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A new stability indicating RP-UPLC method for simultaneous estimation of Doravirine, Lamivudine and Tenofovir disoproxil fumarate in bulk and their combined pharmaceutical formulation

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Abstract

Background: To establish a simple, sensitive, accurate, precise, efficient, economical RP-UPLC method for simultaneous estimation of Doravirine, Lamivudine and Tenofovir disoproxil fumarate in bulk and their combined pharmaceutical formulations. Optimization of Chromatographic separation was achieved on analytical column HSS C18 (100 × 2.1 mm, 1.8 µ) maintained at temperature 30 °C and mobile phase consisting of 0.01 N Potassium dihydrogen orthophosphate buffer (pH-4.8) and acetonitrile in the ratio 60:40 v/v and at a flow rate 0.3 mL/min in isocratic mode. The injection volume was set as 1 µl detection wavelength is 260 nm. The proposed method validation was done as per International Council on Harmonization Q2 (R1) guidelines.

Results: Doravirine, Lamivudine and Tenofovir disoproxil fumarate were eluted at retention times of 1.2, 1.5, and 1.8 min respectively. The proposed method was identified an excellent linearity over concentration range of 12.5–75.0 µg/mL for Doravirine and 37.5–225.0 µg/mL for Lamivudine and 37.5–225.0 µg/mL for Tenofovir disoproxil fumarate. The percentage relative standard deviation for intra-day and inter-day precision of the present method was less than 2% for Doravirine, Lamivudine and Tenofovir disoproxil fumarate. Accuracy of the present method was evaluated by recovery studies which were in the range of 99.62–99.88% for Doravirine and 98.78–99.44% for Lamivudine and 99.67–100.52% for Tenofovir disoproxil fumarate. The limit of detection and limit of quantification were found to be 0.249 µg/mL and 0.756 µg/mL for Doravirine and 0.24 µg/mL and 0.727 µg/mL for Lamivudine and 0.797 µg/mL and 2.966 µg/mL for Tenofovir disoproxil fumarate. Forced degradation studies were carried out under various stress conditions like acid, base, peroxide, thermal, photo and neutral conditions.

Conclusions: The present method makes sure about no degraded impurity peak interference at the retention time of analyte peak hence can be applied for quality control investigation of Doravirine, Lamivudine and Tenofovir disoproxil fumarate in bulk and pharmaceutical formulations.

Keywords: Doravirine, Lamivudine, Tenofovir disoproxil fumarate, Method validation, Forced degradation, Reverse phase ultra-performance liquid chromatographic method

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Background

Doravirine (DOR) is a synthetic derivative of pyridinone moiety significantly hinders the function of the non-nucleoside reverse transcriptase which is responsible for integration and replication of genome of the Human immune virus [1–3]. Chemically DOR is 3-chloro-5-[1-[(4-methyl-5-oxo-1H-1,2,4-triazol-3-yl) methyl]-2-oxo-4-(trifluoromethyl) pyridin-3-yl] oxybenzonitrile. Triphosphate of LMV (3TCTP) is the competitive inhibitor of nucleoside reverse transcriptase [4–6]. LMV chemically is 4-amino-1-[(2R, 5S)-2-(hydroxyl methyl)-1, 3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one. Tenofovir is the active moiety of TDF ceases the replication of viral genome by inhibiting the nucleoside reverse transcriptase competitively [7–9]. TDF chemically is 1-(6-aminopurine-9-yl) propan-2 yl] oxy methyl-(propan-2-yl oxy carbonyl oxy methoxy) phosphoryl] Oxy methyl propan -2yl carbonate. DELSTRIGO™ is a recently approved combined tablet dosage form comprising three active moieties including Doravirine (DOR), Lamivudine (LAM) and Tenofovir disproxil fumarate (TDF). It is a highly potent regimen to improve the quality of life and survival span of HIV-I patients. The chemical structures of DOR, LAM and TDF were given in Fig. 1.

As per literature survey, a quite few analytical methods were disclosed for analysis of DOR, LMV, TDF as single entities. A few analytical methods were developed for simultaneous analysis of LMV with other antiviral agents and TDF with other antiviral agents [10–16]. Certain analytical methods were described to estimate TDF, LMV with other antiretroviral agents [17–26]. An immense exploration of literature exposed that only two HPLC methods were recently reported for simultaneous analysis of DOR, LMV and TDF in a tablet dosage form [27, 28]. In the reported HPLC methods, many significant drawbacks were observed like retention time of LAM (2.45 min) and TDF (7.3 min) and DOR (8.79 min) was longer and more solvent consumption. No UPLC method was noticed for simultaneous estimation of DOR, LAM and TDF in a combined dosage form. Due to lack of methods, there is a scope to develop an economical, efficient UPLC method for simultaneous estimation of DOR, LAM and TDF with high sensitivity and better resolution of drug analytes in the mixture. Hence, current method aimed to develop UPLC method for synchronized estimation of DOR, LAM, TDF analytes in blended pure powder and in film coated tablet dosage form.

Methods

1. **Pure samples**

   The pure form of TDF (99.17%), DOR (99.3%) and LMV (99.4%) were procured from Spectrum pharma research solutions, Hyderabad.

2. **Formulation**

   The DELSTRIGO™ (100 mg of Doravirine, 300 mg of Lamivudine, and 300 mg of Tenofovir disoproxil fumarate) tablets were purchased from local marketing agency upon request.

3. **Chemicals and reagents**

   All the solvents of HPLC grade were procured from local distributor of Merck India Limited, Mumbai. All the chemicals of analytical grade and HPLC grade water were acquired from the Finar chemical distributor.

Instrumentation

The current method was carried out using WATERS Acquity UPLC system which is equipped with Tunable UV detector and HSS (C18 100 × 2.1 mm, 1.8 µ) column used as a stationary phase. Empower 2 software was used to process and integrate the data.

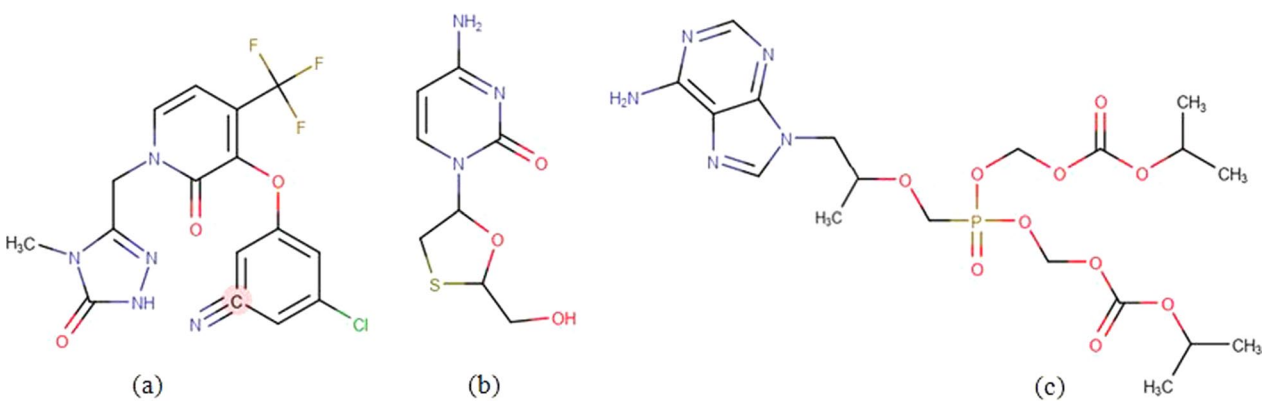


Fig. 1 Chemical structures of **a** Doravirine, **b** Lamivudine and **c** Tenofovir disoproxil fumarate

Preparation of standard solution

25 mg of DOR, 75 mg of LAM and 75 mg of TDF were accurately weighed and transferred into 50 mL volumetric flask and dissolve the above analytes with diluent consisting of equal volumes of water and 0.01 N Potassium dihydrogen ortho phosphate and make up to the volume with the diluent to get the standard stock solution concentration consisting of 500 µg/mL, 1500 µg/mL and 1500 µg/mL for DRV, LMV and TDF respectively. Dilute 1 mL of above solution to 10 mL with diluent to get working standard solution having concentration of 50 µg/mL, 150 µg/mL and 150 µg/mL for DOR, LAM and TDF respectively.

Preparation of sample solution

The tablet (D*elstrigo*™ consisting of 300 mg of Tenofovir disoproxil fumarate, 300 mg of Lamivudine, and 100 mg of Doravirine) powder is equivalent to 75 mg of LAM 75 mg of TDF and 25 mg of DRV were accurately weighed and placed in 50 mL volumetric flask. Above contents of analytes were dissolved with diluents consisting of equal volumes of water and 0.01 N Potassium dihydrogen ortho phosphate in equal proportions. 1 mL of the above solution is further diluted with 10 mL diluent to get a solution having concentration 150 µg/mL, 150 µg/mL and 50 µg/mL for LAM, TDF and DOR respectively. The possible particulate matter in sample was eliminated by using 0.45 µm Nylon filters.

Method development

Chromatographic method conditions:

The current method was carried out by using WATERS UPLC system with Tunable UV detector. DOR, LAM and TDF analytes were successfully separated by using HSS (C18 100 × 2.1 mm, 1.8 µ) column and with mobile phase consisting of 0.01 N Potassium dihyrogen ortho phosphate (pH-4.8) and Acetonitrile in 60:40 v/v pumping at 0.3 mL/min flow rate. Analytes were detected at a wavelength 260 nm. Both analytical column and injection device were maintained at the same temperature 30 °C. Before application of sample and mobile phase into the instrument, filtered through 0.45µ Nylon filters to dispose of particulate matter in it.

Method validation

The following method has been validated using below mentioned validation parameters according to ICH Q2 (R1) guidelines specifications [29].

System suitability

The system suitability of the proposed method has been validated by injecting 6 replicates of standard solution (150 µg/mL of LAM, 150 µg/mL of TDF and 50 µg/mL of DOR) into UPLC system. System suitability parameters like resolution (R), number of theoretical plates (N), tailing factor (T) were assessed by determining the percentage relative standard deviation (%RSD) of parameters for the recorded chromatograms.

Linearity

Linearity of the method assures a direct proportional relationship between input concentrations and the obtained output peak area responses**.** Linearity was estimated by assessing the correlation coefficient (r2) value for the triplicates of the series of working standard solution concentrations about 37.5, 75.0, 112.5, 150.0, 187.5, 225.0 µg/mL for both LAM and TDF and 12.5, 25.0, 37.5, 50.0, 62.5, 75.0 µg/mL for DOR, by plotting a linear response curve in between series of concentrations and obtained peak areas mentioned for each analyte.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were assessed by following formulae

$$\mathrm{LOD} = 3.3\,\sigma/S$$
$$\mathrm{LOQ} = 10\,\sigma/S$$

where σ standard deviation (SD) obtained from the intercept of linear plot ($n=3$). S average slope of the linear plot ($n=3$).

Solution stability

Solution stability was performed by using standard solution stored at room temperature and evaluate the solution at specific intervals of time for 72 h.

Specificity

Specificity of the method was evaluated by analysis of different analytes which are not interfered by the presence of other impurities or degradation products. Specificity was determined by giving subsequent injections of blank, standard solution and placebo spiked in standard solution and make sure that no interferences from blank and placebo at the retention time of DOR, LAM, TDF.

Precision

Precision parameter represents the closeness relationship established among the obtained responses of same

sample under similar conditions. It was done by giving six replicate injections of standard solutions in the same day (intra-day precision) and same standard solution injected two times in a day for three continuous days under similar conditions (inter-day precision). %RSD was calculated for peak areas of recorded chromatograms.

Accuracy

Percentage recovery method was adopted to ensure the accuracy of the proposed method, in which at three percentage levels sample solution was spiked into standard solution. Analysis was done by triplicate injections of each level spiked solution. At the three different levels, mean percentage recovery of DOR, LAM, TDF was calculated.

Robustness

Robustness parameter assures that to maintain originality of the method to produce response by modifying certain method conditions intentionally. In the proposed method, to validate the robustness parameter slight variations employed in method conditions are mobile phase ratio (±10%), flow rate (±10% mL/min) and temperature (±5 °C). %RSD was assessed for the peak areas of recorded chromatograms.

Stability indicating studies

Forced degradation studies were conducted as per Q1A, QIB and Q2B guidelines of ICH [29]. Stability indicating studies were conducted with standard drug solution in order to assess the stability indicating power of the method and to predict the essential requirements of storage conditions for pure drug and its dosage form.

Acid degradations studies

In this study, equal volumes of standard stock solution (1.5 mg/mL of LAM, 1.5 mg/mL of TDF and 0.5 mg/mL of DOR) and 2 N HCl solution were mixed and the resultant solution was refluxed for 60 min at 60 °C. Neutralize the obtained solution with 2 N NaOH and dilution was made to accomplish concentration in the order of 150 µg/mL, 150 µg/mL and 50 µg/mL for TDF, LAM and DOR. Inject 1 µl of above solution into UPLC system to assess the percentage of drugs degraded under acid degradation conditions from the obtained chromatograms.

Alkali degradation studies

In this alkaline study, equal volumes of standard stock solution and 2 N NaOH were mixed properly and reflux the resultant solution for 60 min at 60 °C. The obtained solution was neutralized with 2 N HCl and diluted further to obtain concentration of 150 µg/mL, 150 µg/mL and 50 µg/mL for TDF, LAM and DOR respectively. From the above solution 1 µl was injected into UPLC system and the percentage of drugs degraded was assessed from the attained chromatograms under alkaline conditions.

Oxidative degradation studies

In this study, standard stock solution and 20% hydrogen peroxide solution were mixed in equal proportion and reflux for 60 min at 60 °C. The obtained solution was further diluted to attain 150 µg/mL, 150 µg/mL and 50 µg/mL for TDF, LAM and DOR respectively. A volume of 1 µl was introduced into UPLC system and evaluate the percentage of drugs degraded from the resultant chromatograms.

Photo degradation studies

In this study, expose the standard stock solution to a wavelength of 254 nm in UV chamber for 72 h with dark control. The above solution was to accomplish concentration of 150 µg/mL, 150 µg/mL and 50 µg/mL for TDF, LAM and DOR in that order. 1 µl of the diluted solution was introduced into the UPLC system and assessed the percentage of the drug degraded from the attained chromatograms.

Thermal degradation studies

In this study, held 5 mL of the standard solution in the oven at 105^0C/75% RH for 6 h. A volume of 1 µl resultant solution was injected into the UPLC system and the percentage of the drug degraded was assessed from the attained chromatograms under thermal degradation conditions.

Neutral degradation studies

In this degradation study, standard stock solution and Milli-Q water were mixed properly in equal volumes to get the homogenous solution. Dilute the above solution was further to attain concentration of 150 µg/mL, 150 µg/mL and 50 µg/mL for TDF, LMV and DRV respectively. 1 µl of above solution was introduced and recorded the chromatograms to evaluate the percentage of the drug was degraded under neutral conditions.

Application of the method to Marketed formulation

The current method has been applied to find out the percentage purity of commercial tablets (DELSTRIGO™) by injecting subsequent injections of same concentration of both standard and sample solutions consecutively. The percentage purity of every analyte was evaluated from

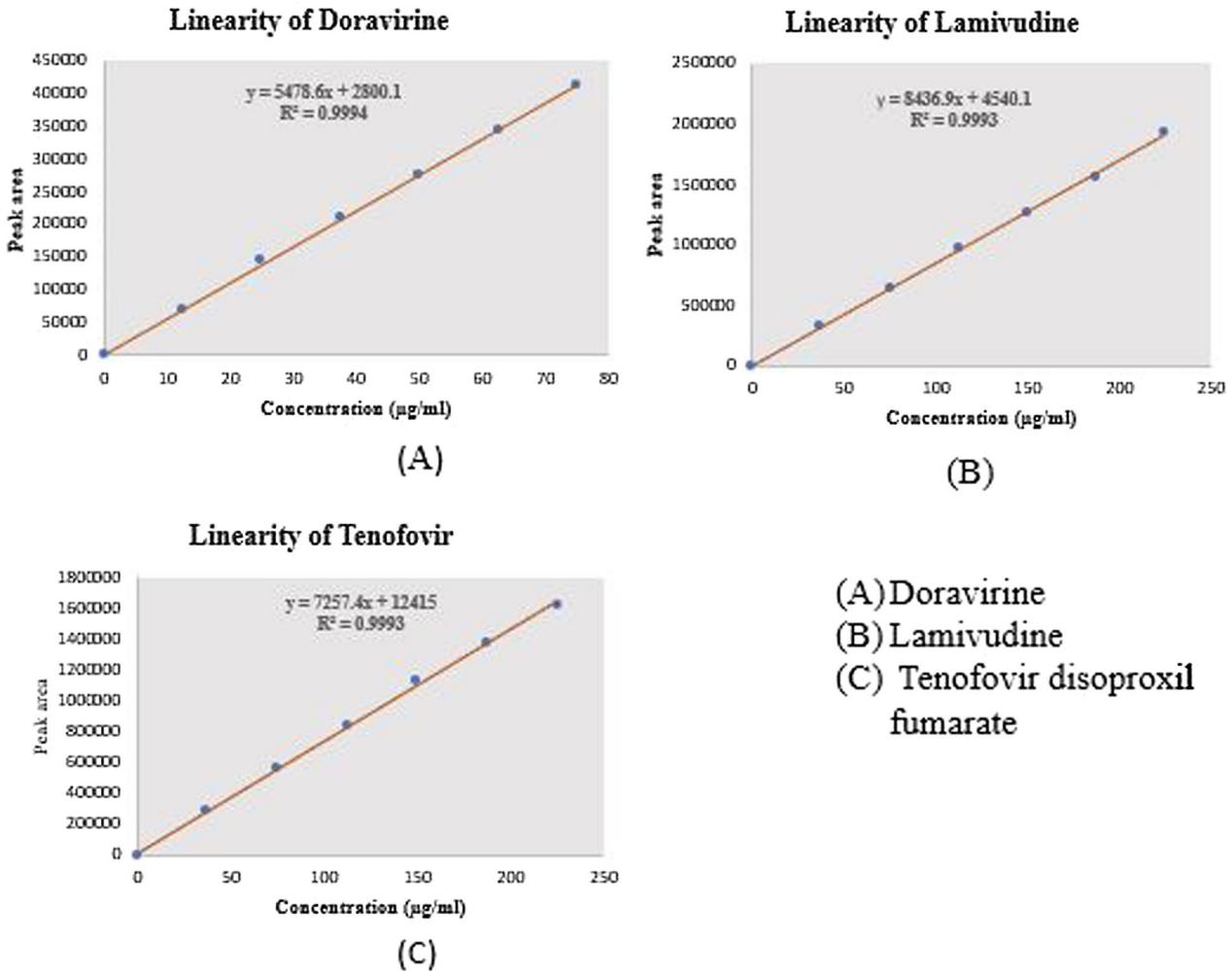


Fig. 2 Chromatogram of the standard solution

the peak areas of three analytes in both standard and sample solution.

Results

Method optimization

An efficient optimized method was achieved from trial-and-error method by using different columns and various mobile phase combinations with different flow rates. Finally, DOR and LAM, TDF were successfully separated with fine resolution at retention time of 1.2 min, 1.5 min and 1.8 min respectively (Fig. 2) by using HSS (C18 100 × 2.1 mm, 1.8µ), mobile phase composition of buffer and Acetonitrile in 60:40 v/v at a flow rate of 0.3 mL/min and a wavelength of 260 nm was selected to detect three different analytes (Table 1).

Method validation

System suitability

The statistical data obtained from the chromatograms of six replicate injections of standard solution proved that %RSD of all parameters like tailing factor, theoretical plate number, resolution have been fulfilled the acceptable limits of various regulatory bodies [30]. The obtained system suitability data were shown in Table 2.

Linearity

The regression equation obtained from the linearity data of DOR is ($Y = 5478.6x + 2800.1$) and for LAM is $y = 8436.9x + 4540.1$ and for TDF is $y = 7257.4x + 12415$. The average correlation coefficient value was calculated from the linearity plot drawn for series of mentioned range of concentrations for DOR, LAM and TDF is 0.9994, 0.9993, 0.9993 respectively,

Table 1 Different trails

Trail	Column	Mobile phase (% v/v)	Flow rate	Observation
1	STD Hibar C18 (100 × 2.1 mm, 2 µm)	Acetonitrile:Water (50:50)	0.3 mL	Baseline noise was observed and platecount of Doravirine peak was less than 2000 (1883)
2	Standard Hibar C18 (100 × 2.1 mm, 2µ)	Methanol:Water (50:50)	0.3 mL	Tenofovir was not eluted and resolution between Doravirine and Lamivudine was less than 2
3	STD Hibar C18 (100 × 2.1 mm, 2 µm)	Acetonitrile:OPA (50:50)	0.3 mL	Doravirine peak plate count was less than 2000 (847.8) and Lamivudine plate count was near to 2000 (2003.6)
4	Standard SB C18 (100 × 2.1 mm, 2µ)	Acetonitrile: Kh2po4 (40:60)	0.3 mL	Doravirine peak plate count was lessthan 2000 (1275.7)
5	HSS C18 (100 × 2.1 mm, 2µ)	Acetonitrile: Kh2po4 (40:60)	0.3 mL	Doravirine, Lamivudine and Tenofovir disoproxil fumarate were eluted at retention time of 1.215, 1.498, 1.823 min respectively

v/v volume by volume, *STD* standard, *SB* stable bonding, *HSS* hollow structural sections, *mm* millimeter, *mL* milliliter, *%* percentage, *µm* micrometer, *OPA* ortho phosphoric acid, *Kh2po4* potassium dihydrogen orthophosphate buffer

Table 2 System suitability results of DOR, LAM and TDF

Drug	Parameter	RT	Peak area	USP Plate Count	USP Tailing
DOR	Mean	1.227	269,930	3562	1.25
	SD	0.007	3869.6	65.634	0.023
	%RSD	0.599	1.4	1.843	1.85
LAM	Mean	1.517	1,247,416	5519.3	1.415
	SD	0.0098	15,064.42	85.104	0.014
	%RSD	0.645	1.207	1.54	0.974
TDF	Mean	1.853	1,143,433	8462.3	1.405
	SD	0.0131	15,455.88	133.04	0.0216
	%RSD	0.708	1.4	1.572	1.54

DOR Doravirine, *LAM* Lamivudine, *TDF* Tenofovir disoproxil fumarate, *SD* standard deviation, *%RSD* relative standard deviation, *RT* retention time

which ensures linearity of the method (Fig. 3). The linearity data was given in Table 3.

Sensitivity

The LOD and LOQ values computed from the stated formulae were found to be 0.25 µg/mL and 0.76 µg/mL for DOR, 0.24 µg/mL and 0.73 µg/mL for LAM and 0.797 µg/mL and 2.97 µg/mL for TDF.

Solution stability

The %RSD of peak area responses of the DOR, LAM and TDF for 48 h was estimated as ≤ 2, which ensures that the solution was stable for 72 h at room temperature.

Specificity

No interferences from blank and placebo was observed at the retention time of the peaks of DOR, LAM and TDF represents the specificity of the method in respect of TDF, LAM and DOR.

Precision

%RSD for the peak area response of the DOR, LAM and TDF in multiple consecutive replicate injections of standard solution was computed as ≤ 2 (Table 4), which significantly assures the precision of the proposed method.

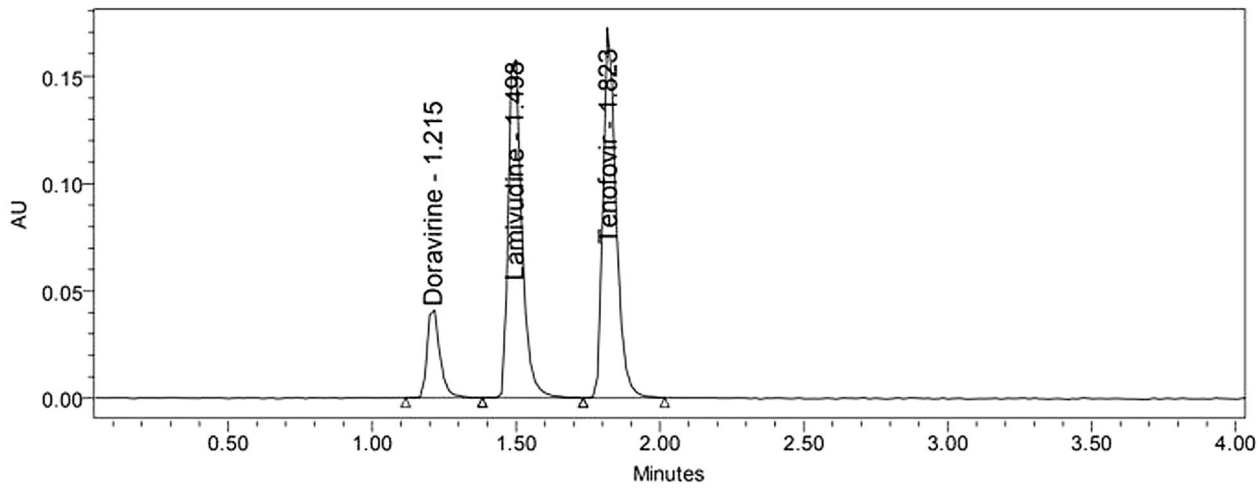
Accuracy

The mean percentage recovery of DOR, LAM and TDF in different spiked levels (i.e., 50%, 100%, 150%) were ascertained to be $100 \pm 2\%$ (Table 5), which represents that the method is highly accurate as per ICH standards.

Table 3 Linear regression data of DOR, LAM and TDF

Parameters (units)	DOR	LAM	TEN
Linearity range (µg/mL)	12.5–75	37.5–225	37.5–225
$r^2 \pm$ SD	0.9992 ± 0.000153	0.9992 ± 0.000404	0.9992 ± 0.0000577
Slope ± SD	5478.6 ± 16.728	8436.9 ± 19.34	7257.37 ± 15.567
Intercept ± SD	2800.367 ± 414.287	4540 ± 613.61	$12{,}415.33 \pm 2153.02$

DOR Doravirine, *LAM* Lamivudine, *TDF* Tenofovir disoproxil fumarate, *n* number of determinations, *µg/mL* microgram per milliliter, *r* correlation coefficient, *SD* standard deviation



Peak name	Retention time	Peak area	USP Plate count	USP Tailing	Resolution
Doravirine	1.215	263191	3926	1.20	-
Lamivudine	1.498	1203252	5926	1.26	3.3
Tenofovir	1.823	1171431	8831	1.36	3.8

Fig. 3 Linearity curves of Doravirine, Lamivudine and Tenofovir disoproxil fumarate

Table 4 Intra-day and inter-day precision data of DOR, LAM and TDF

Precision	S. no	DOR Peak area	LAM Peak area	TDF Peak area
Intraday	Injection-1	268,275	1,230,225	1,172,955
	Injection-2	272,409	1,238,594	1,151,322
	Injection-3	271,214	1,257,594	1,130,281
	Injection-4	266,268	1,230,136	1,128,429
	Injection-6	268,087	1,249,456	1,143,430
	Mean	269,788	1,239,040	1,144,274
	SD	2595.8	12,055.1	16,403.3
	%RSD	1.0	1.0	1.4
Inter-day	**S. no**	**Peak area**	**Peak area**	**Peak area**
Day-1	Injection-1	257,531	1,177,529	1,035,510
	Injection-2	254,276	1,190,106	1,016,879
Day-2	Injection-1	252,049	1,182,299	1,048,079
	Injection-2	260,733	1,172,679	1,016,808
Day-3	Injection-1	255,549	1,171,575	1,047,041
	Injection-2	255,618	1,178,127	1,013,266
	Mean	255,959	1,178,719	1,029,597
	SD	2955.2	6808.3	15,955.2
	%RSD	1.2	0.6	1.5

S. no serial number, *SD* standard deviation, *%RSD* relative standard deviation, *RT* retention time, *DOR* Doravirine, *LAM* Lamivudine, *TDF* Tenofovir disoproxil fumarate

Table 5 Percentage recovery results of DOR, LAM and TDF

DRUG	Level of addition (%)	Amount added (µg/mL)	Drug found (µg/mL) Mean ± SD	Average % recovery Mean ± SD	%RSD
DOR	50	25	24.93 ± 0.222	99.74 ± 0.601	0.603
	100	50	49.81 ± 0.289		
	150	75	74.91 ± 0.385		
LAM	50	75	74.103 ± 0.791	99.01 ± 0.745	0.752
	100	150	149.167 ± 0.077		
	150	225	222.264 ± 1.864		
TDF	50	75	75.39 ± 0.638	100.05 ± 0.892	0.891
	100	150	149.52 ± 1.468		
	150	225	224.96 ± 2.162		

DOR Doravirine, *LAM* Lamivudine, *TDF* Tenofovir disoproxil fumarate, *SD* standard deviation, *%RSD* relative standard deviation, *RT* retention time, *µg/mL* microgram per millilitre

Table 6 Robustness results of DOR, LAM and TDF

Drug name	Variation in parameter		Peak area (mean ± SD)	%RSD
DOR	Flow rate	Low	281,144 ± 4409.7	1.6
	(± 10%)	High	190,472 ± 2766.0	1.5
	Mobile phase	Low	230,244 ± 3529.6	1.5
	Ratio (± 10%)	High	208,540 ± 4330.2	2.0
	Temperature (± 5%)	Low	153,867 ± 2977.1	1.9
		High	140,746 ± 1414.8	1.0
LAM	Flow rate (± 10%)	Low	1,303,776 ± 24,862.5	1.9
		High	880,493 ± 8597.7	1.0
	Mobile phase ratio (± 10%)	Low	2,043,421 ± 29,466.8	1.4
		High	1,730,444 ± 10,228.3	0.6
	Temperature (± 5%)	Low	592,858 ± 8755.3	1.5
		High	584,542 ± 9847.035	1.7
TDF	Flow rate (± 10%)	Low	1,329,263 ± 26,742.6	2.0
		High	1,051,959 ± 10,450.7	1.0
	Mobile phase Ratio (± 10%)	Low	1,135,591 ± 15,566.02	1.4
		High	1,010,700 ± 6822.4	0.7
	Temperature (± 5%)	Low	614,440 ± 8254.7	1.3
		High	594,030 ± 8543.4	1.4

DOR Doravirine, *LAM* Lamivudine, *TDF* Tenofovir disoproxil fumarate, *SD* standard deviation, *%RSD* relative standard deviation

Robustness

Deliberate changes applied to certain method conditions like temperature, flow rate and mobile phase ratio did not affect the system suitability parameters (%RSD less than 2), which significantly represents that robustness of the method. Robustness data was shown in Table 6.

Stability indicating studies

As per researcher view, with the application of different stress conditions up to 20% degradation is effective in stability representing methods [31, 32]. The percentage of drug degraded in different stress conditions was evaluated from the obtained chromatogram peak areas of

Table 7 Summary of degradation data of DOR, LAM and TDF

Degradation condition	DOR		LAM		TDF	
	%Deg	Peak area	%Deg	Peak area	%Deg	Peak area
Acid (0.5 N/60 °C/1 h)	4.05	258,342	4.77	1,190,261	5.19	1,085,169
Base (0.5 N/60 °C/1 h)	3.12	260,826	5.74	1,178,182	4.84	1,089,142
Oxidative (10%w/v H_2O_2/60 °C/1 h)	3.54	259,703	4.91	1,188,582	4.83	1,089,342
Hydrolytic (water/60 °C/1 h)	1.01	266,531	0.87	1,239,093	0.63	1,137,391
Thermal (105 °C/1 day)	1.72	264,614	1.09	1,236,328	1.02	1,132,940
Photolytic (UV radiation at 200-Wh/m^2-dark control)	2.49	262,531	1.83	1,227,093	2.03	1,121,391

DOR Doravirine, *LAM* Lamivudine, *TDF* Tenofovir disoproxil fumarate, *h* hours, *%* percentage, *UV* ultraviolet, *min* minute, *N* normality, *°C* degree celsius, *Wh/m^2* watt-hour per square metre, *% Deg* percentage degradation

DOR, LAM and TDF in both standard and stressed solution. The percentage degradation data of different three analytes were given in Table 7 and concerned chromatograms were represented in Figs. 4, 5, 6, 7, 8, and 9.

Application of the method to marketed formulation

The percentage purity of the DOR, LAM and TDF in commercial tablets was estimated to be 100% ± 10 (Table 8) [33], which indicate that the obtained assay values of DOR, LAM and TDF Were in obedience with the ICH limits. The recorded chromatogram of sample solution was shown in Fig. 10.

Discussion

Proficient and extensive literature survey reveals that no UPLC and two HPLC methods were observed for the estimation of mixture of DOR, LAM and TDF in pure form and in marketed formulation. In the reported HPLC methods, DOR and TDF were eluted at longer retention time and these methods were not considered as economical methods because of low sensitivity and more mobile phase consumption [27, 28]. UPLC when compared with HPLC, has a packed column with less particle size which provides large surface area for analytes to interact and aids in faster elution of analytes and efficiency in separation of analytes. Hence to overcome the drawbacks of HPLC an attempt was made to develop an effective

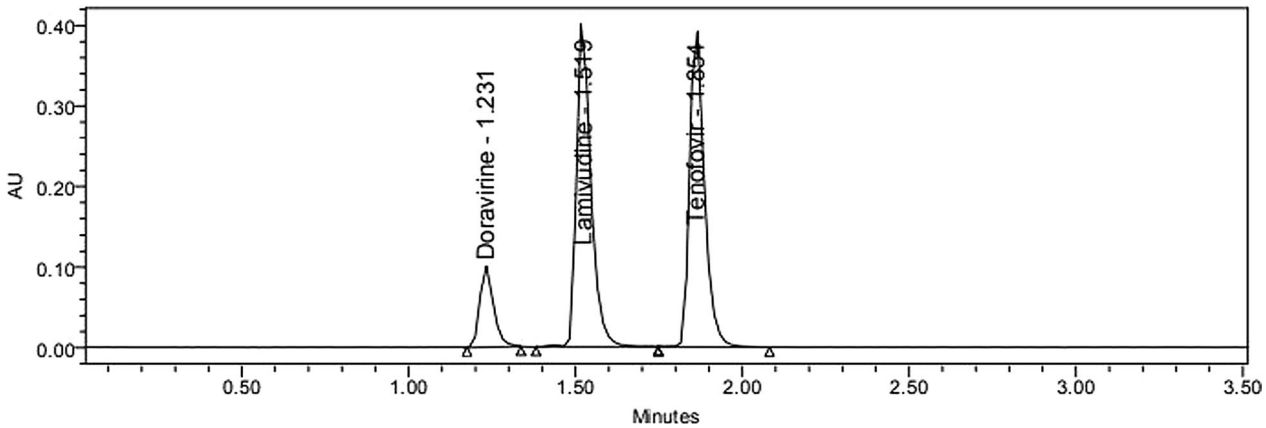


Fig. 4 Chromatogram of the neutral degradation solution

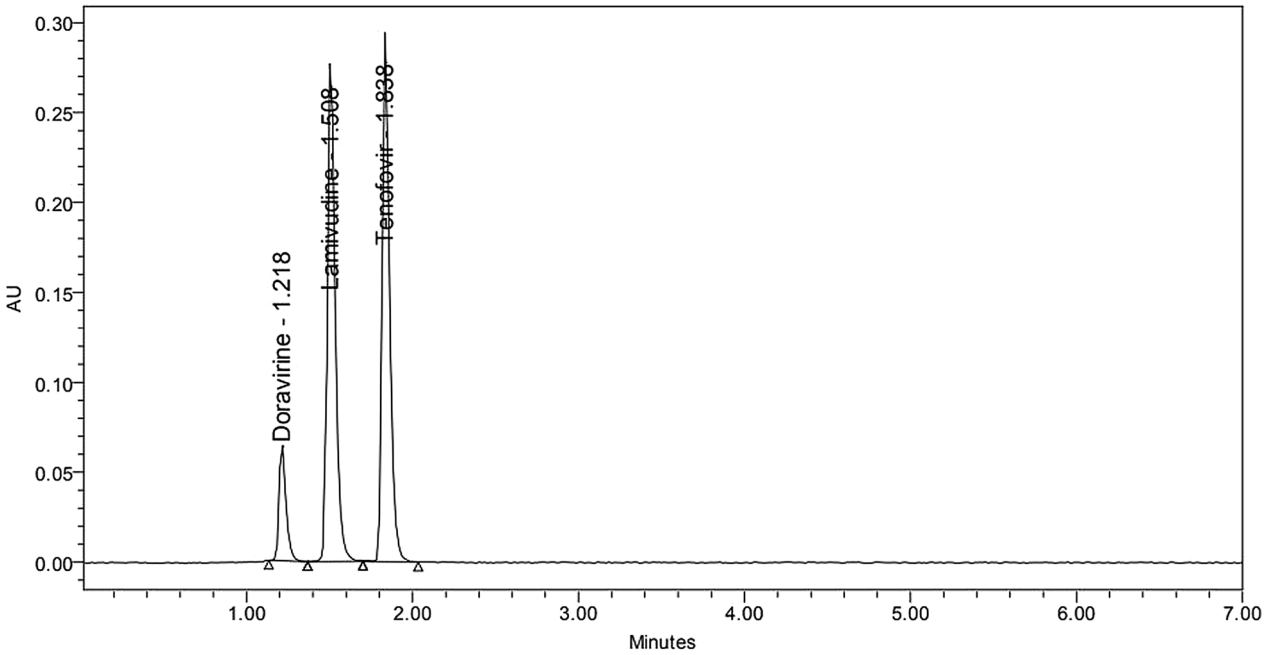


Fig. 5 Chromatogram of the marketed formulation

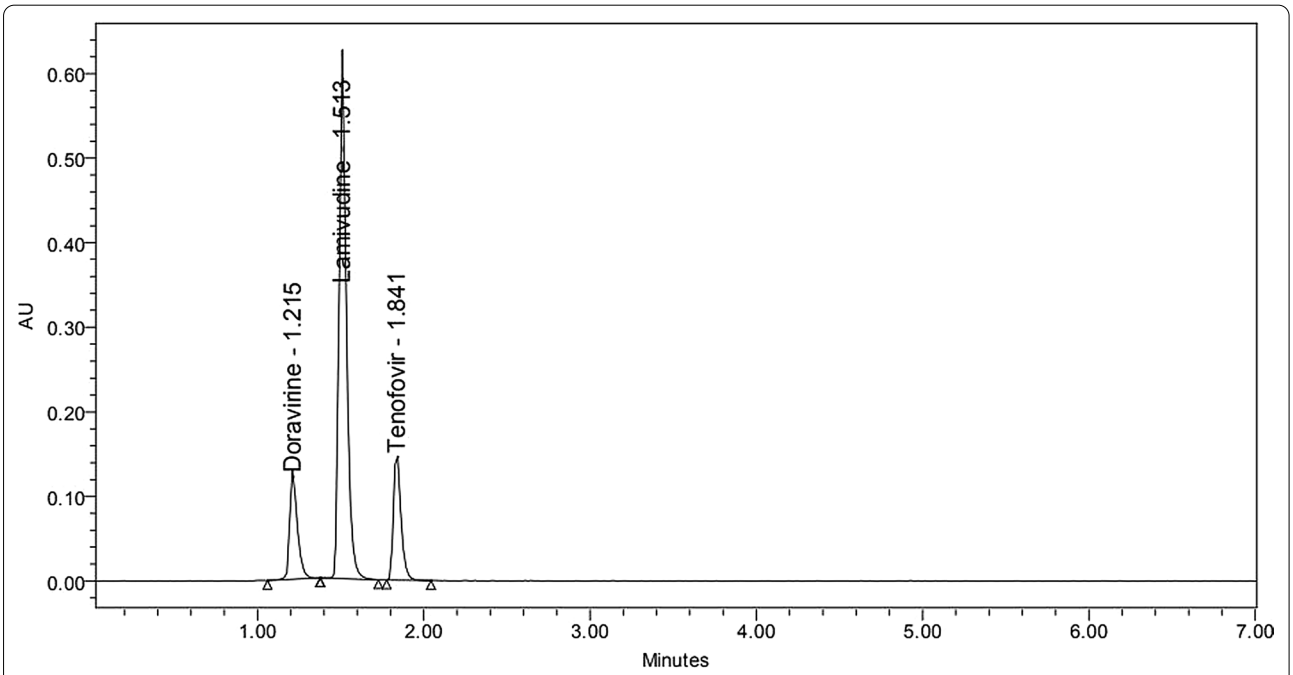


Fig. 6 Chromatogram of the acid degradation solution

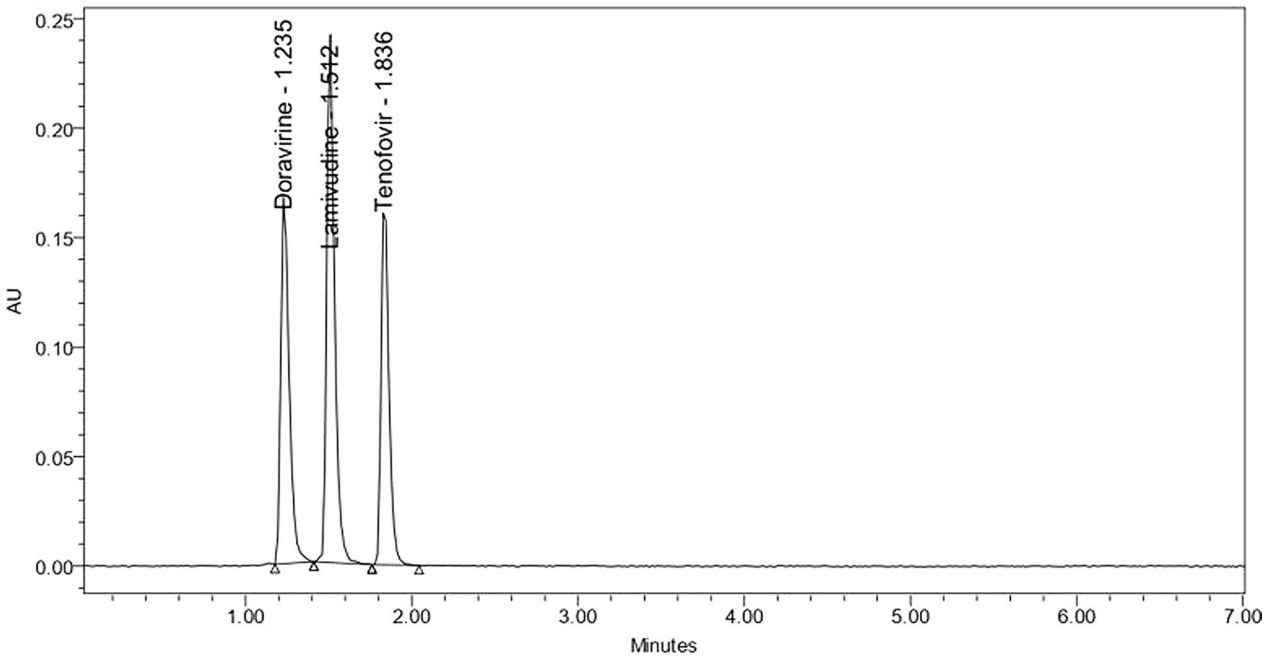


Fig. 7 Chromatogram of the alkaline degradation solution

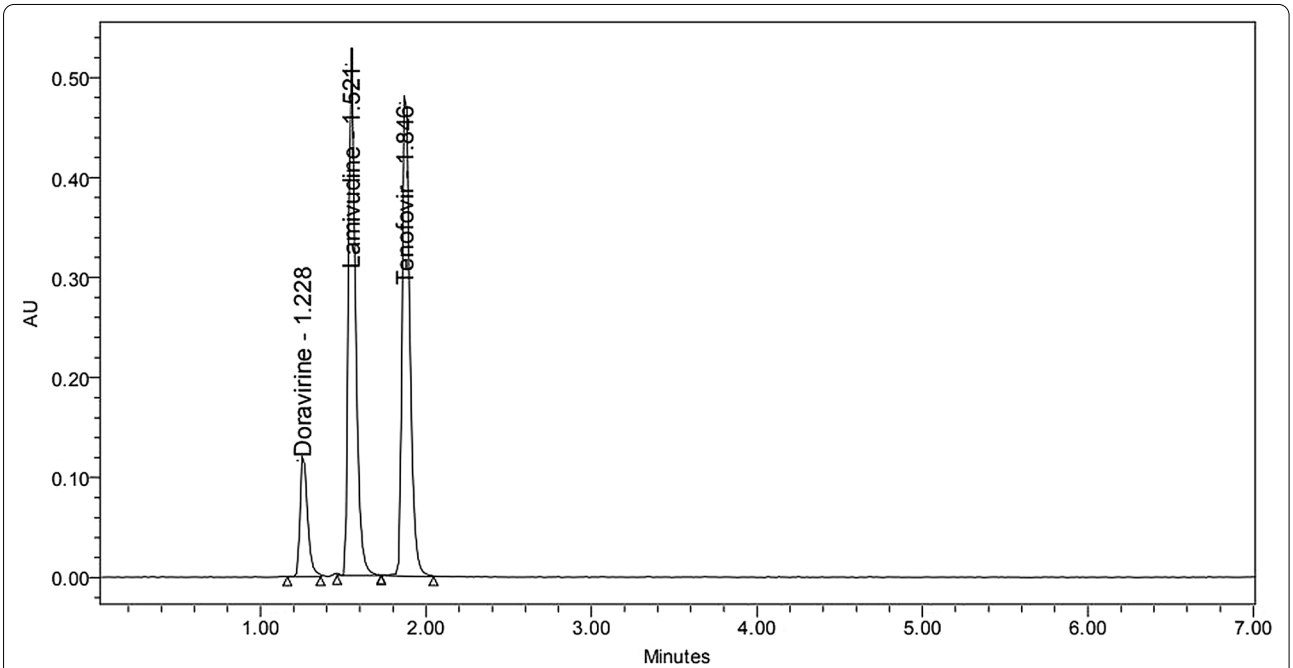


Fig. 8 Chromatogram of the oxidative degradation solution

Table 8 Results of %assay of tablet dosage form

Drug	Peak name	Peak area	Label claim	%assay
DOR	Standard	269,930	100	98.8
	Test	267,191		
LMV	Standard	1,247,416	300	98.7
	Test	1,233,252		
TDF	Standard	1,143,433	300	99.72
	Test	1,141,431		

DOR Doravirine, *LAM* Lamivudine, *TDF* Tenofovir disoproxil fumarate, *%* percentage

RP-UPLC method with more sensitivity and faster elution of elution of analytes with short retention time. In the proposed method a simple mobile phase composition of 0.01 N Potassium dihydrogen ortho phosphate and ACN in (60:40 v/v) ratio was selected for analysis of three drug analytes and DOR, LAM and TDF were eluted at retention time of 1.2 min, 1.4 min and 1.8 min respectively. The current developed method was cost-effective with less retention time and simple mobile phase composition when compared with the reported HPLC methods. More number of samples can be investigated rapidly by using the current method. The statistical data obtained from various validation parameters represents that the current method has ideal specificity, perfect accuracy and reproducible precision with more sensitivity.

Conclusions

A simple, economical and cost-effective RP-UPLC method was established with reproducible precision and superior sensitivity for simultaneous estimation of DOR, LAM and TDF in pure bulk form and their combined film coated tablet form. Investigation of the DOR, LAM and TDF under various stress conditions indicate the stability indication parameter of the method. The method was competently separate DOR, LAM, TDF and the percentage degradation of the respective drugs was evaluated from the recorded chromatograms under various stress conditions. Therefore, the present method has remarkable recognition in the industrial sector for estimation of the DOR, LAM, TDF in the marketed formulation.

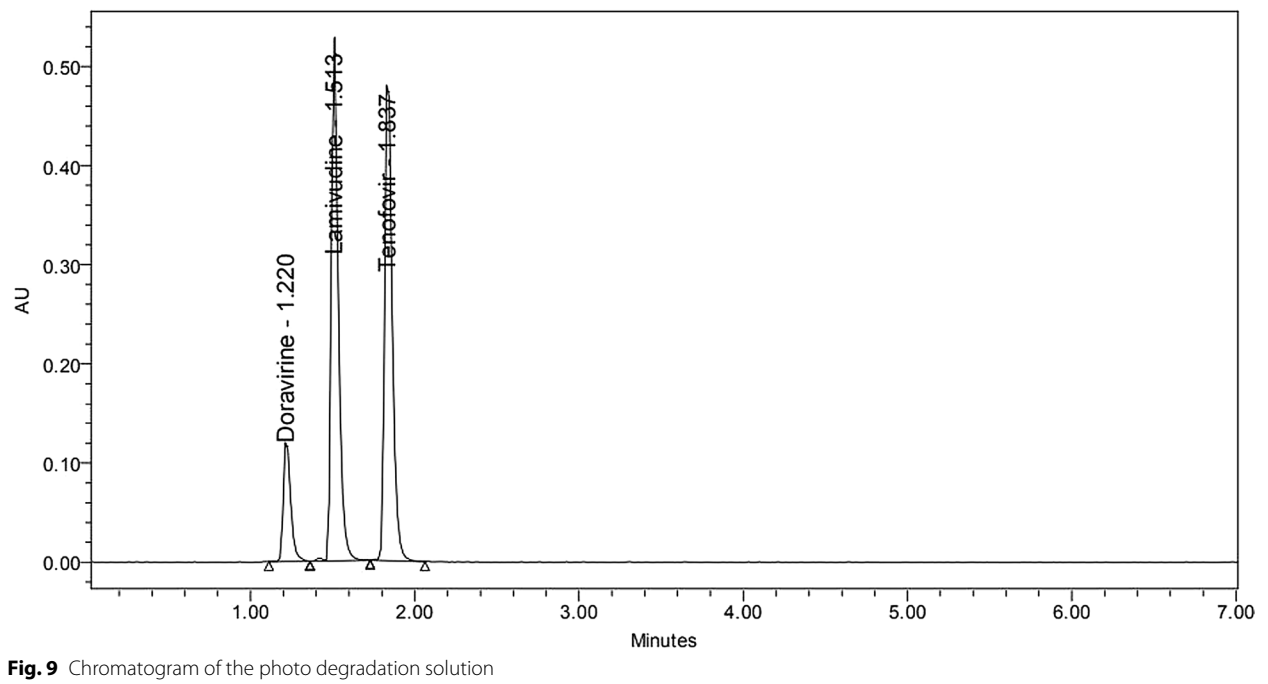


Fig. 9 Chromatogram of the photo degradation solution

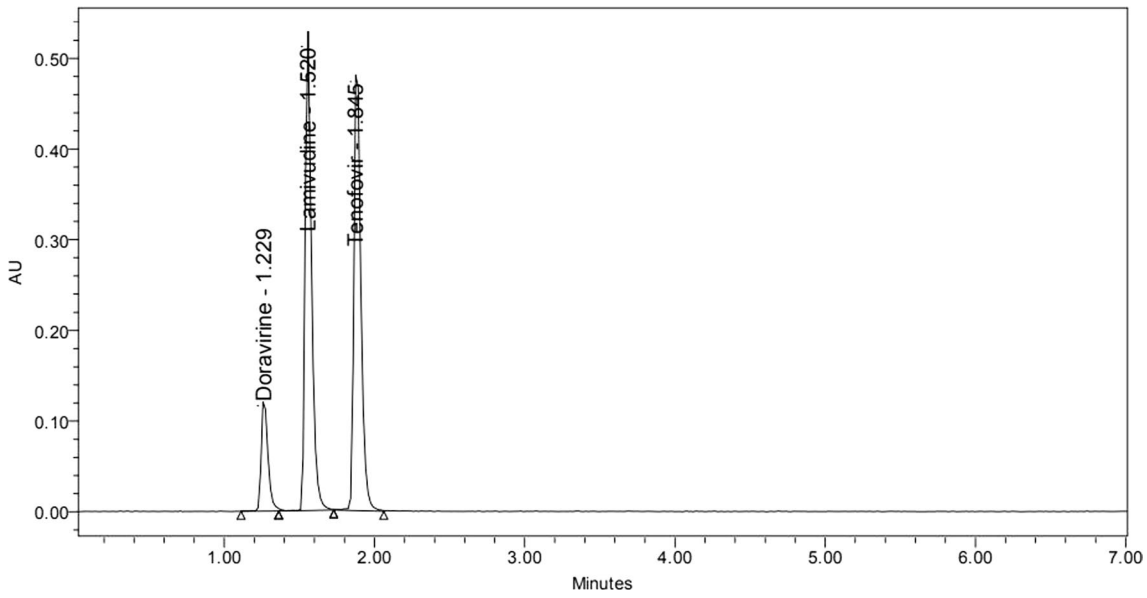


Fig. 10 Chromatogram of the thermal solution

Abbreviations
DOR: Doravirine; LAM: Lamivudine; TDF: Tenofovir disoproxil fumarate; RT: Retention time; LOD: Limit of detection; LOQ: Limit of quantification; SD: Standard deviation; RSD: Relative standard deviation.

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Authors' contributions
SA analyzed and interpreted the data of obtained chromatograms and a major contributor in writing the manuscript. RB performed the bench work and experimental work of the stability indicating liquid chromatographic method development of analytes using UPLC. Both authors read and approved the final manuscript.

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Availability of data and materials
All data and material should be available upon request.

Declarations

Ethics approval consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
No competing interests to declare.

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