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Research Article

A STABILITY-INDICATING HPLC METHOD FOR THE DETERMINATION OF POTENTIAL IMPURITIES IN A NEW FIXED DOSE COMBINATION OF DOLUTEGRAVIR, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE TABLETS USED IN THE FIRST LINE TREATMENT OF HIV-1 INFECTION

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ABSTRACT

Developing a single method for the quantification of related compounds for a combination product containing three active ingredients is difficult task. Separation and compromising run time to elute all known and unknown degradation products are crucial for a combination product. The aim of current work is to develop a new stability indicative method and validate for a fixed dose combination product containing dolutegravir, lamivudine, tenofovir disoproxil fumarate and their potential impurities in a single run by HPLC. The critical separation between dolutegravir, lamivudine, tenofovir disoproxil fumarate and its impurities was successfully attained by a new core-shell bi-phenyl, 250x4.6mm, 5µm column with a run time of 150 min. The run time was 150min. Forced degradation studies were verified to prove the stability-indicating nature of the method. Stability-indicating nature was confirmed by peak purity of all the three active components and impurities. The developed method was validated to prove the potentiality of the method as per ICH guidelines with respect to specificity, linearity, accuracy, precision ad robustness. The sensitivity of the method was proved by establishing limit of detection (LOD) and limit of quantification (LOQ) of for dolutegravir, lamivudine, tenofovir disoproxil fumarate and potential impurities.

Key words: RP-HPLC, Dolutegravir, Lamivudine, Tenofovir disoproxil fumarate, Stress degradation, Method validation.

INTRODUCTION

The development of combination antiretroviral therapy (cART) has been increased drastically in the recent years. cARTS effectively work to improve the immune system and decreasing infections. The purpose of using successful cARTS is to decrease the high pill burden [1,2], drug-drug interaction, short and long term adverse effects. Initially development of combination products includes usage of two nucleoside reversed transcriptase inhibitors (RTI's) such as zidovudine/lamivudine(3TC), abacavir (ABC)/3TC or tenofovir (TDF)/emtricitabine (FTC) or a boosted protease inhibitor (PI) lopinavir/ritonavir (RTV). Tenofovir disoproxil fumarate/ emtricitabine/ efavirenz is the first STR or FDC combination containing three active components[3]. In the current study a new FDC containing dolutegravir 60mg(INSTI's), lamivudine 300mg (NRTI's) and tenofovir disoproxil fumarate 300mg (NRTI's) has been used for identification and quantification of degradation products as well as process impurities.

The chemical name of dolutegravir sodium is Sodium(4R,12aS)-9-{[(2,4-difluorophenyl)methyl]carbamoyl}-4-methyl-6,8-dioxo-3,4,6,8,12,12ahexahydro-2H-pyrido[1`,2`:4,5]pyrazino[2,1-b][1,3]oxazol-7-olate[1,2][4,5]. The chemical name of lamivudine is 4-amino-1-[(2R, 5S)-2-(hydroxymethyl)-1, 3-oxathiolan-5-yl] pyrimidin-2-one[6,7,8]. The chemical name of tenofovir disoproxil fumarate is 9-[(R)-2[[bis[[(isopropoxycarbonyl)oxy]-methoxy]phosphinyl]methoxy] propyl]adenine fumarate [9-13]. Literature reveals that the above three molecules HIV integrase inhibitors, nucleoside reverse transcriptase inhibitors and nucleotide reverse transcriptase inhibitors from three originated medicines being used to treat HIV/AIDS[14,15].

A literature search showed that few methods available for the quantitative determination of dolutegravir [16,17,18,19], lamivudine [20,21,22] and tenofovir disoproxil fumarate[23] alone, a very few methods available for the quantification of emtricitabine and tenofovir disoproxil tablets[24], tenofovir disoproxil fumarate, lamivudine, and efavirenz in combined tablet dosage form [25,26], tenofovir tablets [27], efavirenz, emtricitabine and tenofovir tablets [28]. But no method has been reported till now for the simultaneous quantitative determination of dolutegravir, lamivudine and tenofovir disoproxil fumarate and its related compounds in fixed finished dosage form by HPLC. A RP-HPLC method has been reported for determination of emtricitabine, tenofovir and efavirenz in emtricitabine, tenofovir and efavirenz fixed dosage form. In the present work in addition to degradation impurities, process related impurities were also considered for method development by HPLC. Official pharmacopoeia monographs available only for the estimation of organic impurities for tenofovir tablets and lamivudine impurities but not for dolutegravir.

Hence to develop a single HPLC method that could separate dolutegravir, lamivudine, tenofovir disoproxil fumarate (Figure 1) from its potential impurities namely H-DOL.RC02, H-DOL.RC03, lamivudine carboxylic acid, lamivudine uracil derivative, PMPA, mono-POC-PMPA, tenofovir mixed dimer and tenofovir dimer and process impurities namely H-DOL.RC01, H-DOL.RC04l, lamivudine diastereomer, ipr-POC-PMPA and MOC-POC-PMPA (Figure 2) and also unknown degradation

impurities formed during forced degradation [29,30,31] studies and stability studies. Validation has been covered only for potential impurities and same was reported. ICH guidelines [32,33] were applied to validate the method successfully. Developing a single method for dolutegravir, lamivudine, tenofovir disoproxil fumarate, potential impurities and process impurities by HPLC would reduce solvent consumption and analysis time.

MATERIALS AND METHODS

Chemicals and reagents

Dolutagravir, Lamivudine, Tenofovir disoproxil fumarate standards, standard materials impurities and chemical reagents were obtained from Hetero labs limited, Hyderabad, India. Sodium dihydroge phosphate monohydrate (Merck grade), Orthophosphoric acid (Merck grade), 1-Octane sulfonic acid sodium salt monohydrate (Merck grade), Methanol (Rankem HPLC grade) and Acetonitrile (Rankem HPLC grade) were purchased from Merck chemicals, India and Rankem Chemicals India. High-quality pure water was prepared in-house by use of a Millipore (Billerica, MA, USA) Milli-Q water-purification system.

Instrumentation

Waters HPLC® system (Waters Corp., Milford, MA, USA) equipped with an auto sampler and quaternary gradient pump with an in-line degasser was used. The photodiode array (PDA) detector was employed throughout the analysis. The chromatographic data was acquired using Empower 3 software. The photodiode array (PDA) detector and was utilized for degradation studies.

HPLC Conditions

The chromatographic separation was obtained on a new Core-shell Bi-phenyl polar stationary phase of Phenomenex kinetex Bi-phenyl 250x4.6mm, 5µ column. Mobile phase A sodium dihydrogen phosphate monohydrate (10mM) with 0.5g/L of 1-octane sulfonic sodium salt monohydrate adjusted pH 2.5 with orthophosphoric acid and Mobile phase B was sodium dihydrogen phosphate monohydrate (10mM) with 0.5g/L of 1-octane sulfonic sodium salt monohydrate adjusted pH 2.5, acetonitrile and Methanol in the ratio of 20:60:20%v/v/v. The gradient programme T (min) =% mobile phase B: 0=5, 40=15, 70=45,100=50,105=70,110=70, 115=100, 135=100, 138=5 and 150=5 with flow rate 0.6 mL/min. The injection volume was 10µL and detection wavelength was set at 260nm. The column temperature was maintained at 30°C.Sample cooler was maintained at 5°C.

Preparation of solutions

Preparation of standard solution

A standard solution containing 2.5µg/mL of dolutegravir, 15µg/mL of lamivudine and 15µg/mL of tenofovir disoproxi fumarate was used for calculation of known and unknown impurities.

Preparation of sample solution

A test solution containing of 500µg/mL of dolutegravir, 3000µg/mL of lamivudine and 3000µg/mLof tenofovir disoproxil fumarate was prepared by taking tablet powder equivalent to 50mg of dolutegravir dissolved in 100 mL of 50:50%v/v of 0.1% orthophosphoric acid solution and methanol, sonicated sample solution for 30 minutes. Centrifuged the sample solution at 5000 rpm for 10 minutes. Filtered the sample solution through 0.45µ PVDF (Millipore) filter.

Preparation of spiked sample solution

A sample solution containing 500µg/mL of dolutegravir, 3000µg/mL of lamivudine and 3000µg/mL of tenofovir disoproxil fumarate was spiked with all impurities at 0.5% level was used as spiked sample (Figure 3).

Method validation

Method validation was demonstrated for dolutegravir, lamivudine, tenofovir disoproxil fumarate and their potential impurities using current ICH guidelines. Method validation was demonstrated in terms of Method precision, linearity, accuracy, LOD and LOQ [34].

Specificity

Specificity is the ability of the method that should be well resolved from other active materials, processed impurities, potential impurities, unknown degradation impurities formed during degradation studies. Placebo should not be interference at the retention times of active as well impurities. It was confirmed by injecting individual impurities at specification level and also from spiked sample spiked all potential impurities at specification level.

Forced degradation studies

Forced degradation studies were demonstrated to prove the stability-indicating property of the method. Homogeneity of dolutegravir, lamivudine and teofovir disoproxil peaks in stressed samples were ensured by peak purity. Photo diode array was employed throughout the analysis to assess the peak purity. Stress experiments were performed on equivalent to 50mg of dolutegravir by subjecting with acid hydrolysis (1N HCl /60°C/30min) , base hydrolysis (1N NaoH/RT/2min), oxidative hydrolysis (3%/60°C/30min), thermal (80°C/48hours) [35,36], photolytic (1.2million lux hrs and 200watt hrs/square meter)[37] and humidity (90%RH/168hours) (Figure 4). Stressed samples were diluted for assay calculation of dolutegravir, lamivudine and tenofovir disoproxil against reference standard.

Precision

System precision for the proposed method was confirmed by injecting a standard solution containing dolutegravir, lamivudine and tenofovir disoproxil fumarate at 0.5% of specification level (500µg/mL of dolutegravir, 3000µg/mL of lamivudine and 3000µg/mL of tenofovir disoproxil fumarate) six times into the chromatographic system and reported %RSD.

A spiked sample preparation containing all potential impurities at 0.5% specification level(500µg/mL of dolutegravir, 3000µg/mL of lamivudine and 3000µg/mL of tenofovir disoproxil fumarate) was injected six times to perform the method precision and reported %RSD for all impurities. Intermediate precision was also demonstrated by preparing standard solution preparation and spiked sample preparation same as above on different day, different analyst, different instrument and reported %RSD.

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

A series of solutions containing dolutegravir, lamivudine and tenofovir disoproxil fumarate and all potential impurities was used to established LOD and LOQ at a signal to noise ratio of 3:1 and

10:1 respectively. Precision at LOQ was also established using above sample solutions and reported %RSD for all peaks.

Linearity

The linearity of the method was demonstrated by injecting six solutions containing dolutegravir, lamivudine and tenofovir disoproxil fumarate and all its potential impurities at five levels from LOQ to 150% of specification level (500µg/mL of dolutegravir, 3000µg/mL of lamivudine and 3000µg/mL of tenofovir disoproxil fumarate) and reported correlation coefficient, % intercept, R^2 value and regression equation.

Accuracy

The accuracy of the method was determined in triplicate from LOQ to 150% of specification level (500µg/mL of dolutegravir, 3000µg/mL of lamivudine and 3000µg/mL of tenofovir disoproxil fumarate) by spiking all its potential impurities from LOQ to 150% of specification level and reported % recovery and %RSD at each level.

Robustness

Chromatographic conditions were altered intentionally to prove the robustness of the method, and determined resolution between, dolutegravir, lamivudine, tenofovir disoproxil fumarate and its potential impurities. As the flow rate of the method was 0.6 mL/min, the effect of flow rate was studies between 0.5mL/min to 0.8mL/min instead of ±0.2 units because the lower flow rate limit for HPLC is 0.5mL/min. pH of the method studied by ±0.2 pH units (at pH 2.3 and 2.5). Temperature was studied at ambient, 30°C and 35°C. Remaining chromatographic conditions were kept constant for all the above altered conditions.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Different components exhibit different wavelength maxima and it is the characteristic of that particular component and hence selection of single wavelength is critical for combination product containing three main components, potential impurities and process impurities. Based on the response of dolutegravir, lamivudine, tenofovir disoproxil fumarate, potential impurities and also considering lowest label claim(dolutegravir, lamivudine and tenofovir disoproxil fumarate tablets 50mg/300mg/300mg) 260nm was chosen as detection wavelength.

Different buffers such as potassium phosphate, sodium phosphate, sodium perchlorate, ammonium acetate but these buffers did not offer much resolution. 1-octane sulfonic acid sodium salt, heptanes sulfonic acid sodium salt and pentane sulfonic acid sodium salts were used along with above buffers. Better separation was observed with sodium dihydrogen phosphate monohydrate (10mM) with 0.5g/L of 1-octane sulfonic sodium salt monohydrate. From the preparation of spiked sample it is clear that, resolution is critical between three active components and its all potential and process impurities. Hence columns with stationary phases like C8, C18, phenyl, Cyno, New core shell technology with C8, C18 and bi-phenyl were used to resolve the impurities, finally resolution between three active components and all impurities were found to be good in a new core-shell Kinetex Bi-phenyl with 250 x 4.6mm, 5µ column. The pH values optimized were 2.5, 3.0, 3.5, 5.0, and 7.0. Finally the best results were obtained at pH 2.5±0.05 by adjusting with orthophosphoric acid. Selection of mobile phase and buffer pH was justified by less run time. After several number of experiments, the method has been finalized on Kinetex Bi-phenyl with 250 x 4.6mm, 5µ column using A sodium dihydrogen phosphate monohydrate (10mM) with 0.5g/L of 1-octane sulfonic sodium salt monohydrate adjusted pH 2.5 with orthophosphoric acid and Mobile phase B was sodium dihydrogen phosphate monohydrate (10mM) with 0.5g/L of 1-octane sulfonic sodium salt monohydrate adjusted pH 2.5, acetonitrile and Methanol in the ratio of 20:60:20%v/v/v. The gradient programme T (min) =% mobile phase B: 0=5, 40=15, 70=45,100=50,105=70,110=70, 115=100, 135=100, 138=5 and 150=5 with flow rate 0.6 mL/min. Different column oven temperatures were tried with 30°C, 35°C, and 40°C for better peak shape, base line and resolution. Finally better base line and separation between impurities was observed at a column oven temperature 30°C. A chromatogram obtained from dolutegravir, lamivudine, tenofovir disoproxil fumarate spiked with their fourteen impurities at 0.5% level was shown in Fig.2. The system suitability results were reported in Table 1.

Results of forced degradation

Remarkable degradation was not observed for dolutegravir and lamivudine in acid, base, oxidative, thermal, humidity and photolytic conditions (Fig. 3(b, c, d, e, f, g). For tenofovir disoproxil Mono-POC-PMPA formed in acid, base, peroxide and in thermal conditions Tenofovir mixed dimer and tenofovie dimer observed in thermal condition. The homogeneity of peaks in presence of degradation products in all stressed conditions was assessed by peak purity. Mass balance was found to above 95%. Results of degradation impurities in different conditions, formation unknown degradation impurities and total impurities for dolutegravir, lamivudine and tenofovir disoproxil fumarate reported in Table-2.

Results of method validation

Precision

The repeatability of the method was demonstrated by six sample preparation solutions containing 500 µg mL−1of dolutegravir, 3000µg mL−1 of lamivudine and 3000 mL−1 of tenofovir disoproxil fumarate tablets spiked with 0.5% of all potential impurities (Fig. 2). The %RSD for the area of each peak was calculated and found to be within 5.0 % for method precision and below 1.6% for intermediate precision. The results were tabulated in Table 3(a - c).

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

The LOD and LOQ of the method were determined by injecting standard solutions of progressively decreasing concentration under the chromatographic conditions described above. The LOD was defined as the concentration for which the signal-to-noise ratio was 3:1 and the LOQ was defined as the concentration for which the signal-to-noise ratio was 10:1. The determined limit of detection and limit of quantification values for dolutegravir, lamivudine, tenofovir disoproxil and its potential impurities were reported in Table-3(a - c).

Linearity

The linearity of detector response for dolutegravir, lamivudine, tenofovir disoproxil fumarate and their potential impurities were studied by preparing a series of solutions using dolutegravir, lamivudine, tenofovir disoproxil fumarate and their potential impurities at five different concentrations levels ranging from 0.05% to 150% of test concentration (500µg/mL/3000µg/mL /3000µg/mL). The correlation coefficients, slopes, R^2 value and %-intercepts of the calibration curves were determined. Excellent

correlation observed between peak areas and concentration of analyte (Table-3(a - c)).

Accuracy

The accuracy of an analytical procedure expresses the closeness of results of true value and the value found. The study was executed out by spiking known impurities in triplicate at LOQ, 50%, 100% and 150% of the analyte concentration (500µg/mL for dolutegravir, 3000µg/mL for lamivudine and 3000µg/mL for tenofovir disoproxil fumarate). The % recoveries of all these impurities were found to be in-between the predefined acceptance criterion of 85.0-115.0% and %RSD at each level was found to be within 2.64 (Table-4).

Robustness

The robustness was evaluated at the normal mobile phase flow rate was 0.6 mL min. he flow rate was changed to 0. 5mL and 0.7 mL min−1. Remaining parameters kept same. The effect of the column temperature was studied at 30 and 35 °C. The effect of pH of buffer was studied at varying ±0.2 pH units (at 2.3 and 2.7 buffer pH). The resolution was found more than 2.0 in all conditions

Solution stability and Mobile phase stability

Solution stability was established leaving spiked sample solutions at 2-8°C for 48 h. At room temperature dolutegravir and lamivudine were found to stable. Tenofovir disoproxil fumarate prone to degrade at room temperature. Hence stability studies performed at 5°C. Content of all potential impurities were determined for every 12 h interval up to 48 hrs. The results were within 0.05% for specified and unspecified impurities and 0.2% for total impurities.

Table-1: System suitability results

Compound/impurity	RT (in min)[a]	RRT[b] (n=6)	Peak area (%RS D)[a](n=6)	USP tailing[a] (n=6)
PMPA	17.16	0.20	0.28	1.02
Lamivudine Uracil derivative	23.35	0.42	0.21	1.00
Lamivudine carboxylic acid	39.50	0.68	0.62	1.00
Mono-POC-PMPA	49.12	0.57	2.4	1.05
Lamivudine diastereomer	47.06	0.87	1.07	1.00
Lamivudine	51.70	--	0.44	0.97
H-DOLRC04	63.85	0.69	1.52	0.98
MOC-POC-PMPA	72.8	0.91	1.38	1.00
ipr-POC-PMPA	74.28	0.93	2.87	1.00
H-DOLRC02	77.10	0.85	1.40	0.98
Tenofovir disoproxil	80.98	---	1.15	1.01
n-POC-POC-PMPA	82.37	1.02	1.63	1.00
H-DOLRC01	84.66	0.92	0.39	1.00
Tenofovir mixed dimer	86.50	1.08	1.19	1.00
H-DOLRC03	89.51	0.97	0.29	1.00
Dolutegravir	92.16	---	1.31	0.97
Tenofovir dimer	116.27	1.46	1.52	0.98

[a] Mean (n= 6).

[b] Relative retention times(RRT)were calculated against the retention time(RT)of Lamivudine, Tenofovir disoproxil and Dolutegravir.

Table-2: Summary of forced degradation studies

Degradation mechanism/ condition	Acid(1N HCl/60°C/ 30min	Base(1N NaoH/RT/ 2min	Peroxide (3%/60°C/ 30min)	Thermal/80° C/48hrs	Photolytic (1.2million lux hrs and 200watt hrs/square meter)	Humidity/90% RH, 168 hrs
PMPA	NA	NA	NA	NA	NA	NA
Lamivudine Uracil derivative	NA	NA	NA	NA	NA	NA
Lamivudine carboxylic acid	0.044	0.044	0.041	0.047	0.043	0.042
Mono-POC-PMPA	11.660	8.427	1.384	4.088	0.774	0.826
H-DOLRC02	0.028	0.031	0.034	0.00	0.036	0.034
H-DOLRC03	0.074	0.078	0.074	0.088	0.076	0.076
Tenofovir mixed dimer	0.016	0.024	0.030	1.667	0.032	0.027
Tenofovir dimer	0.025	0.0380	0.052	1.129	0.058	0.050
Lamivudine related total impurities	0.044	0.044	6.658	0.047	0.043	0.042
Tenofovir disoproxil fumarate related total impurities	22.301	20.421	1.468	10.704	0.864	0.903
Dolutegravir related impurities	0.102	0.109	0.108	0.088	0.112	0.110

Table-3(a): LOD, LOQ, Regression and precision data for Dolutegravir

Compound/impurity	Dolutegravir	H-DOLRC02	H-DOLRC03
LOD (µg/mL^{-1})	0.0138	0.0116	0.0142
LOQ (µg/mL^{-1})	0.0418	0.0352	0.0432
Precision at LOQ (%RSD)	2.07	2.22	1.65
Signal/Noise ratio (at LOQ)	12	14	12
Regression equation (Y) Slope(b)	58371.90	51351.74	53455.82
% Intercept(a)	1.04	1.96	0.37
Correlation coefficient	0.99986	0.99955	0.99984
R^2 value	0.99972	0.99911	0.99968
Precision (%RSD)	0.97	2.50	1.60
Intermediate precision (%RSD)	0.97	0.76	1.61

Table-3(b): LOD, LOQ, Regression and precision data for Lamivudine

Compound/impurity	Lamivudine	Lamivudine Uracil derivative	Lamivudine carboxylic acid
LOD (µg/mL^{-1})	0.0059	0.063	0.0050
LOQ (µg/mL^{-1})	0.0180	0.189	0.0151
Precision at LOQ (%RSD)	0.73	1.59	1.16
Signal/Noise ratio (at LOQ)	23	26	15
Regression equation (Y) Slope(b)	27571.41	34928.37	24809.46
%Intercept(a)	0.60	-0.24	0.33
Correlation coefficient	0.99998	0.99998	0.99998
R^2 value	0.99996	0.99996	0.99995
Precision (%RSD)	0.57	0.21	0.23
Intermediate precision (%RSD)	0.84	0.95	0.46

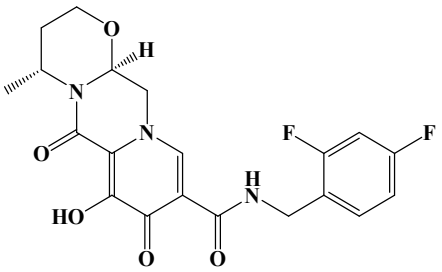
Table-3(c): LOD, LOQ, Regression and precision data for Tenofovir disoproxil

Compound/impurity	Tenofovir disoproxil	PMPA	Mono-POC-PMPA	Tenofovir mixed dimer	Tenofovir dimer
LOD (µg/mL^{-1})	0.251	0.002	0.148	0.241	0.138
LOQ (µg/mL^{-1})	0.761	0.006	0.448	0.730	0.418
Precision at LOQ (%RSD)	2.01	1.32	2.38	5.27	1.03
Signal/Noise ratio (at LOQ)	12	24	17	12	17
Regression equation (Y) Slope(b)	20937.71	47175.36	30825.63	16065.43	16678.16
%Intercept(a)	0.43	-2.43	0.24	1.11	1.24
Correlation coefficient	0.99996	0.99942	0.99998	0.99980	0.99980
R^2 value	0.99992	0.99884	0.99995	0.99960	0.99960
Precision (%RSD)	1.02	5.00	1.99	3.00	0.88
Intermediate precision (%RSD)	1.01	0.93	0.28	0.54	0.31

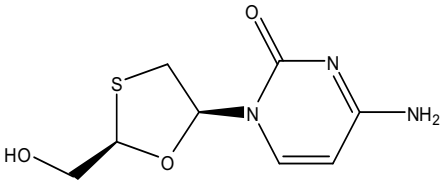
Table-4: Summary of Recovery data

Amount spiked[a]	% Recovery		
	Dolutegravir		
	Dolutegravir	H-DLRC02	H-DLRC03
LOQ	101.0	101.9	92.3
(%RSD)	2.33	1.53	1.38
50%	105.8	92.3	99.0
(%RSD)	0.90	1.13	0.0
100%	98.6	96.4	98.2
(%RSD)	0.0	1.04	0.30
150%	94.2	100.3	97.7
(%RSD)	0.17	0.0	0.36
Amount spiked[a]	% Recovery		
	Lamivudine		
	Lamivudine	Lamivudine carboxylic acid	Lamivudine uracil derivative
LOQ	92.3	95.4	105.8
(%RSD)	1.38	2.42	2.58
50%	99.0	104.0	103.3
(%RSD)	0.0	0.63	0.75
100%	98.2	98.6	104.2
(%RSD)	0.30	0.52	0.96
150%	97.7	101.2	103.3
(%RSD)	0.36	0.70	0.55

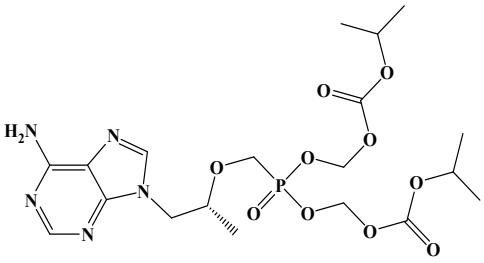
Amount spiked[a]	% Recovery				
	Tenofovir disoproxil				
	Tenofovir disoproxil	Mono-POC-PMPA	Tenofovir mixed dimer	Tenofovir dimer	PMPA
LOQ	99.1	98.2	91.2	94.5	107.6
(%RSD)	2.64	0.41	3.14	0.49	0.68
50%	102.0	103.9	100.8	109.7	105.0
(%RSD)	0.0	0.71	0.40	0.21	0.26
100%	98.3	95.7	100.6	103.2	107.2
(%RSD)	0.30	0.53	0.53	0.45	1.06
150%	97.8	98.6	104.5	106.3	105.9
(%RSD)	0.17	0.23	0.22	0.16	2.53



Dolutegravir

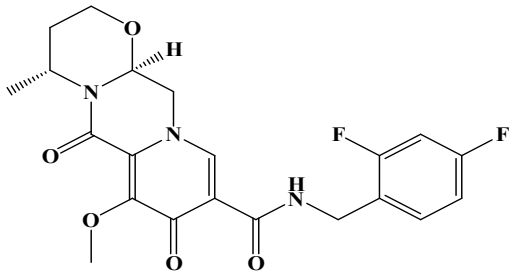


Lamivudine

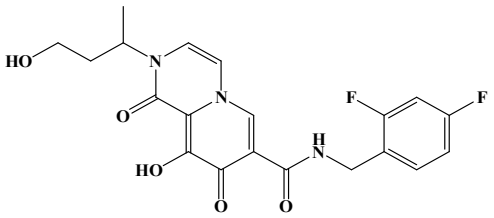


Tenofovir disoproxil fumarate

Figure 1



DOLRC-01



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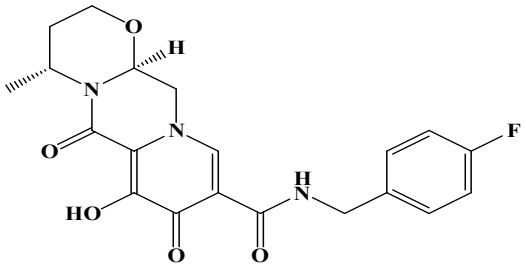

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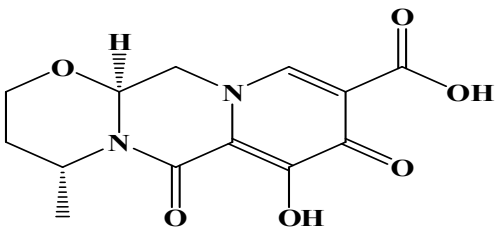

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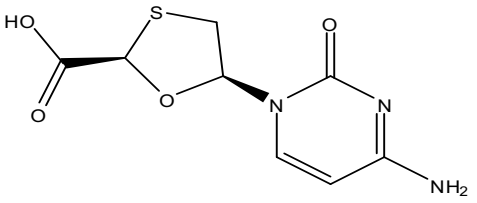
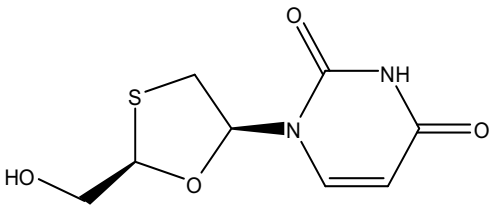
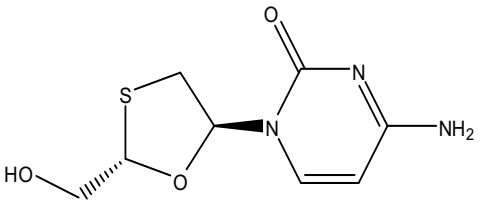


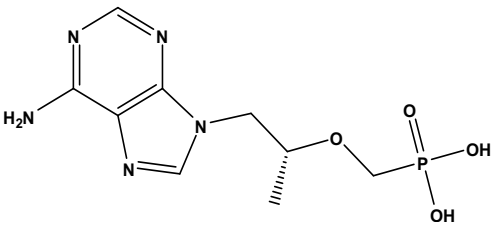
Lamivudine carboxylic acid



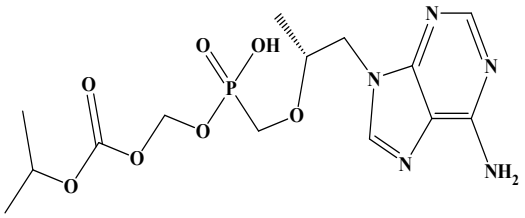
Lamivudine uracil derivative



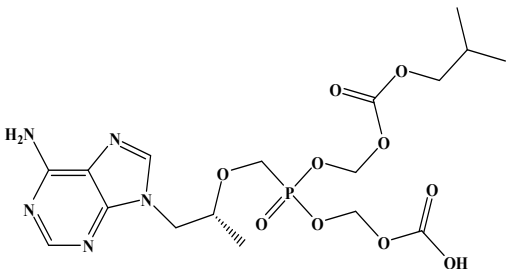
Lamivudine diastereomer



PMPA



Mono-POC-PMPA



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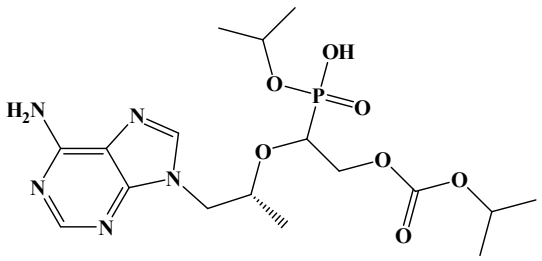
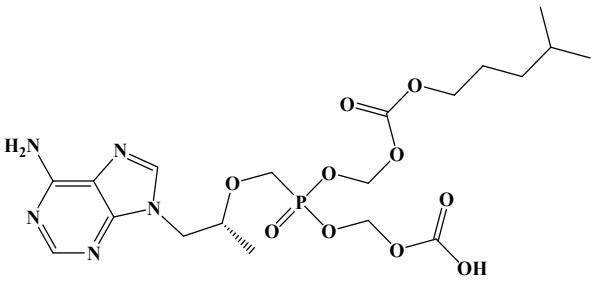
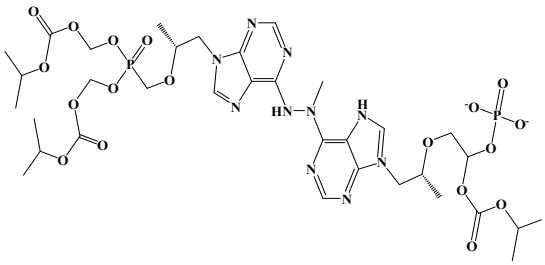


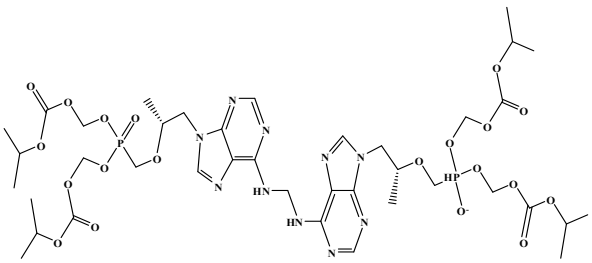
iPr-POC-PMPA



n-POC-POC-PMPA



Tenofovir mixed dimer



Tenofovir dimer

Figure 2

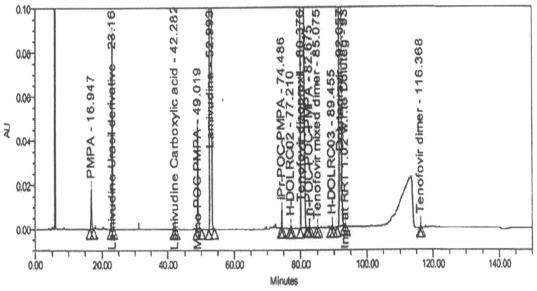
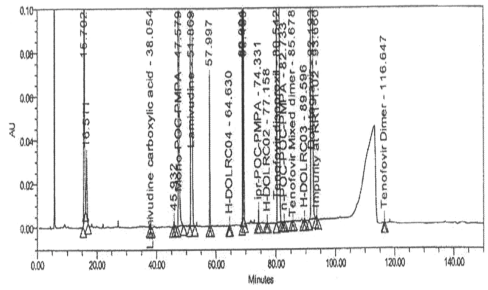
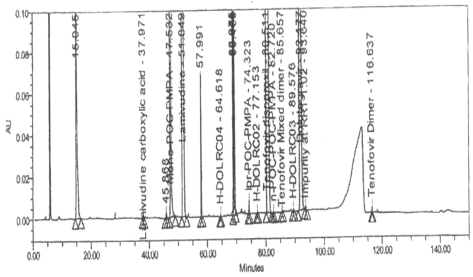


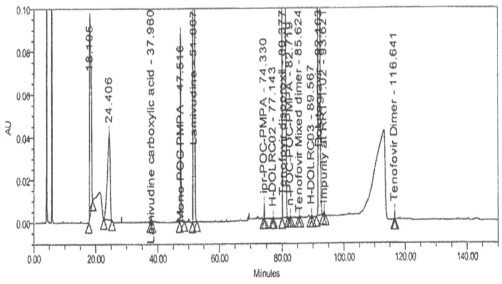
Figure 3: Typical chromatogram of Spiked sample preparation



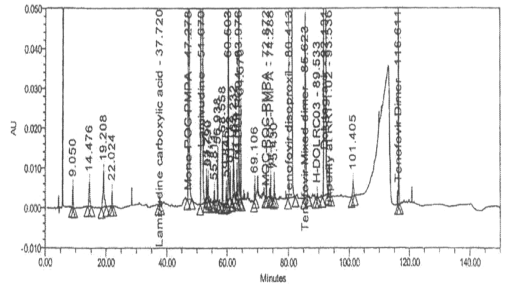
(a) Typical chromatogram of acid stressed sample



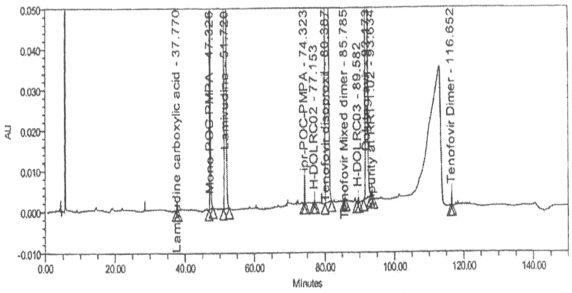
(b) Typical chromatogram of base stressed sample



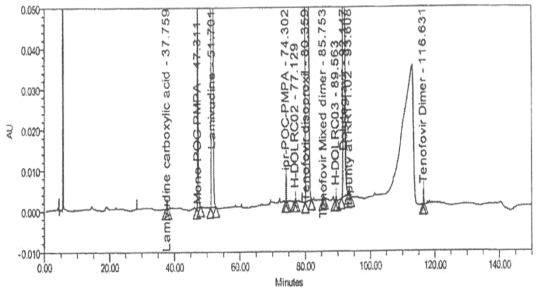
(c) Typical chromatogram of peroxide stressed sample



(d) Typical chromatogram of thermal stressed sample



(e) Typical chromatogram of humidity stressed sample



(f) Typical chromatogram of photolytic stressed sample

Figure 4

CONCLUSION

A new simple, single and selective stability-indicating, gradient RP-HPLC method has been developed for the quantitative determination of dolutegravir, lamivudine, tenofovir disoproxil fumarate and all its potential impurities in drug product. This method is capable of separating all impurities with good resolution within 150 min. The performance of this method in terms of sensitivity and separation was found to be good. Forced degradation studies were conducted, excellent separation achieved between all major known and unknown degradation products. The developed method was found to be specific, precise, accurate and linear and it is useful for quality control analysis of pharmaceutical dosage forms.

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