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SIMULTANEOUS ESTIMATION OF EMTRICETABINE, TENOFOVIR DISOPROXIL FUMARATE AND RILPIVIRINE HCL IN TABLET DOSAGE FORMS BY RP-HPLC

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ABSTRACT

A simple, precise, rapid and accurate RP- HPLC method was developed for the estimation of Emtricetabine (FTC), Tenofovir Disoproxil Fumarate (TDF) and Rilpivirine HCl (RPV)in tablet dosage forms. An Inertsil ODS 3V, 250x4.6 mm, column with 5 µm particle size and the Mobile Phase- A, consisting of 0.03M KH_2PO_4 in water adjusting the pH-3.2 with dilute O-Phosphoric Acid, Mobile Phase-B consisting of Methanol & Water in ratio of 85:15 v/v & Acetonitrile & Buffer in ratio of 70:30 v/v, was used as diluent in the gradient mode. The flow rate was 1.5 ml/min and the effluents were monitored at 265 nm. The retention times were 6.250 min for FTC, 8.386 min for TDF and 10.296 min for RPV successively. The detector response was linear in the concentration of 80-960 µg/mL for FTC, 120-1440 µg/mL for TDF and 10-120 µg/mL for RPV. The respective linear regression equation being Y= 9474.289x + 147734.8116 for FTC, Y = 6903.437x + 202292.0234 for TDF and Y= 25680.392x + 15736.147 for RPV. The Limit of Detection (LOD) is 0.4, 0.06 and 0.5 µg for FTC, TDF and RPVrespectively. The Limit of Quantification (LOQ) is 1.2, 0.18 and 1.5 µg for FTC, TDF and RPV. The percentage assay of FTC, TDF and RPV were 98.60%, 98.68% and 99.39% respectively. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of FTC, TDF and RPV in bulk drug and in its pharmaceutical dosage forms.

Keywords: Emtricetabine (FTC), Tenofovir Disoproxil Fumarate (TDF), Rilpivirine HCl (RPV), RP-HPLC, Estimation, Tablets.

INTRODUCTION

Emtricitabine, chemically, 4-amino-5fluoro-1[(2S, 5R)-2-(hydroxymethyl)-1, 3-oxathiolan-5yl]-1, 2-dihydropyrimidin-2-one, is a (-) enantiomer of a thio analogue of cytidine. (Figure: 1).The empirical formula is $C_8H_{10}FN_3O_3S$ & the molecular weight is 247.248 gms/mol. It is a nucleoside analog [1-2] reverse transcriptase inhibitor. Tenofovir Disoproxil Fumarate (TDF or PMPA), chemically 9-((R)-2-((bis (isopropoxycarbonyl) oxy) methoxy) phosphinyl)methoxy)propyl) adenine Fumarate(Figure: 2). The empirical formula is $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$& the molecular weight is 635.52gms/mol. It is also, a nucleotide analogue reverse transcriptase inhibitor [3-4]. Rilpivirine (RPV/TMC278), chemically Benzonitrile, 4-[[4-[[4-[(1E)-2-Cyanoethenyl]-2, 6-dimethylphenyl] amino]-2-pyrimidinyl] amino]-, hydrochloride (Figure: 3). It is a diarylpyrimidine non-nucleoside [5-6] reverse transcriptase inhibitor. The empirical formula is $C_{22}H_{18}N_6$.HCl & the molecular weight

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is 402.9 gms/mol. The study revealed that once daily regimen containing FTC, TDF and RPV were virologically and immunologically effective [7-8], well tolerated and safe with benefits in the lipid profile in the majority of patients. Literature survey reveals a few chromatographic methods [9-14]to determine FTC, TDF & RPV in tablet dosage form and also in biological fluids. HPLC methods are useful in the determination of drugs in pharmaceutical formulations, especially those containing more than one active component. From the literature, neither liquid chromatography methods nor assay methods have been reported for the simultaneous estimation of FTC, TDF & RPV in pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of FTC, TDF & RPV in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reverse-phase HPLC method for the estimation of FTC, TDF & RPV in bulk drug samples and also in pharmaceutical dosage forms.

EXPERIMENTAL

Materials / Chemicals and Reagents

FTC, TDF & RPV were obtained as a gift samples from M/s Mylan Pharmaceuticals Pvt. Ltd, Hyderabad, Andhra Pradesh. Acetonitrile, Methanol and water used were of HPLC grade (Qualigens). Potassium Dihydrogen Orthophosphate and Ortho- Phosphoric Acid were obtained from SDFCL, Mumbai. Commercially available tablets (Complera®- Gilead Sciences, Inc.) were procured from local market.

Chromatography Instrument

Quantitative HPLC was performed on liquid Chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 10 µl, and 2693 pump. An Inertsil ODS 3V, RP-C_{18} Column (250x4.6 mm i.d; particle size 5 µ) was used. The HPLC system was equipped with Empower 2 Software. The column was maintained at 40^{o} C and eluted under isocratic conditions over 15.0 min at a flow rate of 1.5 ml/min.

HPLC Conditions

The contents of the Mobile Phase A - consisting of 4.08 gms of 0.03M KH_2PO_4 in 1000 ml of water adjusting the pH:3.2 with dilute O-Phosphoric Acid, Mobile Phase B consisting of Methanol & Water in ratio of 85:15 v/v & Acetonitrile & Buffer in ratio of 70:30 v/v, was used as diluent in the gradient mode.They were filtered before use, through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.5 ml/min. The run time was set at 25.0 min and the column temperature was ambient. Prior to the injection (10 µl) of the drug solution, the column was equilibrated for at least 30 min with the mobile phases flowing through the system. The eluents were monitored at 265 nm.

Preparation of the Primary Standard/Stock Drug Solution

A standard stock solution of the drug was prepared by dissolving 400 mg of FTC, 600 mg of TDF & 50 mg of RPV in 50 ml volumetric flask containing 15 ml of diluent (Acetonitrile: Buffer 70:30 v/v), sonicated for about 15 min and then made up to 50 ml with Methanol to get standard stock solution of 0.8 mg/mL of FTC, 1.2 mg/mL of TDF & 0.1 mg/mL of RPV.

Preparation of the Working Standard Drug Solution

5ml of the above stock solutions were taken in 50 ml volumetric flask and made up to 50 ml with diluents (Acetonitrile: Buffer - 70:30 v/v) to get a concentration of each 800 µg/mL of FTC, 1200 µg/mL of TDF and 100 µg/mL of RPV respectively.

Preparation of Sample solution

Twenty tablets (Complera® - Gilead Sciences, Inc.) were weighed, and then powdered. A sample of the powdered tablets, equivalent to mixture containing concentration of each 0.8 mg/mL of FTC, 1.2 mg/mL of TDF & 0.1 mg/mL of RPV active ingredients, were mixed with 15 ml of Acetonitrile: Buffer - 70:30 v/v as diluent in 50 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drugs, and then filtered through a 0.45 µm membrane filter, followed by addingMethanol up to 50 ml to obtain a stock solution. 5ml of the above sample stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent to get a concentration of each 800 µg/mL of FTC, 1200 µg/mL of TDF and 100 µg/mL of RPV respectively.

Linearity

Aliquots of standard FTC, TDF & RPV stock solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of FTC, TDF & RPV were in the range of 40-1200 µg/mL, 60-1800 µg/mL and 5-150 µg/mL respectively. Each of these drug solutions (10 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 265 nm and the Calibration graphs were obtained by plotting peak area versus concentration of FTC, TDF & RPV (Figure: 4). The plot of peak areas of each sample against respective concentration of FTC, TDF & RPV were found to be linear in the range of 80-960 µg/mL, 120-1440 µg/mL and 10-120 µg/mL with correlation coefficient of 0.9993. Linear regression least square fit data obtained from the measurements are given in Table 1. The respective linear regression equation being Y= 9474.289x + 147734.8116 for FTC, Y = 6903.437x + 202292.0234 for TDF and Y= 25680.392x + 15736.147 for RPV. The regression

characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table 1.

Accuracy

Accuracy was evaluated in triplicate by addition of three different amounts of FTC, TDF & RPV, to a previously analyzed sample and comparing the amounts of analytes recovered with the amounts added. The amounts added were equivalent to 80, 100, and 120% of the amount originally present. %Recovery and RSD (%) were calculated for amount added. From the data obtained, it is obvious that the method is remarkably accurate, which ensures that this method produces reliable results as depicted in Table2.

Precision

The precision of the method was ascertained, separately from the peak area obtained by actual determination of six replicas of a fixed amount of the drug and formulation.

The HPLC systems were set up, describing chromatographic conditions, mentioned as above and following the system equilibration of the working standard solution containing 800 µg/mL of FTC, 1200 µg/mL of TDF and 100 µg/mL of RPV was injected six times and the response peak areas were recorded. The precision was repeated with the formulated sample for the same concentrations by injecting the working sample solutions containing 800 µg/mL of FTC, 1200 µg/mL of TDF and 100 µg/mL of RPV.The sample (Complera® - Gilead Sciences, Inc.) was processed six times for the response of peak area. The % Relative Standard Deviation (RSD) and % range of error (at 0.05 and 0.01 confidence levels) were calculated and presented in Tables 3&4 respectively.

Limits of Detection and Quantitation

Limit of Detection (LOD) of the method was determined as the lowest concentrations of active pharmaceutical ingredients producing a signal-to-noise (S/N) ratio of about 3. The Limit of Quantitation (LOQ) was determined as the lowest concentrations of active pharmaceutical ingredients capable of being quantified with acceptable accuracy and precision producing signal-to-noise (S/N) ratio of about 10.

Method Applicability

The present developed method was evaluated by applying to Pharmaceutical dosage forms for the estimation of FTC, TDF & RPV by our research group.

Assay

10 µl of sample solution (Complera® - Gilead Sciences, Inc.) was injected into the injector of liquid chromatograph. The retention times were found to be 6.250 min for FTC, 8.386 min for TDF and 10.296 min for RPV successively. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table 2.

Recovery Studies

Accuracy was determined by recovery studies of FTC, TDF & RPV; known amount of standard was added to the pre-analyzed sample and subjected to the proposed HPLC analysis. Results of recovery studies are shown in **Table 2**. The study was done at three different concentration levels.

RESULTS AND DISCUSSION

HPLCMethod Development and Optimization

In response to lack of simple, reliable and easy-to-use method for the determination of FTC, TDF & RPV concentrations in pharmaceutical matrices, an isocratic Reversed-Phase HPLC method was developed for quantification of above mentioned, API. We examined several HPLC method variables with respect to their corresponding effects on the result of analysis. To optimize the chromatographic conditions, different combinations of Methanol-Water, and Acetonitrile-Water and Acetonitrile-Di-Potassium Phosphate buffer were tested. Water with Phosphate buffer system [pH 3.2] was promisingly preferred, because it resulted in greater resolution of API after several preliminary investigatory runs, compared with other mobile phases (Table7). The other parameters in this factorial design were temperature, flow rate, detection wavelength and volume of injection. Buffer molarity was changed and optimum buffer strength was selected as 0.03M on the basis of theoretical plate number. At 265 nm, UV responses of all three active pharmaceutical analytes were good and free form interferences. Under these conditions, the analyte peaks were well defined and free from tailing. Considering the whole body of the data obtained from this extensive study, the set of conditions indicated earlier in this article was selected for further validation. Typical chromatogram of FTC, TDF & RPV (Standard and Working Sample) has been shown in Figure 5 & 6.

The system suitability tests were carried out on freshly prepared standard stock solutions of FTC, TDF & RPV. Parameters that were studied to evaluate the suitability of the system were discussed and presented in Table 5 [15].

Method Validation Tests

Recommended method validation characteristics including Method precision (RSD, %), Method accuracy (Recovery % and RSD, %), Linear range (Correlation Coefficient), and LOD & LOQ, were investigated systematically.

Linearity

The plot of peak areas of each sample against respective concentrations were found to be linear, in the range of 80-960 µg/ml for FTC, 120-1440 µg/ml for TDF

and 10-120 µg/ml for RPV with Correlation Coefficient of 0.9993 (Table 1). Linear regression least square fit data obtained from the measurements are given in Table 1. The respective linear regression equation being Y= 9474.289x + 147734.8116 for FTC, Y = 6903.437x + 202292.0234 for TDF and Y= 25680.392x + 15736.147 for RPV. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table 1. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table1.These results show that there was an excellent correlation between peak areas and analyte concentration.

Accuracy

Recovery of the individual substances at 80%, 100%, and 120% of specified concentrations were between 90.83% -107.00%, which proves the accuracy of the method. From these data, RSD was always less than 1%, which indicates it is obvious that the method is remarkably accurate, produces reliable results (Table 2).

Precision

The low value (<1%) of RSD indicates the repeatability of the method. These data indicate a considerable degree of precision and reproducibility for the method both during one analytical run and between different runs (Table 3 &4).

Robustness

Robustness was studied out to evaluate the effect of small but deliberate variations in the chromatographic conditions at three different levels, i.e. –2, 0, +2. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the columns by ±2 nm (263 nm and 267 nm), mobile phase buffer to Acetonitrile ratio (68:32 and 72:28, *v*/*v*), mobile phase pH by ±0.2 units (pH 3.0 and 3.4), and mobile phase flow rate by 0.8 mL min−1 (0.6 and 1.0 mL min−1) had no significant effect on the retention time and chromatographic response of the method, indicating that the method was robust. The results are shown in Table6.

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

The Limit of Detection (LOD) found was 0.4, 0.06 and 0.5 µg for FTC, TDF & RPV respectively. The Limit of Quantification (LOQ) analyzed was 1.2, 0.18 and 1.5 µg for FTC, TDF & RPV respectively. These values reflect the high sensitivity of the method, which is of great importance in most studies and also indicating the method can be used for detection and quantification of analytes in a very wide concentration range.

Specificity

No evidence of signals, in the corresponding times of the chromatogram were monitored as a sign of potential interfering peaks, were found when the pharmaceutical metered dose inhalers were tested. Hence, this method can be used reliably for the estimation of respected active pharmaceutical ingredients in a variety of dosage forms.

Table 1. Linear Regression Data of Calibration Curves

Parameter	Emtricetabine (FTC)	Tenofovir Disoproxil Fumarate (TDF)	Rilpivirine HCl (RPV)
Concentration range(µg/mL)	80-960	120-1440	10-120
Slope (m)	9474.289	6903.437	25680.392
Intercept (Y)	374713.3003	147718.0793	26099.0326
Standard error of estimate (c)	147734.8116	202292.0234	15736.147
Correlation coefficient (r)	0.999	0.999	1
Linear regression (r^2)	0.998	0.997	1
%RSD	0.4	0.3	0.2

Table 2. Assay & Recovery Accuracy Studies of Emtricetabine (Ftc), Tenofovir Disoproxil Fumarate (Tdf) &Rilpivirine Hcl (Rpv) In Tablet Dosage Forms

Tablet formulation	Amount claim (mg/tablet)	Amount claim (mg/tablet)	Amount claim (mg/tablet)	Amount Obtained (mg)* by proposed method			** % Recovery by the Proposed method		
	Emtricetabine	Tenofovir Disoproxil Fumarate	Rilpivirine HCl	Emtricetabine	Tenofovir Disoproxil Fumarate	Rilpivirine HCl	Emtricetabine	Tenofovir Disoproxil Fumarate	Rilpivirine HCl
1). 120%	200	300	25	205.00	321.5	26.5	95.41	96.75	103.33
2).100%	200	300	25	200.05	300.2	25.1	100.66	96.58	107.00
3). 80%	200	300	25	200.75	300.2	25.1	90.83	99.91	103.80
Average Mean	200	300	25	201.93	307.3	25.56	95.63	97.74	104.71

***Average of three determinations** After spiking the sample**

Continued….

Accuracy Parameter	Emtricetabine		Tenofovir Disoproxil Fumarate		Rilpivirine HCl	
Assay (120%)	129.65%		130.02%		132.00%	
Assay (100%)	110.68%		110.27%		112.23%	
Assay (80%)	89.74%		90.93%		91.97%	
	Standard	**Spiked**	**Standard**	**Spiked**	**Standard**	**Spiked**
% RSD (120%)	1.7	2.1	1.5	2.4	1.7	3.2
% RSD (100%)	1.5	0.4	2.0	0.8	0.4	0.7
% RSD (80%)	0.8	0.1	0.7	0.2	0.8	0.3
	Area		**Area**		**Area**	
Standard Deviation (120%)	168971.6	224001.3	163018.0	269635.1	57673.1	114363.4
Standard Deviation (100%)	125153.2	32919.3	174609.5	77676.3	10477.4	22036.9
Standard Deviation (80%)	52850.3	9801.4	50719.7	14419.9	17834.6	8532.9

Table 3. Precision of Recommended Procedure Using Standard Drugs: Emtricetabine (FTC), Tenofovir Disoproxil Fumarate (TDF) & Rilpivirine HCl (RPV)

Sr. No	Inj. No	Name of the Drug & Conc. (800 µg/ml)	Retention time in minutes	Peak Area	Name of the Drug & Conc. (1200 µg/ml)	Retention time in minutes	Peak Area	Name of the Drug & Conc. (100 µg/ml)	Retention time in minutes	Peak Area
1	1	FTC	6.278	7696446	TDF	8.439	8209779	RPV	10.487	2341031
2	2	FTC	6.288	7683636	TDF	8.452	8202956	RPV	10.505	2339563
3	3	FTC	6.270	7810952	TDF	8.429	8330977	RPV	10.432	2357141
4	4	FTC	6.280	7771205	TDF	8.432	8277249	RPV	10.442	2340346
5	5	FTC	6.265	7697062	TDF	8.414	8262495	RPV	10.406	2358919
6	6	FTC	6.269	7692024	TDF	8.420	8233647	RPV	10.402	2371258
7	Mean		6.275	7725220.8	----	8.431	8252850.5	----	10.446	2351376.2
8	Standard Deviation		0.009	52757.1	----	0.013	47969.6	----	0.042	13066.2
9	% RSD		0.14	0.7	----	0.16	0.6	----	0.40	0.6

Table 4. Precision of Recommended Procedure Using Sample - Complera®

Sr. No	Inj. No	Name of the Drug & Conc. (800 µg/ml)	Retention time in minutes	Peak Area	Name of the Drug & Conc. (1200 µg/ml)	Retention time in minutes	Peak Area	Name of the Drug & Conc. (100 µg/ml)	Retention time in minutes	Peak Area
1	1	FTC	6.270	7631294	TDF	8.417	8207710	RPV	10.381	2341114
2	2	FTC	6.268	7654884	TDF	8.413	8211530	RPV	10.362	2330163
3	3	FTC	6.261	7591030	TDF	8.402	8181076	RPV	2329106	2329106
4	4	FTC	6.254	7611878	TDF	8.397	8232932	RPV	10.320	2330239
5	5	FTC	6.250	7649795	TDF	8.386	8214311	RPV	10.296	2337993
6	6	FTC	6.255	7575501	TDF	8.394	8178072	RPV	10.302	2330659
7	Mean		6.260	7619063.4	----	8.402	8204272.0	----	10.333	2333212.3
8	Standard Deviation		0.008	31975.9	----	0.012	21029.2	----	0.034	5036.20
9	% RSD		0.13	0.4	----	0.14	0.3	----	0.33	0.2

Table 5. Validation Summary / System Suitability

Parameter	Emtricetabine (FTC)	Tenofovir Disoproxil Fumarate(TDF)	Rilpivirine HCl (RPV)
Theoretical Plates(N)	52146.37	57811.40	32612.16
Tailing factor	1.17	1.13	0.94
Retention time(min)	6.250	8.386	10.296
Resolution	4.95	1.97	1.79
Area	15476664	5906395	17314485
% Peak Area	99.95	99.20	99.77
LOD (µg/mL)	0.4	0.06	0.5
LOQ (µg/mL)	1.2	0.18	1.5

Table 6. Results from testing of the Robustness of the method (n=3, 100% of the Working Standard Solution & Sample solution contains: 800 µg/mL of Emtricetabine (FTC), 1200 µg/mL of Tenofovir Disoproxil Fumarate (TDF) & 100 µg/mL of Rilpivirine HCl (RPV)

Condition Studied in Robustness	Modification In OFAT analysis	Mean Peak Area ± S.D				% RSD (Peak Area)			Mean Retention Time (in min) ± S.D			% RSD (Retention time)		
		Parameter Fixation	FTC	TDF	RPV	FTC	TDF	RPV	FTC	TDF	RPV	FTC	TDF	RPV
Column(s) (Inertsil ODS 3V)	Hypersil &, Hypurity C_{18}	Std	8463052.3 ± 18085.3	8970840.3 ± 16617.9	2760882.0 ± 27396.4	0.2	0.2	1.0	6.243 ± 0.004	8.435 ± 0.002	9.963 ± 0.003	0.06	0.02	0.03
		Sample	8762072.9 ± 56212.5	9175763.1 ± 49267.6	2764749.3 ± 9872.3	0.6	0.5	0.4	6.248 ± 0.003	8.437 ± 0.003	9.956 ± 0.004	0.05	0.04	0.04
Flow rate (1.5 ml/min)	1.7 ml/min & 1.3 ml/min	Std – Increase	7875785.7 ± 40250.1	8379263.6 ± 21605.2	2674545.1 ± 42642.0	0.5	0.3	1.6	6.077 ± 0.010	8.210 ± 0.011	9.629 ± 0.015	0.17	0.13	0.16
		Std- Decrease	8369865.3 ± 9744.1	8848632.6 ± 21116.6	2788811.6 ± 10874.0	0.1	0.2	0.4	6.421 ± 0.005	8.675 ± 0.004	10.298 ± 0.005	0.08	0.05	0.05
		Sample- Increase	7919375.0 ± 56417.9	8435143.4 ± 94213.4	2713178.0 ± 25926.5	0.7	1.1	1.0	6.071 ± 0.003	8.201 ± 0.003	9.615 ± 0.004	0.05	0.04	0.04
		Sample- Decrease	8368828.1 ± 14031.9	8877022.9 ± 15152.4	2790186.9 ± 1891.1	0.2	0.2	0.1	6.418 ± 0.002	8.666 ± 0.001	10.265 ± 0.013	0.03	0.01	0.13
pH (3.2)	3.4 & 3.0	Std - Increase	7799013.2 ± 40496.7	8264510.8 ± 51342.4	2423076.7 ± 16822.5	0.5	0.6	0.7	6.238 ± 0.002	8.355 ± 0.003	10.085 ± 0.004	0.03	0.04	0.04
		Std- Decrease	7739708.5 ± 210382.3	8054720.0 ± 162256.1	2839016.5 ± 31401.3	2.7	2.0	1.1	6.220 ± 0.005	8.284 ± 0.004	8.916 ± 0.005	0.08	0.05	0.05
		Sample - Increase	7850416.8 ± 10154.6	8320070.9 ± 21303.8	2449562.6 ± 8466.0	0.1	0.3	0.3	6.240 ± 0.008	8.354 ± 0.008	10.078 ± 0.009	0.13	0.10	0.09
		Sample - Decrease	7905239.6 ± 54423.8	8323149.1 ± 77212.9	2936545.6 ± 17942.6	0.7	0.9	0.6	6.223 ± 0.009	8.282 ± 0.008	8.913 ± 0.007	0.14	0.10	0.08

Table 7.Mobile Phase Composition In Gradient Mode / Programme For RP-HPLC

Time in Minutes	% of Mobile Phase-A	% of Mobile Phase-B
0	90	10
3	90	10
4	30	70
6	10	90
11	10	90
13	90	10
15	90	10

Mobile Phase – A : 4.08 gms of 0.03M KH_2PO_4 in 1000 ml of water adjusting the pH:3.2 with dilute O-Phosphoric Acid
Mobile Phase – B : Methanol & Water in the ratio of 85:15 v/v;
Diluent: Acetonitrile & Buffer in the ratio of 70:30 v/v.

Fig 1. Emtricetabine

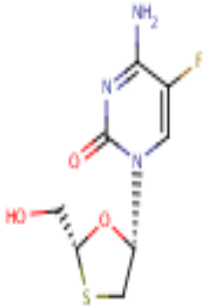


Fig 2. Tenofovir Disoproxil Fumarate

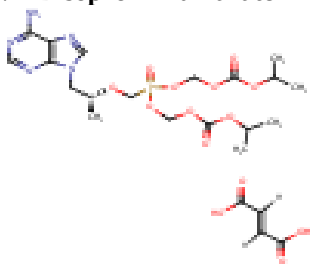


Fig 3. Rilpivirine HCl

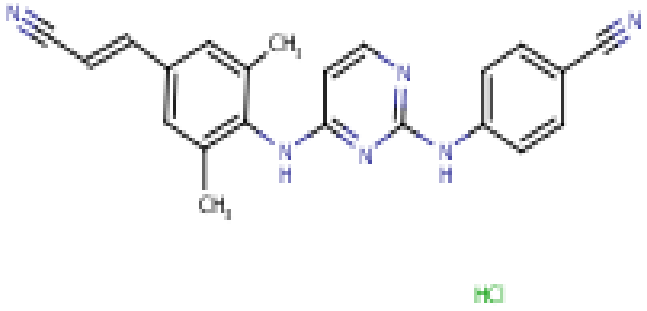


Fig 4. Calibration Curves of the Emtricetabine (FTC), Tenofovir Disoproxil Fumarate (TDF) and Rilpivirine HCl (RPV) by RP-HPLC

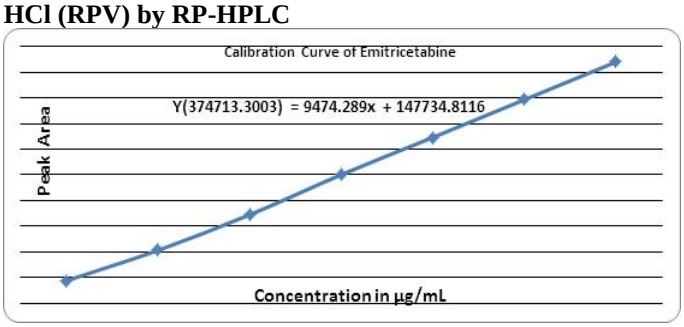


Fig 5. Typical Chromatogram of Emtricetabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl (Standard & Working Sample) by RP-HPLC

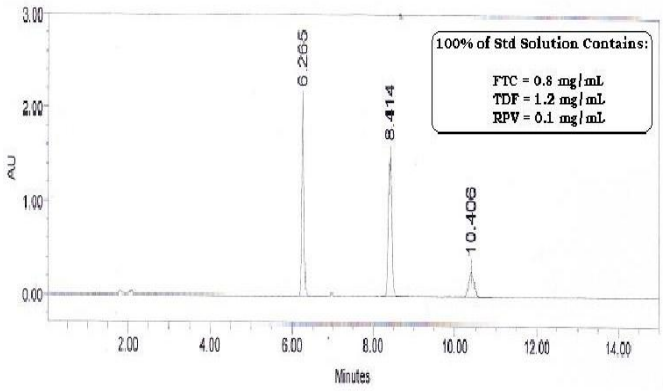
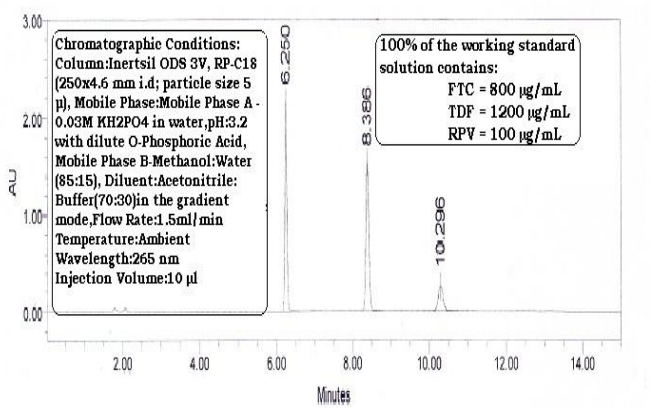


Fig 6.Typical Chromatogram of Emtricetabine, Tenofovir Disoproxil Fumarate and Rilpivirine HCl (Standard & Working Sample) by RP-HPLC



CONCLUSION

A simple and easily available HPLC method was developed in this study for the quantification of FTC, TDF & RPV in pharmaceutical matrices. The main advantages of this method are its considerably shorter run times, easy-to-use and its simplicity. All of these properties are very important in practice, particularly when a large number of samples are to be analyzed. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. The results of validation tests were, collectively, indicative for a method with a relatively wide linear range, acceptable precision and accuracy and practically reliable sensitivity. The method enables simple, selective, sensitive, and specific analysis of FTC, TDF & RPV and can be used for routine analysis in pharmaceutical quality control within a short time.

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