ORIGINAL ARTICLE

Simultaneous Estimation of Dolutegravir Sodium, Emtricitabine, and Tenofovir Disoproxil Fumarate by UPLC

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Abstract

Introduction: A new reversed-phase ultra-fast liquid chromatography (RP-UPLC) method has been developed for the simultaneous quantification of Dolutegravir sodium, Emtricitabine, and Tenofovir disoproxil fumarate. Dolutegravir, Emtricitabine, and Tenofovir disoproxil fumarate are anti-retroviral drugs. Dolutegravir sodium is an human immunodeficiency virus integrase inhibitor. Emtricitabine and Tenofovir disoproxil fumarate are reverse transcriptase enzyme inhibitors. **Materials and Methods:** Shimadzu NexeraX2 Model UPLC system with PDA detector and Shim-pack C18 column was employed for the chromatographic study. Mobile phase consisting of 0.1% Tri ethyl amine (Adjusted to pH 6.0 with ortho phosphoric acid): Acetonitrile (55: 45) was used with 1.0 mL/min flow rate and UV detection at 260 nm. **Results and Discussion:** Beer-Lambert's law was obeyed over the concentration range 5–400, 2–150 and 5–500 µg/mL with linear regression equation y = 1211.7× + 506.73 (R^2 = 0.9998), y = 3330.4×–1162.3 (R^2 = 0.9999), and y = 1262.7× + 990.03 (R^2 = 0.9998) for tenofovir disoproxil fumarate, dolutegravir sodium, and emtricitabine, respectively, and the method was validated as per ICH guidelines. The total run time was 5 min. The limit of quantitation values was found to be 1.9113, 4.8752, and 4.7654 µg/mL and that of the limit of detection values 0.6287, 0.1598, and 0.1568 µg/mL for tenofovir disoproxil fumarate, dolutegravir sodium, and emtricitabine respectively. The proposed RP-UPLC method is simple, precise, and accurate. This method can be used for the regular analysis of pharmaceutical dosage forms. **Conclusion:** The proposed RP-UPLC method is simple, precise, and accurate. This method can be used for the regular analysis of pharmaceutical dosage forms.

Key words: Dolutegravir sodium, emtricitabine, reversed-phase ultra-fast liquid chromatography, tenofovir disoproxil fumarate, validation

INTRODUCTION

Dolutegravir[1,2] sodium is an anti-retroviral drug acting as an human immunodeficiency virus (HIV) integrase inhibitor. Dolutegravir acts against HIV-Type 1 infection. It has molecular formula, $C_{20}H_{18}F_2N_3NaO_5$, and molecular weight 441.37 g/mol. Emtricitabine[3] is an anti-retroviral drug. It acts by inhibiting the reverse transcriptase enzyme. It has molecular formula $C_8H_{10}FN_3O_3S$ and molecular weight 247.25 g/mol. Tenofovir disoproxil fumarate[4] is an antiretroviral agent acts as a reverse transcriptase inhibitor. It has molecular formula $C_{19}H_{30}N_5O_{10}P$. $C_4H_4O_4$ and molecular weight 635.52 g/mol. Tenofovir disoproxil fumarate is a salt of fumaric acid. Therefore fumarate formula was written separately as shown in the chemical structure. The chemical structures of dolutegravir sodium, emtricitabine, and tenofovir disoproxil fumarate are shown in Figure 1.

Sharma and Gupta[5] developed a RP-HPLC method for the simultaneous estimation of emtricitabine and tenofovir disoproxil fumarate in tablet dosage forms using Luna C18 column with UV detection at 260 nm. A mixture of acetonitrile: potassium dihydrogen phosphate buffer (pH 3.0 ± 0.05 adjusted with orthophosphoric acid): Triethylamine (70: 30: 0.5) was used as mobile phase and the flow rate was 1.5 mL/min. Emtricitabine and tenofovir disoproxil fumarate were eluted at 1.78 and 2.27 min, respectively, and the linearity was obeyed over the concentration range

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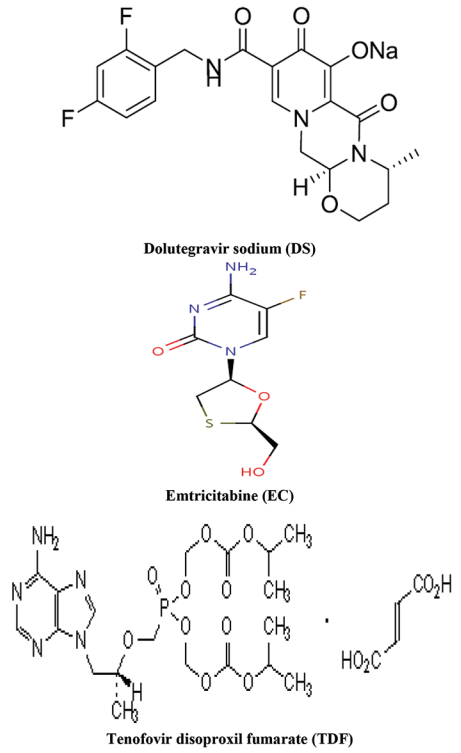

Figure 1: Structures of anti-retroviral drugs

5–50 µg/mL for both emtricitabine tenofovir disoproxil fumarate.

Delahunty *et al*. developed a LC-MS/MS method[6] for the simultaneous estimation of tenofovir and emtricitabine in human plasma using Synergi polar-RP analytical column on isocratic mode using internal standards. Mobile phase consisting of 3% acetonitrile and 1% acetic acid in ultrapure water was used with flow rate 200 µL/min for the chromatographic separation. The detection was attained by ESI positive ionization tandem mass spectrometry and the linearity was 10–1500 ng/mL for both tenofovir and emtricitabine.

Janhavi *et al*. developed a simultaneous HPTLC-densitometric method[7] for the analysis of tenofovir and emtricitabine tablet dosage forms. Aluminum foil plates pre-coated with silica gel 60F254 and mobile phase mixture consisting of toluene: Methanol: Ethyl acetate: Acetic acid (4: 2: 5: 0.1) was chosen for the chromatographic study (UV detection at 270 nm). The RF was found to be 0.52 ± 0.05 for tenofovir and 0.40 ± 0.02 for emtricitabine. The linearity reported was 120–600 ng/spot for tenofovir and 80–560 ng/spot for emtricitabine.

Different spectrophotometric[8-10] methods were also developed for the simultaneous estimation of tenofovir and emtricitabine in tablets. No liquid chromatographic method has been developed so far for the simultaneous estimation of combined tablet dosage forms of dolutegravir sodium, emtricitabine, and tenofovir disoproxil fumarate. In the present study, a new RP-UPLC method has been proposed for the simultaneous quantification of dolutegravir sodium, emtricitabine, and

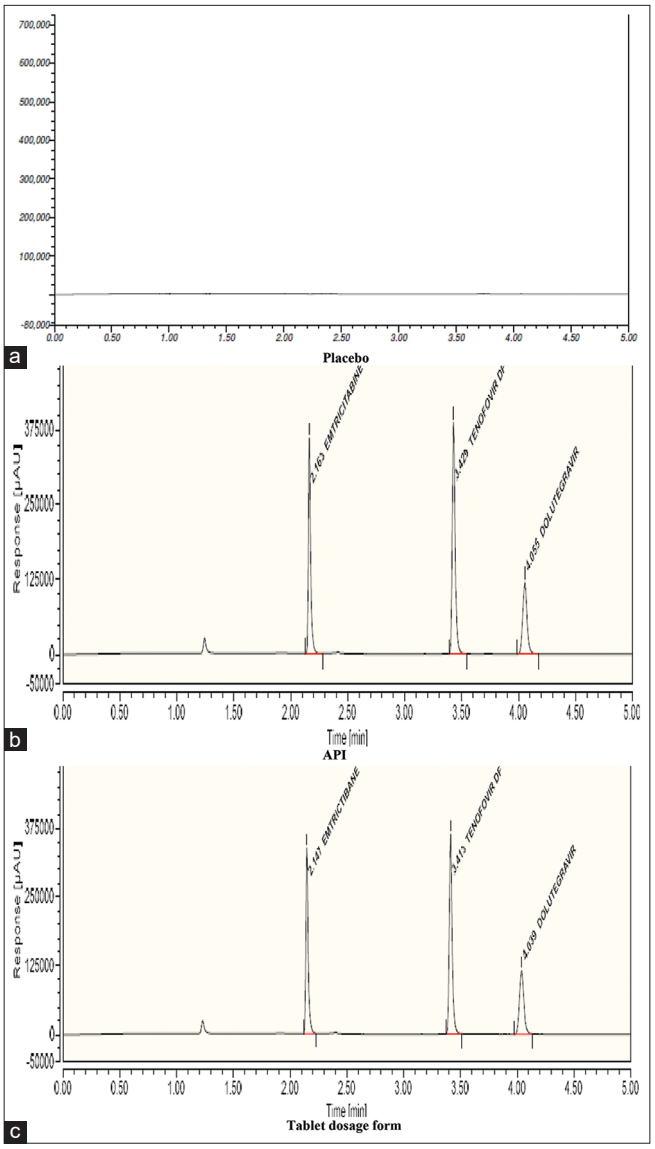

Figure 2: (a-c) Representative chromatograms of the combination of dolutegravir sodium, emtricitabine and tenofovir disoproxil fumarate

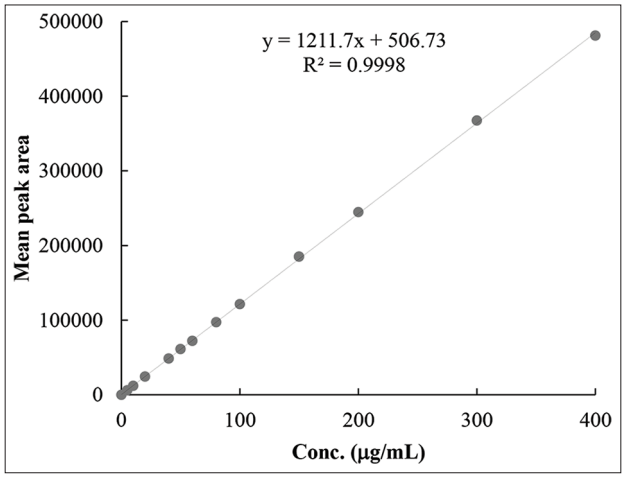

Figure 3: Calibration curve of tenofovir disoproxil fumarate

tenofovir disoproxil fumarate in pharmaceutical dosage forms and validated as per ICH guidelines.

MATERIALS AND METHODS

HPLC grade acetonitrile, triethylamine (AR grade), and *o*-phosphoric acid (AR grade) were procured from Merck.

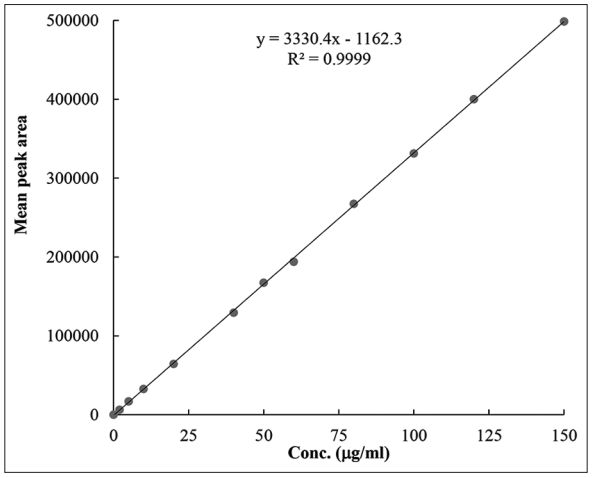

Figure 4: Calibration curve of dolutegravir sodium

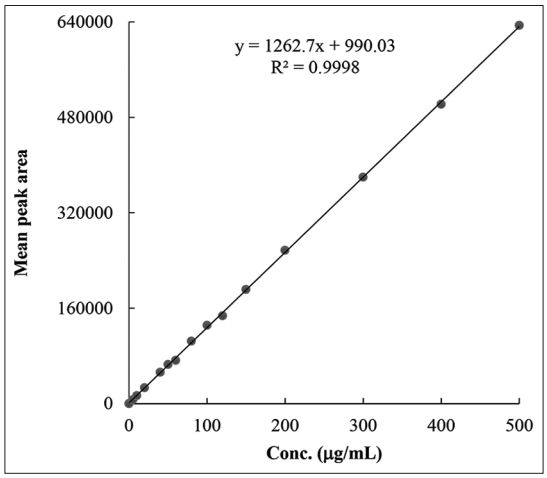

Figure 5: Calibration curve of emtricitabine

Instrumentation and chromatographic conditions

Shimadzu NexeraX2 Model UPLC system with PDA detector and Shim-pack C18 column was employed for the chromatographic study. Mobile phase consisting of 0.1% Tri ethyl amine (Adjusted to pH 6.0 with orthophosphoric acid): Acetonitrile (55: 45) was used with 1.0 mL/min flow rate and UV detection at 260 nm.

Preparation of stock and working standard solutions

Stock solution of tenofovir disoproxil fumarate was prepared by transferring 250 mg accurately to a 25 mL volumetric flask and dissolved in acetonitrile and dilutions that were made as per the requirement with the mobile phase. Stock solution of dolutegravir sodium was prepared by transferring 50 mg accurately to a 50 mL volumetric flask and dissolved in acetonitrile and dilutions were made with the mobile phase. Stock solution of emtricitabine was prepared by transferring 100 mg accurately to a 100 mL volumetric flask and dissolved in acetonitrile and dilutions that were made as per the requirement with the mobile phase. All these solutions were filtered through 0.42 µm nylon filter before injecting in to the system.

Method validation[11]

Linearity, precision, and accuracy

A series of tenofovir disoproxil fumarate (5–400 µg/mL), dolutegravir sodium (2–150 µg/mL), and emtricitabine (5–500 µg/mL) solutions were prepared from the stock solution (1000 µg/mL) and diluted with the mobile phase and 1.0 µL each was injected into the UPLC system (n = 3) and the average peak area was calculated from the respective chromatograms. Calibration graph was drawn by plotting the concentration of the drug on the X-axis and the corresponding mean peak area on the Y-axis.

The intraday precision studies were conducted on the same day at different equal time intervals and the interday precision studies were conducted on 3 successive days (Day 1, Day 2 and Day 3) and the data were analyzed.

Table 1: Literature survey

Method	Reagent/mobile phase (v/v)	λ (nm)	Linearity (µg/mL)	Drugs	Ref
RP-HPLC	Acetonitrile: Phosphate buffer (pH 3.0): TEA (70: 30: 0.5)	260	5–50 5–50	Tenofovir Emtricitabine	5
LC-MS/MS (Human plasma)	3% Acetonitrile: 1% Acetic acid	-	0.01–1.5	Tenofovir Emtricitabine	6
HPTLC	Toluene: Methanol: Ethyl acetate: Acetic acid (4: 2: 5: 0.1)	270	1–100	Tenofovir Emtricitabine	7
RP-UPLC	0.1% Tri ethyl amine (Adjusted to pH 6.0 with *o*-phosphoric acid): Acetonitrile (55: 45)	260	5–400 2–50 5–500	Tenofovir Dolutegravir Emtricitabine	Present method

Accuracy studies were performed by spiking the formulation solution with 50%, 100%, and 150% of API solution and % recovery was calculated.

The percentage relative standard deviation was calculated in all the validation parameters.

Assay of tablet dosage forms

The tablet dosage form consisting of emtricitabine (200 mg), tenofovir disoproxil fumarate (25 mg), and dolutegravir sodium (50 mg) are available with brand name, Spegra from Emcure Pharmaceuticals Ltd (India). 20 Tablets containing emtricitabine, tenofovir disoproxil fumarate, and dolutegravirsodium were weighed, powdered, and tablet powder equivalent to emtricitabine (200 mg), tenofovir disoproxil fumarate (25 mg), and dolutegravirsodium (50 mg) was first extracted with acetonitrile and sonicated for 30 min. The contents were filtered and required concentrations were prepared using the mobile phase. 1 µL of all these solutions were injected after passing through the 0.42 µ membrane filter in to the UPLC system and the respective chromatogram was recorded. The percentage purity was determined from the peak area of the chromatogram with the help of linear regression equation.

RESULTS AND DISCUSSION

A new RP-UPLC method for the simultaneous determination of tenofovir disoproxil fumarate, dolutegravir sodium, and emtricitabine in tablets using Shimadzu NexeraX2 Model UPLC system with PDA detector and Shim-pack C18 column was employed for the present study and the total run time was 5 min. Initially, 10 µg/ml each of tenofovir disoproxil fumarate, dolutegravir sodium, and emtricitabine were injected individually with the mobile phase, phosphate buffer: Acetonitrile in 50: 50 ratio, but emtricitabine peak was not symmetrical and the tailing factor >2. Therefore, the aqueous phase was slightly modified to pH 4.6 with the help of orthophosphoric acid, but the theoretical plates were <2000. Finally, 0.1% Tri ethyl amine was introduced instead of phosphate buffer with pH maintenance at 4.6, by which the three drugs were eluted with good resolution and acceptable system suitability parameters. Mobile phase consisting of 0.1% Tri ethyl amine (Adjusted to pH 6.0 with orthophosphoric acid): Acetonitrile (55: 45) was used with 1.0 mL/min flow rate and UV detection at 260 nm. Previously, established analytical methods were reviewed briefly in Table 1. The chromatograms of placebo and that of the drugs are shown in Figure 2. Tenofovir disoproxil fumarate was eluted at 3.429 min, dolutegravir sodium was eluted at 4.055 min, and emtricitabine was eluted at 2.163 min with acceptable system suitability parameters.

Linearity, precision, and accuracy

Tenofovir disoproxil fumarate, dolutegravir sodium, and emtricitabine obey Beer-Lambert's law over the concentration range 5–400, 2–150, and 5–500 µg/mL [Table 2] with linear

Table 2: Linearity

Conc. (µg/mL)	*Mean peak area		
	TDF	DS	EC
0	0	0	0
2	-	6246	-
5	6013	16892	6551
10	12121	32541	13514
20	24298	64296	26543
40	48569	129238	52644
50	61105	167234	65681
60	72268	193897	72546
80	97256	267283	104854
100	121452	331284	131227
120	-	399948	147358
150	184978	498638	191535
200	244583	-	257234
300	367452	-	379546
400	481025	-	502015
500	-	-	634112

*Mean of three replicates

Table 3: Precision study (40 µg/mL)

Intraday precision			
S. No.	Peak area		
	TDF	DS	EC
1	48569	129238	52644
2	48668	129114	52584
3	48541	129357	52602
4	48729	129227	52714
5	48458	129452	52627
6	48601	129354	52631
Mean	48594.33	129290.33	52633.67
SD	95.5817	120.3971	44.9029
% RSD	0.1967	0.0931	0.0853
Interday precision			
Day 1	Peak area		
	TDF	DS	EC
Day 1	48569	129238	52644
Day 2	48781	129836	52521
Day 3	48699	129649	52484
Mean	48683	129574.33	52549.67
SD	106.9018	305.9123	83.7636
% RSD	0.2196	0.2361	0.1594

Table 4: Accuracy study

Level %	Spiked conc. (µg/mL)			Formulation (µg/mL)			% Recovery* (% RSD)		
	TDF	DS	EC	TDF	DS	EC	TDF	DS	EC
50	12.5	25	100	25	50	200	99.32 (0.41)	98.93 (0.82)	99.94 (0.54)
100	25	50	200	25	50	200	98.89 (0.72)	99.65 (0.54)	99.51 (0.63)
150	37.5	75	300	25	50	200	99.52 (0.62)	99.81 (0.93)	99.84 (1.24)

*Mean of three replicates

Table 5: System suitability parameters (Acceptance criteria)

Drug	TDF	DS	EC	Acceptance criteria
Rt (min) (API)	3.429	4.055	2.163	>2.0
Rt (min) (dosage form)	3.413	4.039	2.147	>2.0
Theoretical plates (API)	98756	38954	81454	>2000
Theoretical plates (dosage form)	97963	39763	82698	>2000
Tailing factor (API)	1.27	1.12	1.38	<2.0
Tailing factor (dosage form)	1.31	1.15	1.29	<2.0
Resolution	35.16	11.21	-	>2.0

regression equation y = 1211.7× + 506.73 (R^2 = 0.9998) [Figure 3], y = 3330.4×–1162.3 (R^2 = 0.9999) [Figure 4], and y = 1262.7× + 990.03 (R^2 = 0.9998) [Figure 5] for tenofovir disoproxil fumarate, dolutegravir sodium, and emtricitabine, respectively. The limit of quantitation values was found to be 1.9113, 4.8752, and 4.7654 µg/mL and that of the limit of detection values 0.6287, 0.1598, and 0.1568 µg/mL for tenofovir disoproxil fumarate, dolutegravir sodium, and emtricitabine respectively.

Precision study was performed by injecting the mixture of TDF, DS, and EC (n = 6) into the UPLC system and the chromatographs were recorded. The mean peak area, standard deviation, and the relative standard deviation were calculated from the respective linear regression equations. The % RSD in intraday precision was found to be 0.1967, 0.0931, and 0.0853, whereas for interday precision, it was found to be 0.2196, 0.2361, and 0.1594 for, tenofovir disoproxil fumarate, dolutegravir sodium, and emtricitabine, respectively [Table 3] which was found to be <2.0% indicating that the method is precise. In the accuracy study, the % RSD was found to be 0.41–0.72 for tenofovir disoproxil fumarate, 0.54–0.93 for dolutegravir sodium, and 0.54–1.24 for emtricitabine, respectively (<2.0) indicating that the method is accurate [Table 4]. The system suitability parameters observed for the API as well as the tablet dosage form are shown in Table 5.

Assay of tablet dosage forms

The combination of tenofovir disoproxil fumarate (25 mg), dolutegravir sodium (50 mg), and emtricitabine (200 mg) is available with brand name, Spegra as tablets from Emcure Pharmaceuticals Ltd (India). The tablet dosage forms were tested for the assay content using the proposed RP-UPLC method. The percentage of purity of tenofovir disoproxil fumarate, dolutegravir, and emtricitabine was found to be 99.75, 99.84, and 99.63, respectively, and the typical chromatogram consisting of tenofovir disoproxil fumarate, dolutegravir, and emtricitabine is shown in Figure 2c.

CONCLUSION

The present proposed RP-UPLC method for the estimation of tenofovir disoproxil fumarate, dolutegravir, and emtricitabine is simple, precise, and accurate and the method was validated as per ICH guidelines. There is no interference of excipients during the assay.

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