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Concurrent estimation of lamivudine, tenofovir disoproxil fumarate, and efavirenz in blended mixture and triple combination tablet formulation by a new stability indicating RP-HPLC method

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Abstract

Background: An easy, defined, rapid, and accurate reverse phase high-performance liquid chromatography method was developed and subsequently validated for the concurrent estimation of lamivudine, efavirenz, and tenofovir disoproxil fumarate in their pure blend and combined tablet formulation. An efficient and appropriate separation of the three analytes was attained with Zorbax eclipse XDB-Phenyl column, with a mobile phase of methanol: buffer (0.1% v/v formic acid in water) (73:27 v/v) at a flow rate of 1mL/min and isocratic elution by using 260nm as detection wavelength. Equal ratio of acetonitrile and water was used as diluent.

Results: The retention times of lamivudine, tenofovir disoproxil fumarate, and efavirenz were found at 2.6, 4.4, and 5.9 min respectively. The linear response for lamivudine, tenofovir disoproxil fumarate, and efavirenz was in the range of 15.0–45.0μg/mL, 15.0–45.0μg/mL, and 20.0–60.0 μg/mL respectively. The method validation was done in accordance to ICH guidelines and all validation parameters in compliance with ICH standards. The degradants produced by stress testing were well resolved from the peaks of active analytes, which stipulates the stability-indicating property of the method.

Conclusion: The method has the ability to separate lamivudine, efavirenz, and tenofovir disoproxil fumarate concurrently in blended powder and their combined tablet. All degradants produced by application of stress conditions were separated with high resolution and determined with good sensitivity that ensures the stability-indicating property of the method. Thus, the projected method has high probability to adopt in the pharmaceutical industrial sector.

Keywords: Lamivudine, Efavirenz, Tenofovir disoproxil fumarate, Stress testing, Stability indicating, Isocratic elution

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Background

The antiviral therapy with combination of two or more drugs is a great advancement in the treatment of hepatitis B and human immunodeficiency virus diseases. Multi-drug combination therapy is compliance by the patients due to decreased pill load per day. The three-drug combination of lamivudine (LAM), tinofovir disproxil fumarate (TDF), and efavirez (EVZ) is a competent highly active anti-retroviral therapy which comprise of one non-nucleoside reverse transcriptase inhibitor and two nucleoside reverse transcriptase inhibitor drugs [1, 2]. LAM, chemically, is 4-amino-1-[(2R, 5S)-2-(hydroxyl methyl)-1,3-oxathiolan-5-yl]-1, 2-dihydropyrimidin-2-one [3]. It ceases DNA replication by inhibiting the reverse transcriptase enzyme competitively [4]. TDF, chemically, is 1-(6-aminopurine -9-yl) propan-2 yl] oxymethyl-(propan-2-yl oxycarbonyl oxymethyl) phosphoryl] Oxymethyl propan -2yl carbonate [5]. It is a prodrug of tenofovir, which ceases DNA replication by inhibiting the reverse transcriptase enzyme competitively [6–9]. Chemically EVZ, (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-1H-3,1-benzoxazin-2-one, is a non-nucleoside reverse transcriptase inhibitor, which inhibits the DNA polymerase non-competitively [10, 11]. The chemical structures of LAM, TDF, and EVZ are shown in Fig. 1.

A competent analytical method is important for an analyte to determine alone or in blend with other analytes concurrently. The broad literature search disclosed that a small number of analytical methods like UV and RP-HPLC methods were at hand for determination of LAM. TDF and EVZ individually and in combined dosage form [3, 5, 12–16]. In addition to those methods,

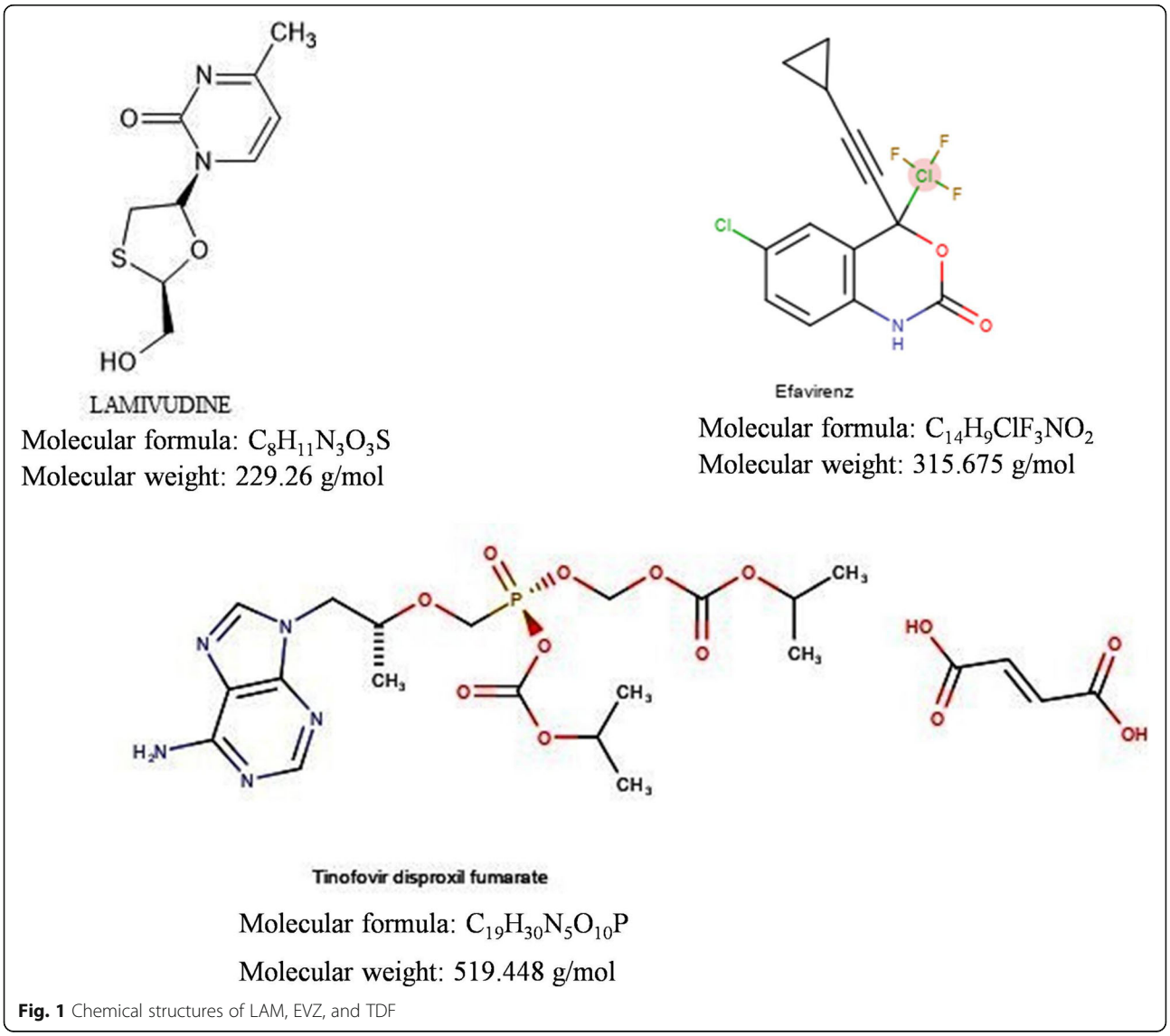


Fig. 1 Chemical structures of LAM, EVZ, and TDF

Table 1 Different trials

Trial	Column	Buffer	Mobile phase	Flow rate ml/min	Observation
1	Zorbax eclipse XDB-Phenyl (250×4.6mm, 5μm)	0.1% (V/V) formic acid	Buffer: ACN (50:50 v/v)	1.23	Peak tailing (1.3) and long retention time (9.52 min) of EVZ
2	Zorbax SB C8 (100×4.6mm, 5μm)	0.1% (V/V) formic acid	Buffer: ACN (35:65 v/v)	1.23	Resolution (1.5) was not good between LAM and TDF
3	Inertsil C8 (150×4.6mm, 5μm)	0.1% (V/V) formic acid	Buffer: ACN (35:65 v/v)	1.23	Resolution (1.6) was not good between LAM and TDF
4	Zorbax eclipse XDB-phenyl (250×4.6mm, 5μm)	0.1% (V/V) formic acid	Buffer: ACN (30:70 v/v)	1.23	Resolution was good, but retention time of EFV was long (8.056 min)
5	Phenyl XDB (250×4.6mm, 5μm)	0.1% (V/V) formic acid	Buffer: methanol (50:50 v/v)	1	Long retention time of EFV (7.201 min)
6	Phenyl XDB (250×4.6mm, 5μm)	0.1% (V/V) formic acid	Buffer: methanol (27:73 v/v)	1	Good resolution between LAM and TDF (9.13), TDF and EVZ (7.55), and less retention time (LAM-2.6 min, TDF-4.4 min, EVZ-5.9 min)

XDB extra dense bonding, *ACN* acetonitrile, *EFV* efavirenz

few RP-HPLC methods were described for determination of LAM, TDF with dolutegravir, and other antiviral agents [17–21]. As of yet, a competent stability-indicating RP-HPLC method was not available for the concurrent evaluation of LAM, EVZ, and TDF in pure blend and combined tablet formulation. Hence, research attempts have been done to develop an efficient, highly responsive and cost-effective RP-HPLC method to estimate the percentage purity and to evaluate the stability of LAM, EVZ, and TDF in blended bulk powder and tablet formulation concurrently. To assure the developed method, validation of the method was done as indicated by Q2R1 guidelines of ICH.

Methods

Pure drug samples

Pure and active form of LAM (99.4%), EVZ (99.4%), and TDF (99.3%) were procured as gift samples from Aurobindo Pharma, Hyderabad.

Formulation

Film-coated tablet dosage forms (Symfi Lo is a fixed-dose combination product containing 400 mg of EVZ, 300 mg of LAM, and 300 mg of TDF) were purchased from local pharmacy in Hyderabad.

Chemicals and reagents

All HPLC grade and analytical grade solvents were purchased by local distributor of Merck India.

Instrument specifications

The method was done by using WATERS HPLC (2695model, PDA detector, and the Empower 2 solution software). In addition, an analytical balance (Denver instrument, TB-215D), a digital pH meter (MEZARIT), and water (Milli-Q) were used. Forty-five micrometers povidone filters were used to filter all the solutions and the solvents before introduced into the instrument.

Chromatographic method conditions

An efficient and appropriate separation of the three analytes was done with Zorbax eclipse XDB-Phenyl (250 × 4.6mm, 5μ) column, using a mobile phase consist of methanol: buffer (0.1% v/v formic in water) (73:27 v/v) at a flow rate of 1mL/min and a detection wavelength of

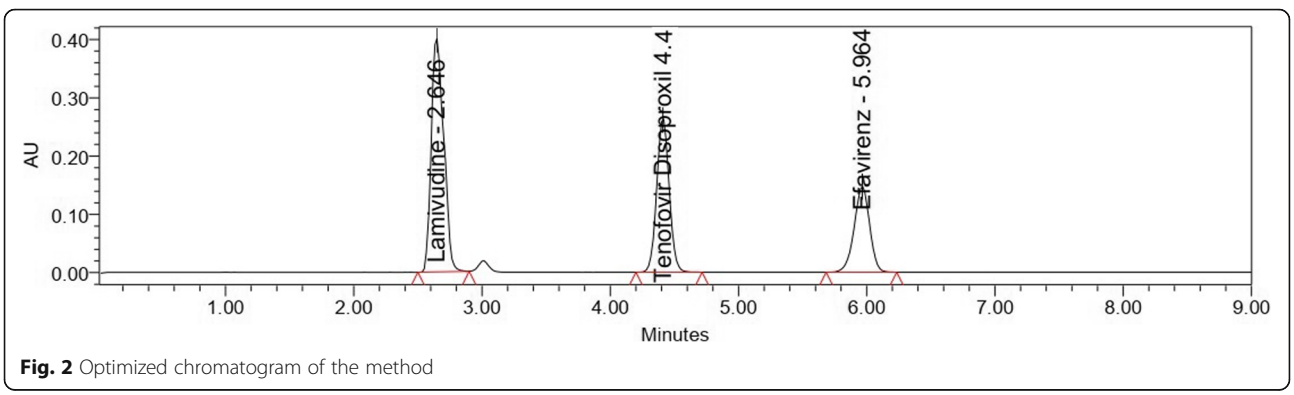


Fig. 2 Optimized chromatogram of the method

Table 2 Results of system suitability parameters of standard solution

	Parameter	tR	Area	USP tailing	USP plate count	Average resolution
LAM	**Mean**	2.65	2,702,001	1.21	2863.8	-
	SD	0.01	9637.52	0.01	27.23	
	%RSD	0.24	0.35	1.0	0.95	
TDF	**Mean**	4.40	1,779,305	1.0	9659.2	9.13
	SD	0.01	4811.89	0.01	152.76	
	%RSD	0.28	0.27	0.52	1.581	
EVZ	**Mean**	5.93	1,237,753	0.94	11737.6	7.55
	SD	0.02	2925.29	1.24	127.73	
	%RSD	0.42	0.23	1.32	1.08	
Acceptance limit	**%RSD-** (≤ 2)			(≤ 2)	> 2000	˃2

SD standard deviation, *%RSD* relative standard deviation

260.0 nm. Equal ratio of acetonitrile and water was used as diluents.

Preparation of standard solution

One hundred milligrams of LAM, 100.0mg of TDF, and 133.3mg of EVZ pure powders were accurately weighed and dissolved with diluent in a 100-mL volumetric flask. One milliliter of the above solution was diluted to 10mL. Three milliliters of the resultant solution was further diluted to 10mL to get a concentration of 30.0μg/mL, 40.0μg/mL, and 30.0μg/mL for LAM, EVZ, and TDF respectively.

Preparation of sample solution

The crushed tablet powder equivalent to 100mg of LAM, 100mg of TDF, and 133.3mg of EVZ pure powders was accurately weighed and dissolved with diluent in 100-mL volumetric flask. One milliliter of the above solution was diluted to 10mL. Three milliliters of the resultant solution was further diluted to 10mL to get a concentration of 30.0μg/mL, 40.0μg/mL, and 30.0μg/mL for LAM, EVZ, and TDF respectively.

Method validation

Validation is written evidence that provides assurance of the method or process with high degree level. To validate analytical method, Q2R1 guidelines of the ICH were taken into consideration.

System suitability test

The system suitability test of the present method was established by injecting standard solution in 6 consecutive injections, and the parameters such as percentage relative standard deviation (%RSD), tailing factor (T), resolution (R), and number of theoretical plates (N) were assessed for the chromatograms thus attained.

Linearity

The linearity of an analytical method signifies the direct proportional relationship between experimental results and the given concentrations. It was established for the solutions having concentrations ranging from 15.0 to 45.0 μg/mL of both LAM and TDF and 20.0 to 60.0μg/mL of EVZ. A calibration curve was plotted between concentration and peak area to determine regression coefficient (R^2).

Precision

The precision of the method expresses the close agreement between the observed responses of homogenous samples on multiple sampling at identical conditions.

Table 3 Concentration and peak areas of linearity level solution of LAMI, TDF, and EVZ

%Level	LAM		TDF		EVZ	
	Concentration (μg/mL)	Peak area	Concentration (μg/mL)	Peak area	Concentration (μg/mL)	Peak area
50	15.0	1,343,818	15.0	913,725	20.0	613,391
75	22.5	2,110,176	22.5	1,430,523	30.0	934,415
100	30.0	2,690,079	30.0	1,832,027	40.0	1,216,978
125	37.5	3,422,954	37.5	2,339,804	50.0	1,552,558
150	45.0	4,176,437	45.0	2,842,257	60.0	1,903,481
Regression coefficient (R^2)		0.998		0.998		0.998

R^2 regression coefficient

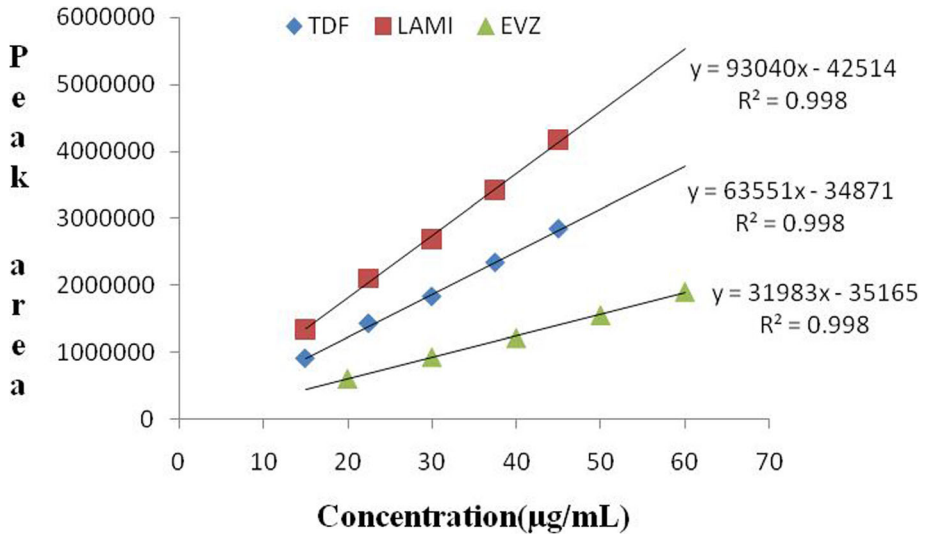


Fig. 3 Linearity curve of LAM, EVZ, and TDF

In general, it can be performed on the same day and on different consecutive days represented as intra-day (repeatability) precision and inter-day precision (intermediate precision) respectively. Repeatability was assessed by injecting standard solution for five times in a single day, and intermediate precision was assessed by injecting same standard solution for two times per day for three successive continuous days. %RSD values were assessed for peak areas attained in chromatograms.

Accuracy

Substantially, the accuracy was validated by percentage recovery studies where specified amount of sample solution was spiked into standard solution. In general, spiking can be done at 50, 100, and 150% level concentrations of standard solution. Each level solution was injected for three times. The % mean recovery of sample at each spiked level was calculated.

Specificity

Specificity is the ability of the analytical method to assess the analyte of interest in the presence of other substances including degradation products and excipients without interferences. It was performed by introducing consecutive injections of placebo, blank, standard, and placebo mixed with standard. The obtained chromatograms were examined for interference from the peaks of other substances with peaks of analytes to be determined.

Sensitivity

The LOQ and LOD were reckoned by using the following formulae:

Table 4 Results of percentage recovery

Drug name	% level	Amount added (µg/ml)	Amount recovered (µg/ml)	% recovery	Acceptance limit
LAM	50	15.0	14.86	99.06	98.0–102.0%
	100	30.0	30.01	100.0	
	150	45.0	44.9	99.7	
TDF	50	15.0	15.0	100.0	
	100	30.0	29.97	99.9	
	150	45.0	45.0	100.0	
EVZ	50	20.0	19.9	99.5	
	100	40.0	39.6	99.0	
	150	60.0	60.2	100.3	

The percentage recovery at each percentage level within the acceptable limit

Table 5 Results of intraday and inter-day precision of standard solution

Precision	S.NO	LAM		TDF		EVZ	
		tR	Peak area	tR	Peak area	tR	Peak area
Intraday	1	2.646	2,717,067	4.411	1,786,776	5.964	1,235,921
	2	2.658	2,696,080	4.418	1,776,006	5.955	1,235,063
	3	2.642	2,704,851	4.396	1,780,791	5.924	1,240,927
	4	2.644	2,692,168	4.391	1,774,513	5.913	1,235,911
	5	2.644	2,699,839	4.390	1,778,441	5.909	1,240,942
	Mean	2.647	2,702,001	4.401	1,779,305	5.933	1,237,753
	SD	0.01	9637.5	0.01	4811.9	0.03	2925.3
	%RSD	0.24	0.36	0.29	0.27	0.42	0.24
Inter-day							
Day 1	1	2.646	2,717,067	4.411	1,786,776	5.964	1,235,921
	2	2.65	2,632,428	4.388	1,799,791	5.899	1,202,415
Day 2	1	2.648	2,637,342	4.391	1,805,023	5.907	1,203,026
	2	2.652	2,647,962	4.398	1,812,939	5.917	1,209,358
Day 3	1	2.65	2,641,549	4.398	1,806,413	5.914	1,205,118
	2	2.65	2,644,845	4.393	1,810,105	5.903	1,207,765
	Mean	2.649	2,653,532	4.397	1,803,508	5.917	1,210,601
	SD	0.002	31,605	0.008	9349	0.024	12,690
	%RSD	0.08	1.19	0.18	0.52	0.40	1.05

SD standard deviation, *%RSD* relative standard deviation, *tR* retention time

$$\mathrm{LOD} = 3 \times \sigma / S$$

$$\mathrm{LOQ} = 10 \times \sigma / S$$

where σ is the SD of intercept and S is the slope of the linear curve.

Robustness

Method's robustness was assessed by changing the optimized conditions of the method to a little extent intentionally. In this case, optimized conditions like ratio of the mobile phase (± 1mL), flow rate of mobile phase (± 0.1 mL/min), and detection wavelength (± 2nm) were intentionally altered to a little extent.

Forced degradation studies

In forced degradation (FD) studies, chemical stability of the analyte can be assessed in the presence of more intensive conditions like acid and alkali hydrolysis, oxidative degradation, and thermal and photo stability as provided by ICH quality guidelines.

Table 6 Results of robustness of standard solution

Variation in parameter		LAM		TDF		EVZ	
		%RSD of peak area	Plate count	%RSD of peak area	Plate count	%RSD of peak area	Plate count
Mobile phase ratio (±1ml)	26:74	1.3	2905	0.97	9659	0.67	11,823
	28:72	1.4	2817	1.35	9253	1.60	11,742
Flow rate (±0.1mL/min)	0.9 mL/min	0.52	2896	0.27	9675	0.47	11,963
	1.1 mL/min	0.51	2841	0.24	9352	0.23	11,452
Wavelength (±2nm)	258	0.35	2831	0.27	9341	0.31	11,994
	262	0.36	2912	0.27	9368	0.23	11,535
	%RSD		**1.46**	**%RSD**	**1.89**	**%RSD**	**1.88**

%RSD relative standard deviation time
Slight change in method parameter could not influence the USP plate count and tailing factor

Table 7 Results of forced degradation studies

Drug name	Standard	Acid hydrolysis	Base hydrolysis	Oxidative degradation	Photo degradation	Thermal degradation
Peak area of the solution						
LAM	2,717,067	2,434,053	2,578,801	2,616,177	2,694,163	2,679,066
TDF	1,786,776	1,641,002	1,472,204	1,753,290	1,758,594	1,776,392
EVZ	1,235,921	1,177,547	1,055,922	1,194,572	1,212,763	1,222,960
% Degradation						
LAM	-	10.4	5.0	3.7	0.85	1.4
TDF	-	8.1	17.6	1.87	1.58	0.6
EVZ	-	4.7	14.5	3.34	1.87	1.0

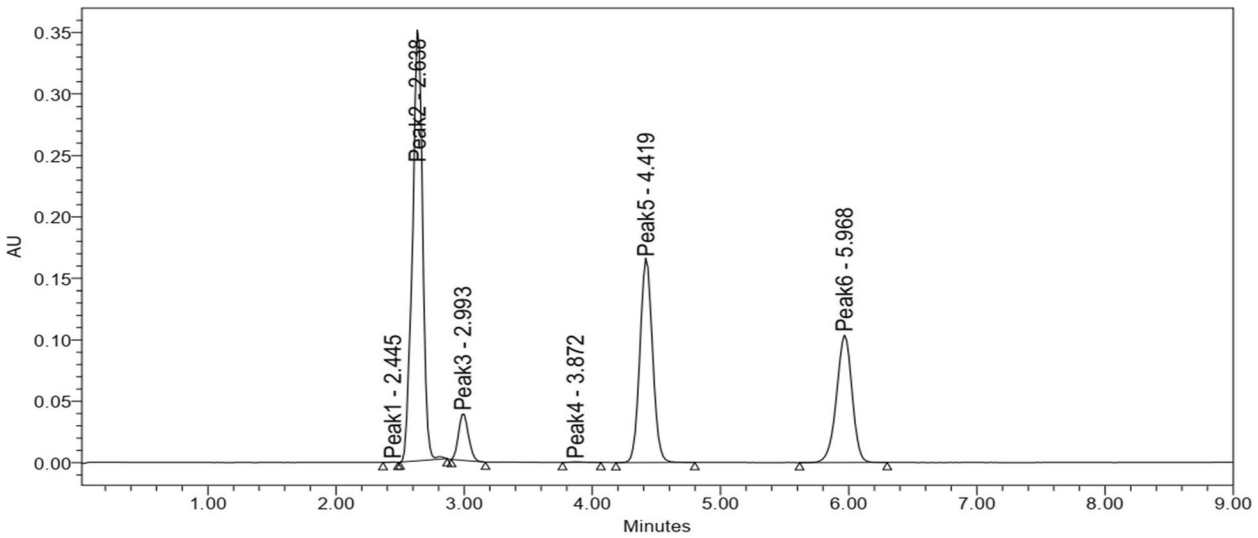
Percentage degradation was less than 20% with different stress conditions

Acid and alkali hydrolysis

Ten milliliters of standard stock solution was mixed with 2mL of 0.1N HCl and 2mL of 0.1N NaOH separately, reflux the prepared solutions for 2 h at 70°C, and kept it aside at room temperature for 24 h. The resultant solutions were neutralized and further diluted in such a way to get a concentration of 30μg/mL, 40μg/mL, and 30μg/mL for LAM, EVZ, and TDF respectively.

Oxidative degradation

Ten milliliters of standard stock solution was mixed with 2mL of 3% hydrogen peroxide and reflux the prepared solutions for 2h at 70 °C, and kept it aside



Peak	tR	Peak area	Resolution (R)
Peak1	2.445	940	-
Peak2 (Lamivudine)	2.638	2434053	2.1
Peak3	2.993	206629	2.9
Peak4	3.872	3825	4.2
Peak5 (Tenofovir disoproxil fumarate)	4.419	1641002	2.6
Peak6 (Efavirez)	5.968	1177547	7.51

Fig. 4 Chromatogram of acid degradation

for 24 h and further diluted in such a way to get a concentration of 30μg/mL, 40μg/mL, and 30 μg/mL for LAM, EVZ, and TDF respectively.

Thermal degradation

The standard stock solution was placed in hot air oven at 80°C/75% relative humidity for 24 h. One milliliter of the above exposed solution was further diluted in such a way to get a concentration of 30μg/mL, 40μg/mL, and 30μg/mL for LAM, EVZ, and TDF respectively.

Photo degradation

The standard stock solution was in ultraviolet chamber at 254.0 nm for 24 h. One milliliter of above exposed solution was further diluted in such a way to get a concentration of 30μg/mL, 40μg/mL, and 30μg/mL for LAM, EVZ, and TDF respectively.

The above mentioned FD solutions were injected and computed for the percentage degradation of LAM, EVZ, and TDF. As per most of the researcher's suggestions, the considerable degradation of analyte is around 20% for the validation of stability-indicating HPLC method.

Assay

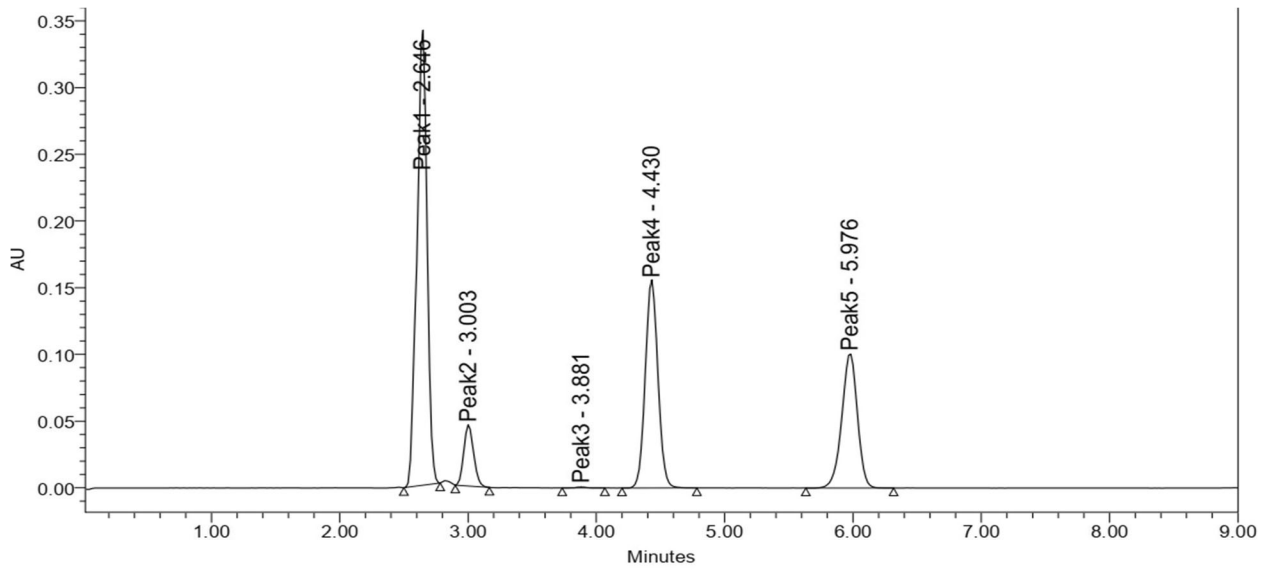
The assays of the LAM, EVZ, and TDF in commercial tablets were determined by injecting sample solution.

Results

The foremost step in the method development is to determine the solubility of the analyte. It was observed that LAM and TDF were freely soluble in water and acetonitrile and slightly soluble in methanol, and EVZ was soluble in methanol and acetonitrile and insoluble in water. On the basis of solubility of LAM, EVZ, and TDF, water and acetonitrile in 1:1 ratio was chosen as diluent.

Method optimization

Method optimization was completed after several trials. In this research, several trials have been done with different columns, mobile phases, and flow rates to attain a method with good resolution (R), N, T, and % RSD. At last, a method with Zorbax eclipse XDB-Phenyl column, with a mobile phase of methanol: buffer (0.1% v/v formic in water) (73:27 v/v) at a flow rate of 1mL/min and detection wavelength of 260.0nm was elected as optimized



Peak	tR	Peak area	Resolution (R)
Peak1 (Lamivudine)	2.638	2578801	-
Peak2	3.003	246463	2.81
Peak3	3.881	3707	4.21
Peak4 (Tenofovir disoproxil fumarate)	4.430	1472204	2.62
Peak5 (Efavirez)	5.976	1055922	7.60

Fig. 5 Chromatogram of base degradation

conditions (trial 6). The different trial conditions and their observation are shown in Table 1. The optimized chromatogram is shown in Fig. 2.

Method validation

System suitability

All parameters of the system suitability test were in compliance with standards of ICH (Table 2).

Linearity

The linear response of the LAM, TDF, and EVZ were in the series of 15.0 to 45.0 μg/mL, 15.0 to 45.0 μg/mL, and 20.0 to 60.0μg/mL. Linearity was confirmed by calculating R^2 value from linear plot constructed between concentration and peak area (Table 3, Fig. 3). The R^2 values of the three analytes were in compliance with ICH limits.

Accuracy

The mean percentage recovery of the all the three analytes in spiked solutions of three specified levels were found to be 100%±2 (Table 4), which illustrates that method was highly accurate as it is in compliance with standards laid down by ICH.

Precision

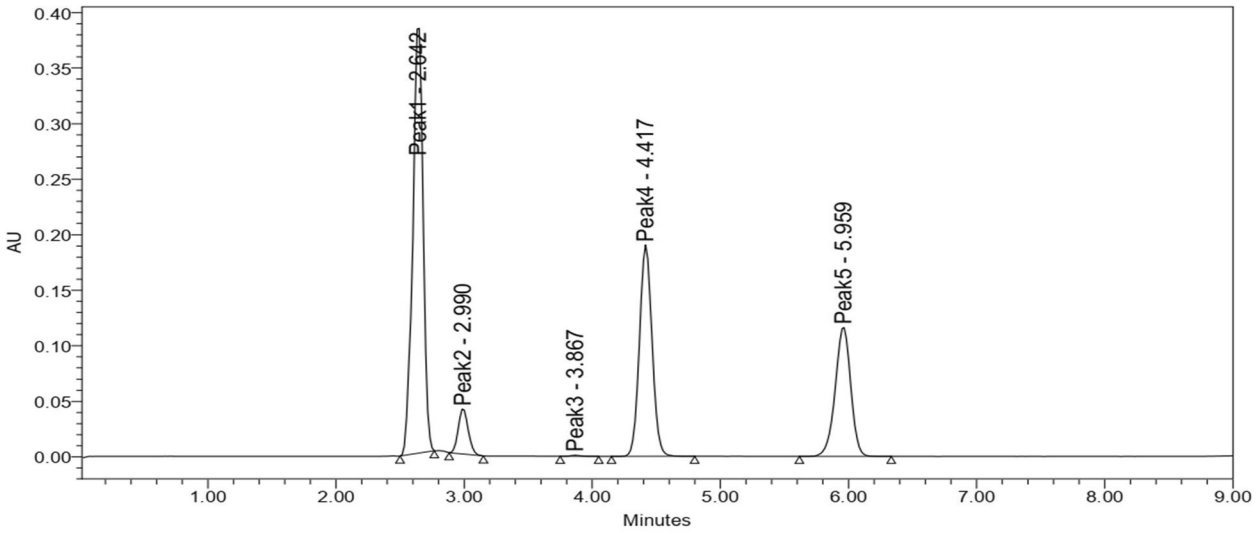
%RSD value of the peak areas of the LAM, EVZ, and TDF in replicate injections of the standard solution was assessed as ≤ 2 (Table 5), which describes that the current method was highly precise.

Sensitivity

The detection limit (LOD) and quantification limit (LOQ) responses were assessed to be 1.9μg/ml and 5.8μg/ml for LAM, 1.7μg/mL and 5.2μg/mL for TDF, and 2.3 μg/mL and 6.8μg/mL for EVZ respectively.

Robustness

Intentional changes in optimized procedure conditions to little extent could not influence the system suitability parameter results which were in compliance with the standards of ICH (Table 6), stipulates that the method has considerable robustness.



Peak	tR	Peak area	Resolution (R)
Peak1 (Lamivudine)	2.642	2616177	-
Peak2	2.990	221450	2.80
Peak3	3.867	5547	4.25
Peak4 (Tenofovir disoproxil fumarate)	4.417	1753290	2.59
Peak5 (Efavirez)	5.959	1194572	7.62

Fig. 6 Chromatogram of oxidative degradation

Forced degradation

The percentage degradations of the three analytes in stressed solutions are given in Table 7. Acid hydrolysis, alkali hydrolysis, oxidative degradation, photo degradation, and thermal chromatograms were mentioned in Figs. 4, 5, 6, 7, and 8 respectively. Those results were stipulating the stability-indicating property of the method.

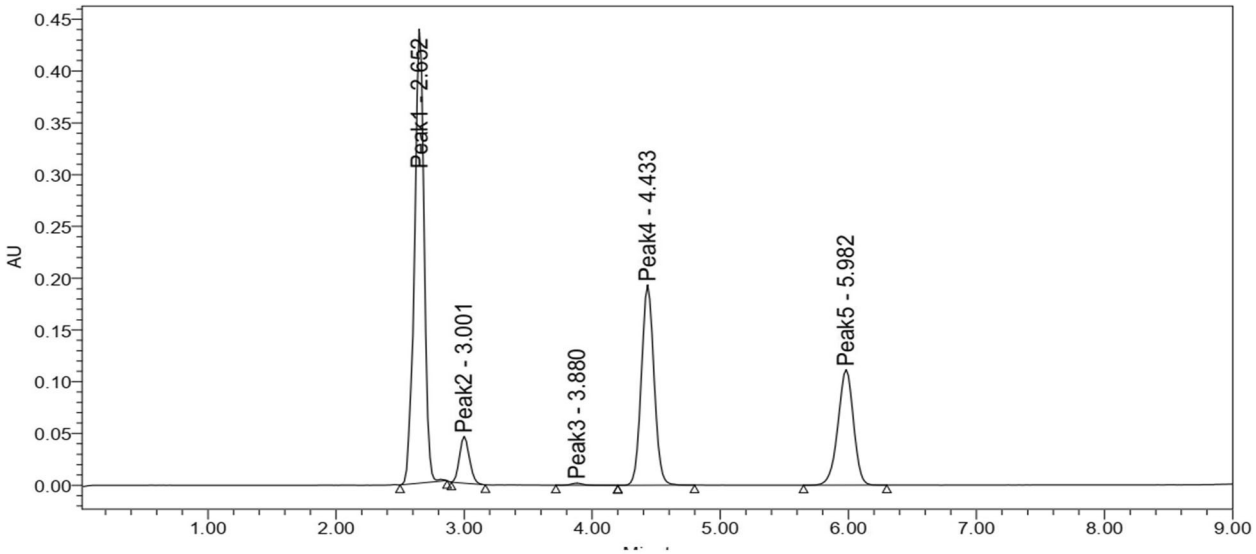
Assay

The percentage assay of the LAM, EVZ, and TDF in the combined tablet form were within 100%±15 limit (Table 8), which are in compliance with standards of ICH.

Discussion

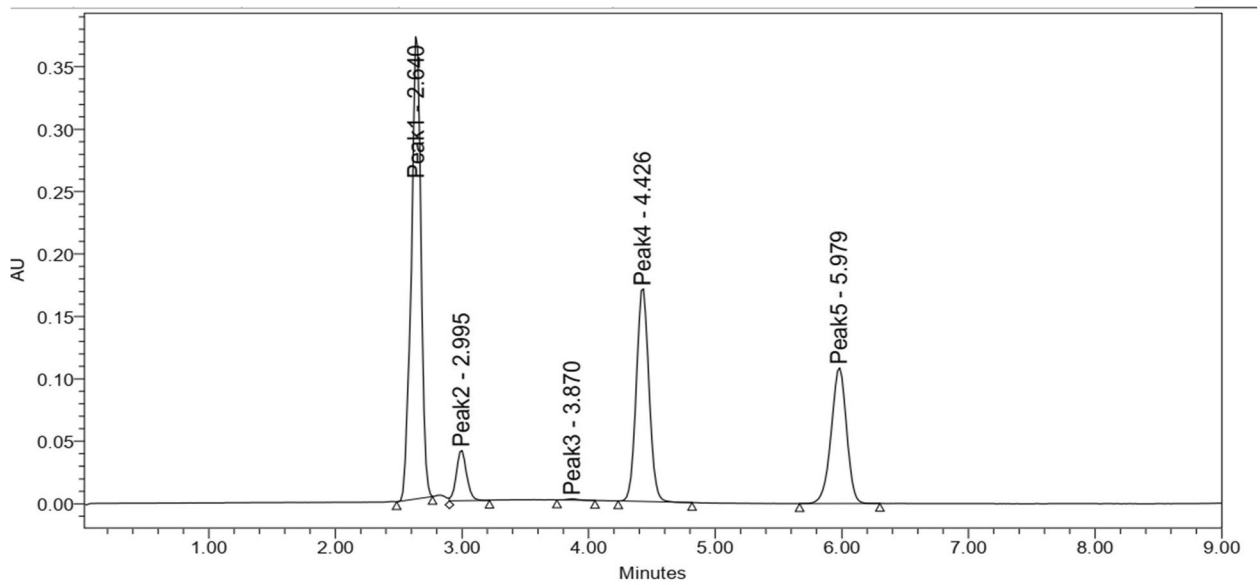
Many RP-HPLC procedures were available for simultaneous determination of LAM, EVZ, and TDF in combined tablets [12–19]. But no RP-HPLC method was subsisted with a simple solvent system, and less retention time existed. Along with those, the reported methods have drawbacks like less sensitivity and without FD studies. Therefore, endeavored to ascertain a stability-indicating method with simple solvent system [methanol 0.1% formic acid (73:27 v/v)] and less RT (less than 6min). The RT of the analytes was less than the previously subsisted method which is regarded as cost-effective due to decrease in elution time and volume of solvent to be consumed. Therefore, analysis of sample time is reduced, and more number of samples can be analyzed.

The developed method was validated as per Q2R1 guidelines of ICH. The developed method has good LOD and LOQ of 1.9μg/ml and 5.8μg/ml for LAM, 1.7μg/mL and 5.2μg/mL for TDF, and 2.3 μg/mL and 6.8μg/mL for EVZ respectively. Those values revealed that the current method has good sensitivity than the previously reported methods. The FD studies helped in determination of the amount of the drug degraded by application of different stressed conditions, which represents the stability-indicating property of the method. The developed method validation parameters were in compliance with Q2R1 specifications of ICH.



Peak	tR	Peak area	Resolution (R)
Peak1 (Lamivudine)	2.652	2694163	-
Peak2	3.001	243233	2.83
Peak3	3.880	12664	4.31
Peak4 (Tenofovir disoproxil fumarate)	4.433	1758594	2.62
Peak5 (Efavirez)	5.982	1212763	7.60

Fig. 7 Chromatogram of photo degradation



Peak	tR	Peak area	Resolution (R)
Peak1 (Lamivudine)	2.640	2679066	-
Peak2	2.995	127122	2.79
Peak3	3.870	6812	4.20
Peak4 (Tenofovir disoproxil fumarate)	4.426	1776392	2.62
Peak5 (Efavirez)	5.979	1222960	7.51

Fig. 8 Chromatogram of thermal degradation

Conclusion

An easy, accurate, unambiguous, insightful, and precised RP HPLC-PDA method with isocratic mobile phase was created to determine LAM, EVZ, and TDF concurrently in blended powder and their combined tablet. The current method has the ability to separate LAM, EVZ, and TDF, with effective resolution with less retention times. All degradants produced by application of FD degradation were separated with high resolution and determined with good sensitivity that ensures the stability-indicating property of the method. Thus, the projected method has high probability to adopt in the pharmaceutical industrial sector.

Table 8 Results of % assay of the tablet dosage form

Drug	Peak name	Retention time	Peak area	USP tailing	USP plate count	%Assay
LAM	Standard	2.64	2,691,283	1.22	2902	99.2
	Test	2.65	2,690,079	1.21	2993	
TDF	Standard	4.41	1,832,027	1.04	9631	99.6
	Test	4.40	1,831,359	1.04	9057	
EVZ	Standard	5.964	1,217,278	0.94	11,737	99.2
	Test	5.903	1,217,069	0.94	11,261	

Average weight of the tablet, 1520mg
% Purity of LAMI standard (API), 99.4
% Purity of TDF standard (API), 99.4
% Purity of EFV standard (API), 99.3

Abbreviations
LAM: Lamivudine; TDF: Tinofovir disproxil fumarate; EVZ: Efavirez; XDB: Extra dense bonding; FD: Forced degradation; SD: Standard deviation; RSD: Relative standard deviation; LOD: Limit of detection; LOQ: Limit of quantification

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RG, VK, GS, and DB contributed equally in design of the work, acquisition and interpretation of data, and manuscript preparation. All authors have read and approved the manuscript.

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

Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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