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Original Contribution

STABILITY INDICATING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF LAMOTRIGINE IN PHARMACEUTICALS

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ABSTRACT

A simple, precise and accurate stability-indicating isocratic reverse phase ultra-performance liquid chromatographic (RP-UPLC) method was developed for the determination of lamotrigine (LMT) in bulk drug and in its tablets. The method was developed using Waters Aquity BEH C_{18} (100 mm × 2.1 mm, 1.7 µm) column with mobile phase consisting of a mixture of potassium dihydrogen phosphate buffer of pH 3.5 and an equal ratio of methanol and acetonitrile (50:50 v/v). The eluted compound was detected at 228 nm with a UV detector. The standard curve of mean peak area versus concentration showed an excellent linearity over a concentration range 1.0 – 75 µg/mL. Within-day and between-days RSD were less than 1.3% pronounces precision of the method. The accuracy of the method was further ascertained by recovery studies via standard addition procedure and the recoveries obtained were 98 - 100%. Forced degradation of the bulk sample was conducted in accordance with the ICH guidelines. Acidic, basic, hydrolytic, oxidative, thermal and photolytic degradation were used to assess the stability indicating power of the method. LMT was found to degrade significantly in acidic, basic and oxidative stress conditions and stable in thermal, hydrolytic and photolytic conditions.

Key words: Lamotrigine, UPLC, Stability indicating assay, Pharmaceuticals.

INTRODUCTION

Lamotrigine (LMT), chemically known as [6-(2, 3- dichlorophenyl)-1,2,4-triazine-3,5-diamine] **(Figure 1)**, is a broad spectrum antiepileptic, used as monotherapy and as an adjunct with other antiepileptics for treatment of partial and generalized toxic-clonic seizures. It's use to treat neurological lesions and as a tranquilizer has also been studied [1, 2].

Owing to its use as an antiepileptic drug, it has attracted the attention of many analysts. LMT is official in United States Pharmacoepia (USP) [3], which describes a chromatographic technique with monobasic potassium phosphate buffer, triethylamine and acetonitrile as mobile phase. The methods for LMT analysis utilized a variety of chromatographic techniques in body-fluids [4-21]. Most of these chromatographic methods describes assay of LMT either in plasma or serum and few are immunoassays. Few methods have been reported for its determination in pharmaceuticals and include titrimetry with acetous perchloric acid in anhydrous acetic acid medium [22], visible spectrophotometry [23-25], UV-spectrophotometry [26, 27], planar chromatography [4], thin layer chromatography and high performance liquid chromatography [28] and adsorptive stripping voltammetry [29]. Simultaneous determination of LMT, oxcarbazepine and zonisamide has been reported by Elizabeth *et al* [30] using HPLC and gas chromatographic techniques. Recently, Anantha Kumar *et al* [31] have reported RP-HPLC method in which the assay was carried out on a Luna C_{18} column by employing a mixture of KH_2PO_4 (pH 7.3) and methanol (60:40) as

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mobile phase and UV detection at 305 nm. The method is reported to be linear over the concentration range 10-70 µg/mL LMT.

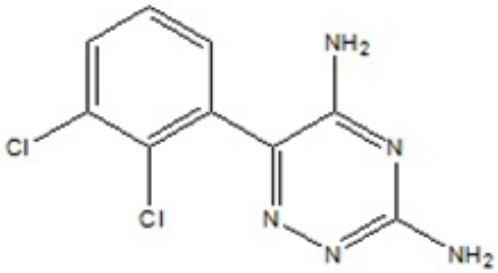


Figure 1. Chemical structure of LMT

Ultra performance liquid chromatography (UPLC) is an innovative technique that has brought revolution in liquid chromatography by outperforming conventional HPLC. UPLC decreases sample run times by a factor of 10, uses up to 95% less solvent and significantly improves productivity in laboratories. The replacement of standard 5 µm particles in conventional HPLC column with sub 2 µm particles in UPLC has resulted in significant improvements in resolution, sensitivity and speed in chromatographic separations besides minimizing band spreading contributions of both the instrument column.

UPLC system has been demonstrated to decrease time and cost per analysis significantly besides improving the quality of results with wider linear dynamic range. UPLC photodiode array (PDA) detector detects and quantifies lower levels of analyte and trace impurities at level of 0.004%. It is easy to detect components with UPLC which are difficult by conventional HPLC. In spite of these significant advantages, to the best of our knowledge, a validated RP-UPLC method for the determination of LMT has not been reported.

For LMT so far two UPLC methods are proposed in the literature [20, 21] and they are neither applicable for quantification of drug in tablets nor used as routine quality control procedure.

The aim of this work was to develop and validate a sensitive, accurate and precise UPLC method for the determination of LMT in bulk drug and in its tablet dosage form. In addition, the bulk drug was subjected to forced degradation in accordance with the ICH guidelines. Acidic, basic, hydrolytic, oxidative, thermal and photolytic degradation were used to assess the stability indicating power of the method.

EXPERIMENTAL PROCEDURE

MATERIALS AND REAGENTS

Pure active ingredient sample of LMT was kindly supplied by Cipla India Ltd, Mumbai, India, as gift and used as received. Commercial dosage forms used: Lamosyn 100 (Sun pharmaceuticals Ltd, Mumbai, India) and Lametec 50-DT (Cipla India Ltd, Mumbai, India), tablets were purchased from local commercial sources. HPLC grade acetonitrile and methanol were purchased from Merck India Pvt. Ltd. Mumbai, India. Potassium dihydrogen orthophosphate and formic acid were from Qualigens Fine Chemicals. Mumbai, India. Doubly distilled water was used throughout the investigation.

Chromatographic conditions and equipment

UPLC was performed using a Waters Acquity system equipped with binary solvent delivery pump, an auto sampler and tunable UV (TUV) detector. The output signal was monitored and processed using Empower-2 software. The Chromatographic column used was Acquity UPLC BEH C-18 (100 mm, 2.1 mm and 1.7 µm particle size). Isocratic elution process was adopted throughout the analysis.

Mobile phase preparation

Dissolved 1.4 g of potassium dihydrogen orthophosphate in 1000 ml of water and adjusted the pH to 3.5 using 10% formic acid. A 600 ml portion of this resulting buffer was mixed with 400 ml of solvent mixture containing equal volumes of methanol and acetonitrile, shaken well and filtered using a 0.22 µm Nylon membrane filter. This solution was also used as diluent in all subsequent preparations of the sample.

Instrumental parameters

The isocratic flow rate of mobile phase was maintained at 0.40 ml/min. The column temperature was adjusted to 35 °C. The injection volume was 4.0 µl. Eluted compound was monitored at 228 nm and the run time was 5.0 min. The retention was about 1.9 min.

Procedures

Preparation of stock solution

A stock standard solution of LMT (1 mg mL^{-1}) was prepared by dissolving an accurately

weighed 100 mg of pure drug in 100 ml volumetric flask using the mobile phase.

Procedure for preparation of calibration curve

Working solutions containing 1-75 µg/mL LMT were prepared by serial dilutions of aliquots of the stock solution. Aliquots of 4 µL were injected (six injections) and eluted with the mobile phase under the reported chromatographic conditions. The average peak area *versus* the concentration of LMT in µg/mL was plotted. Alternatively, the corresponding regression equation was derived using mean peak area-concentration data and the concentration of the unknown was computed from the regression equation.

Preparation of tablet extracts and assay procedure

Twenty tablets each containing 100 mg or 25 mg of LMT were weighed and transferred into a clean, dry mortar and powdered. Portion of the powdered tablet equivalent to 100 mg of LMT was transferred in to a 100 ml volumetric flask and 60 ml of the mobile phase was added. The content was sonicated for 20 min to achieve complete dissolution of LMT, and the solution was then diluted to volume with the mobile phase to yield a concentration of 1000 µg/mL and filtered through a 0.22 µm nylon membrane filter. The filtrate was then diluted with the mobile phase to get a 50 µg/mL solution. The solution obtained was injected to the UPLC column.

Stress study

Aliquots of pure drug solution equivalent to 50 µg/mL LMT were transferred into four different 50 mL volumetric flasks and added 10 ml of 1 M HCl, 1 M NaOH, Water and 5% H_2O_2 separately, and the flasks were heated for 2 h on a water bath maintained at 80 °C. Then the solutions were cooled and neutralized by adding base or acid, the volume in each flask was brought to the mark with mobile phase, and the required quantity (4.0 µL) was injected for analysis. Solid state thermal degradation was carried out by exposing pure drug to dry heat at 105 °C for 3 h. For photolytic degradation studies, pure drug in solid state was exposed to 1.2 million flux hours in a photo stability chamber. The sample after exposure to heat and light was used to prepare 50 µg/mL solutions in mobile phase and the chromatographic procedure was followed.

PROCEDURE FOR VALIDATION

Accuracy and precision

To determine the accuracy and intra-day precision, pure LMT solutions at three different concentrations were analyzed in seven replicates during the same day. Mobile phase was injected as blank solution before sample injection and the RSD (%) values of peak area and retention time were calculated.

Limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ were obtained by signal to noise (S/N) ratio method. LOQ and LOD were obtained by a series of dilutions of the LMT stock solution. Precision study was performed at LOQ level also. LOQ solution was injected seven times (n=7) and calculated the % RSD values for the obtained peak area and retention time.

Linearity

Linearity solutions were prepared from LOQ level to 150% of the actual sample concentration (50 µg/mL LMT). A total of five concentrations of the solutions (25, 37.5, 50, 62.5 and 75 µg/mL levels) were made separately and injected in triplicate.

Robustness and ruggedness

To determine the robustness of the method the experimental conditions were deliberately changed. The flow rate of the mobile phase (0.4±0.04 ml/min), column oven temperature (35±5 °C), mobile phase composition (66:40, 54:40, 60:36 and 60:44; buffer: solvent mixture *v/v*) and detection wavelength (228±1 nm) were the varied parameters. In each case the %RSD values were calculated for the obtained peak area and retention time. The number of theoretical plates and tailing factors were compared with those obtained under the optimized conditions. Three different columns of same dimensions were used for the analyses. The study was performed on same day and on three different days by three different analysts for three different concentrations of LMT (triplicates injections). The area obtained from each concentration was compared with that of the optimized one. The relative standard deviation values were evaluated for each concentration.

Solution stability and mobile phase stability

Stability of LMT solution was performed by injecting the sample into the chromatographic system. The peak area was recorded in the time intervals of 0, 12 and 24 hrs and the RSD values were calculated. The mobile phase stability was

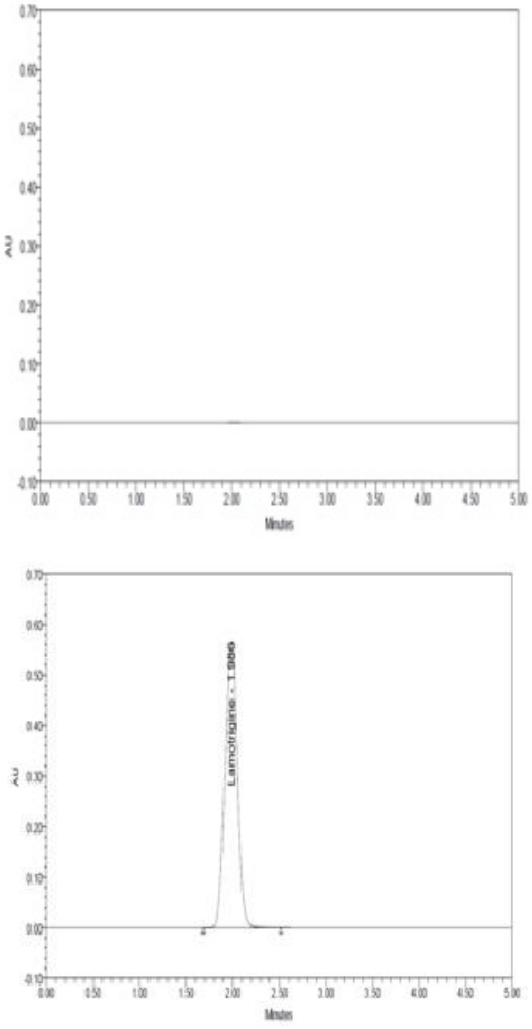
studied by injecting a freshly prepared sample solution at the same time intervals (0, 12 and 24 hours) and RSD values of the peak areas were calculated.

RESULTS AND DISCUSSION

Method development

Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, column temperature, pH of mobile phase and diluents were optimized. Several proportions of buffer, and solvents (water, methanol and acetonitrile) were evaluated in-order to obtain suitable composition of the mobile phase. Choice of retention time, tailing, theoretical plates and run time were the major tasks while developing the method. Alternate combinations of gradient and isocratic methods were also performed to obtain a suitable peak. Finally isocratic method was found better to use for the assay.

When LMT solutions were injected with methanol and acetonitrile solvent mobile phases individually, the resultant peak showed either tailing or much shortened retention time factor. As the buffer ratio increased, peaks eluted with abnormal shape. Acquity BEH C18, 50 mm × 2.1 mm, 1.7 µm column used for the elution, but the peak eluted before 1.5 minutes with a tailing factor of 2. Experiment with Phenyl 100 mm × 2.1 mm, 2 µm column ended with inconsistent retention time and peak fronting. The column temperature was varied from 20 to 45 ºC in a 5 ºC increment with the same column, the peak shape was found unaltered. Buffer and methanol: acetonitrile solvents ratio were changed and ended up with less number of theoretical plates. Different buffers like sodium dihydrogen othophospahe, dipotassium hydrogen orthophosphate and disodium hydrogen orthophosphate of different pH were tried and the results revealed that the use of potassium dihydrogen orthophosphate was most suitable. The pH of the mobile phase was varied from 2 to 6. At pH greater than 3.5, the peak eluted very early and resulted in less number of theoretical plates. At lower and higher flow rates, no peak elution and inefficiency of the system (pressure >15000 psi), respectively, were observed. Under these optimized conditions (40:60 solvent mixture: buffer of pH 3.5 v/v, Acquity BEH C18, 100 mm × 2.1 mm, 1.7 µm column, 35 º C, detection at 228 nm) the system was found more suitable for the validation study with the tailing less than 1.2, number of theoretical plates > 2000 and % RSD for peak area less than 1.0. The typical chromatograms obtained for blank and pure LMT from final UPLC conditions are depicted in **Figure 2**.



a

b

Figure 2. Chromatogram obtained for (a) mobile phase blank and (b) pure lamotrigine

Forced degradation

All forced degradation samples were analyzed at 50 µg/mL concentration level. The observation was made based on the peak area of the respective sample. Degradation was not observed when LMT was subjected to light (1.2 million lux hours), thermal (105 ºC for 3 hours) and hydrolysis (Water) conditions. Significant degradation was observed when the drug was subjected to acid and basic hydrolysis and oxidation (1 N HCl, 1 N NaOH and 5% H_2O_2 for 2 hours). The chromatograms obtained for LMT after subjecting to degradation are presented in **Figure 3**. Assay study was carried out by the comparison with the peak area of LMT sample without degradation.

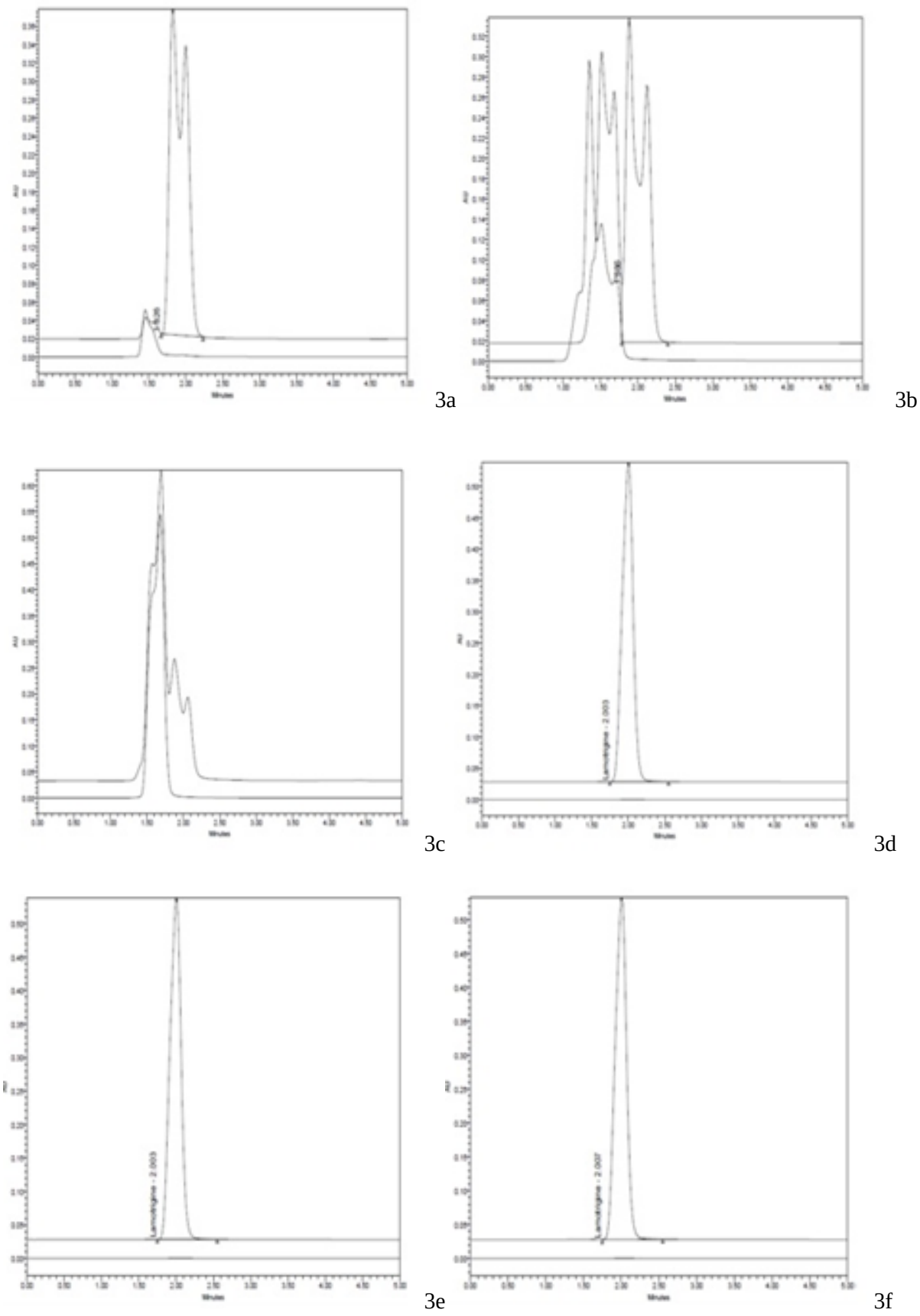


Figure 3. Typical chromatograms obtained for lamotrigine after subjecting to (a) acid, (b) basic, (c) oxidative, (d) hydrolytic, (e) thermal and (f) photo degradation

VALIDATION OF THE METHOD

The described method for the assay of LMT has been validated as per the current ICH Q2 (R1) Guidelines [32].

Analytical parameters

A calibration curve was obtained for LMT from 50% to 150% of its stock solution. A linear correlation was obtained between the peak area and the concentration in the range of 1.0 – 75 µg/mL LMT from which the linear regression equation was computed and found to be:

$$Y = m C + a, (r = 0.9999)$$

where Y is the mean peak area, C is the concentration of LMT in µg/mL and r is the correlation coefficient. The LOD and LOQ values, slope (m), y-intercept (a) and their standard deviations are evaluated and presented in **Table 1**. These results confirm the linear relation between concentration of LMT and the peak areas as well as the sensitivity of the method.

Table 1. *Linearity and regression parameters*

Parameter	Value
Linear range, µg/mL	1.0 -75
Limits of quantification, (LOQ), µg/mL	0.10
Limits of detection, (LOD), µg/mL	0.03
Regression equation	
Slope (b)	107523.8
Intercept (a)	-55901.9
Correlation coefficient (r)	0.9999
Standard deviation of b, (S_b)	525.64
Standard deviation of a, (S_a)	25448.51

Accuracy and precision

The percent relative error which is an index of accuracy is ≤ 1.5 and is indicative of high accuracy. The calculated percent relative standard deviation (%RSD) can be considered to be satisfactory. The peak area based and retention time based RSD values were < 1. The results obtained for the evaluation of precision and accuracy of the method is compiled in **Table 2 and 3.**

Table 2. *Results of accuracy study (n=7)*

Concentration of LMT injected, µg/mL	Intra-day		Inter-day	
	Concentration of LMT found, µg/mL	RE[a], %	Concentration of LMT found, µg/mL	RE[a], %
25	24.84	0.64	24.34	2.64
50	49.07	1.86	50.54	1.34
75	74.68	0.43	74.19	1.08

[a] Relative error

Table 3. *Results of precision study*

Chromatographic conditions		Mean peak area ±SD*	% RSD	Mean t_R±SD*	% RSD	Mean Theoretical plates ±SD*	% RSD	Mean Tailing factor ±SD*	% RSD
Temperature	35ºC	5337699 ±24019	0.45	2.00± 0.002	0.10	3824± 48.36	1.72	1.10 ± 0.007	0.64
	40ºC	5229586 ±2629	0.05	1.98± 0.001	0.05	5253 ± 50.25	2.16	1.01 ± 0.005	0.50
	45ºC	5316145 ±12759	0.24	1.97± 0.002	0.10	4943 ± 68.98	1.41	1.12 ± 0.006	0.54
Mobile phase composition	40:60	5217656 ±8870	0.17	2.00± 0.002	0.10	5185 ± 48.36	0.93	1.06 ± 0.007	0.66
	50:50	5218928±8436	0.16	1.985±0.002	0.10	5264±48.36	0.92	1.02±0.003	0.29
	60:40	5316145 ± 7679	0.14	1.97± 0.002	0.10	5299 ± 68.98	1.30	1.01 ± 0.006	0.59
Flow rate (ml min^{-1})	0.35	6176700 ± 6014	0.10	2.00± 0.002	0.10	5185 ± 48.36	0.93	1.06 ± 0.007	0.66
	0.40	5222143±8594	0.16	1.996±0.002	0.10	5174±60.47	1.17	1.04±0.003	0.29
	0.45	4700348 ± 7054	0.15	1.99± 0.003	0.15	5157 ± 98.57	1.91	1.02 ± 0.008	0.78
Wavelength (nm)	226	4694298 ±3558	0.08	1.99± 0.008	0.40	5138 ± 96.48	1.88	1.01 ± 0.001	0.10
	228	5268406±6674	0.13	1.976±0.002	0.10	5118±56.67	1.11	1.01±0.003	0.30
	230	5113645 ±3658	0.07	1.99± 0.001	0.05	5125 ± 45.66	0.89	1.01 ± 0.005	0.50

*Mean value of three determinations

Robustness and ruggedness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. At the deliberate varied chromatographic conditions (flow rate, temperature, and mobile phase composition), the analyte peak %RSD, tailing factor and theoretical plates remained near to the actual values. The RSD values ranged from 0.1 to 1.2% resumes the robustness of the proposed method. In method ruggedness, different columns (same lot), at different day by different analyst were performed. The results were summarized in **Table 4 and 5**.

Stability of the solution

At the specified time interval, % RSD for the peak area obtained from drug solution stability and mobile phase stability were within 1%. This shows no significant change in the elution of the peak and its system suitability criteria (%RSD, tailing factor, theoretical plates). The results also confirmed that the standard solution of drug and mobile phase were stable at least for 24 hours during the assay performance.

Table 4. *Method robustness*

Concentration injected µg/mL	Intra-day precision (n=7)			Inter-day precision (n=5)		
	Mean area ± SD	RSD %[a]	RSD %[b]	Mean area ± SD	RSD%[a]	RSD%[b]
25	2706422 ± 1246	0.38	0.38	2697635± 1284	0.32	0.56
50	5344217 ± 1923	0.76	0.45	5339877 ± 6767	0.24	0.54
75	8073493± 1445	0.47	0.37	8076876 ± 3786	0.67	0.64

[a] Relative standard deviation based on peak area

[b] Relative standard deviation based on retention time

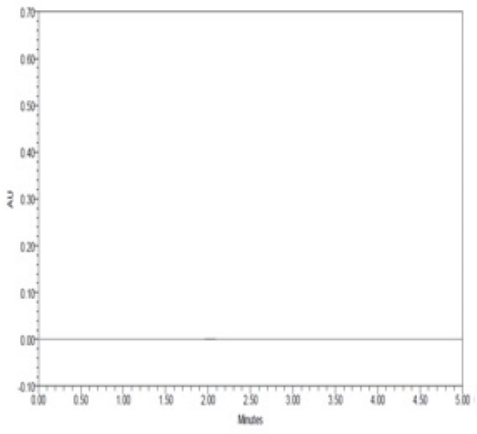
Table 5. *Method ruggedness*

Variable	Mean Peak area ±SD*	RSD%	Mean t_R ± SD*	RSD%	Mean Theoretical plates ± SD	RSD%	Mean Tailing factor± SD*	RSD
Analyst (n=3)	5271157±6714	0.13	1.996±0.002	0.11	5416±70.63	1.30	1.11±0.004	0.36
Column (n=3)	5282015±6318	0.12	1.995±0.003	0.15	5388±86.33	1.60	1.08±0.005	0.46

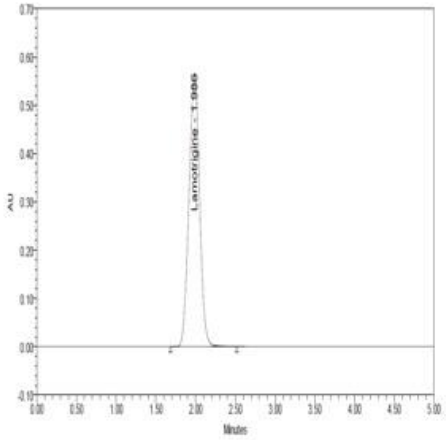
*Mean value of three determinations

Selectivity

Selectivity of the method was evaluated by injecting the mobile phase, placebo blank, pure drug solution and tablet extract. No peaks were observed for mobile phase and placebo blank and no extra peaks were observed for tablet extracts **(Figures 4a and 4b)**.



4a



4b

Figure 4. Typical chromatogram obtained for (a) placebo blank (b) tablet extract

Application to tablet

A 50 µg/mL solution of tablets was prepared as per '*preparation of tablet extracts and assay procedure'* and injected in triplicate to the UPLC system. The mean peak area of the tablets extract was found to be equivalent to the pure drug and the results were compared with those of a reference method [3]. The reference method involved a chromatographic technique with monobasic potassium phosphate buffer, triethylamine and acetonitrile as mobile phase.The accuracy and precision of the proposed method was further evaluated by applying Student's t- test and variance ratio F-test, respectively. The t- and F- values at 95% confidence level did not exceed the tabulated values and this further confirms that there is no significant difference between the reference and proposed methods. **Table 6** illustrates the results obtained from this study.

Table 6. *Results of analysis of tablets by the proposed methods and statistical comparison of the results with the reference method*

Formulation brand name	Nominal amount, mg	% LMT found[c] ± SD		t- value	F- value
		Reference method	Proposed method		
Lamosyn 100[a]	100.0	100.8± 0.68	99.86 ± 0.52	2.45	1.71
Lametec 25[b]	25.0	100.6± 0.72	99.65 ± 0.86	2.21	1.42

[a] Marketed by Sun pharmaceuticals Ltd, Mumbai, India

[b] Cipla India Ltd, Mumbai, India

[c] Mean value of five determinations. Tabulated t-value at 95% confidence level is 2.78; Tabulated F-value at 95% confidence level is 6.39

Recovery study

A standard addition procedure was followed to further evaluate the accuracy of the method. The solutions were prepared by spiking pure drug into a pre-analyzed tablet powder at three different levels and injected to chromatographic column. The recovery of the known amount of added analyte was computed. The percentage recovery of LMT from pharmaceutical dosage forms ranged from 99.49% - 101.4%. The results given in **Table 7** reveal good accuracy of the proposed method.

Table 7. *Results of recovery study by standard addition method*

Tablet studied	LMT in tablet, µg/mL	Pure LMT, µg/mL	Total found, µg/mL	Pure LMT recovered %±SD
Lamosyn 100	29.96	15.0	45.59	97.56±0.68
	29.96	30.0	60.05	100.3±0.86
	29.96	45.0	76.13	102.6±0.58
Lametec 25	29.59	15.0	44.77	101.4± 0.80
	29.59	30.0	60.85	104.2±0.57
	29.59	45.0	74.87	100.7±0.66

CONCLUSIONS

A rapid isocratic RP-UPLC method was developed for quantitative analysis of lamotrigine in pharmaceutical dosage forms is precise, accurate, linear, robust and specific. Satisfactory results were obtained from validation of the method. The retention time obtained (1.9 min) enables rapid determination of the drug which is important in routine analysis. The method exhibited an excellent performance in terms of sensitivity and speed. The method is stability indicating and can be used for routine analysis of production samples and can be used for the assay of lamotrigine either in pure drug or pharmaceutical formulations. Degradation study of the method reveals that the product is highly unstable in acidic, basic and oxidative media and stable in hydrolytic, photolytic and elevated temperature.

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