DEVELOPMENT, VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TENOFOVIR DISPROXIL FUMARATE, EMTRICITABINE AND RILPIVIRINE HYDROCHLORIDE IN BULK, FORMULATION AND USED IN NANOSUSPENSION

Vijaya Sri K.[a*], Madhuri M.[a], Anusha V.[a] and Siresha V.R.K.[a]

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ABSTRACT

The present research work illustrates the development and validation of RP HPLC method for simultaneous estimation of tenofovir disproxil fumarate, emtricitabine and rilpivirine hydrochloride in bulk and formulated in a pharmaceutical dosage form as a nanosupension. Antiretroviral drug treatment is the primary line of therapy for treating HIV. The multicomponent system formulated as a nanosuspension evidenced increased hydrophilicity, potency and decreased side effects. The separation was carried out by using efficient BDS hypersil C_{18} HPLC column with empower software. Combination method of Precipitation—ultrasonic homogenization was used for the preparation of the nanosuspension. The mobile phase used was methanol, water, acetonitrile (80:13.4:6.6) V/V and flow rate 1mL /min. The developed method was thus validated as per ICH guidelines for various parameters whose results advocated the reliability of the method. The results for parameters viz. retention times of tenofovir disproxil fumarate, emtricitabine and rilpivirine were 3.09 min, 2.78 min and 3.68 min, linearity range was between 7.5-90, 5-60, 0.625-7.5µg/mL, respectively. Thus the new RP-HPLC method is optimum, reliable and can be used for the simultaneous estimation of tenofovir disproxil fumarate, emtricitabine and rilpivirine hydrochloride.

Keywords: Tenofovir disproxil fumarate, Emtricitabine Rilpivirine hydrochloride RP-HPLC-PDA; Method validation; Nanosuspension, particle size.

INTRODUCTION

The primary line type of treatment for HIV/AIDS is antiretroviral drug therapy which aims to keep the amount of HIV in the body at a low level. Tenofovir disproxil fumarate, emtricitabine and rilpivirine hydrochloride combination is a novel formulation consisting of two nucleoside analogs HIV-1 reverse transcriptase inhibitors, (tenofovir disproxil fumarate, emtricitabine) and one non-nucleoside reverse transcriptase inhibitor (rilpivirine).[1,2] Tenofovir disproxil fumarate is chemically known as 9-[(R)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy] phosphinyl]- methoxy]propyl]adenine fumarate (1:1). Tenofovir disproxil fumarate is an acyclic nucleoside phosphonate diester analog of adenosine mono phosphate. Emtricitabine, a synthetic nucleoside analog of cytidine, is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate. Emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 RT by competing with the natural substrate deoxycytidine 5'-triphosphate and by being incorporated into nascent viral DNA which results in chain termination. Rilpivirine hydrochloride is chemically known as 4-[[4-[[4-[(E)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2pyrimidinyl] amino]benzonitrilemono hydro chloride. Rilpivirine is a diarylpyrimidine non-nucleoside reverse transcriptase inhibitor of HIV-1. The structures of tenofovir disproxil fumarate, emtricitabine and rilpivirine hydrochloride are shown in Fig. 1. These types of multicomponent formulations have gained a lot of importance due to greater patient acceptability, increased potency and decreased side effects. They are estimated by HPLC methods as consume less time and are economical.

Literature survey reveals that there are several UV, HPLC, UPLC, LC-MS methods available for the estimation of tenofovir disproxil fumarate, emtricitabine and rilpivirine hydrochloride individually[4-8] and for combinations[9-20] of tenofovir disproxil fumarate and emtricitabine in bulk, pharmaceutical dosage forms and biological samples. Till date there is no reported HPLC method for nanosuspensions to simultaneously estimatie

[a] Department of Pharmaceutical Analysis, Malla Reddy College of Pharmacy , (Affiliated to Osmania University), Maisammaguda, Secunderabad-500 014, Telangana, India

*For Correspondence: E-mail: vijayasree_2002@yahoo.com

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Table I: Different mobile phases of tenofovir, emtricitabine and rilpivirine

Sr. No	MOBILE PHASE	OBSERVATION	REMARK
1	Mobile phase: methanol: water (80:20) Flow rate: 1 mL min^{-1}	Rilpivirine was not eluted completely	Not satisfactory
2	Mobile phase: methanol: phosphate buffer (50:50), Flow rate: 1 mL min^{-1}	Rilpivirine was not eluted completely	Not satisfactory
3	Mobile phase: methanol, water containing 1% O-phosphoric acid (70:30) Flow rate: 1 mL min^{-1}	Rilpivirine was not eluted completely	Not satisfactory
4	Mobile phase: Methanol, water, acetonitrile (50:20:30) Flow rate: 1 mL min^{-1}	Rilpivirine was not eluted completely, Dentate peak for emtricitabine	Not satisfactory
5	Mobile phase: Methanol, water, acetonitrile (70:20:10) Flow rate: 1 mL min^{-1}	Dentate peak for emtricitabine	Not satisfactory
6	Mobile phase: Methanol, water, acetonitrile (90:7.7:3.3) Flow rate: 1 mL min^{-1}	Resolution was low	Not satisfactory
7	Mobile phase: Methanol, water, acetonitrile (80:13.4:6.6) Flow rate: 1 mL min^{-1}	Resolution good	Satisfactory

Table II: System suitability parameters

Drug name	Retention time	Resolution	Theoretical plates (N)	Tailing factor
Emtricitabine	2.785	5.450	8147	1.145
Tenofovir	3.095	2.368	7963	1.212
Rilpivirine	3.677	3.298	4464	1.499

Table III: Purity table

Drug name	Purity angle	Purity Threshold
Tenofovir	0.175	0.283
Emtricitabine	0.106	0.267
Rilpivirine	0.070	0.284

Table IV: Repeatability

Drug name		Tenofovir	Emtricitabine	Rilpivirine
Concentration		45 µg/mL	30 µg/mL	3.75 µg/mL
Peak Area	1	1946965	1518333	460780
	2	1964001	1498709	464387
	3	1946958	1518328	460778
	4	1952345	1512822	464101
	5	1951429	1524609	462275
	6	1952640	1512891	464588
Area mean*±SD		1952390±6240	1514282±8786	462818±1780
%RSD		0.319	0.418	0.385

**Mean of 6 determinations*

Table V: Intraday and interday precision

Drug name	Conc (µg/mL)	Intraday precision		Interday precision	
		Area mean*±SD	%RSD	Area mean*±SD	%RSD
Tenofovir	7.5	447046.3±405.15	0.906	447046±125.40	0.028
	45	1948452±2595.77	0.133	1951380±1351.60	0.069
	90	3740121±8549.01	0.229	3750081±12404.12	0.331
Emtricitabine	5	316086.3±262.26	0.829	316087.7±1452.20	0.459
	30	1520423±3633.84	0.239	1523325±2812.58	0.185
	60	3015339±9597.26	0.318	3011084±17668.54	0.587
Rilpivirine	0.625	88123±72.77	0.083	88122±107.89	0.122
	3.75	461278±863.71	0.187	461126.7±734.03	0.159
	7.5	922338±2003.40	0.217	925404.3±3728.71	0.403

**Mean of 3 determinations*

Table VI: Results of Accuracy

Drug name	Spiked level (%)	Formulation conc (µg/mL)	Pure drug conc (µg/mL)	Total conc (µg/mL)	Amount found* (µg/ml) ±SD	%Recovery ±SD	%RSD
Tenofovir	50	30	15	45	45.27±0.148	100.60±0.328	0.326
	100	30	30	60	60.19±0.697	100.32±1.161	1.157
	150	30	45	75	75.29±0.332	100.39±0.443	0.441
Emtricitabine	50	20	10	30	29.71±0.245	99.043±0.816	0.824
	100	20	20	40	39.75±0.205	99.398±0.512	0.515
	150	20	30	50	49.78±0.361	99.57±0.722	0.725
Rilpivirine	50	2.5	1.25	3.75	3.76±0.019	100.37±0.514	0.512
	100	2.5	2.5	5	5.01±0.051	100.38±1.029	1.025
	150	2.5	3.75	6.25	6.265±0.010	100.23±0.167	0.167

Table VII: Assay results of tablet formulation

Drug name	Amount taken (µg/mL)	Amount found* (µg/mL) ±SD	%Purity ±SD	%RSD
Tenofovir	300	300.128±1.200	100.043±0.400	0.400
Emtricitabine	200	200.303±0.850	100.151±0.425	0.424
Rilpivirine	25	25.166±0.104	100.66±0.416	0.413

**Mean of 3 determinations*

Table VIII: Robustness-Change in flow rate

Drug name	Concentration (µg/mL)	Change in flow rate (mL/ min)			Area mean*±SD	%RSD
		0.95	1	1.05		
Tenofovir	30	1420198	1398678	1366554	1395143±26996	1.935
Emtricitabine	20	1029809	1002317	997317	1009814±17495	1.733
Rilpivirine	2.5	314231	308901	301981	308371±6142	1.992

**Mean of 3 determinations*

Table IX: Robustness-Change in Detection wavelength

Drug name	Conc (µg/mL)	Change in wavelength (nm)			Area mean*±SD	%RSD
		259	261	263		
Tenofovir	30	1399950	1398678	1374760	1391129±14191	1.020
Emtricitabine	20	1017336	1002317	1000655	1006769±9189	0.913
Rilpivirine	2.5	305749	308901	317189	310613±5909	1.902

**Mean of 3 determinations*

Table X: Robustness-Change in methanol ratio

Drug name	Concentration (µg/mL)	Change in methanol ratio(%)			Area mean*±SD	%RSD
		79	80	81		
Tenofovir	30	1386133	1398678	1408344	1397718±11137	0.797
Emtricitabine	20	996509	1002317	1017119	1005315±10627	1.057
Rilpivirine	2.5	304869	308901	312552	308774±3843	1.245

**Mean of 3 determinations*

Table XI: Ruggedness

Drug name	Parameter	Concentration (µg/mL)	Area mean* (n=3) ±SD	%RSD
Tenofovir	Analyst 1	30	1365307±11533.6	0.845
	Analyst 2	30	1371634±1844.4	0.134
Emtricitabine	Analyst 1	20	1030110±9190.2	0.892
	Analyst 2	20	1034928±4896.4	0.473
Rilpivirine	Analyst 1	2.5	302041±1331.8	0.441
	Analyst 2	2.5	301255±1388.9	0.461

**Mean of 3 determinations*

Table XII: Statistical data tenofovir, emtricitabine and rilpivirine by RP-HPLC method

Parameter	Tenofovir	Emtricitabine	Rilpivirine
Linearity range (µg/mL)	7.5-90	5-60	0.625-7.5
Regression equation (y= mx + c)	y = 39680x + 150997	y = 49415x + 41881	y = 120614x + 10509
Slope	39680	49415	120614
Intercept	150997	41881	10509
Correlation coefficient (r)	0.9997	0.9997	0.9995
Accuracy (%)	100.32-100.69	99.39-100.37	100.37-100.42
Precision (%RSD)	0.319	0.418	0.385
Limit of detection	2.076	1.181	0.202
Limit of quantification	6.291	3.579	0.613

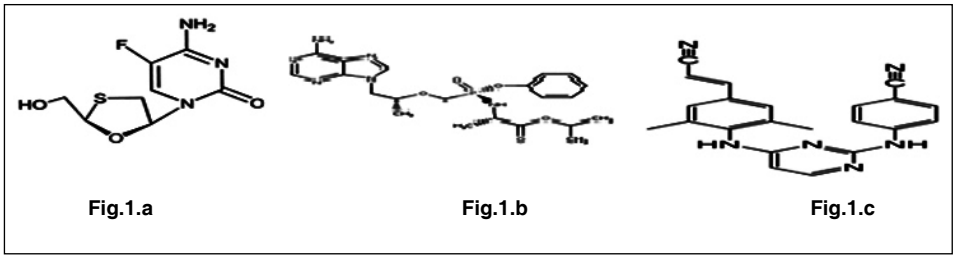

Fig.1.a Fig.1.b Fig.1.c

Fig. 1: Structures of a. tenofovir disproxil fumarate, b. emtricitabine and c. rilpivirine hydrochloride

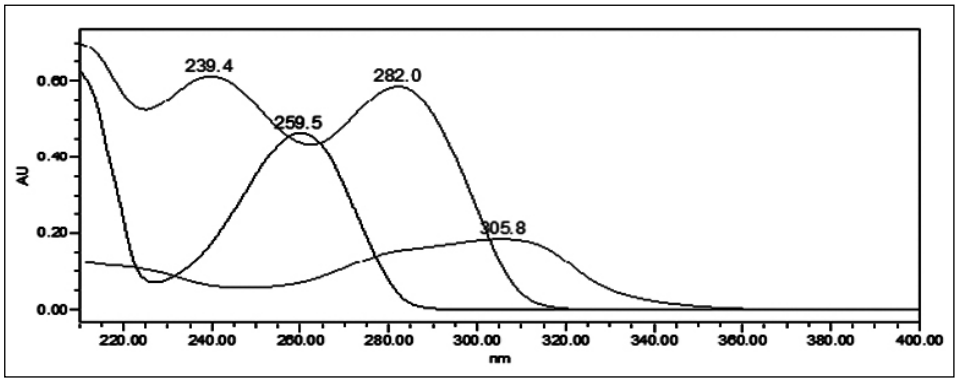

Fig. 2: Overlay spectra of tenofovir, emtricitabine and rilpivirine

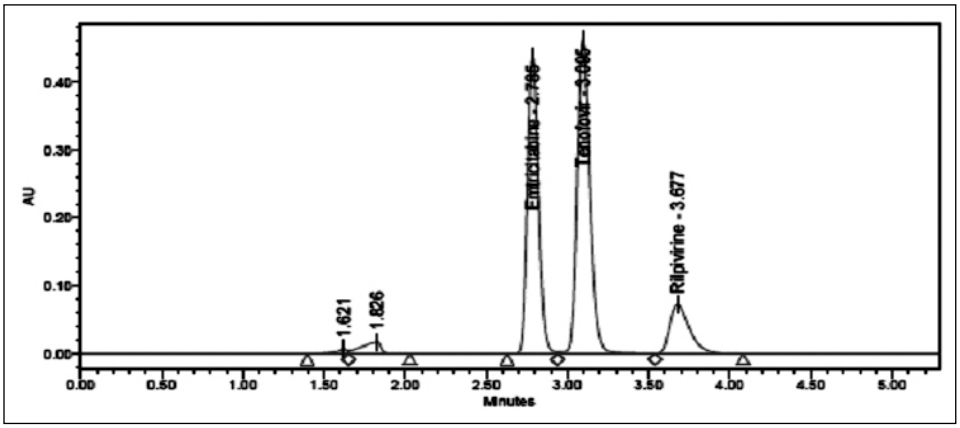

Fig. 3: Chromatogram for System suitability

tenofovir disproxil fumarate, emtricitabine and rilpivirine hydrochloride.

Product development scientists often encounter significant difficulties in solving the problem of poor water solubility of drug candidates in the development of pharmaceutical dosage forms. Nanosizing greatly increases the surface area of drug particles, improves its dissolution rate, and thus enhances bioavailability[21-22]. Nanosuspensions can successfully formulate the brick dust molecules for improved dissolution and good absorption. Recently estimation of few drugs formulated as nanosuspensions have been reported by HPLC[23-24].

The proposed RP-HPLC method is fast, reproducible, and simple. By adopting this method one can elute all the three drugs in 5 minutes. The proposed method can be utilized for routine analysis in bulk, pharmaceutical dosage forms and in nano-formulations.

MATERIALS AND METHODS

Tenofovir disproxil fumarate, Emtricitabine, Rilpivirine Hydrochloride gift samples were received from Hetero Labs. Acetetonitrile and methanol was purchased from Merck Chemical Company. PVP-K30, Sodium lauryl sulphate were procured from commercial sources. The 0.45 µm pump Nylon filter was obtained from Advanced Micro Devices (Ambala Cantt, India) & Whatman No 5 filter paper was obtained from Modern Science Lab, (Nashik, India). Other chemicals used were analytical or HPLC-grade.

Instrumentation

Waters HPLC system coupled with a photo diode array detector (PDA) and Empower software was use. Chromatographic separation was achieved using a BDS hypersil C_{18} (4.6 x 250mm, 3µm) column.

Preparation of Standard Stock Solution

Stock I solution: Standard stock solution of emtricitabine, tenofovir disproxil fumarate and rilpivirine were prepared separately by dissolving 30mg of tenofovir disproxil fumarate, 20mg of emtricitabine in diluent and made the volume up to 10mL with diluent and dissolving 25mg of rilpivirine in diluent and made the volume up to 100ml with diluent.

Stock II solution: From the above stock solutions 1mL of each of these aliquots were pipetted out in a 10mL volumetric flask separately and the volume was made up

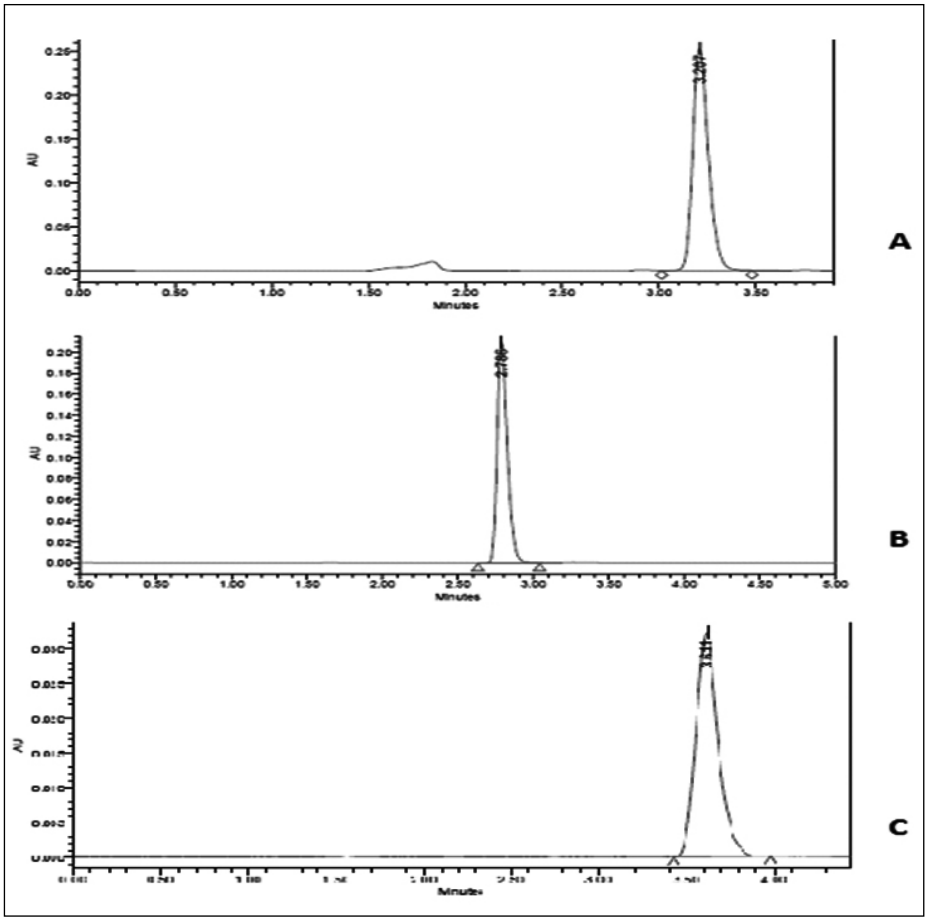

Fig. 4: Chromatogram of standard solution of A. Tenofovir disproxil fumarate B. Emtricitabine C. Rilpivirine

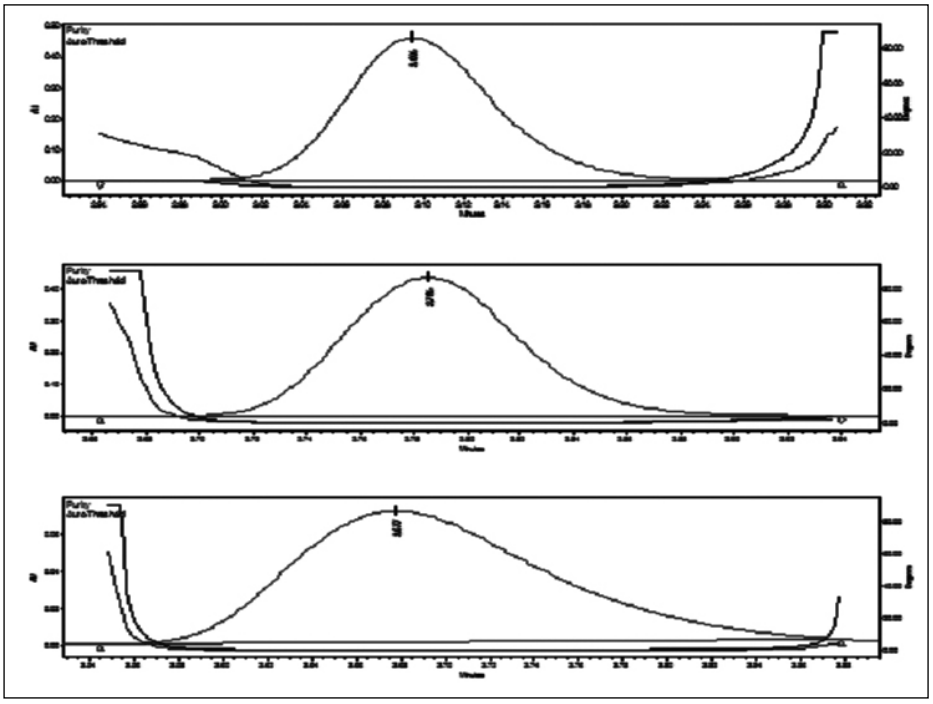

Fig. 5: Purity plot for Tenofovir disproxil fumarate, Emtricitabine, Rilpivirine

to the mark with diluent to obtain the final concentration of 300µg/mL of tenofovir disproxil fumarate, 200µg/mL of emtricitabine and 25µg/mL of rilpivirine.

Preparation of Sample Solution

Twenty tablets were accurately weighed and ground into a fine powder using a glass mortar and pestle. The powder equivalent to about 30 mg of tenofovir disproxil fumarate (20 mg of emtricitabine and 2.5 mg of rilpivirine) was transferred to a 100mL volumetric flask. Approximately 75mL of diluent was added to the flask and mixed well by sonicating it for 15 min. Then the volume was adjusted with diluent. The solution was filtered using whatmann filter 5 into a volumetric flask. The resulting solution was used as a working sample solution which contained 300µg/mL of tenofovir disproxil fumarate, 200µg/mL of emtricitabine and 25µg/mL of rilpivirine.

Validation of RP-HPLC method

When a method has been optimized it must be validated before practical use. By following the ICH guidelines for analytical method validation Q2 (R1), the validation characteristics were addressed.

System suitability testing

System suitability standard solution which contained 30µg/mL of tenofovir disproxil fumarate, 2 µg/mL of emtricitabine and 2.5µg/mL of rilpivirine was prepared by appropriately diluting and mixing the corresponding stock standard solutions. System suitability was determined from six replicate injections of the system suitability standard before sample analysis. Parameters such as a number of

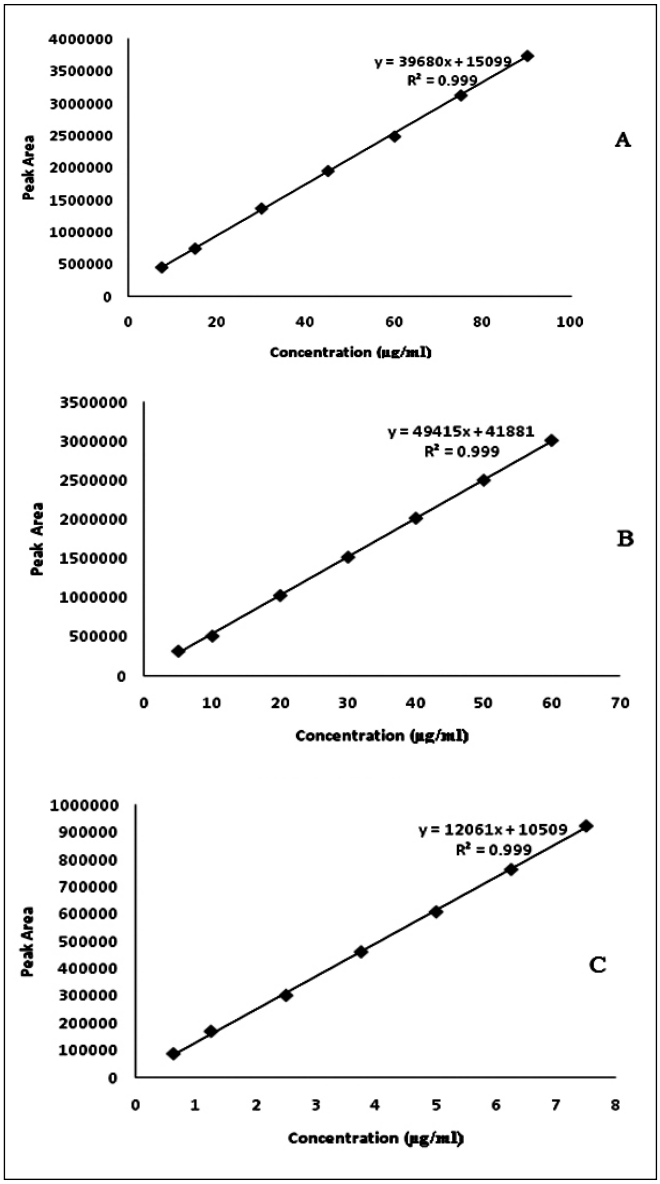

Fig. 6: Calibration curve for A. Tenofovir disproxil fumarate B. Emtricitabine C. Rilpivirine

theoretical plates (N), tailing factor and retention time, and resolution were calculated.

Specificity

Specificity studies were carried out in order to demonstrate the absence of interference with the elution of the drugs and its impurities.

Identification of tenofovir disproxil fumarate, emtricitabine and rilpivirine

The individual standard solutions of the three drugs were injected and chromatograms were recorded. The drugs were taken as a mixture and injected for record the chromatogram. The sample solution (pharmaceutical dosage form) was then injected and chromatogram was recorded.

Peak Purity

The specificity of the method was studied by assessment of peak purity of tenofovir disproxil fumarate, emtricitabine and rilpivirine using Waters empower software and photo diode array detector. Peak purity was calculated to determine if the peaks were spectrally homogeneous. Spectral heterogeneity can indicate the presence of coelution. A coelution of two or more spectrally distinct compounds can produce a spectrally heterogeneous peak.

Linearity

Linearity was performed by preparing 7.5-90µg/mL for tenofovir disproxil fumarate, 5-60 µg/ml for emtricitabine, 0.625-7.5µg/mL for rilpivirine from mixed stock II solution (300µg/mL of tenofovir disproxil fumarate, 200µg/mL of emtricitabine and 25µg/mL of rilpivirine) by appropriate dilution. Each of these mixed drug solutions (20µL) were injected into the chromatographic system thrice. The peak area and retention time were recorded and the mean values of peak areas were plotted against concentrations.

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method. Repeatability, Intraday precision and Inter day precision was calculated.

Repeatability

Repeatability of the instrument was checked by repeated scanning and measurement of the absorbance of solution (n=6) for tenofovir disproxil fumarate (45 µg/mL), emtricitabine (30µg/mL) and rilpivirine (3.75µg/mL) without changing the parameters of the proposed UV method. % RSD was calculated.

Intraday precision

Intraday precision was found by carrying out the analysis of the standard drugs on the same day at three different concentrations of standard solution of tenofovir (7.5, 40, 90µg/mL), emtricitabine (5, 30, 60µg/mL) and rilpivirine (0.625, 3.75, 7.5 µg/mL). Each concentration was injected in triplicate and % RSD was calculated.

Inter day precision

Inter day precision was found out by carrying out the analysis of the standard drugs on consecutive day

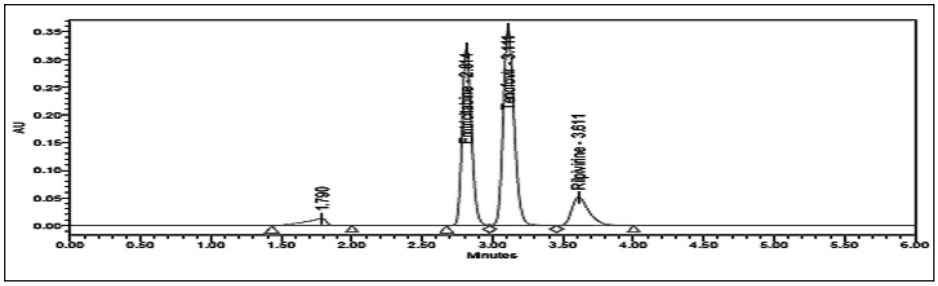

Fig. 7: Chromatogram at 50% spiked level

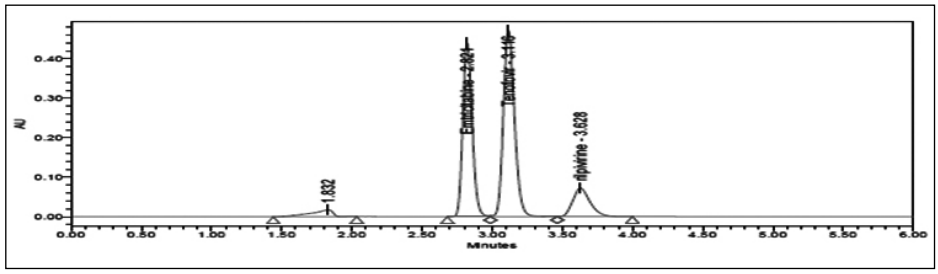

Fig.8: Chromatogram at 100% spiked level

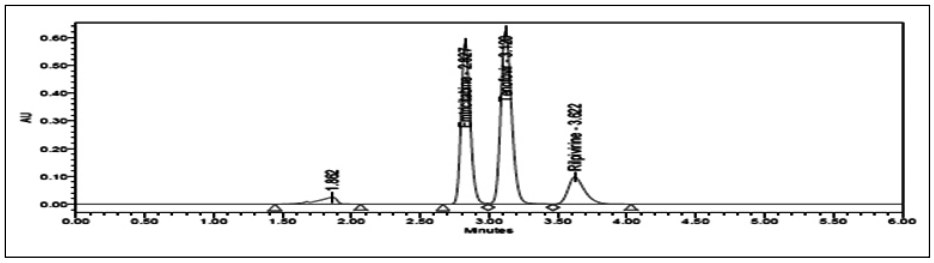

Fig. 9: Chromatogram at 150% spiked level

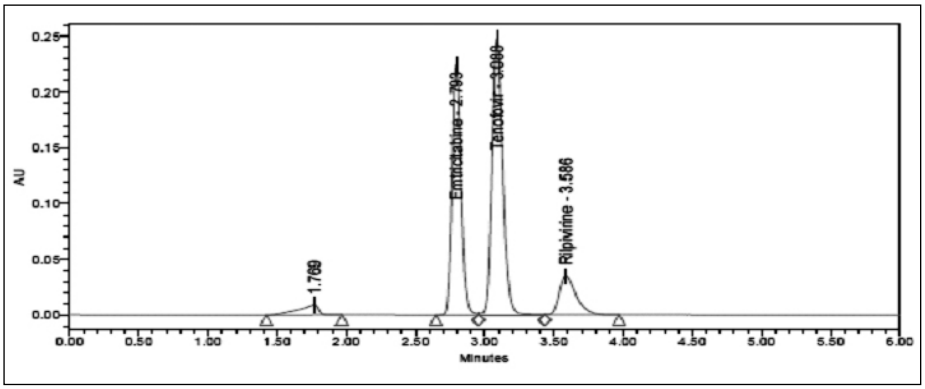

Fig.10: Chromatogram of Formulation

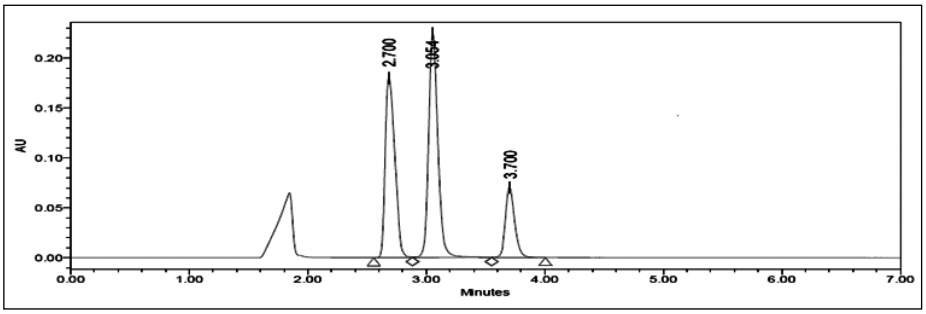

Fig.11: Nano suspension chromatogram

at three different concentrations of standard solution of tenofovir (7.5, 40, 90µg/mL), emtricitabine (5, 30, 60µg/mL) and rilpivirine (0.625, 3.75, 7.5). Each concentration was injected in triplicate and % RSD was calculated.

Accuracy

Accuracy of the proposed method was determined using recovery studies by the spiking method. The recovery studies were carried out by adding known amounts (50, 100 and 150%) of the pure drug to the pre-analysed formulation. Different concentrations of sample solution of tenofovir (30µg/mL), emtricitabine (20µg/mL) and rilpivirine (2.5µg/mL) and add the standard solutions of tenofovir (15, 30, 45 µg/mL), emtricitabine (10, 20, 30 µg/mL) and rilpivirine (1.25, 2.5 and 3.75 µg/mL). The solutions were prepared in triplicates and the % recovery was calculated.

Analysis of formulation

From the working sample solution 1mL was pipetted out and diluted to 10mL with diluent. The final solution was injected in the HPLC, chromatogram was recorded and the area was measured.

Robustness

Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is carried out by changing the flow rate of mobile phase from 0.95 to 1.05 mL/min, changing the detection wavelength from 259 to 263 nm and varying the methanol ratio between to 79 to 81%. From the results, the %RSD is determined.

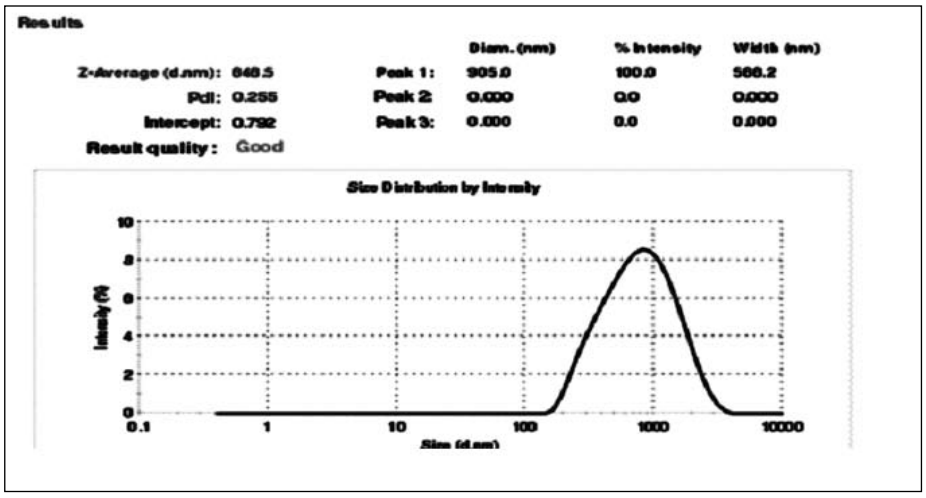

Fig. 12: Particle size determination of tenofovir disproxil fumarate emtricitabine rilpivirine nanosuspension

Ruggedness

Standard solution of mixture of tenofovir disproxil fumarate, emtricitabine, disproxil fumarate and rilpivirine was analysed by analyst 1 and analyst 2 at similar operational and environmental conditions using the developed method.

Limits of detection and quantification (LOD & LOQ)

The limit of quantification (LOQ) and limit of detection (LOD) were based on the residual standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization Guidelines Q2 (R1).

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where,

σ = residual standard deviation of the response

S = slope of the calibration curve

Preparation of the nanosuspension

Tenofovir disproxil fumarate, emtricitabine and rilpivirine hydrochloride nanosuspension were prepared by the combination method of precipitation—ultrasonic homogenization. 300 mg of tenofovir disproxil fumarate, 200 mg of emtricitabine and 25 mg of rilpivirine hydrochloride was dissolved in 2mL of methanol, then sonicated for 15 minutes by using ultrasonic bath sonicator. Accurately weighed quantities of (0.030 mg) PVP K-30 and (0.05mg) of sodium lauryl sulphate were dissolved in aqueous vehicle distilled water. The organic phase was injected drop by drop into the aqueous phase containing 25mL of the above solution with stirring for 30min. The nanosuspension was kept in ultrasonic homogenizer (Model No: 3000) and the solution was homogenized for 30min. The temperature was maintained at 8°C in a refrigerator in order to avoid particle aggregation. 0.1mL of nanosuspension was dissolved in 100mL of volumetric flask and the volume was made mobile phase.

Particle Size

Particle size growth is mainly responsible for agglomeration. Precise sizing techniques can provide useful information about the particle size distribution in nanosuspension[18]. The particle size was measured using a particle size analyzer, HORIBA scientific SZ-10. Appropriate scattering intensity was at 25°C was measured by placing the sample in a disposable sizing cuvette at a count rate of 372.0 (kcps) for 20 s.

RESULTS AND DISCUSSION

Selection of mobile phase and experimental conditions

Several mobile phases were evaluated for the analysis. The mobile phase methanol & 2:1 ratio of Methanol, water, acetonitrile (80:13.4:6.6) Flow rate: 1 mL min showed good separation and good peak symmetry and shown in Table I.

Wavelength selection

The ultraviolet spectra of tenofovir disproxil fumarate, emtricitabine and rilpivirine showed the maximum absorption wave length at 260 nm for tenofovir, 282 nm for emtricitabine and 306 nm for rilpivirine. Therefore, 261nm wavelength was selected, after comparing the spectra, to achieve the highest sensitivity for the studied compounds. The overlay spectra of tenofovir disproxil fumarate, emtricitabine and rilpivirine is shown in Fig. 2.

Selection of flow rate

Increase in the flow rate results in a decrease in the retention time. Hence optimum flow rate of 1 mL/min was chosen to avoid overlap between the peaks and to obtain acceptable resolution values.

METHOD VALIDATION

System suitability

The system suitability test ensures the validity of the analytical procedure as well as confirms the resolution between different peaks of interest. All critical parameters tested, met the acceptance criteria. The chromatogram is illustrated in Fig. 3 The results are reported in Table II.

Blank interference

Chromatogram of the blank was recorded and it did not show any peak at the retention time of the analyte peaks.

Identification of tenofovir disproxil fumarate, emtricitabine and rilpivirine

The retention time of tenofovir disproxil fumarate, emtricitabine and rilpivirine were found to be 3.207, 2.786 and 3.552 in the individual standard solutions as illustrated in Fig. 4. The retention times of tenofovir disproxil fumarate, emtricitabine and rilpivirine were found to be 3.001, 2.779 and 3.683min in mixed standard solutions as recorded and illustrated in Fig. 6.

Peak Purity

The specificity of the method was studied by assessment of peak purity of tenofovir disproxil fumarate, emtricitabine, and rilpivirine using waters empower software and photo diode array detector. These results demonstrate that there was no interference from other excipients in the tablet formulation. Thus confirms the specificity of the method for the three drugs. Purity angles were less than the purity thresholds which indicates that the peaks were spectrally homogeneous. The results are shown in Table III and the purity plots of tenofovir disproxil fumarate, emtricitabine, and rilpivirine are showning Fig. 5 respectively.

Linearity and range

Linearity was found in the range of 7.5-90µg/mL for tenofovir disproxil fumarate, 5-60µg/mL for emtricitabine and 0.625-7.5µg/mL for rilpivirine. The resultant calibration curves of tenofovir disproxil fumarate, emtricitabine and rilpivirine were shown in Fig. 6, respectively.

The slope, intercept and correlation coefficient of tenofovir disproxil fumarate were found to be 39680, 150997 and 0.9997, respectively. The slope, intercept and correlation coefficient of emtricitabine were found to be 49415, 41881 and 0.9997, respectively. The slope, intercept and correlation coefficient of rilpivirine were found to be 120614, 10509 and 0.9995, respectively.

PRECISION

Repeatability (Method precision)

Repeatability was determined for all the drugs and %RSD values were found to be 0.319 for tenofovir disproxil fumarate, 0.418 for emtricitabine and 0.385 for rilpivirine and the results are tabulated in Table IV.

Intraday Precision and Interday precision

Intraday precision was determined for all the three drugs and %RSD values were found to be 0.133-0.906 for tenofovir disproxil fumarate, 0.239-0.829 for emtricitabine and 0.083-0.217 for rilpivirine and results are tabulated in Table V. Interday day precision was determined for all the three drugs and %RSD values were found to be 0.028-0.331 for tenofovir disproxil fumarate, 0.185-0.587 for emtricitabine and 0.122-0.403 for rilpivirine and the results are tabulated in Table V.

Accuracy

A good percentage of recovery indicates that the proposed method is accurate and it can be used for the simultaneous estimation of Emtricitabine, Tenofovir and Rilpivirine. Satisfactory recovery in the range of 99.38 -100.60 is obtained by the proposed method and shown in Fig. 7-9. The percentage recovery of the results obtained is listed in Table VI.

Analysis of Formulation

Estimation of emtricitabine, tenofovir disproxil fumarate and rilpivirine in tablet dosage form was done and the % purity were 100.043, 100.151 and 100.66% respectively. The assay results are mentioned in Table VII. The area under curve of sample (formulation) was shown in Fig. 10.

Robustness Studies

The drug solution was subjected to small, deliberate changes like flow rate, wavelength and mobile phase ratio and results are shown in Table VIII, IX and X, respectively and, the results obtained were not affected by varying the conditions and were in accordance with the results in original conditions. This indicates the method was robust.

Ruggedness Studies

Ruggedness was performed by two different analysts and the results for varied analysts in terms of %RSD were found to be 0.845, 0.134 for tenofovir disproxil fumarate, 0.892, 0.473 for emtricitabine and 0.441, 0.461 for rilpivirine. The results of the different analysts are given in Table XI.

Limit of Detection & Limit of Quantification

The LOD and LOQ were found to be 2.076, 6.291 for Tenofovir disproxil fumarate, 1.181, 3.579 for Emtricitabine and 0.202, 0.613 for Rilpivirine.

Rilpivirine hydrochloride nanosuspension

The nanosuspension prepared by precipitation—ultrasonic homogenization method are discrete, uniform, nanometeric average particle in the size of 656 nm are mentioned in Fig. 12. The prepared tenofovir disproxil fumarat, emtricitabine, and rilpivirine hydrochloride nanosuspension area under curve chromatogram are shown in Fig. 11

Optimum conditions, optical characteristics and Statistical data of the Regression equation in HPLC method

The optical characteristics such as linearity range, LOD and LOQ in each method were calculated. Besides these parameters the regression equation like slope (b), intercept (a) and correlation coefficient(r) were calculated and shown in Table XII.

CONCLUSION

A simple and effective HPLC method was developed and validated for the simultaneous determination of tenofovir disproxil fumarat, emtricitabine, and rilpivirine. The prepared nanosuspension particle was nanosize. This method was considered specific, linear, accurate, precise, and robust for a quick determination of above drugs according to the ICH guideline.

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