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Reverse phase-liquid chromatography assisted protocol for simultaneous determination of lamivudine and tenofovir disoproxil fumarate in combined medication used to control HIV infection: an investigative approach

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Abstract

Background: Human immunodeficiency virus (HIV) causes severe life-threatening condition, i.e., AIDS. HIV destabilises an individual's ability to prevent infection. Therefore, the combine medication lamivudine (LVD) and tenofovir disoproxil fumarate (TDF) are prescribed to suppress the amount of HIV infection in individual's body; thus, the individual's immune system could function properly. Consequently, the objective of present research work was to investigate robust and sensitive liquid chromatography avenue for simultaneous determination of lamivudine and tenofovir disoproxil fumarate in pure material and combined dosage form.

Results: The reversed-phase chromatographic separation has been performed through Hypersil BDS C_{18} column using solvent system composed of 10 mM potassium dihydrogen phosphate (pH 4.0): acetonitrile (60:40% *v/v*). The determination was executed at 30 °C at 1 mL/min rate for flow of solvent system through column. The eluents of column were monitored at 265 nm using Photodiode Array detector has revealed admirable retention times, i.e., 4.67 and 8.78 min for both drugs, respectively. The calibration curve demonstrated excellent linearity in the range of 10–50 μg/mL for lamivudine and tenofovir disoproxil fumarate with better determination coefficients was more than (r^2 0.999).

Conclusion: The estimable method was effectively validated with respect to accuracy, precision, sensitive (limit of detection and limit of quantitation), robustness, ruggedness, and for selectivity and specificity. The value less than 2 for percentage relative standard deviation for accuracy, precision, robustness, and ruggedness satisfying the acceptance criteria as per procedure of International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use.

Keywords: HIV medication, Lamivudine, Tenofovir disoproxil fumarate, Liquid chromatography, Robustness

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Background

Nucleoside and nucleotide-analogue reverse-transcriptase inhibitors (NRTIs) executed decisive function for the management of human immunodeficiency virus (HIV) infection. The diphosphate and triphosphate active metabolite of NRITs are responsible for blocked the HIV reverse transcriptase enzymatic activity through adding into the analogue of nucleotide effecting termination of DNA chain or through opposing with natural active part of the virus. This course of the action stops the movement of viral RNA into double-stranded DNA [1, 2]. The combination of medicines that are used for management of HIV is known as antiretroviral medicinal agents or phenomenon is known as antiretroviral therapy (ART). The management of HIV infection through the ART is long lasting process; since ART been shown to decrease the rate of disease, death, and the concern of drug toxicity. The principle function of ART are that to control the HIV-relevant morbidity, enhancing optimal immunity function, extending safety and significant decrease of the probability of HIV transmission in many other patients [1].

Lamivudine (LVD) is a cytidine analogue has more active for hepatitis B virus (HBV), HIV-1 and -2. By competing with the substrate, i.e., deoxycytosine triphosphate and activating the termination of the DNA strand by integrating it into viral DNA, it primarily prevents involvement in HIV-1 RT. In fixed dose combination formulations, LVD is usually combined with other NRIT's medicinal agents. For adults with HIV, the prescribed amount of LVD is 300 mg once daily or 150 mg of orally taken twice a day. Generally, LVD is good absorbed in the gastrointestinal tract and concentration of peak plasma is attained in about 0.5–1.5 h and absolute bioavailability is 82–88%. A round 70% of LVD is excreted unchanged in the urine through a combination of active tubular secretion and glomerular filtration [3–6]. For patients with co-infection with HIV-1 and HBV, 3TC can be used in a combined ART regimen incorporating tenofovir disoproxil fumarate (TDF) as the dosage recommended for HIV-1 treatment. TDF is a prodrug of tenofovir (TF) which is taken orally; after absorption, TDF is quickly changed into its active form, i.e., TF. It is a nucleotide agent which specifically blocks the reverse transcriptase of both HBV and HIV. TDF is prescribing with number of other antiviral medicinal agents as a part of fixed-dose combination medication. It is gradually orally absorbed and, through both glomerular filtration and active tubular secretion, is removed renally. It could be offered without respect to food perhaps once a day treatment. In patients with renal dysfunction, dose modification is required [7–9]. Thus, TDF is used with LVD or emtricitabine and along with third drug candidate for management of HIV infection.

Chemically, LVD is 4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one show in Fig. 1, while chemically TDF is [[(2*R*)-1-(6-aminopurin-9-yl)propan-2-yl]oxymethyl-(propan-2-yloxycarbonyloxymethoxy)phosphoryl]oxymethyl propan-2-yl carbonate; (*E*)-but-2-enedioic acid shown in Fig. 1. Molecular weight of LVD and TDF is 229.25 gm/mol and 635.5 gm/mol, respectively.

Literature survey demonstrated that numerous reports have been published so far for analysis of LVD and TDF individually and combined with other drug candidates, i.e., combination of three drug candidates. To our knowledge, there are only few HPLC methods so far published for simultaneous analysis of LVD and TDF [10–17]; these reports are having some rewards and consequences. None of published reports systematically evaluates the robustness parameters; since robustness test which become fundamental part of any analytical approach during method development and it becomes assess meticulously so that when analytical protocol is transferred from one quality control lab to another one the results should not be varied. Because of the stringent regulatory criteria, particularly with regard to pharmaceutical analysis, the findings of the research must be documented with an appropriate consistency. Robustness evaluation was initially undertaken to classify significant variables that may have an effect on the outcomes, i.e., to determine the reproducibility of method. At the end of the validation of the method, just before the inter-laboratory study, this research was then carried out. However, since modifying or refining and re-validating a method that is observed to be non-robust is necessary, this leads to an increase in time and cost of growth. Robustness is, however, confirmed earlier in validation cycle [18, 19].The fixed dose formulation of LVD and TDF is among the most favored drug preparation for the prevention of HIV infection and acquired immunodeficiency syndrome. Thus, the application of FDC-ART has strengthened and mitigated the monitoring and treatment of people with AIDS and chronic disabilities. It also offers greater convenience, lower costs, and therefore increased performance and safety. Therefore, in proposed investigation, it was planned to explore the robust and versatile reverse-phase liquid chromatography approach for the simultaneous analysis of LVD and TDF in pure form and pharmaceutical preparation.

Methods

Pure samples

Lamivudine (purity—99.53%) and tenofovir disoproxil fumarate (purity—99.40%) were acquired as gift sample from Cipla Pvt. Ltd. Pune, India.

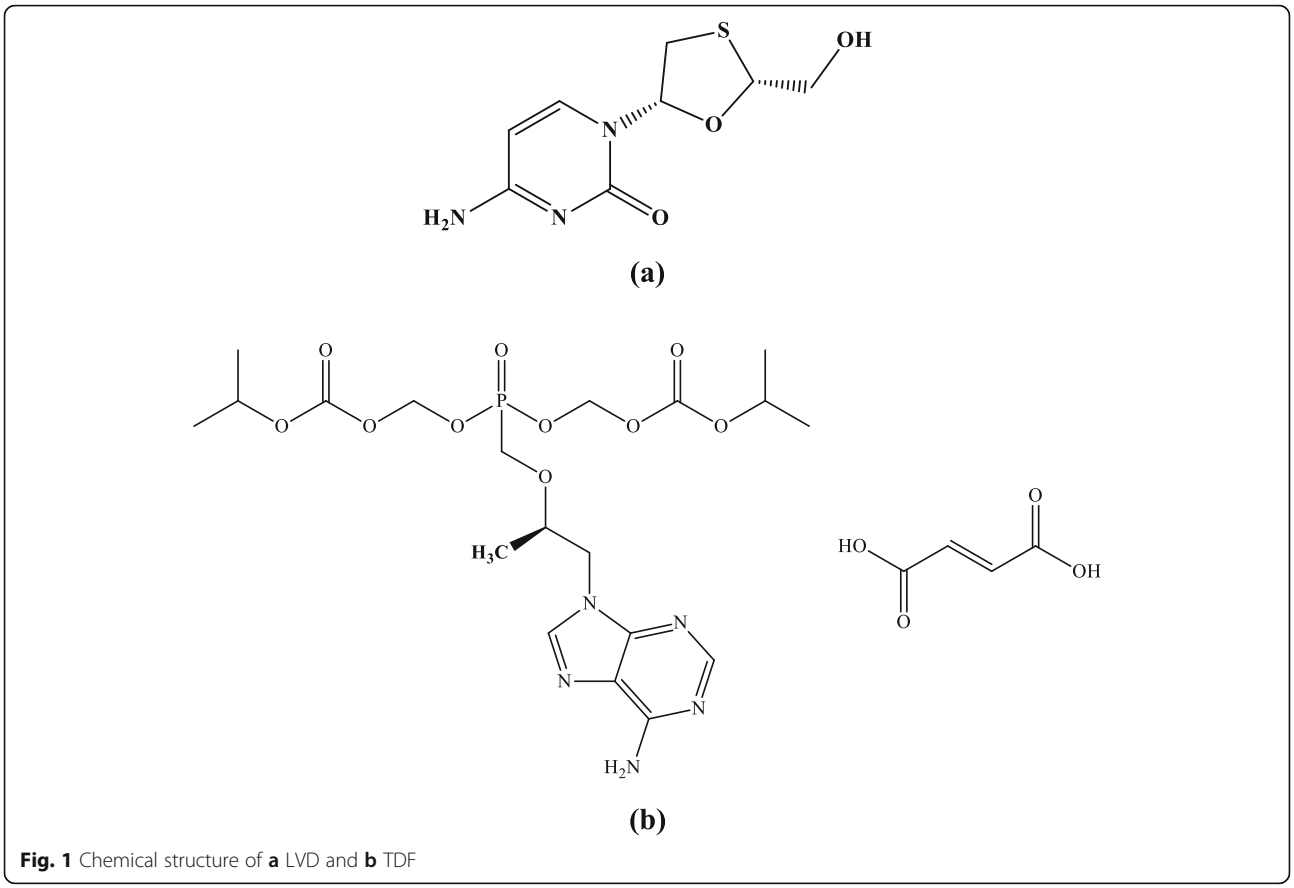


Fig. 1 Chemical structure of **a** LVD and **b** TDF

Formulation

The tablet matrix of Tenvir-L consisting of 300 mg of LVD and 300 mg of TDF was procured from local Indian market.

Chemical and reagents

Acetonitrile (ACN), methanol, orthophosphoric acid, and other reagent and chemicals were procured from Merck, Mumbai, India. Water for HPLC also procured from Merck, Mumbai, India.

Instrumentation

Chromatographic resolution was executed with the help of LC 20AD system (Shimadzu Corporation, Japan) which composed of LC-20 AD (binary solvent delivery pump) connected to 20 μL injection loop (a Rheodyne injector). SPD-M20A photodiode array detector and CTO 10 AS vp (thermostated column oven compartment). The data were collected and analyzed with LC-solution (Shimadzu Corporation, Japan). Ultrasonication of samples was conducted by means of an Ultrasonicator; ENERTECH Electronics Pvt. Ltd., India.

Chromatographic conditions

The chromatographic resolution of present analysis was studied on Hypersil BDS C_{18}(250 mm × 4.6 mm) particle size 5 μm. Hypersil BDS column was equilibrated with solvent system comprised of 10 mM potassium dihydrogen phosphate: acetonitrile (60:40% *v/v*). Lastly, pH 4.0 was adjusted by mean of orthophosphoric acid. A 20-μL solution of standard was injected. The analysis was established at 30 °C at 1 mL/min rate for flow of mobile system through Hypersil BDS column. The analytes of column were monitored at 265 nm using PDA.

Preparation of buffer solution for mobile phase

Potassium dihydrogen phosphate was used for solvent system. The 10 mM potassium dihydrogen phosphate solution of buffer was prepared by accurately solubilising predetermined amount of potassium dihydrogen phosphate in double distilled water (1000 mL) and solution of buffer was adjusted to pH 4.0 using orthophosphoric acid. Further, prepared buffer solution was filtered using 0.45 μm filter.

Preparation of standard stock solution

One milligram per milliliter stock solution of LVD and TDF were workout separately in 10 mL of calibrated

flask in ACN. From prepared stock solution aliquots of 1 mL each were moved to neat and dry10 mL of calibrated flask and diluted up to the mark with same to achieved 100 μg/mL concentration for LVD and TDF, respectively.

Preparation of sample of LVD and TDF in marketed formulation

The present validated investigational RP-LC method was employed for quantification of LVD and TDF in tablet matrix. The tablet matrix of Tenvir-L consisting of 300 mg of LVD and 300 mg of TDF was procured from local Indian market. To estimating analyte in the marketed matrix; 20 tablets were weighed precisely ground into fine powder. Accurately measured tablet powder equivalent to (300 mg LVD and 300 mg TDF) was moved into a 100 mL of calibrated flask, 30 mL ACN was added and the calibrated flask was sonicated for 20 min. Correspondingly, volume of calibrated flask was made using ACN up to the mark to obtained 3000 μg/mL concentration of stock solution of tablet matrix. Then, after resulting solution of tablet matrix filtered using 0.45 μm filter, suitable volume was moved from resulting solution of tablet matrix into calibrated flask of 10 mL and the volume of it was diluted up to the mark using the solvent system to achieved 30 μg/mL sample solution concentration.

Validation of investigated reversed phase-liquid chromatography method

The present RP-LC method has been validated for the confirmation of LVD and TDF in marketed product. The investigated method has been effectively validated for the different parameters namely system suitability, accuracy, precision, sensitive (limit of detection and quantification), robustness, ruggedness, and for selectivity and specificity as stated in the Q2R1 procedure International Council for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use [20].

System suitability

System suitability assessment is typically aimed to preventing the perceived instability of chromatographic elements like detector, type of column, and pump from negatively affecting official methods [21]. The 10 μg/mL concentration of LVD and TDF solution was introduced and examined as six replicates. Theoretical plate number, tailing factor, resolution, and relative standard deviation (% RSD) of retention time (R_t) and peak area values for LVD and TDF were estimated.

Calibration curve

Calibration curve for LVD and TDF for present investigation have been constructed in optimized chromatographic conditions. For calibration curve, appropriate volumes were moved from the previously prepared stock solution of 100 μg/mL in the range of 1–5 mL was moved to series of calibrated flask of 10 mL and volume was marked up to the mark using solvent system to give concentration in the range of 10–50 μg/mL for LVD and TDF, respectively. Through Hamilton Syringe's help a fixed volume of 20 μL is injected into the LC. All measurements for every single concentration were replicated six times. Regression analysis for obtained results was conducted through the least-square method.

Accuracy

To evaluate the closeness of the measured value to the true value, the accuracy of an analytical method is established. A method's accuracy is generally assessed through the drug candidate's percentage recovery, which is spiked into a placebo matrix. Percent recovery of the commenced investigation has been performed at 80, 100, and 120% levels. It was done by the addition to the pre-studied sample a known amount of standard drug and further it was re-examined through the same investigation.

Precision

Precision of the commenced investigation was accomplished through the intra-day and inter-day precision and repeatability (system precision) and was assessed as RSD percentage. According to ICH Q2R1 procedure, RSD percentage value must be less than 2%. Three distinct concentrations 20, 30, and 40 μg/mL were selected for intra-day and inter-day precision and repeatability of present investigation was performed using 20 μg/mL.

Sensitivity

Sensitivity the commenced investigation was evaluated for LOD and LOQ. LOD and LOQ for LVD and TDF have being estimated by injecting the 10–30 μg/mL low concentrations solution of the LVD and TDF through the investigated method. The formulae's were used to approximate the LOD = 3.3×ASD/S and LOQ = 10×ASD/S. Where, average standard deviation and slope of the linearity curve are ASD and S.

Robustness

According to ICH procedure, the concept of robustness of the analytical protocol is an appraisal potential to remain unaffected due to minor yet deliberate differences in the analytical protocol parameters. To evaluate the robustness of the investigation, flow rate of solvent system, column oven temperature, and pH of solvent system were selected as independent variables. The impact of each independent variable was observed over the responses, i.e., tailing factor, R_t, theoretical plates.

Robustness evaluation has been investigated using concentration of 30 μg/mL.

Ruggedness

The ruggedness of the investigated protocol is the level of repeatability, under the same analytical and environmental conditions, of the test outcomes produced by the estimate of the sample of interest by two independent researchers. Ruggedness evaluation has been investigated using concentration of 30 μg/mL.

Selectivity and specificity

Specificity is a step for detecting of analytical sample of interest quantitatively throughout the context of ingredients that could be required to escape in the sample solid form; thus, the selectivity is the step for qualitatively distinguishing the analyte in the existence of ingredients officially available in the solid form. Selectivity and specificity of the present investigation evaluated using checking the resolution factor of LVD and TDF chromatographic peaks were assessed though the UV spectra generated by a UV detector.

Results

Optimization of proposed investigation

In present investigation, our endeavored is to established and validated RP-LC investigation for quantification of LVD and TDF in combined tablet medication used to control HIV infection. To established effective RP-LC investigation, two insights have been taken into consideration for development and validation. The two insights included the interpretation of physical-chemical properties of LVD and TDF that enables the choosing of convenient conditions of RP-LC from broad literature studies. Establishment of RP-LC investigation was hence concentrated on the collection of detailed knowledge of LVD and TDF inclusive of chemical structure, dissociation constant, partition coefficient, molecular weight, solubility, and absorption UV-spectrum. The LVD and TDF are freely soluble into methanol and ACN; hence, the ACN was selected for stock standard preparation for LVD and TDF due to its high chemical stability and low viscosity. Chemically, LVD is 4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one show in Fig. 1, while chemically TDF is [[(2*R*)-1-(6-aminopurin-9-yl)propan-2-yl]oxymethyl-(propan-2-yloxycarbonyloxymethoxy)phosphoryl]oxymethyl propan-2-yl carbonate;(*E*)-but-2-enedioic acid shown in Fig. 1. Molecular weights: 229.25 gm/moL and 635.5 gm/moL; UV absorption spectrum was found to be 265 nm which is further used as detection wavelength for LVD and TDF. The method was initiated and optimized after obtaining this preliminary information by modifying different LC parameters as defined in the method section.

For optimization of chromatographic conditions, various combination and diverse ratios of solvent systems like methanol: water, ACN: water, methanol: ACN and methanol: ACN: water of different pH were tested for separate the both analytes but it was observed that these combinations methanol: ACN: water was not able to resolved the peaks of both analytes to obtained appropriate system suitability test. Therefore, in order to achieve the excellent resolution of both analytes system of various buffers were attempted effectively. Hence, lastly, a solvent system comprised of 10 mM potassium dihydrogen phosphate: acetonitrile (60:40% *v/v*) and pH of aq. phase was maintained through orthophosphoric acid up to 4.0. The excellent chromatographic resolution of both analytes has been achieved on Hypersil BDS C_{18} (250 mm× 4.6 mm) particle size, i.e., 5 μm. The analysis was established at 30 °C at 1 mL/min rate for flow of mobile system through Hypersil BDS column. The analytes of column were monitored at 265 nm using PDA. The standard chromatogram for LVD and TDF are depicted in Fig. 2.

Validation of method

These optimized chromatographic conditions were selected for validation of proposed investigation.

System suitability

Parameters of system suitability like theoretical plate number, tailing factor, resolution, capacity factor, R_t, and peak area were studied using injecting the 10 μg/mL solution of LVD and TDF (20 μL) for six times. The outcomes of system suitability parameters are in the acceptable limit and demonstrated in Table 1. The both analytes, i.e., LVD and TDF were continuously well resolved and retained at 4.67 and 8.78 min with RSD % less than 1% depicting strong reproducibility of the duplicate injections used on the integral LC system according to USP. In all chromatographic cycles, theoretical plate number still crossed over 2000 maintaining strong column efficacy across the entire separation process of investigation. Finally, the present investigation displayed superior resolution, i.e., 2.59 ± 0.05 and 2.88 ± 0.03 for LVD and TDF, respectively. Limit of acceptance for tailing factors were 1.34 and 1.17, and tailing factor have not ever crossed 1.5 with an outstanding peak symmetry.

Calibration curve

The linearity of investigated method is the calculation of how good a response against drug concentration calibration curve better reflects a straight line. To construct calibration curve, five drug concentrations in the range of 10–50 μg/mL for LVD and TDF were prepared and constant volume of 20 μL is injected into the system. The calibration plot is linear over the concentration

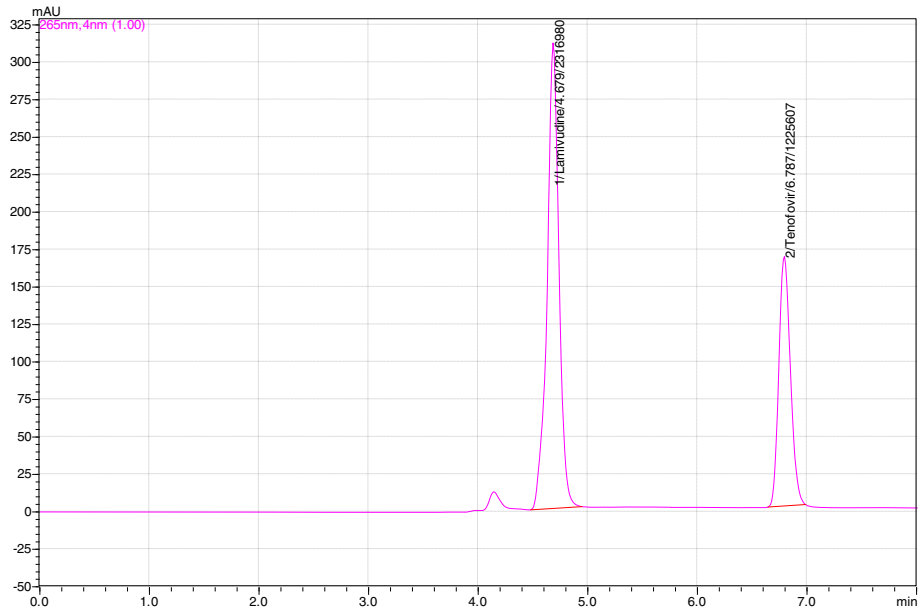


Fig. 2 Standard chromatogram for LVD and TDF

range of 10–50 μg/mL. The straight line equation of the calibration plot based on the peak outcome were y = 54100x − 3539 with a (r^2) determination coefficient 0.999 for LVD and y = 19821x + 1385 with a (r^2) determination coefficient of 0.999. The findings of the linear regression study revealed the importance of the approach proposed. The calibration curve for LVD and TDF are depicted in Fig. 3.

Accuracy

Accuracy of proposed investigation is assessed using the percentage recovery. Percent recovery of the commenced investigation has been performed at 80, 100, and 120% levels. For accuracy determination three distinct concentrations of standard LVD and TDF were prepared and spiked with placebo samples. Three times every concentration is injected and recovery was calculated. The percentage recovery of proposed investigation ranged from the 99.19–100.05 percentage with RSD % in the ranged of 0.20–0.72 percentage indicates the accuracy of the investigation. The percentage recovery observations are tabulated in Table 2.

Precision

The precision of the conducted examination was established through intra-day and inter-day precision and repeatability and was calculated as a percentage of RSD. For intra-day and inter-day precision three distinct concentrations 20, 30, and 40 μg/mL were prepared through diluting the stock solution of LVD and TDF using solvent system and every concentration is injected for three times in the same day and for the successive days, accordingly the RSD % values were determined. While repeatability of present investigation was assessed by injecting 20 μL solution six times of a concentration of 20 μg/mL, accordingly the RSD % values were determined. RSD percentage level for precision study less than 2% thus according ICH Q2R1 protocol indicating precision of the present investigation. The precision studies outcomes are tabulated in Table 3.

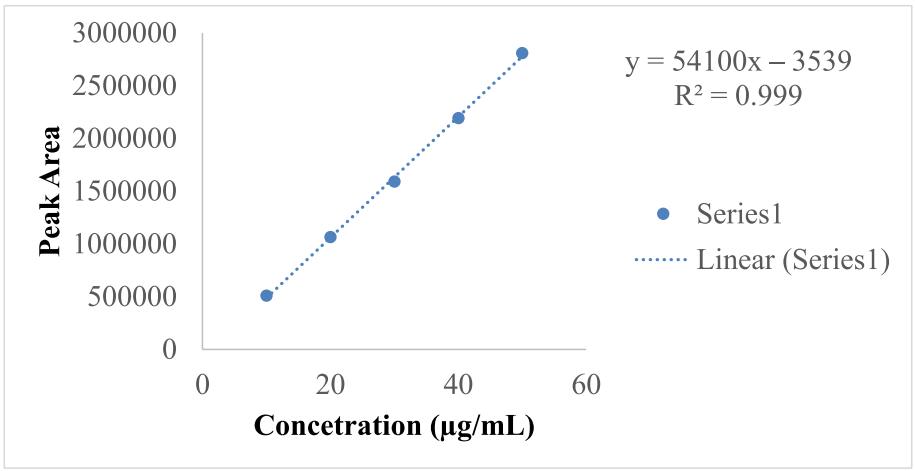
Sensitivity

Sensitivity of present investigation is calculated in terms of LOD and LOQ. To estimated LOD and LOD; five distinct concentrations 10, 15, 20, 25, and 30 μg/mL have been work out through diluting the stock solution of LVD and TDF using solvent system and injecting 20 μL solution to LC system six times for each concentration.

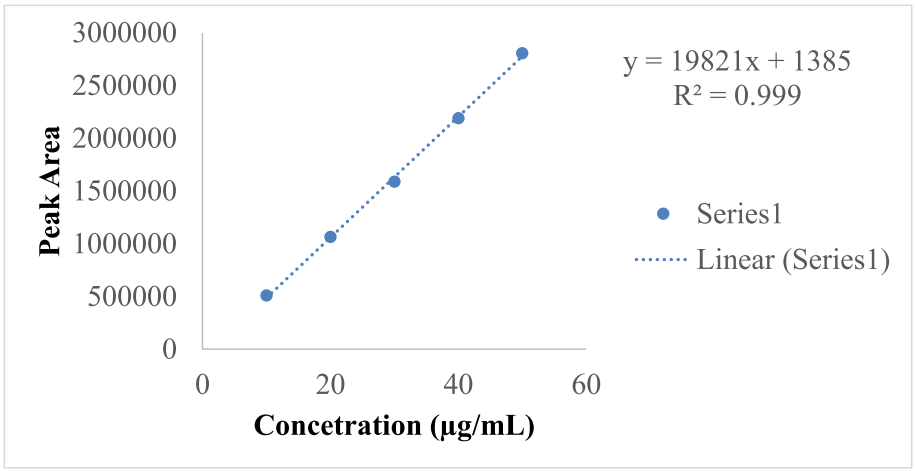
Table 1 System suitability test

Tests/parameters	Retention time (*Rt*)		Theoretical plates		Tailing factor		Resolution
Analytes	**LVD**	**TDF**	**LVD**	**TDF**	**LVD**	**TDF**	**2.59 ± 0.05**
Average (n = 6)	4.67	8.78	7896	2549	1.34	1.17	2.88 ± 0.03

n = number of determinations



(a)



(b)

Fig. 3 Calibration curve for LVD (**a**) and TDF (**b**)

The LOD and LOQ were estimated 0.10, 0.25, and 0.32, 0.45 µg, respectively, for LVD and TDF.

Robustness

Robustness of proposed investigation was studied by using the concentration of 30 µg/mL. It was investigated by changing the rate of flow of solvent system (0.8–1.2 mL/min), temperature of column oven (25–35 °C) and pH of solvent system (3.0–4.0) and according their impact was studied on tailing factor, R_t, theoretical plates of LVD and TDF, respectively. From it was observed that by introducing small but deliberate variations to the chosen independent variable, no remarkable effects were observed on the responses. Hence, suggested that proposed investigation was robust upon introducing small but deliberate changes on chromatographic conditions. The robustness evaluation observations are tabulated in Table 4.

Ruggedness

Ruggedness of proposed investigational protocol was executed through concentration of 30 µg/mL. The results were obtained satisfactory upon examined the study through two independent researchers under same analytical and environmental conditions. Thus, it was concluded that method was rugged. The ruggedness evaluation observations are tabulated in Table 5.

Selectivity and specificity

The specificity of the present investigation was executed using evaluation of resolution factor of analytes peaks from all the peaks of presence of other components. The results of specificity evaluation indicated that the investigation was specific enough for analytes. The resolution factor for the drug peaks was more than 2.67 and 2.54 for LVD and TDF, respectively.

Table 2 Investigation of accuracy study

Initial amount [μg/mL]	Amount added [μg/mL]	Amount found [μg/mL]		% Recovery		% RSD	
		LVD	TDF	LVD	TDF	LVD	TDF
Level of recovery study 80%							
20	16	36.10	35.90	100.62	99.66	0.49	0.44
20	16	35.95	36.04	99.68	100.51		
20	16	35.98	35.99	99.87	99.88		
Mean ± SD 100.05 ± 0.49100.01 ± 0.44							
Level of recovery study 100%							
20	20	39.87	39.77	99.38	99.28	0.72	0.83
20	20	39.96	40.00	99.80	100.00		
20	20	39.68	39.56	98.40	98.35		
Mean ± SD 99.19 ± 0.71 99.21 ± 0.82							
Level of recovery study 120%							
20	24	44.05	43.95	100.20	99.80	0.20	0.10
20	24	43.95	44.00	99.79	100.00		
20	24	44.00	43.95	100.00	99.85		
Mean ± SD 99.99 ± 0.20 99.88 ± 0.10							

**SD* standard deviation, *%RSD* percent relative standard deviation

Table 3 Precision analysis

Intra-day precision			Inter-day precision		
Concentrations	**% amount found [*n* = 3]**	**% RSD**	**Concentrations**	**% amount found [*n* = 3]**	**% RSD**
For LVD					
20	98.05	0.26	**20**	98.85	
	101.88			99.05	0.10
	101.80			98.99	
Mean ± SD	**98.32 ± 0.25**		**Mean ± SD**	**98.96 ± 0.10**	
30	101.88	0.66	30	100.78	
	100.56			100.24	0.28
	100.98			100.68	
Mean ± SD	**101.14 ± 0.67**		**Mean ± SD**	**100.56 ± 0.28**	
40	101.8	1.64	40	100.54	1.15
	98.56			98.25	
	99.65			99.34	
Mean ± SD	**100.00 ± 1.64**		**Mean ± SD**	**99.37 ± 1.14**	
For TDF					
20	100.65		20	100.25	0.49
	99.95	0.35		99.37	
	100.35			100.19	
Mean ± SD	**100.31 ± 0.35**		**Mean ± SD**	**99.93 ± 0.49**	
30	101.35		30	100.15	0.40
	100.56	0.39		99.34	
	100.86			99.68	
Mean ± SD	**100.92 ± 0.39**		**Mean ± SD**	**99.72 ± 0.40**	
40	101.17		40	100.95	0.68
	100.48	0.44		99.87	
	100.34			99.69	
Mean ± SD	**100.66 ± 0.44**		**Mean ± SD**	**100.17 ± 0.68**	

**n* number of determinations, *SD* standard deviation, *%RSD* percent relative standard deviation

Table 4 Robustness evaluation

Independent variables	Explored range	LVD			TDF			Optimized value
		Tailing factor	Retention time (R_t)	Theoretical plates	Tailing factor	Retention time (R_t)	Theoretical plates	
Flow rate (mL/min)	0.8–1.2	1.36	4.59	7895	1.17	8.45	2549	1 mL/min
		1.30	4.60	7896	1.14	8.50	2534	
		1.45	4.61	7894	1.15	8.53	2541	
Column oven temperature (°C)	25–35	1.35	4.56	7859	1.14	8.35	2514	30 °C
		1.34	4.62	7887	1.12	8.38	2516	
		1.33	4.59	7864	1.15	8.34	2534	
pH of solvent system	3.0–4.0	1.33	4.57	7814	1.16	8.39	2536	pH 4.0
		1.29	4.55	7858	1.15	8.41	2574	
		1.36	4.61	7865	1.17	8.38	2539	

Validation parameters for proposed investigation are summarized in Table 5.

Application of investigational approach for estimation of analyte in marketed preparation

The established method was effectively explored for the estimation of LVD and TDF in Tenvir-L marketed preparation. The assay of LVD and TDF in Tenvir-L was found to be 99.78 ± 0.24% and 99.50 ± 0.17% presented in Table 5. Optimally, a greater percentage of an analyte assay is needed when establishing analytical protocol, since it encourages other analysts to analyze the similar drug candidates as well in routine, or in different other kinds of pharmaceutical dosage forms. Moreover, present investigation is compared with published HPLC report [16] for percent assay. Hence, the amount of drug candidates estimated through the published report and present investigation was found to be 100.00%, 99.85%, and 99.65%, correspondingly which means that the present investigation is comparable.

Table 5 Summary of validation parameters for proposed investigation

Parameters	RP-HPLC	
	LVD	TDF
Linearity		
Range (μg/mL)	10–50	
Determination coefficient (r^2)	0.995	0.996
Accuracy		
Mean percent recovery (%)	99.19–100.05	99.21–100.01
RSD %	00.20–0.72	0.10–0.83
Precision		
Intra-day precision (RSD %)	0.26–1.64	0.10–1.15
Inter-day precision (RSD %)	0.35–0.44	0.40–0.68
Repeatability (RSD %)	0.57	0.71
Sensitivity		
LOD (μg)	0.10	0.25
LOQ (μg	0.32	0.45
Robustness	Robust	
Ruggedness		
Analysts-I (mean ± SD)	99.24 ± 0.14	99.45 ± 0.19
Analysts-II (mean ± SD)	99.95 ± 0.25	99.69 ± 0.27
Specificity	Specific	
% Assay of drugs	99.78 ± 0.24	99.50 ± 0.17

*SD Standard deviation, %RSD Percent relative standard deviation

Discussion

It is vitally important to validate an analytical approach of analysis for a LVD and TDF in pure sample and pharmaceutical matrix to ensure the accurate chromatographic recovery and separation. The proposed RP-LC method meant for the analysis of LVD and TDF in combined tablet dosage form using Hypersil BDS column with PDA detector to monitored analytes. The mobile phase composed of 10 mM potassium dihydrogen phosphate (pH 4.0): acetonitrile (60:40% *v/v*) was employed for separation analysis with 1 mL/min rate of flow from column. The results recorded from the investigation that established and validated RP-LC approach is precise, sensitive and robust [18]. The study assessing specificity demonstrated that there was complete absence of any interruption of the components of tablet matrix and no other components eluting at the R_t of LVD and TDF. Subsequently, the established method was validated in accordance with ICH guidelines and all the indicators of validation were evaluated using this investigation were recorded within the adequate limit as stated by the ICH [20]. Moreover, the superiority of the proposed approach can also be confirmed on the basis of its high sensitivity (LOD and LOQ), which is clear from lower values of LOD and LOQ 0.10, 0.25, 0.32, and 0.45 μg, respectively,

for LVD and TDF. In brief, the findings confirm the high level of use of the investigated approach for estimating LVD and TDF in pharmaceutical matrix.

Conclusion

The proposed investigation leads to the application of RP-LC approach for simultaneous determination of LVD and TDF is investigated and validated for calibration curve, accuracy, precision, sensitivity, robustness, ruggedness, selectivity, and specificity for enormously estimation in combined marketed preparation and in other pharmaceutical preparation. This investigation implicates the easy single step for sample and stock preparation LVD and TDF; moreover, direct introduction of solution into the system. The total analysis time was less than 9 min; which demonstrated the minimum wastage solvent system. Consequently, the investigation is quite sensitive to detect and quantify the analytes microgram quantity. That is why the proposed investigation could be implemented for routine analysis of LVD and TDF in different marketed preparation as well as for estimation of metabolites of respective drugs.

Abbreviations

NRTIs: Nucleoside and nucleotide-analogue reverse-transcriptase inhibitors; HIV: Human immunodeficiency virus; ART: Antiretroviral therapy; HBV: Hepatitis B virus; LVD: Lamivudine; TDF: Tenofovir disoproxil fumarate; ACN: Acetonitrile; RP-HPLC: Reverse-phase high-performance liquid chromatography; ICH: International Conference on Harmonization; LOD: Limit of detection; LOQ: Limit of quantification

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Authors' contributions

All the authors have contributed to the study design. SS and JPA have performed the HPLC method development and validation study under the guidance of VSA and RRT. RSC, SRC, and ASP have drafted the manuscript as per the journal submission format. All authors read and approved the final manuscript.

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Availability of data and materials

All data and materials are available upon request.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable as our study does not include patients.

Competing interests

The authors declare that they have no competing interests.

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