg/ml for Emtricitabine, 75-450µg/ml for Tenofovir Disoproxil Fumarate and 6.25-37.5µg/ml for Rilpivirine with correlation coefficient 0.999. The percentage recovery was found to be 99.71 % to 99.96%, 99.68% to 100.05% and 99.82% to 100.08% for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine respectively. Validation parameters such as specificity, linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. The developed method was successfully applied for the quantification of bulk and active pharmaceutical ingredient present in tablet dosage form.

Keywords: Emtricitabine, Tenofovir Disoproxil Fumarate, Rilpivirine, RP-HPLC, ICH.

INTRODUCTION

Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. Emtricitabine works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. Emtricitabine is chemically known as 4-amino-5-fluoro-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one was shown in Figure 1, is an analogue of cytidine^[1]. Tenofovir disoproxil fumarate (a prodrug of tenofovir), belongs to a class of antiretroviral drugs known as nucleoside analogue reverse transcriptase inhibitors (NRTI), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. Tenofovir is chemically known as ({[(2R)-1-(6-amino-9H-purin-9-yl) propan-2-yl] oxy} methyl) phosphonic acid^[1] was shown in Figure 2. In vivo Tenofovir disoproxil fumarate is converted to Tenofovir, an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate. Rilpivirine is non-nucleoside reverse transcriptase inhibitor (NNRTI) which is used for the treatment of HIV-1 infections in treatment-naive patients. It is a diaryl pyrimidine, a class of molecules that resemble pyrimidine nucleotides found in DNA. Because of its flexible

chemical structure, resistance of rilpivirine is less likely to develop than other NNRTI's. Rilpivirine is chemically known as 4-{{4-{{4-{{(1E)-2-cyanoeth-1-en-1-yl}-2, 6-dimethylphenyl} amino) pyrimidin-2-yl} amino} benzonitrile^[1] was shown in Figure 3. Literature review reveals that very few analytical methods has been reported for the determination of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine which includes UPLC^[2], HPLC^[3,4], LCMS^[5] and UV-Spectrophotometric^[6]. The present study was aimed to develop a novel, simple, economic and validated method for the simultaneous estimation of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl according to ICH guidelines^[7].

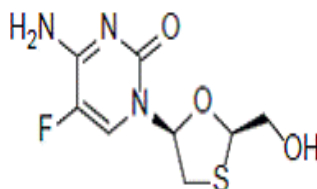


Figure 1: Chemical structure of Emtricitabine

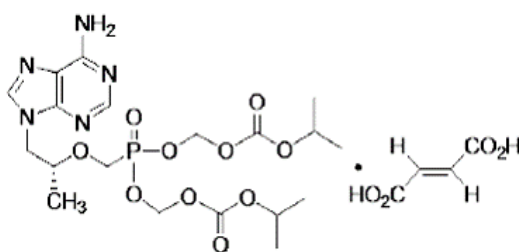


Figure 2: Chemical structure of Tenofovir Disoproxil Fumarate

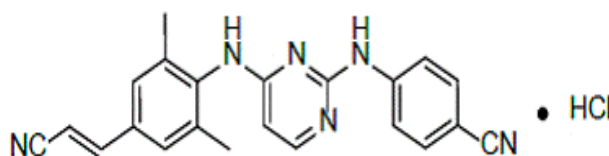


Figure 3: Chemical structure of Rilpivirine HCl

MATERIALS AND METHODS

Chemicals and Reagents:

Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl bulk drugs was kindly provided as gift sample by Hetero Drugs Limited, Hyderabad, India. Analytical grade of Potassium dihydrogen phosphate purchased from Rankem Ltd., India and HPLC grade of Acetonitrile purchased from Merck Specialities Private Limited, India. HPLC grade of Water and Ortho phosphoric acid purchased from Rankem Ltd., India. A COMPLERA tablet contains Emtricitabine 200 mg, Tenofovir DF 300 mg and Rilpivirine HCl 25 mg, and is obtained from a local pharmacy manufactured by Gilead Sciences, Inc.

Instrumentation:

The analysis was performed by using a chromatographic system from Waters Alliance e2695 HPLC system with 2998 PDA detector. The HPLC system was equipped with Empower 2 software. Semi-micro analytical balance (India), an Ultrasonic bath sonicator (Frontline FS 4, Mumbai, India), Digital pH meter (Systronics model 802) and Whatmann filter paper No. 41 (Whatmann International Ltd., England) were used in the study.

Chromatographic conditions:

Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl was analysed in an Inertsil ODS 3V C₁₈ (250mm×4.6 mm, 5µm particle size) column for the chromatographic separation. The mobile phase was composed of 0.01M Potassium dihydrogen phosphate (pH adjusted to 4 with orthophosphoric acid) and Acetonitrile (30:70, v/v). Filtered through 0.45µm nylon membrane filter under vacuum filtration and pumped at ambient temperature, at a flow rate of 1 ml/min with UV detection wavelength at 265nm. Injection volume was 20µl. The run time was 8 min and the retention time of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine was found to be

1.976min, 2.661min and 4.316min respectively with resolution of 3.1 and 6.8. The resulting HPLC chromatogram was shown in Figure 7.

Chromatographic Parameters:

Equipment : Waters Alliance e2695 HPLC system with 2998 PDA detector
Column : Inertsil ODS 3V C₁₈ (250mm×4.6 mm, 5µm particle size)
Flow rate : 1ml/min
Wavelength : 265 nm
Injection volume : 20 µl
Column oven : Ambient
Run time : 8 Minutes

Preparation of Phosphate buffer:

A 0.01M Phosphate buffer was prepared by dissolving 1.368gm of Potassium dihydrogen phosphate in 1000ml of HPLC grade water and pH was adjusted to 4 with orthophosphoric acid. The buffer was filtered through 0.45µm nylon membrane filter to remove all fine particles and gases.

Preparation of mobile phase:

The above prepared Phosphate buffer and Acetonitrile HPLC grade were mixed in the proportion of 30:70 v/v and was filtered through 0.45µm nylon membrane filter and degassed by sonication.

Preparation of diluent:

Mobile phase was used as diluent.

Preparation of standard stock solutions of Emtricitabine, Tenofovir DF and Rilpivirine HCl:

Standard stock solutions of Emtricitabine, Tenofovir DF and Rilpivirine HCl were prepared by dissolving 200mg of Emtricitabine, 300mg of Tenofovir DF and 25mg of Rilpivirine HCl in 100ml of diluent into a 100ml clean dry volumetric flask and the standard solutions was filtered through 0.45 µm nylon membrane filter and degassed by sonicator to get the concentration of 2000µg/ml of Emtricitabine, 3000µg/ml of Tenofovir DF and 250µg/ml of Rilpivirine HCl.

Preparation of standard solutions of Emtricitabine, Tenofovir DF and Rilpivirine HCl for assay:

From the above standard stock solution of 2000µg/ml of Emtricitabine, 3000µg/ml of Tenofovir DF and 250µg/ml of Rilpivirine HCl further pipette 1 ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 200µg/ml of Emtricitabine, 300µg/ml of Tenofovir DF and 25µg/ml of Rilpivirine HCl.

Selection of wavelength:

In simultaneous estimation of Emtricitabine, Tenofovir DF and Rilpivirine HCl isosbestic wavelength is used. Standard stock solutions of Emtricitabine, Tenofovir DF and Rilpivirine HCl were prepared by dissolving 200mg of Emtricitabine, 300mg of Tenofovir DF and 25mg of Rilpivirine HCl in 100ml of diluent into a 100ml clean dry volumetric flask and the standard solutions was filtered through 0.45 µm nylon membrane filter and degassed by sonicator to get the concentration of 2000µg/ml of Emtricitabine, 3000µg/ml of Tenofovir DF and 250µg/ml of Rilpivirine HCl. From the above standard stock solution of 2000µg/ml of Emtricitabine, 3000µg/ml of Tenofovir DF and 250µg/ml of Rilpivirine HCl further pipette 1 ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 200µg/ml of Emtricitabine, 300µg/ml of Tenofovir DF and 25µg/ml of Rilpivirine HCl. The wavelength of maximum absorption (λ_{max}) of 200µg/ml of Emtricitabine, 300µg/ml of Tenofovir DF and 25µg/ml of Rilpivirine HCl were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against mobile phase as blank. The isosbestic wavelength (λ_{max}) was found to be 265nm for the combination shown in Figure 4.

Preparation of sample solutions of Emtricitabine, Tenofovir DF and Rilpivirine HCl:

Twenty tablets were accurately weighed and powdered and tablet powder equivalent to 200mg of Emtricitabine, 300mg of Tenofovir DF and 25mg of Rilpivirine HCl were taken into 100ml clean dry volumetric flask, diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent. Further pipette out 1ml from the above Emtricitabine, Tenofovir DF and Rilpivirine HCl sample stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to get the concentration of 200µg/ml of Emtricitabine, 300µg/ml of Tenofovir DF and 25µg/ml of Rilpivirine HCl. 20µL from standard and sample solution were injected into the chromatographic system and the peak areas were measured for Emtricitabine, Tenofovir DF & Rilpivirine

HCl was shown in Figure 7 and 8 and the % Assay was calculated by comparing the peak area of standard and sample chromatogram by using the formula given below and the assay results was shown in Table 1.

$$\text{Assay \%} = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}}{\text{WT}} \times \frac{\text{P}}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = Average peak area of sample preparation

AS= Average peak area of standard preparation

WS = Weight of standard taken in mg

WT=Weight of sample taken in mg

P = Percentage purity of working standard

DS= Dilution factor for standard preparation

DT=Dilution factor for sample preparation

VALIDATION OF THE PROPOSED METHOD^[8]

The developed method for the simultaneous estimation of Emtricitabine, Tenofovir DF and Rilpivirine HCl was validated as per the ICH guidelines for the parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

System Suitability:

At first the HPLC system was optimized as per the chromatographic conditions. One blank followed by six replicates of a single calibration standard solution of 200µg/ml of Emtricitabine, 300µg/ml of Tenofovir DF and 25µg/ml of Rilpivirine HCl was injected to check the system suitability. To ascertain the system suitability for the proposed method, the parameters such as retention time, theoretical plates, peak asymmetry and resolution were taken and results were presented in Table 2.

Specificity:

The effect of excipients and other additives usually present in the combined tablet dosage form of Emtricitabine, Tenofovir DF and Rilpivirine HCl in the determination under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting the blank and placebo solution into the HPLC system. The representative chromatogram of blank and placebo was shown in Figure 5 and 6.

Linearity for Emtricitabine, Tenofovir DF and Rilpivirine HCl:

Aliquots of 0.25, 0.5, 0.75, 1, 1.25 and 1.5ml of mixed standard working solutions of Emtricitabine, Tenofovir DF and Rilpivirine HCl was pipette out from the standard stock solution of 2000µg/ml of Emtricitabine, 3000µg/ml of Tenofovir DF and 250µg/ml of Rilpivirine HCl and transferred into a series of 10ml clean dry volumetric flask and make volume up to the mark with the same diluent to get the concentration of 50, 100, 150, 200, 250 and 300µg/ml of Emtricitabine, 75,150,225,300,375and450µg/ml of Tenofovir DF and 6.25, 12.5, 18.75, 25, 31.25, and 37.5µg/ml of Rilpivirine HCl. The calibration standard solutions of Emtricitabine, Tenofovir DF and Rilpivirine HCl were injected using a 20µl Hamilton Rheodyne injector and the chromatograms were recorded at 265nm and a calibration graph was obtained by plotting peak area versus concentration of Emtricitabine, Tenofovir DF and Rilpivirine HCl respectively. The linearity data is presented in Figure 9 and Table 3. Acceptance Criteria: Correlation coefficient should be not less than 0.999

Accuracy studies for Emtricitabine, Tenofovir DF and Rilpivirine HCl:

The accuracy of the method was determined by calculating recovery of Emtricitabine, Tenofovir DF and Rilpivirine HCl by the method of standard addition. Known amount of standard solution of Emtricitabine, Tenofovir DF and Rilpivirine HCl at 50%, 100% and 150% was added to a pre quantified sample solution and injected into the HPLC system. The mean percentage recovery of Emtricitabine, Tenofovir DF and Rilpivirine HCl at each level was calculated and the results were presented in Table 4, 5 and 6.

Preparation of pre quantified sample solution for accuracy studies:

Tablet powder equivalent to 200mg of Emtricitabine, 300mg of Tenofovir DF and 25mg of Rilpivirine HCl were taken into 100ml clean dry volumetric flask and diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent. Further pipette out 0.5ml from the above Emtricitabine, Tenofovir DF and Rilpivirine HCl sample stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to get the concentration of 100µg/ml of Emtricitabine, 150µg/ml of Tenofovir DF and 12.5µg/ml of Rilpivirine HCl.

Preparation of standard solution of Emtricitabine, Tenofovir DF and Rilpivirine HCl for accuracy studies:

Standard stock solutions of Emtricitabine, Tenofovir DF and Rilpivirine HCl were prepared by dissolving 200mg of Emtricitabine, 300mg of Tenofovir DF and 25mg of Rilpivirine HCl in 100ml of diluent into a 100ml clean dry volumetric flask and the standard solutions were filtered through 0.45 µm nylon membrane filter and degassed by sonicator to get the concentration of 2000µg/ml of Emtricitabine, 3000µg/ml of Tenofovir DF and 250µg/ml of Rilpivirine HCl.

Preparation of 50% standard solution:

From the standard stock solution of 2000µg/ml of Emtricitabine, 3000µg/ml of Tenofovir DF and 250µg/ml of Rilpivirine HCl further pipette 0.25ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 50µg/ml of Emtricitabine, 75µg/ml of Tenofovir DF and 6.25µg/ml of Rilpivirine HCl.

Preparation of 100% standard solution:

From the standard stock solution of 2000µg/ml of Emtricitabine, 3000µg/ml of Tenofovir DF and 250µg/ml of Rilpivirine HCl further pipette 0.5ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 100µg/ml of Emtricitabine, 150µg/ml of Tenofovir DF and 12.5µg/ml of Rilpivirine HCl.

Preparation of 150% standard solution:

From the standard stock solution of 2000µg/ml of Emtricitabine, 3000µg/ml of Tenofovir DF and 250µg/ml of Rilpivirine HCl further pipette 0.75ml and transferred into a 10ml volumetric flask and dilute up to the mark with diluent to get the concentration of 150µg/ml of Emtricitabine, 225µg/ml of Tenofovir DF and 18.75µg/ml of Rilpivirine HCl.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Precision studies for Emtricitabine, Tenofovir DF and Rilpivirine HCl:**Method precision (Repeatability):**

Tablet powder equivalent to 200mg of Emtricitabine, 300mg of Tenofovir DF and 25mg of Rilpivirine HCl were taken into 100ml clean dry volumetric flask, diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent. Further pipette out 1ml from the above Emtricitabine, Tenofovir DF and Rilpivirine HCl sample stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to get the concentration of 200µg/ml of Emtricitabine, 300µg/ml of Tenofovir DF and 25µg/ml of Rilpivirine HCl. A homogenous sample of a single batch analysed six times and was checked whether the method is giving consistent results. The %RSD for the area of six replicate injections was calculated as mentioned in Table 7a and 7b.

Acceptance Criteria: The % RSD for the peak area of six sample injections should not be more than 2%.

System precision:

The system precision was carried out to ensure that the analytical system is working properly. The standard preparation concentration of 200µg/ml of Emtricitabine, 300µg/ml of Tenofovir DF and 25µg/ml of Rilpivirine HCl was injected six times into the HPLC and the %RSD for the area of six replicate injections was calculated as mentioned in Table 8.

Acceptance Criteria: The % RSD for the peak area of six standard injections should not be more than 2%.

Intermediate precision/ruggedness:

The intermediate precision (also known as Ruggedness) of the method was evaluated by performing precision on different lab by different analyst and different days. The standard preparation concentration of 200µg/ml of Emtricitabine, 300µg/ml of Tenofovir DF and 25µg/ml of Rilpivirine HCl was injected six times into the HPLC and the %RSD for the area of six replicate injections was calculated as mentioned in Table 9a and 9b.

Acceptance Criteria: The % RSD for the peak area of six standard injections should not be more than 2%.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as $3.3 \times SD/S$ and $10 \times SD/S$ respectively as per ICH guidelines, Where SD is the standard deviation of the response (Y-intercept) and S is the slope of the calibration curve. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD of Emtricitabine, Tenofovir DF and Rilpivirine HCl was calculated

and shown in Table 10. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Emtricitabine, Tenofovir DF and Rilpivirine HCl was calculated and shown in Table 10.

Robustness:

As part of the Robustness, deliberate change in the flow rate and mobile phase proportion of $\pm 10\%$ was made to evaluate the impact on the method. The results reveal that the method is robust. The results are summarized in Table 11 and 12.

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Emtricitabine, Tenofovir DF and Rilpivirine HCl were obtained with a mobile phase containing a mixture of 0.01M Potassium dihydrogen phosphate (pH adjusted to 4 with orthophosphoric acid) and Acetonitrile (30:70, v/v) was delivered at a flow rate of 1 ml/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 265nm based on peak area. The retention time of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl was found to be 1.976min, 2.661min and 4.316min respectively with resolution of 3.1 and 6.8 was shown in Figure 7. Linearity was established for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl in the range of 50-300 $\mu\text{g/ml}$ for Emtricitabine, 75-450 $\mu\text{g/ml}$ for Tenofovir Disoproxil Fumarate and 6.25-37.5 $\mu\text{g/ml}$ for Rilpivirine HCl with correlation coefficient 0.999 and mean accuracies were found to be 99.71 % to 99.96%, 99.68% to 100.05% and 99.82% to 100.08% for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine respectively, which indicates accuracy of the proposed method. The % RSD values of accuracy for Emtricitabine, Tenofovir DF and Rilpivirine HCl were found to be $< 2\%$. The % RSD values of method precision are 0.15%, 0.18% and 0.09% for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl respectively and % RSD values of system precision are 0.15%, 0.25% and 0.09% for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl. The % RSD values of reproducibility are 0.23%, 0.21% and 0.21% for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl respectively, reveal that the proposed method is precise. LOD values for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl were found to be 0.16 $\mu\text{g/ml}$, 0.26 $\mu\text{g/ml}$ and 0.07 $\mu\text{g/ml}$ respectively and LOQ values for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl were found to be 0.49 $\mu\text{g/ml}$, 0.80 $\mu\text{g/ml}$ and 0.21 $\mu\text{g/ml}$ respectively was shown in Table 10. The results reveal that the method is robust enough was shown in Table 11 and 12. These data show that the proposed method is specific and sensitive for the determination of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl. The results of system suitability testing are given in Table 2.

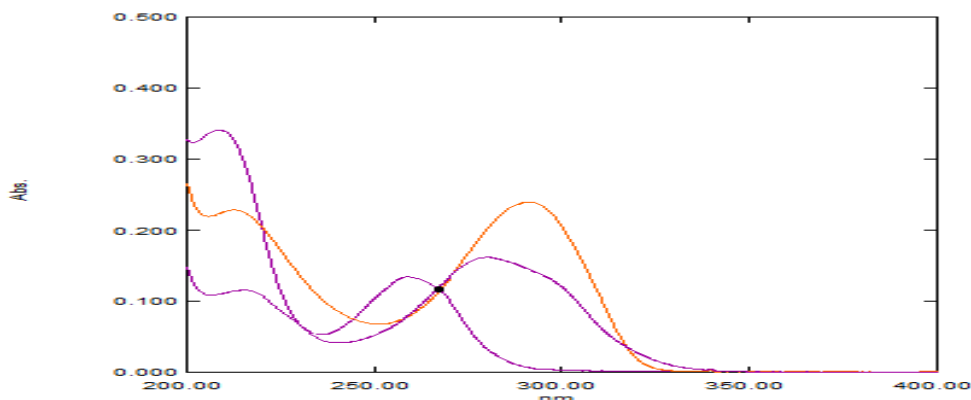


Figure 4: Isosbestic point of Emtricitabine, Tenofovir DF and Rilpivirine HCl at 265nm

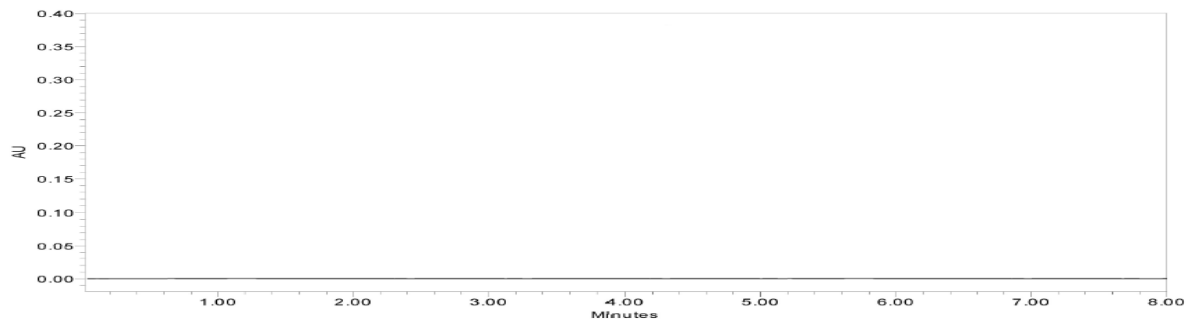


Figure 5: Chromatogram of Blank

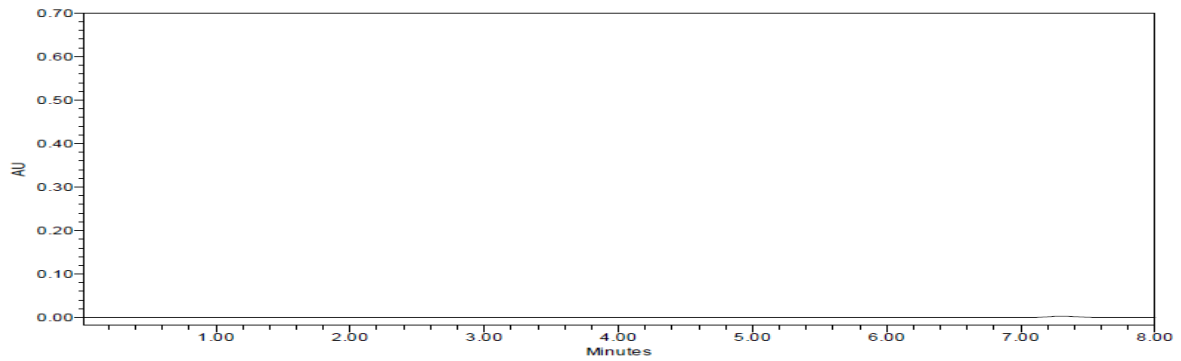


Figure 6: Chromatogram of Placebo

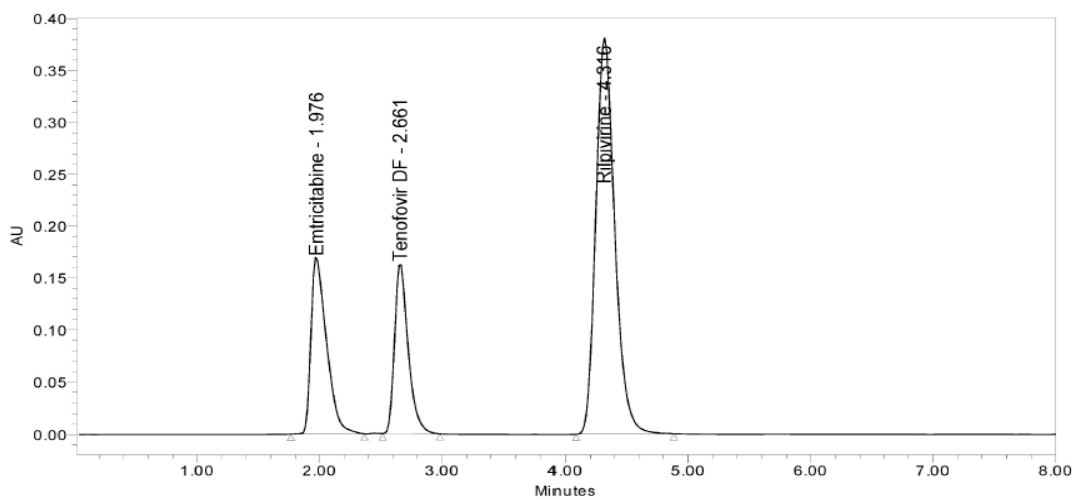


Figure 7: Standard chromatogram of Emtricitabine, Tenofovir DF and Rilpivirine HCl

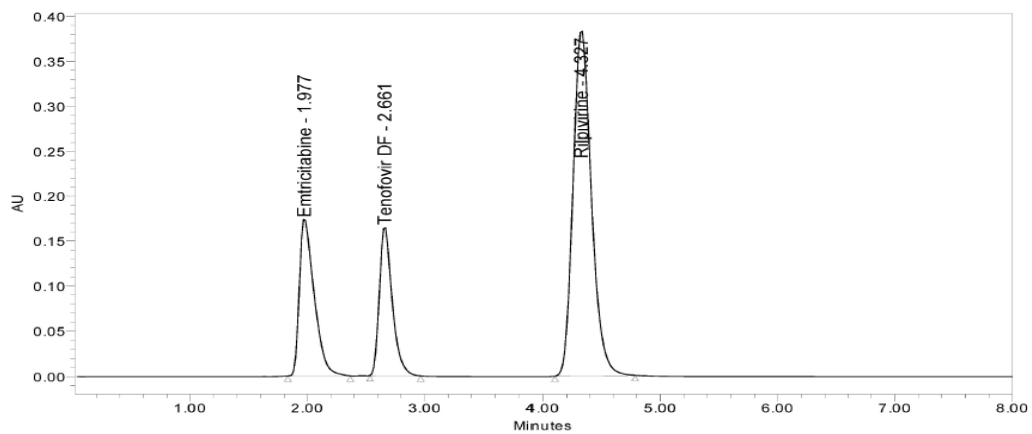


Figure 8: Sample chromatogram of Emtricitabine, Tenofovir DF and Rilpivirine HCl

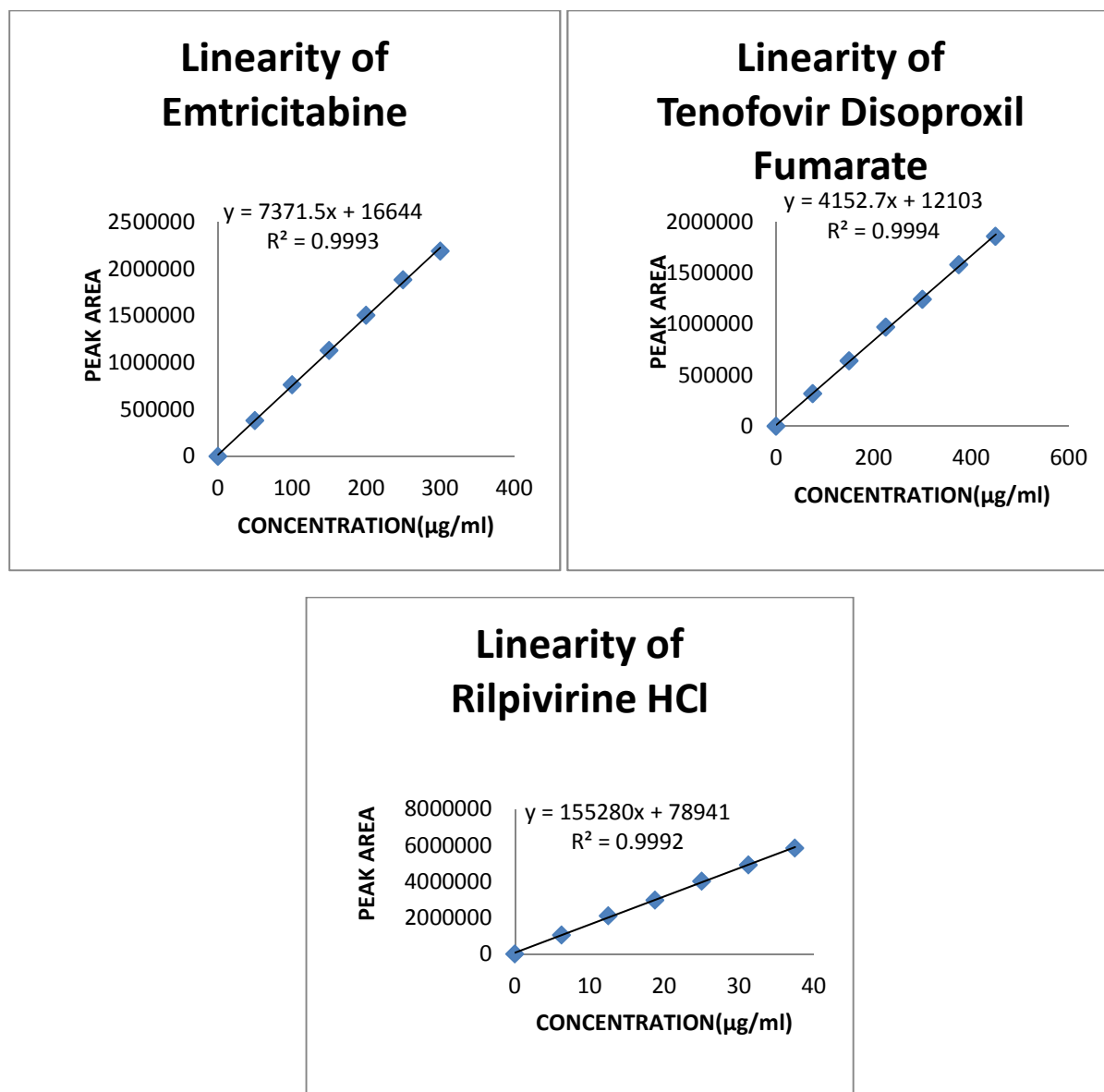


Figure 9: Linearity graph of Emtricitabine, Tenofovir DF and Rilpivirine HCl

Table 1: Assay of Marketed Formulation of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl

Drug	Compera Tablet Label Claim (mg)	Amount Found (mg)	% Label Claim ± % RSD (n=3)
Emtricitabine	200	200.06	100.03±0.49
Tenofovir DF	300	300.69	100.23±0.10
Rilpivirine HCl	25	24.99	99.96±0.13

Table 2: System Suitability Test Parameters for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl

Parameter (n=6)	Emtricitabine	Tenofovir DF	Rilpivirine HCl
Retention Time (Mins)	1.976	2.661	4.316
Theoretical plates	2110	2819	3726
Tailing factor	1.66	1.4	1.2
Resolution		3.1	6.8

Table 3: Linearity data for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl

Linearity of Emtricitabine		Linearity of Tenofovir Disoproxil Fumarate		Linearity of Rilpivirine HCl	
Concentration (µg/ml)	Peak Area	Concentration (µg/ml)	Peak Area	Concentration (µg/ml)	Peak Area
50	381625	75	320211	6.25	1054625
100	763170	150	640202	12.5	2109249
150	1130303	225	973091	18.75	2982076
200	1507070	300	1245443	25	4026511
250	1883838	375	1583016	31.25	4915813
300	2190605	450	1863283	37.5	5844768

Table 4: Recovery Study Data of Emtricitabine

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S ₁ :50%	50	50.05	100.11	Mean-99.96 S.D-0.13 %RSD-0.13
S ₂ :50%	50	49.92	99.85	
S ₃ :50%	50	49.96	99.92	
S ₄ :100%	100	99.53	99.53	Mean-99.71 S.D-0.27 %RSD=0.27
S ₅ :100%	100	99.57	99.57	
S ₆ :100%	100	100.03	100.03	
S ₇ :150%	150	149.69	99.79	Mean-99.8 S.D-0.06 %RSD-0.06
S ₈ :150%	150	149.61	99.74	
S ₉ :150%	150	149.8	99.87	

Table 5: Recovery Study Data of Tenofovir Disoproxil Fumarate

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S ₁ :50%	75	74.72	99.62	Mean-99.68 S.D-0.12 %RSD-0.12
S ₂ :50%	75	74.87	99.83	
S ₃ :50%	75	74.7	99.6	
S ₄ :100%	150	150.87	100.58	Mean-100.05 S.D-0.53 %RSD=0.52
S ₅ :100%	150	149.28	99.52	
S ₆ :100%	150	150.09	100.06	
S ₇ :150%	225	224.96	99.98	Mean-99.99 S.D-0.12 %RSD-0.12
S ₈ :150%	225	224.74	99.88	
S ₉ :150%	225	225.30	100.13	

Table 6: Recovery Study Data of Rilpivirine HCl

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S ₁ :50%	6.25	6.23	99.81	Mean-99.82 S.D-0.06 %RSD-0.06
S ₂ :50%	6.25	6.24	99.89	
S ₃ :50%	6.25	6.23	99.76	
S ₄ :100%	12.5	12.43	99.44	Mean-100.08 S.D-0.55 %RSD=0.55
S ₅ :100%	12.5	12.55	100.43	
S ₆ :100%	12.5	12.54	100.37	
S ₇ :150%	18.75	18.78	100.16	Mean-100.04 S.D-0.19 %RSD-0.19
S ₈ :150%	18.75	18.71	99.81	
S ₉ :150%	18.75	18.77	100.15	

Table 7 a: Method Precision Data for Emtricitabine and Tenofovir Disoproxil Fumarate

S.No.	Emtricitabine				Tenofovir Disoproxil Fumarate			
	Concentration (µg/ml)	Retention time (min)	Peak Area	% Assay	Concentration (µg/ml)	Retention time (min)	Peak Area	% Assay
1	200	1.980	1567126	99.75	300	2.663	1290554	100.46
2	200	1.977	1565687	99.61	300	2.663	1294583	100.71
3	200	1.977	1566715	99.97	300	2.663	1290636	100.16
4	200	1.980	1563086	99.83	300	2.663	1293145	100.44
5	200	1.977	1564475	99.71	300	2.664	1295901	100.40
6	200	1.980	1560781	99.56	300	2.666	1297458	100.33
Average		1.9785	1564645	99.74	Average	2.664	1293713	100.42
SD		0.0015	2404.824	0.15	SD	0.0011	2804.964	0.18
%RSD		0.076	0.15	0.15	%RSD	0.041	0.22	0.18

Table 7 b: Method Precision Data for Rilpivirine HCl

Rilpivirine HCl				
S.No.	Concentration (µg/ml)	Retention time (min)	Peak Area	% Assay
1	25	4.326	4252542	99.70
2	25	4.325	4254181	99.68
3	25	4.327	4250027	99.79
4	25	4.326	4256384	99.91
5	25	4.329	4253125	99.87
6	25	4.331	4253860	99.77
Average		4.327	4253353	99.79
SD		0.0020	2092.92	0.09
%RSD		0.047	0.05	0.09

TABLE 8: System Precision Data for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl

Emtricitabine				Tenofovir Disoproxil Fumarate			Rilpivirine HCl		
S.No.	Conc. (µg/ml)	Retention time (min)	Peak Area	Conc. (µg/ml)	Retention time (min)	Peak Area	Conc. (µg/ml)	Retention time (min)	Peak Area
1	200	2.305	1567914	300	4.108	1282101	25	4.108	4256697
2	200	2.301	1568744	300	4.11	1282937	25	4.11	4259284
3	200	2.305	1564009	300	4.105	1285951	25	4.105	4250328
4	200	2.304	1562567	300	4.109	1284895	25	4.109	4251864
5	200	2.304	1565936	300	4.108	1288116	25	4.108	4250071
6	200	2.301	1564570	300	4.111	1290598	25	4.111	4255049
Average		2.30333333	1565623	Average	4.109	1285766	Average	4.109	4253882
SD		0.0018619	2372.35	SD	0.00207	3196.844	SD	0.00207	3733.488
%RSD		0.08	0.15	%RSD	0.05	0.25	%RSD	0.05	0.09

TABLE 9 a: Ruggedness Data for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl

Ruggedness Data for Emtricitabine									
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2				
Conc. (µg/ml)	Analyst-1		Analyst-2		Analyst-1		Analyst-2		
	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	
200	100.01	99.67	99.75	99.78	99.65	99.89	101.25	99.93	
200	100.06	99.64	99.84	99.71	99.65	99.91	101.22	99.94	
200	99.76	99.78	99.81	99.52	99.77	100.80	101.19	100.43	
200	99.67	100.19	99.79	99.03	100.18	100.82	101.15	100.65	
200	99.88	99.75	99.80	99.01	99.77	100.82	100.93	100.44	
200	99.79	99.76	99.87	99.02	99.77	100.85	100.94	100.46	
Average	99.86	99.79	99.81	99.34	99.79	100.51	101.11	100.30	
SD	0.15	0.2	0.04	0.37	0.2	0.48	0.14	0.3	
%RSD	0.15	0.2	0.04	0.37	0.2	0.47	0.14	0.3	
Intermediate precision within-laboratories variations (n=24)									
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2				
Average	99.7				Average	100.43			
SD	0.19				SD	0.28			
%RSD	0.19				%RSD	0.28			
Reproducibility between laboratories (n=48) (% Assay)									
Average	100.06								
SD	0.23								
%RSD	0.23								
Ruggedness Data for Tenofovir Disoproxil Fumarate									
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2				
Conc. (µg/ml)	Analyst-1		Analyst-2		Analyst-1		Analyst-2		
	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	
300	100.46	99.76	100.14	99.17	99.83	99.88	99.41	99.41	
300	100.71	99.40	100.22	99.82	99.79	99.89	99.68	99.44	
300	100.16	100.38	99.77	100.40	99.76	99.39	99.84	99.28	
300	100.44	99.70	99.95	99.8	99.77	99.90	99.58	99.50	
300	100.4	99.87	99.52	99.81	99.81	99.80	99.48	99.6	
300	100.33	99.68	99.67	99.77	99.84	99.92	99.38	99.76	
Average	100.41	99.79	99.87	99.79	99.8	99.79	99.56	99.49	
SD	0.18	0.32	0.27	0.38	0.03	0.2	0.17	0.16	
%RSD	0.17	0.32	0.27	0.39	0.03	0.2	0.17	0.16	
Intermediate precision within-laboratories variations (n=24)									
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2				
Average	99.96				Average	99.66			

SD	0.28	SD	0.14
%RSD	0.28	%RSD	0.14
Reproducibility between laboratories (n=48) (% Assay)			
Average	99.81		
SD	0.21		
%RSD	0.21		

TABLE 9 b: Ruggedness Data for Rilpivirine HCl

Ruggedness Data for Rilpivirine HCl								
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
Conc. (µg/ml)	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
25	99.7	100.86	99.70	99.65	99.89	99.63	100.38	99.76
25	99.68	100.78	100.19	99.85	99.92	99.15	100.47	99.54
25	99.79	100.67	99.50	100.82	99.80	99.17	100.95	99.49
25	99.91	100.90	99.65	99.80	99.69	99.20	100.30	99.45
25	99.87	101.02	99.79	100.81	99.73	99.86	100.87	99.79
25	99.77	100.67	99.80	99.78	99.82	99.32	100.39	99.57
Average	99.78	100.81	99.77	100.11	99.81	99.39	100.56	99.6
SD	0.09	0.13	0.23	0.54	0.08	0.29	0.27	0.14
%RSD	0.09	0.13	0.23	0.54	0.08	0.29	0.27	0.14
Intermediate precision within-laboratories variations (n=24)								
Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
Average	100.12				Average	99.84		
SD	0.24				SD	0.19		
%RSD	0.24				%RSD	0.19		
Reproducibility between laboratories (n=48) (% Assay)								
Average	99.98							
SD	0.21							
%RSD	0.21							

Table 10: Summary of Validation Parameter for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl

Parameters	RP-HPLC method					
	Emtricitabine		Tenofovir Disoproxil Fumarate		Rilpivirine HCl	
Linearity range (µg/ml)	50-300		75-450		6.25-37.5	
Slope	7371		4152		15528	
Intercept	16644		12103		78941	
Correlation coefficient	0.999		0.999		0.999	
LOD (µg/ml)	0.16		0.26		0.07	
LOQ (µg/ml)	0.49		0.80		0.21	
Method Precision (% RSD, n=6)	0.15		0.18		0.09	
System precision (% RSD, n=6)	0.15		0.25		0.09	
Ruggedness (% RSD, n=24)	Lab-1	Lab-2	Lab-1	Lab-2	Lab-1	Lab-2
	0.19	0.28	0.28	0.14	0.24	0.19
Reproducibility (% RSD, n=48)	0.23		0.21		0.21	
% Accuracy	99.71-99.96		99.68-100.05		99.82-100.08	
Robustness (% RSD, n=3)	Less Flow rate	More Flow rate	Less Flow rate	More Flow rate	Less Flow rate	More Flow rate
	0.16	0.49	0.17	0.67	0.57	0.86
	Less Organic phase	More Organic phase	Less Organic phase	More Organic phase	Less Organic phase	More Organic phase
	0.23	0.88	0.49	0.54	0.38	0.50

Table 11: Summary of Robustness (Change in Flow Rate) for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl

Drug	Change in Flow rate (ml/min)	Retention Time (Mins)	Robustness (0.9 ml/min to 1.1 ml/min)				
			Average peak area (n=3)	SD	% RSD	USP Plate Count	Asymmetry
Emtricitabine	0.9	2.450	2108868	3489.164	0.16	2052	1.8
	1.0	1.976	1505604	12982.58	0.86	2110	1.66
	1.1	1.661	1413420	6995.101	0.49	2126	1.5
Tenofovir Disoproxil Fumarate	0.9	3.305	1725249	2922.121	0.17	2837	1.5
	1.0	2.661	1246476	7071.198	0.56	2819	1.4
	1.1	2.232	1127115	7559.275	0.67	2621	1.3
Rilpivirine HCl	0.9	5.378	5653056	32356.73	0.57	3778	1.2
	1.0	4.316	4103179	34851.1	0.85	3726	1.2
	1.1	3.624	3829954	33002.12	0.86	3449	1.2

Table 12: Summary of Robustness (Change in Mobile Phase) for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl

Drug	Change in Mobile Phase	Retention Time (Mins)					
			Average peak area (n=3)	SD	% RSD	USP Plate Count	Asymmetry
Emtricitabine	10% less Organic (37:63 v/v)	1.657	1439403	3345.916	0.23	2971	1.7
	Actual (30:70 v/v)	1.976	1505604	12982.58	0.86	2110	1.66
	10% more Organic (23:77 v/v)	1.673	1373705	12156.07	0.88	2276	1.4
Tenofovir Disoproxil Fumarate	10% less Organic (37:63 v/v)	2.406	1178986	5763.37	0.49	2692	1.3
	Actual (30:70 v/v)	2.661	1246476	7071.198	0.56	2819	1.4
	10% more Organic (23:77 v/v)	2.116	1144323	6224.77	0.54	2600	1.3
Rilpivirine HCl	10% less Organic (37:63 v/v)	4.224	4041187	15239.82	0.38	3536	1.2
	Actual (30:70 v/v)	4.316	4103179	34851.1	0.85	3726	1.2
	10% more Organic (23:77 v/v)	3.194	3807210	19101.63	0.50	3356	1.2

CONCLUSION

RP-HPLC method for simultaneous estimation of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl in their combine dosage form was established and validated as per the ICH guidelines. Linearity was achieved for Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl in the range of 50-300µg/ml for Emtricitabine, 75-450µg/ml for Tenofovir Disoproxil Fumarate and 6.25-37.5µg/ml for Rilpivirine HCl with correlation coefficient 0.999. The percentage recovery of drug was achieved in the range of 98-102% which was within the acceptance criteria. The percentage RSD was NMT 2 % which proved the precision of the developed method. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust. Hence it can be used for the routine analysis of Emtricitabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl in their bulk and combine dosage form.

Acknowledgement

The authors are thankful to Malla Reddy College of Pharmacy for providing the chemicals and instruments and Hetero Drugs Limited, Hyderabad, India for providing the samples for research.

REFERENCES

- [1] Nimish Patel, Christopher D Miller: *HIV/AIDS - Research and Palliative Care Dove Press Journal*. **2014**, 61–71.
- [2] Kavitha K. Y, Geetha. G, Hariprasad.R, Venkatnarayanan. R, Kaviarasu. M.: *IRJP*. **2013**: 4(1), 150-151.
- [3] Narendra Devanaboyina, Satyanarayana T and Ganga Rao B: *International Journal of Research in Pharmaceutical and Biomedical Sciences*. **2012**:3(1), 360-367.
- [4] Appala RN, Shabana B.: *Research J Pharm and Tech*. **2008**:1(4),522-5.
- [5] Noel AG, Vikas VV, Ashutosh P, Santosh SJ, Sagar AP.: *Journal of Pharmaceutical and Biomedical Analysis*. **2008**: 48(3), 918-26.
- [6] Anindita Behera, Aurobinda Parida, Amit Kumar Meher, Dannana Gowri Sankar, Swapan Kumar Moitra, Sudam Chandra Si: *International Journal of PharmTech Research*. **2011**:3(3), 1874-1882.
- [7] Shabir GA: *J Chromatogr A*. **2003**: 987, 57–66.
- [8] International conference of Harmonization (ICH) – Guidance for Industry – Q2B validation of Analytical procedures: Methodology, **1996**, ICH.