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Comparative study of UV spectroscopy, RP-HPLC and HPTLC methods for quantification of antiviral drug lamivudine in tablet formulation

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Abstract

Background In the current study, estimation of lamivudine (LMU) by UV spectroscopy, reverse-phase HPLC (RP-HPLC) and HPTLC methods in tablet formulation was developed, and comparative studies between the methods were investigated by analytical results and statistical test analysis of variance (ANOVA) to find out best method. In the UV spectral method, LMU was quantified at 271 nm absorption maxima using methanol as the solvent. In the RP-HPLC method, the Shimadzu C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) was employed for chromatographic separation. The mobile phase used consists of methanol: water (70:30 v/v) in an isocratic mode with a 1.0 mL/min flow rate. In the HPTLC method, the chromatogram was developed on a pre-coated plate of silica gel 60 F254 with a mobile phase composition of chloroform: methanol (8:2 v/v). The quantification was performed at an absorbance mode of 271 nm by densitometry. The methods were validated according to the International Conference on Harmonization (ICH) guideline Q2 (R1). The degradation conditions were employed as per ICH guidelines Q1A(R2) and Q1B which include acid, alkaline, neutral, thermal and photostability to determine the intrinsic stability of the drug in varied environmental conditions.

Results LMU absorption maxima was found to be 271 nm. The retention time of LMU was 3.125 min, and the total analysis time was 5 min. The R_f value of LMU was 0.49–0.62. The methods were linear within 2–12 µg/mL range. The correlation coefficient (r^2) for UV, HPLC and HPTLC was 0.9980, 0.9993 and 0.9988, and percent recoveries were calculated as 98.40–100.52%, 99.27–101.18% and 98.01–100.30%, respectively, with percentage relative standard deviation (RSD) less than 2% showing that methods were precise and accurate.

Conclusion Developed UV, RP-HPLC and HPTLC methods are free from intervention caused by excipients present in tablets and thus can be used for regular quantitative analysis of LMU in tablet formulation. Based on analytical results and statistical tests, ANOVA, it is inferred that the HPLC method is best for LMU quantification tablet formulation due to its high reproducibility, good retention time and sensitivity; it has a higher percent recovery and has less analysis time, i.e., 5 min. The degradation peaks were well separated from the LMU peak indicating stability of the HPLC method.

Keywords Lamivudine, UV spectroscopy, RP-HPLC, HPTLC and stability indicating

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Background

Lamivudine, Fig. 1, (4-amino-1-[(2R, 5S)-2-(hydroxyethyl)-1, 3-oxathiolan-5-yl] pyrimidin-2-one) is a reverse transcriptase inhibitor. It is used for HIV (human immunodeficiency virus) and hepatitis B infections. It is used for both HIV-1 and HIV-2. LMU is a synthetic nucleoside analogue of cytidine. Lamivudine triphosphate (L-TP) was formed by going through intracellular phosphorylation. An active 5′-triphosphate metabolite that competes with it is incorporated into the DNA of the virus. They impede reverse transcriptase enzymes competitively. The inserted nucleoside analogue has a 3′-OH group missing which functions as a chain breaker of DNA synthesis, which is necessary for the production of the 5′ to 3′ phosphodiester linkage needed for DNA chain extension. LMU is marketed under the brand name Lamivir 150-mg film-coated tablet [1–3].

An extensive literature survey disclosed that LMU has been determined independently or in combination with other drugs by UV spectroscopy [4, 5], RP-HPLC [6–13] and HPTLC [14–16]; however, there was not a single research work that has been done reporting that LMU individually was determined simultaneously by all three methods, i.e., UV spectrophotometry, RP-HPLC and HPTLC, and investigating the best method among them. Further to carry out stability indicating study of the selected superior method for separating the active analyte present in the pharmaceutical dosage is carried out, which makes the present research work unique and novel. Hence, we have strived to develop precise, accurate, sensitive and inexpensive methods and compare them based on analytical results such as sensitivity, % recovery and % assay of the drug. Validation of the method was performed according to Q2 (R1) ICH guidelines [17].

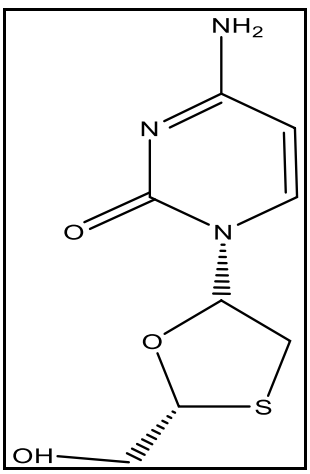

Fig. 1 LMU Chemical Structure

Methods

Materials

LMU was procured from *Cipla Ltd, Kurkumbh*, Maharashtra, India, as a gift sample. The marketed pharmaceutical tablets of Lamivir 150 mg (manufactured by Cipla Ltd) were purchased from a nearby pharmacy. Double distilled water was obtained from the Millipore unit. HPLC-grade chloroform and methanol were acquired from *Sisco Research Laboratories Pvt. Ltd, Mumbai, India*.

Instrumentation

A double-beam UV-1800 Shimadzu UV spectrophotometer along with a pair of matched quartz cells 10 mm is used. The HPLC system was a Shimadzu model no. DGU-20A5R which consists of a PDA detector. HPTLC was carried out on a pre-coated silica gel 60 F254 TLC Merck plate using a sample syringe of Camag 100 µl with an applicator of Linomat 5 and a twin-trough chamber; densitometry was executed with a CAMAG TLC Scanner 3 with Visioncats software. Shimadzu ATX224 digital analytical balance and PCi analytics ultrasonic bath were employed for weighing the samples and for sonication, respectively.

Sample preparation for UV, RP-HPLC and HPTLC method development

Standard solution preparation

Five milligrams of LMU was precisely weighed and poured into a 50-mL volumetric flask. Methanol was used as a solvent to make up the volume. 1 mL of stock solution was poured into a 10-mL volumetric flask, and the volume was raised to have a final concentration of 10 µg/mL.

Sample solution preparation

Approximately 20 tablets were weighed, and an average weight was determined for each tablet. A powder equal to five milligrams was weighed and poured into a 50-mL volumetric flask, consequently adding 15 mL of methanol and sonicating for 30 min. Later on, the volume was raised to the mark and filtered from the Whatman filter paper No. 41. Appropriately the solution was diluted to get a concentration of 10 µg/mL.

UV method development

Determination of LMU maximum absorbance (λmax)

The standard solution of LMU in the region of 200–400 nm is scanned. An absorption maximum was determined to be 271 nm, which was selected as the analytical wavelength for further analysis. The spectrum was recorded as shown in Fig. 2.

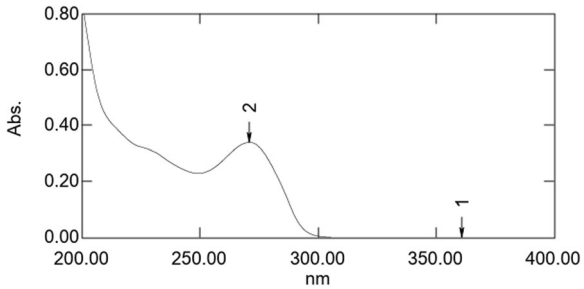

Fig. 2 UV spectrum of LMU standard solution. The λmax was determined to be 271 nm

HPLC method development

Optimization of HPLC method

To achieve optimized chromatographic conditions, the below parameters were modified in each trial. The trial runs are shown in Table 1 (Additional file 1).

From the trial, the best possible chromatographic condition was selected based on peak shape that is sharp evaluated by theoretical plates and tailing factor which were within specified limit, and retention time is 3.125 min which is much less. Therefore, separation of LMU was performed on a Shimadzu C_{18} (250 mm × 4.6 mm i.d., 5 µm particle size) consisting of methanol: water (70:30) as a mobile phase; by using a membrane filter it was filtered and degassed. The flow rate was retained at 1.0 mL/min. The injection volume was kept at 10 µl at a column oven temperature of 30 °C, and effluents were checked at 271 nm. The mode of separation was isocratic. The chromatogram of LMU and its 3D image are shown in Figs. 3 and 4**,** respectively.

Peak purity

The peak purity of the LMU peak was examined in a degradation solution using a photodiode array detector. Peak purity for each solution was passed at the threshold level. The peak purity report is depicted in Table 2, and peak purity spectra and profile are depicted in Figs. 5 and 6.

Table 1 Trial run for optimization of chromatographic conditions

Mobile phase	Flow rate (mL/min)	Retention time (min)	Comment
Methanol:Water (50:50)	0.8	4.135	Sharp peak appears, but retention time was more
Methanol:Water (60:40)	0.8	3.795	Sharp peak appears, but retention time was more
Methanol:Water (60:40)	1.0	3.534	Sharp peak appears, but retention time was more
Methanol:Water (65:35)	1.0	3.351	Sharp peak appears, but retention time was more
Methanol:Water (70:30)	1.0	3.125	Sharp peaks appear with less retention time

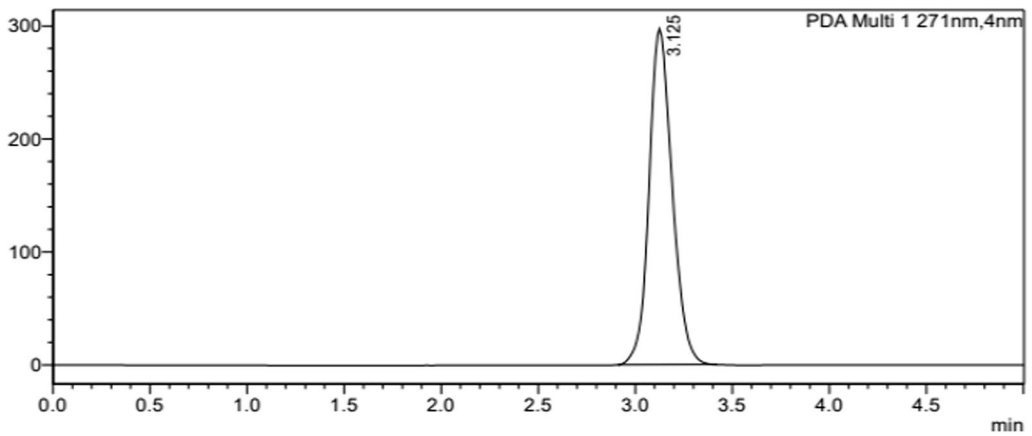

Fig. 3 Chromatogram showing the separation of LMU

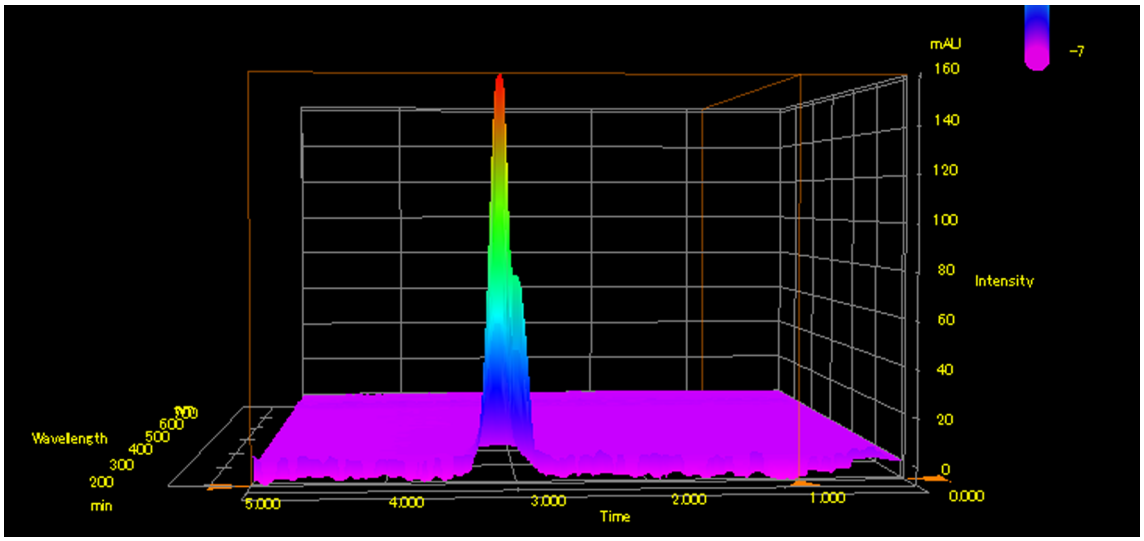

Fig. 4 3D image of LMU chromatogram

Table 2 Peak purity description

Impurity	Not detected
Peak purity index	0.284
Single-point threshold	1.00

HPTLC method development

Optimization of mobile phase by TLC

Different compositions of the mobile phase were tried first on the TLC plate. The trial runs are presented in Table 3. From the trial data, chloroform: methanol (8:2) is selected as a suitable mobile phase since it shows a detectable significant spot of LMU (Additional file 1). This optimized mobile phase is used for HPTLC method development.

The LMU standard solution of 2 µl was employed as spot bands of 4 mm to the HPTLC plates under the stream of nitrogen using LINOMAT V. Application locations were at least 15 mm from the edges and 10 mm from the foot of the plate. The development chamber was kept for saturation with chloroform: methanol (8:2 v/v) before each run for 20 min. Development of the plate was performed to migrate a distance of 7 cm by the ascending technique. The analyses were performed in a temperature-controlled laboratory (20–24 °C). Densitometry scanning was carried out using a deuterium lamp in absorbance mode at 271 nm. The chromatogram is depicted in Fig. 7.

Forced degradation studies by the HPLC method

Forced degradation includes the degradation of active substances and drug products which results in

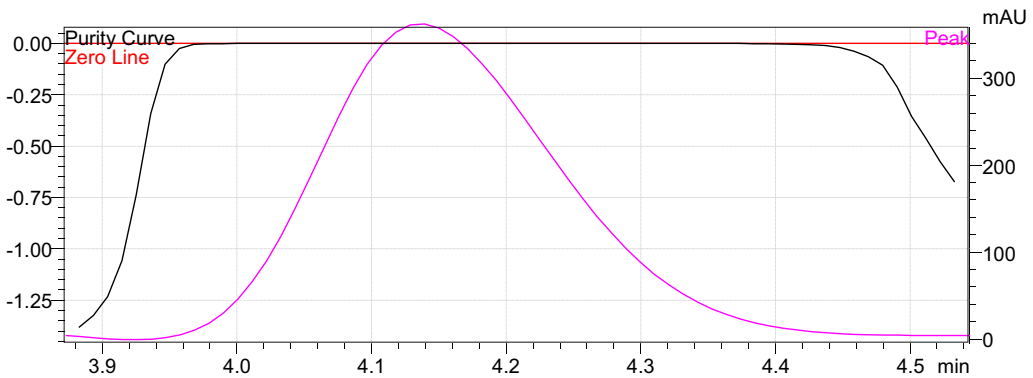

Fig. 5 Peak purity spectra of LMU

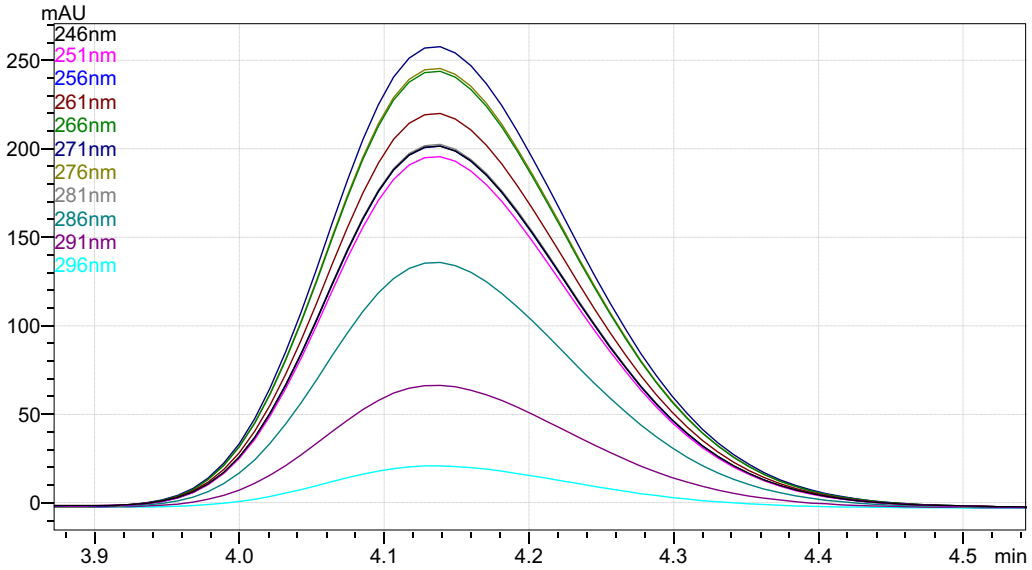

Fig. 6 Peak profile of LMU

Table 3 Trial run for optimization of mobile phase for HPTLC

Trial run	Volume of toluene (mL)	The volume of ethyl acetic acid (mL)	Volume of methanol (mL)	Volume of glacial acetic acid (mL)	Volume of chloroform (mL)	Rf value	Comment
1	4	4	1.5	0.5	–	0.46	Spot shows tailing
2	6	2	2	–	–	0.71	Spot shows tailing
3	4	2	4	–	–	–	No spot appears
4	5	2	3	–	–	–	No spot appears
5	–	–	2	–	8	0.60	Significant spot appears without tailing

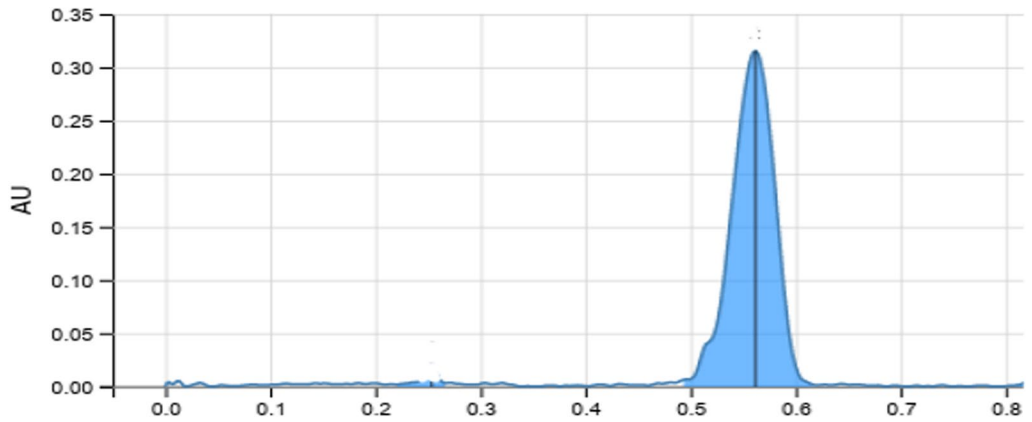

Fig. 7 HPTLC chromatogram of LMU. Rf value 0.49–0.62

degradation products that are studied to evaluate the intrinsic stability of the molecule. Degradation conditions such as acidic, alkaline, thermal, neutral and photostability were represented by ICH guidelines Q1A, Q1B [18, 19] and Q2 (R2). In a stability-indicating method, the acceptable degradation percentage should not exceed 20% .

Results

Method validation

System suitability parameters

After equilibrating the column with the mobile phase, the standard solution was autoinjected five times and the chromatograms were noted. The data are presented in Table 4.

Linearity

The standard stock solution of LMU was serially diluted to yield 6 distinct concentrations, i.e., 2, 4, 6, 8, 10 and 12 µg/mL. At 271 nm, their absorbance was measured against a blank. For HPLC, similar dilutions were performed and these solutions were autoinjected with optimized chromatographic conditions. For HPTLC, a volume of 2 µl of each serially diluted solution was employed on the HPTLC plate to carry 2, 4, 6, 8, 10 and 12 µg/mL of LMU per spot. The UV, HPLC and HPTLC methods confirmed linearity in the 2–12 µg/mL range, and the linearity equations were $y=0.0421x-0.0016$, $y=33177x - 534$ and $y=0.001x+0.0018$ with r^2 of 0.9980, 0.9993 and 0.9988, respectively. Table 5 shows the results. The calibration plots for UV, HPLC and HPTLC are depicted in Figs. 8, 9 and 10, respectively.

Table 4 System suitability results (RP-HPLC)

Sr. No	Peak area	Tailing factor	Theoretical plates
1	2,413,422	1.162	2860
2	2,431,570	1.147	2908
3	2,459,066	1.142	2846
4	2,467,969	1.140	2884
5	2,431,480	1.146	2870
% RSD	0.91	0.75	0.83
Limit	NMT 2%	NMT 1.5	NLT 2000

Table 5 Calibration curve data by UV, HPLC and HPTLC

UV		HPLC		HPTLC	
Conc (µg/mL)	Absorbance	Conc (µg/mL)	Area	Conc (µg/mL)	Area
2	0.090	2	66,582	2	0.00377
4	0.158	4	133,162	4	0.0055
6	0.251	6	199,746	6	0.00775
8	0.338	8	258,328	8	0.00966
10	0.411	10	332,908	10	0.01145
12	0.510	12	399,492	12	0.01331
r^2	0.9980	r^2	0.9993	r^2	0.9988

Accuracy

Accuracy was measured at 50%, 100% and 150% by spiking a standard solution of LMU (0.5, 1.0, 1.5 µg/mL) into the sample solution. The recoveries were ascertained in the range of 98.40–100.52%, 99.27–101.18% and 98.01–100.30% by UV, HPLC and HPTLC, respectively. The % RSD was found to be less than 2, indicating that there was no interference of the excipients while determining LMU. Therefore, the method is accurate. The accuracy data are depicted in Table 6.

Precision

For this study, six standard solutions of LMU were analyzed by UV, HPLC and HPTLC methods. The % RSD was less than 2, which significantly assures the precision of the proposed methods. Intraday and interday data are reported in Table 7.

Robustness

LMU's six working standard solutions were used for analysis. In the proposed UV method, to validate the robustness parameter, slight variation was employed in wavelength (± 2 nm); for HPLC robustness was assessed by introducing little, variation in the percent of methanol (± 2%), flow rate (± 0.2 mL/min), sonication time (± 5 min) and using different Whatman filter no. (40, 42). Similarly, for HPTLC, there was a small change in chamber saturation time (± 5 min) and the mobile phase composition (± 0.5). The data are presented in Table 8. The % RSD was not more than 2, hence significantly representing methods to be robust (Table 9).

Sensitivity

Sensitivity data

Results of analysis of tablet formulation of LMU by UV, HPLC and HPTLC methods

LMU standard and sample solutions absorbance was measured at 271 nm. In HPLC, both standard and sample solutions were autoinjected into the HPLC system, similarly for HPTLC 2 µl of standard, and sample solutions were employed as bands 4 mm on the HPTLC plate. The amount of LMU present per tablet was determined by UV, HPLC and HPTLC by comparing the absorbance and peak area of the sample with that of the standard,

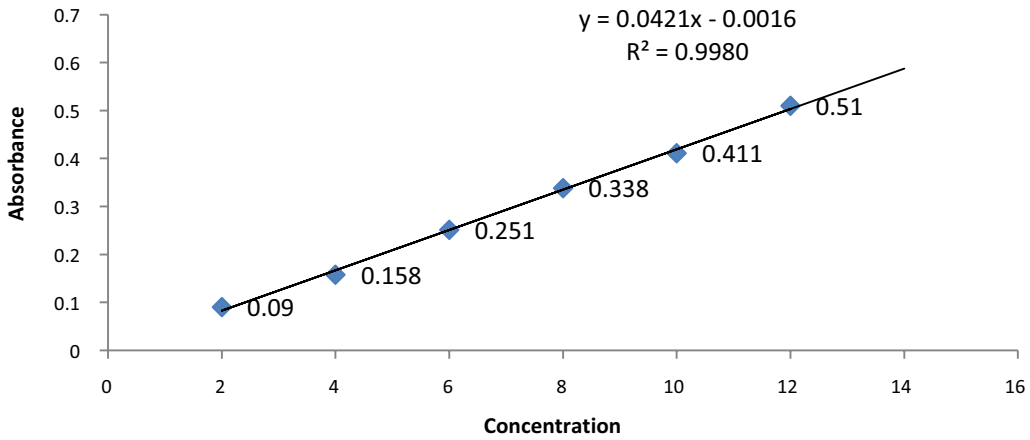

Fig. 8 Calibration curve of LMU by UV

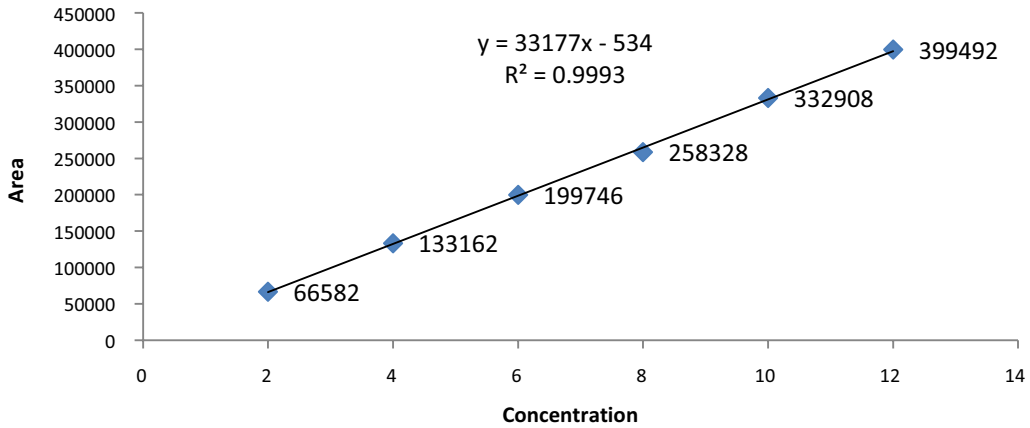

Fig. 9 Calibration curve of LMU by HPLC

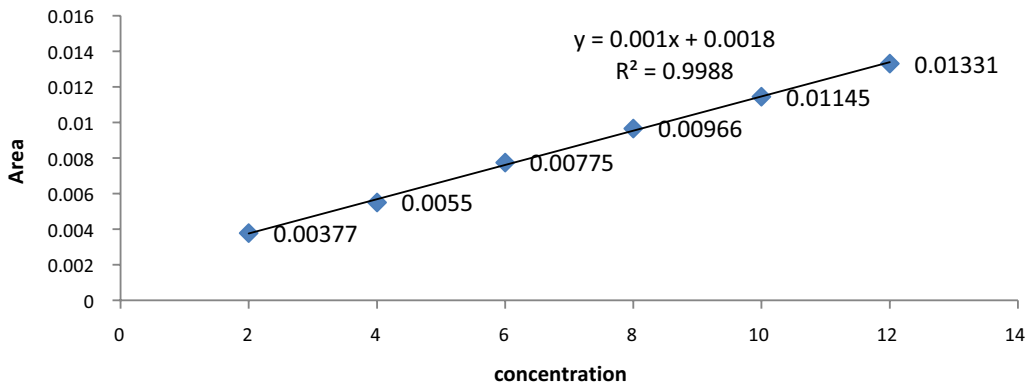

Fig. 10 Calibration curve of LMU by HPTLC

Table 6 Accuracy study results

Conc. (%)	Expected amount (µg/mL)	UV			HPLC			HPTLC		
		The actual amount found (µg/mL)	% Recovery	% RSD	Actual amount found (µg/mL)	% Recovery	% RSD	Actual amount found (µg/mL)	% Recovery	% RSD
50	15	14.96	99.2	0.51	14.97	99.80	0.27	14.96	99.20	1.46
50	15	14.90	99.33		14.89	99.27		14.92	99.50	
50	15	14.92	98.4		14.95	99.65		14.97	99.40	
100	20	20.05	100.52	1.57	19.95	99.75	0.18	20	100	0.97
100	20	19.79	98.95		19.98	99.90		19.85	98.50	
100	20	20.05	100.5		20.02	101.18		20.03	100.30	
150	25	24.80	98.67	0.83	24.89	99.56	0.75	24.79	98.01	0.27
150	25	25.02	100.13		24.95	99.80		24.84	99	
150	25	24.68	98.72		24.99	99.96		24.92	99.47	

Table 7 Precision data

UV method				
Concentration (10 µg/mL)	**Intraday study (n = 6)**		**Interday study (n = 6)**	
	0 Hour	**6 Hours**	**2nd Day**	**3rd Day**
*Mean absorbance	0.415	0.400	0.409	0.421
Standard deviation	0.0039	0.0037	0.0038	0.0042
% RSD	0.94	0.91	0.94	0.99
HPLC method				
Concentration (10 µg/mL)	Intraday study (n = 6)		Interday study (n = 6)	
	0 Hour	6 Hours	2nd Day	3rd Day
*Mean area	286,645.5	292,654.5	336,242.8	284,331.8
Standard deviation	2106.99	1933.56	3151.59	3535.95
% RSD	0.74	0.66	0.94	1.24
HPTLC method				
Concentration (10 µg/mL)	Intraday study (n = 6)		Interday study (n = 6)	
	0 Hour	6 Hours	2nd Day	3rd Day
*Mean area	0.01144	0.01152	0.01156	0.01162
Standard deviation	2.88	2.73	3.16	2.92
% RSD	0.25	0.24	0.27	0.26

n = number of measurements, *mean of six observations

Table 8 Robustness data

Parameters	***Mean of six observation**	**Standard deviation**	**% RSD**
UV			
Wavelength at 269 nm	0.687	0.0062	0.90
Wavelength at 273 nm	0.658	0.0059	0.89
HPLC			
Mobile phase Methanol:water (68:32)	283,160	1519.04	0.54
Mobile phase Methanol:water (72:28)	296,229	2833.96	0.96
Flow rate (0.8 mL/min)	363,663	2110.50	0.58
Flow rate (1.2 mL/min)	246,713	2380.96	0.96
Sonication time: 25 min	246,817	1728.86	0.70
Sonication time: 35 min	247,887	2041.86	0.83
Whatman Filter no. 40	252,347	4160.51	1.67
Whatman Filter no. 42	230,568	3367.76	1.43
HPTLC			
Mobile phase Chloroform:methanol(9.5:0.5)	0.01143	2.5166	0.22
Mobile phase Chloroform:methanol(8.5:2.5)	0.01144	3.5119	0.31
Chamber saturation time: 15 min	0.01146	1.5275	0.13
Chamber saturation time: 25 min	0.01148	2.3094	0.20

Table 9 Sensitivity data

Sensitivity	**UV method (µg/mL)**	**HPLC Method (µg/mL)**	**HPTLC method (µg/mL)**
LOD	0.58	0.33	0.44
LOQ	1.75	1.01	1.32

respectively. The obtained results of % labeled claim are reported in Table 10. Percent content obtained by UV HPLC and HPTLC was statistically compared by ANOVA, as depicted in Table 11.

Statistical comparison between UV, HPLC and HPTLC methods for % contents

ANOVA test From the statistical data, it could be inferred that the F value is greater than the F critical value indicating there is a remarkable differentiation between the mean % content determined by UV, HPLC and HPTLC methods, and hence, the null hypothesis is rejected.

Forced degradation study

For this study, drug was given treatment with various degradation conditions. 1 mL from stock solutions (1000 µg/mL) was treated separately with 1 mL

Table 10 Results of analysis of tablet formulations

Method	Brand name	Label claim	Manufacturer name	Batch No	% Label claim estimated (% content)	Mean	% RSD
UV	Lamivir 150	150 mg Lamivudine	Cipla	SA12696	98.76	99.02	0.36
					99.34		
					98.53		
					99.38		
					98.82		
					99.01		
HPLC	Lamivir 150	150 mg Lamivudine	Cipla	SA12696	99.97	99.90	0.83
					98.79		
					100.87		
					99.16		
					100.77		
					99.85		
HPTLC	Lamivir 150	150 mg Lamivudine	Cipla	SA12696	99.24	99.54	0.23
					99.57		
					99.45		
					99.51		
					99.93		
					99.53		

Table 11 Observations and results of ANOVA test for % contents study

Parameter	Variance			*F* Value	*P* Value	F critical
	UV	HPLC	HPTLC			
% Content	0.1133	0.6970	0.0504	4.5764	0.0280	3.6823

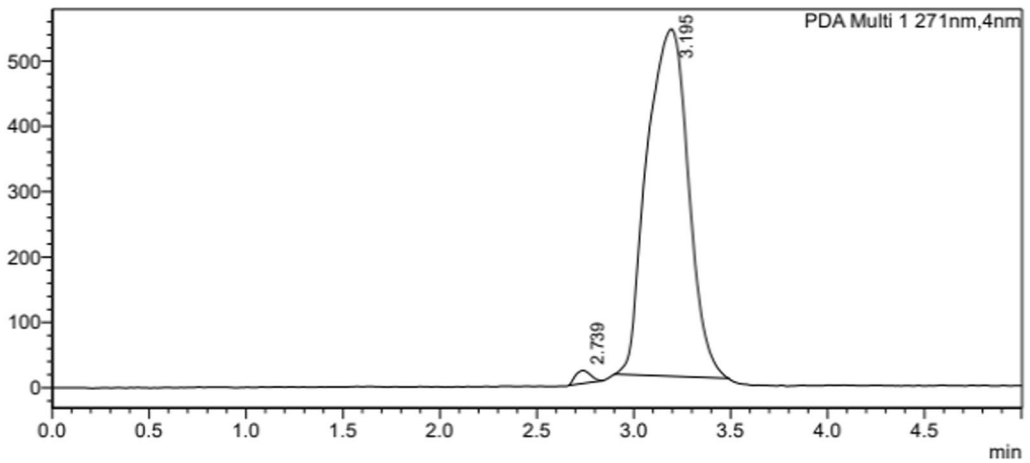

Fig. 11 Acid degradation chromatogram

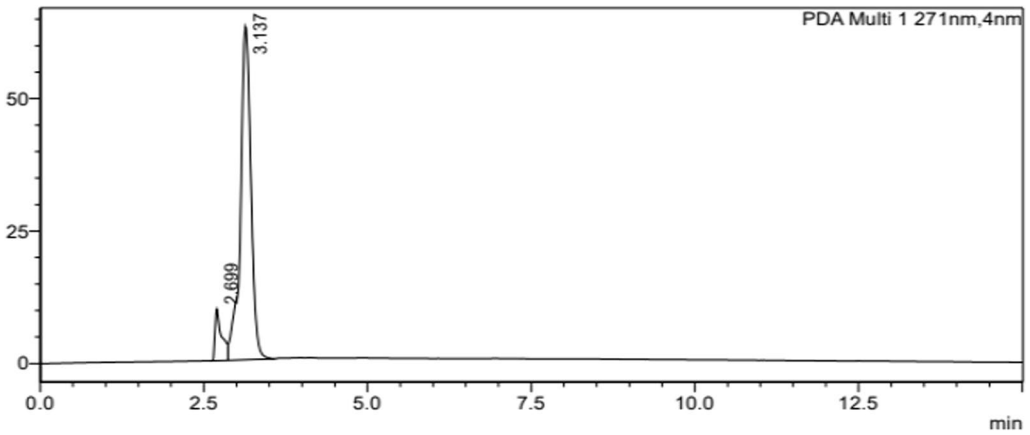

Fig. 12 Alkali degradation chromatogram

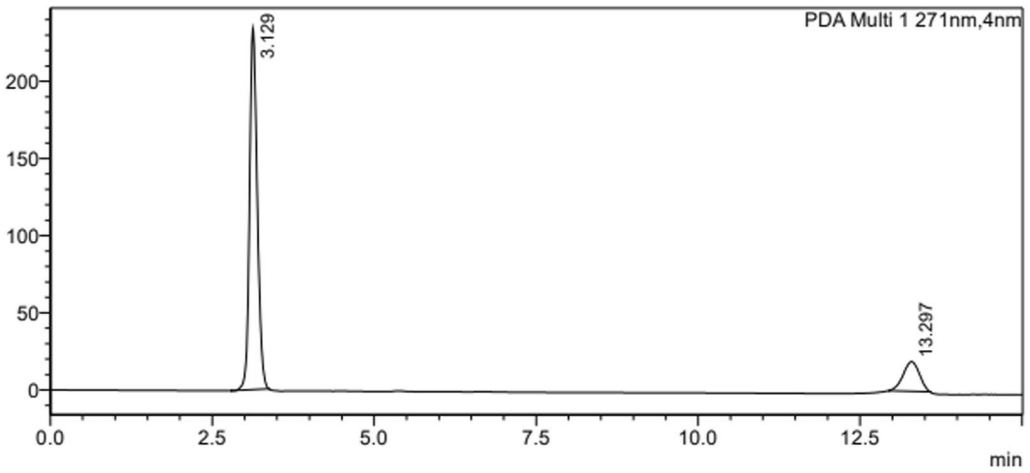

Fig. 13 Neutral degradation chromatogram

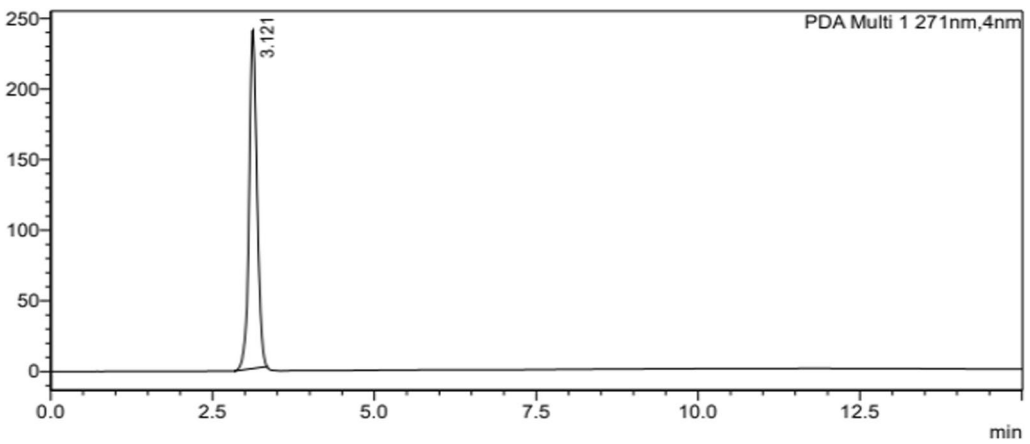

Fig. 14 Thermal degradation chromatogram

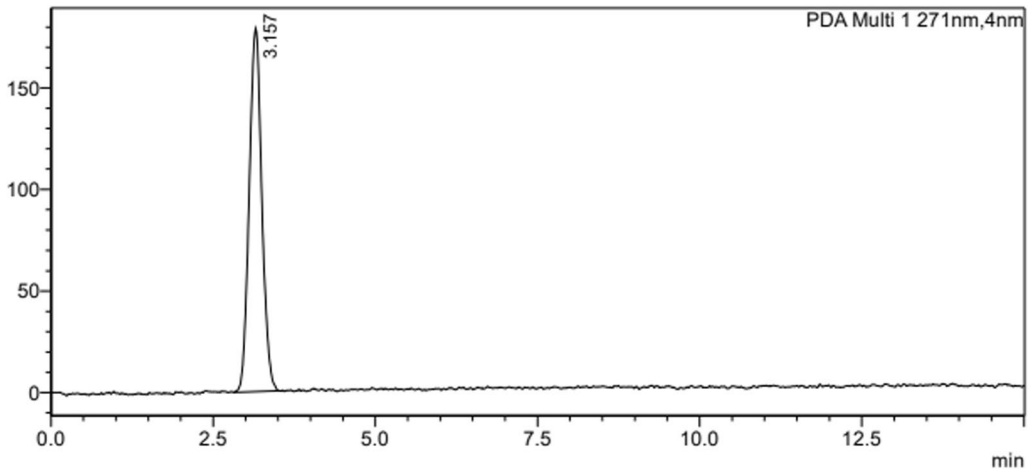

Fig. 15 Photodegradation chromatogram

of 1N hydrochloric acid (heated in a water bath for 2 h at 60 °C), 1 mL of sodium hydroxide (heated in a water bath for 2 h at 60 °C), neutral degradation (refluxing with water for 6 h at 60 °C), dry heat degradation (exposure of drug powder in the oven at 60 °C for 10 days) and photostability degradation (exposure of drug powder in sunlight for 10 days). Samples were taken at regular intervals to monitor degradations. There is no interaction of the degradation peak with that of the LMU peak. Hence, the proposed HPLC method was stability indicating and specific (Figs. 11, 12, 13, 14 and 15). The peak purity index values of LMU peak and degradation peaks were found to be within acceptable limits, Table 13.

Discussion

The present research work aims to compare all three developed methods UV, RP-HPLC and HPTLC on grounds of sensitivity, accuracy, percent recovery and percent purity and to evaluate the best method among them, since to our knowledge, no such venture had been made earlier. The inference from the study could be briefed as the UV method could be implemented in laboratories that lack high-tech analytical instruments, in developing countries where affording expensive instruments is a big deal along with the availability of highly skilled person so UV spectroscopy is a method of choice, which is cheapest and does not require so-skilled person in comparing with HPLC and HPTLC methods which are complicated, expensive and time-consuming. The HPTLC method utilized not more than 30 mL of mobile phase thus reducing mobile phase consumption when compared with HPLC method, also less mobile phase consumption indicate the eco-friendly nature of the method. After optimization of the method by TLC to develop that new method on HPTLC it takes an average of 1 h which is much less relative to HPLC but HPTLC shows less sensitivity as per analysis data reported in Table 12. Handling HPTLC requires a skilled person and is expensive. The RP-HPLC method is more sensitive at 0.33 µg/mL and has a high % recovery of 99.27–101.18% and a % label claim of 99.90% in comparison with the other two methods as depicted in the analysis data of Table 12. Complex samples having many ingredients can be separated easily via HPLC showing high separation capacity, since being autosampler enables batch analysis of multiple components; it is an extremely precise and reliable technique. It is an expensive method that requires a large amount of expensive organic solvents and needs regular

Table 12 Comparative study of UV, HPLC and HPTLC methods

Parameters	UV method	HPLC method	HPTLC method
Linearity equation	y = 0.0421x – 0.0016	y = 33177x – 534	y = 0.001x + 0.0018
Correlational coefficient(r^2)	0.9980	0.9993	0.9988
% Recovery	98.40–100.52%	99.27–101.18%	98.01–100.3%
Sensitivity LOD	0.58	0.33	0.44
LOQ	1.75	1.01	1.32
% label claim	99.02	99.90	99.54

Table 13 Result of forced degradation study

Sample name	% Degradation	Peak purity index	Single-point threshold
Drug	–	0.284	1.00
Acid degradation	1.38	0.309	1.00
Alkaline degradation	9.19	0.128	1.00
Neutral degradation	14.83	0.883	1.00
Thermal degradation	No degradation	–	–
UV degradation	No degradation	–	–

maintenance of the system. Also, the current research work provides an alternative method to the Anbazhagan S et al. approach [5] where the simultaneous quantification of three drugs was carried out by all three methods while in the present research work the focus is on LMU alone which was never quantified individually by all three methods before as per literature review. Therefore, in comparison with the Anbazhagan S et al. research work, the current research work requires less consumption of solvents for dilutions, chemicals and glassware thus promising the cost-efficiency of the present method, also total analysis time for HPLC is just 5 min with a retention time of LMU 3.125 min while in Anbazhagan S et al. research work it was 10.81 min with retention time of LMU 4.330 min indicating shorter period of analysis, and hence, rapid analysis of more number of samples could be done. ANOVA was applied to validate the information that there is a remarkable differentiation between mean % content determined by UV, HPLC and HPTLC methods. The *P* value (0.0280) is smaller than the alpha value ($\alpha = 0.05$), i.e., significance level therefore rejecting the null hypothesis, and the proposed null hypothesis was there is no remarkable difference. From the forced degradation studies, it could be inferred that all the degradant peaks and LMU peaks were well separated from each other. Peak purity for each solution was passed at the threshold level. Therefore, the proposed HPLC method is confirmed to be stability indicating (Table 13).

Conclusions

The proposed UV, RP-HPLC and HPTLC methods for the quantification of LMU in tablet formulation were linear having a concentration range (2–12 µg/mL) and had perfect accuracy ranging from 98 to 102%, precision with sensitivity and robust in nature. The % RSD was less than 2% thus compliance with ICH guidelines. The proposed methods are free from intervention due to excipients in tablets and thus could be used for regular determination of LMU in tablets. In conclusion, as per the aim of the study comparison between the methods was carried out, and based on analytical results and statistical tests, ANOVA shows that HPLC is the best method for the quantification of LMU in tablets due to its high reproducibility, sensitivity, good retention time; it has a higher percent recovery and has less analysis time, i.e., 5 min. Hence, forced degradation study by RP-HPLC was carried out by employing many stress conditions to assess the method's stability. The developed RP-HPLC method successfully separates the drug and its degradation products with good resolution so the method is proved to be stability indicating. The present research work is going to be extended to perform the impurity profile of LMU and to detect the pathway of degradation for the same.

Abbreviations

LMU	Lamivudine
ANOVA	Analysis of variance
% RSD	Percent relative standard deviation
LOD	Limit of detection
LOQ	Limit of quantification

Supplementary Information

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Supplementary Material 1.

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KS analyzed, interpreted the data and performed experimental work and a major contributor in writing the manuscript. VS and PR performed the bench work, methodology and writing the rough draft. The overall work was carried out under the guidance of PS. All authors read and approved the final manuscript.

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