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Research article

Quantitative determination of related substances for Lamotrigine extended release tablet by RP-HPLC

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ABSTRACT

Lamotrigine extended release tablet dosage form LAMICTAL XR used as an anticonvulsant in the treatment of generalized tonic clonic, absence seizures and partial seizures. The objective of the present study is to develop and validate analytical method for the estimation of related substances in the LAMICTAL XR from GSK; however it is very important to have simple, sensitive, robust and validated analytical method. Hence a precise RP-HPLC analytical method developed for the determination of Related substances in LAMICTAL XR tablet dosage form with gradient elution pattern having mobile phase A as buffer pH 8.0 and mobile phase B as an Acetonitrile at 1.5 mL/min flowrate, using Hypersil BDS C18 column, ambient column temperature and PDA detector with wavelength 220 nm. The analytical method is validated as per ICH guidelines including its forced degradation studies. The method was found to be linear in the range of 0.2 ppm to 2.5 ppm with correlation coefficient 0.999. Accuracy performed at LOQ to 250% level and recovery was found to be in the range of 95% to 105%. Therefore the developed related substances method provides a safe, easy and reproducible for the stability studies and QC release testing for the estimation of related substances.

1. Introduction

Lamotrine (C9H7Cl2N5) is chemically 3, 5- diamino- 6- (2,3-dichlorophenyl)- 1,2,4-trizine with 256Int. J. Adv. Res. Pharm. Bio-Sci..09 molecular weight [1]. The Lamotrigine's mechanism of action involves inhibition of voltage sensitive Na channels; therefore stabilize the presynaptic membrane and mainly prevents the release of glutamate excitatory neurotransmitters. Lamotrigine was found to be effective in animal models, for the treatment of generalized tonic-clonic, absence seizure and partial seizure [2]. The structure of Lamotrigine and its related impurities are as follows:

Lamotrigine

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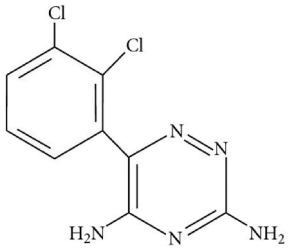
Lamotrigine

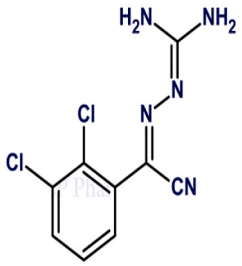


Lamotrigine impurity B

Lamotrigine impurity B

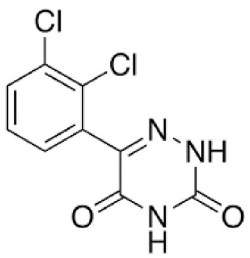
Lamotrigine impurity C

Lamotrigine impurity C



Lamotrigine impurity D

Lamotrigine impurity D



Complete literature survey reveals that several methods are reported for the determination of assay and related substances. The analytical method for determination of assay of lamotrigine tablet in human serum and saliva is reported but did not focused on impurities [3]. Various HPLC methods for the estimation of % assay of lamotrigine in bulk and tablet formulation were reported but no complete analytical validation is reported [4,5]. The LOD and LOQ for Lamotrigine were found to be 0.007 ppm and 0.15 ppm respectively but the same method when utilized for related substance study the LOD and LOQ was not achieved [6]. The analytical method for determination of assay of lamotrigine various tablet dosage form were meant for to determine the percentage assay of

lamotrigine but do not give any idea regarding the impurities or degradation pattern [7–9,10]. The RP-HPLC method is reported for lamotrigine and its related substances in the tablet dosage form but no focus observed on the lamotrigine related impurity C [11]. The spectrophotometric, TLC and HPLC methods are available for the determination of lamotrigine and its impurity but were found to be least sensitive [12–14,15]. HPLC method is reported for the quantitation of plasma lamotrigine concentrations in epilepsy patients. Simultaneous estimation of lamotrigine and other combinations in human plasma by HPLC was reported but no clarification on force degradation studies is stated by the authors [16]. HPLC methods are reported for the estimation of lamotrigine in various biological fluids [17–28]. The spectrophotometric methods using chromogenic reagents were developed for the lamotrigine estimation in the urine samples and various dosage forms [29–31]. To the best of author's knowledge no simple, sensitive, precise analytical method found for the determination of Lamotrigine and its related substances. The objective of the specified research study was to develop and validate rapid, simple and selective RP-HPLC method for the determination of LAMICTAL XR tablet dosage form.

The proposed validated RP-HPLC analytical method is validated as per ICH guidelines [32] and therefore can be applied for determination of Lamotrigine (LAMICTAL XR) routine, stability studies and QC testing for the determination of related substances.

2. Materials and methods

2.1. Instrumentation

Shimadzu HPLC system with auto injector PDA detector, weighing balance and pH meter from Mettler Toledo. Ultrasonicator, Thermo lab's humidity cum photo stability chamber. Columns utilized for analytical method development are Zorbax Eclipse XBDC18 (250 × 4.6 mm x 5 μm) and Inertsil ODS 3V (150 × 4.6 mm x 5μ) GL Sciences Inc., Japan, RP-8 Xterra and RP-18 Xterra (250 × 4.6 mm x 5 μm) Waters, Alliance., Japan, C 18 Hypersil BDS (250 × 4.6 mm x 5 μm) Thermofisher scientific., Mumbai.

2.2. Chemicals and reagents

The purity of Lamotrigine reference standard 99.8%, IMP-C 98.88%, IMP-B 92.76%, IMP-D purity 100.0%, & controlled drug samples were obtained from Macleods research centre, Mumbai, India. Milli-Q water, HPLC grade methanol and acetonitrile, Potassium dihydrogen orthophosphate, orthophosphoric acid, triethylamine were utilized. AR grade HCl, NaOH and H2O2 were procured for forced degradation studies.

2.3. Analytical method development

2.3.1. Selection of chromatographic method

Lamotrigine is polar in nature therefore, reverse phase, ion exchange or ion pair chromatography method can also be used. Here, the HPLC analytical method was opted for the primary separation due to its simplicity, suitability and ruggedness. For solvent selection; drug solubility was observed in mobiles phase. So in this study mobile phase is utilized as a diluent. The solubility of the API and its impurities were observed in mobile phase.

2.3.2. Preparations of analytical solutions

Lamotrigine standard solution

Accurately weighed 50.015 mg 50 mg of Lamotrigine reference standard in to 100 mL VF then 5 mL of this solution diluted to 50 mL and make up the volume with diluent.

Imp.B, Imp.C and Imp.D stock solutions

Accurately weighed 1.018 mg 1 mg of standard impurity B, C and D were weighed and transferred into 20 mL VF volume made up to the mark with the diluent, diluted 5 mL of this solution to 50 mL of VF and made up the volume with diluents.

Impurity C solution

5 mL of Imp.C stock solution was diluted to 50 mL with the diluent (1 ppm) approximately 1%.

Reference solution (a): It is prepared by dissolving 80.025 mg 80 mg of Lamotrigine in-house reference standard in 200 mL of diluent. 5 mL of this solution diluted to 200 mL with diluent; the solution concentration was found to be 1 μg/mL.

Reference solution (b): Reference solution (b) was prepared by dissolving 5.011 mg 5 mg of each Impurity A standard, Impurity B standard and Impurity C standard in 25 mL of diluent in 50 mL VF and the final volume was made up to 50 mL with same diluents.

Reference solution (c) (sample solution spiked with impurities): It is prepared by dissolving 50.019 mg 50 mg of Lamotrigine in-house reference standard in 50 mL VF containing 0.75 mL of reference solution b and the final volume was made up to 50 mL with diluents.

Sample solution: Test solution was prepared by dissolving 50.023 mg 50 mg of sample added 25 mL diluent sonicated to dissolve and make up the volume to 50 mL with diluent.

2.4. Calculation

$$\%\ Impurities = \frac{A_T}{A_S} \times \frac{Wt.std}{200} \times \frac{5}{200} \times \frac{5}{50} \times \frac{25}{Wt.spl} \times \frac{P}{100} \times \frac{Avg.Wt}{L.C.} \times \frac{1}{RRF} \times 100 \times 1000$$

Where,

A_T: area of known/unknown imp peak in test sample
AS: mean area of peak due to Lamotrigine in sample chromatogram
Wt. std: weight of Lamotrigine working std used for std preparation (mg)
Wt. Spl: sample weight (mg)
Avg.Wt: average weight of Lamictal XR (mg)
LC: Label claim (mg)
P: % purity of Lamotrigine working standard
RRF: Relative response factor; for unknown Imp RRF is 1

2.5. Selection of analytical wavelength

Lamotrigine and its impurities were scanned at UV PDA detector overlaid to determine the detection wavelength. The best possible wavelength was found to be 220 nm.

2.6. Mobile phase and gradient selection

Mobile phase A: On the basis of trials and pKa of the drug, different buffer concentrations were tried and potassium dihydrogen orthophosphate pH 8.0 with OPA selected for good peak shapes with proper resolutions. Mobile phase B: Acetonitrile; the optimized gradient pattern is shown (Table 1).

2.7. Selection of column

The development carried out with various C8 columns using different mobile phase concentrations and column oven temperatures. The poor resolution between Imp.C and Imp.B and broad peak shape for Lamotrigine implies that C8 column is not suitable; Hence C18 column was selected for further development. The RP-18Xterra, 250 × 4.6 mm, 5 μm column the negative drift of baseline was observed in gradient pattern (Fig. 1a). With Inertsil ODS 250 × 4.6 mm, 5 μm the impurity peak shapes was good, but in acid sample API peak was experiencing overloading on column (Fig. 1b). The Eclipse-XDB, C18 250 × 4.6 mm, 5 μm column all the peak shape were improved but retention time of impurity B and impurity C were very closer (Fig. 1c, d and 1e). The peak shape and resolution of lamotrigine among all components improved with Zorbax eclipse XBD C18, 250mm × 4.6 mm, 5 μm column. All peak shapes were good but resolution between Impurity B and Impurity C was not satisfactory but at 35 °C column oven temperature all the peaks were resolved in C18 Hypersil BDS column (Fig. 1f and g).

2.8. Optimization of chromatographic conditions

The RP-HPLC method was optimized for Lamotrigine & its related substances in pharmaceutical formulation The Hypersil BDS C18, (250 mm × 4.6 mm), 5 μm is used as an analytical column, Phosphate buffer pH 8.0 ± 0.2 and acetonitrile in gradient programme is utilized as a mobile phase. The column temperature 35 °C and sample temperature is kept at ambient temperature. Flow rate was 1.5 mL/min and Injection volume 10 μL. The retention time of Lamotrigine was found to be about 7.08min, at wavelength 220 nm

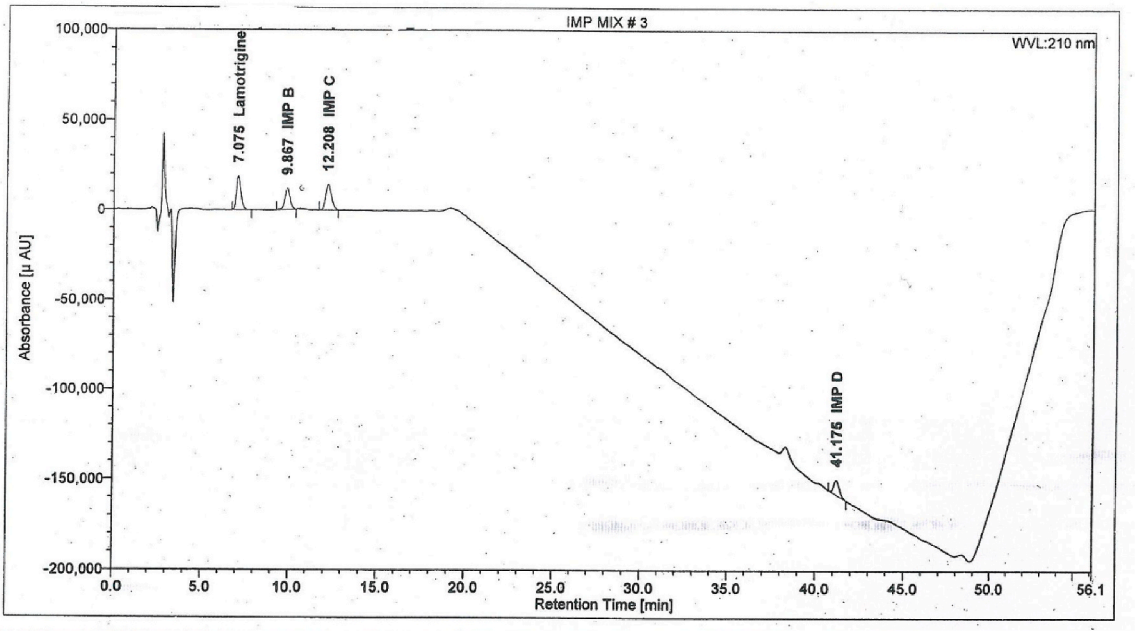
3. Results and discussion

3.1. Analytical validation

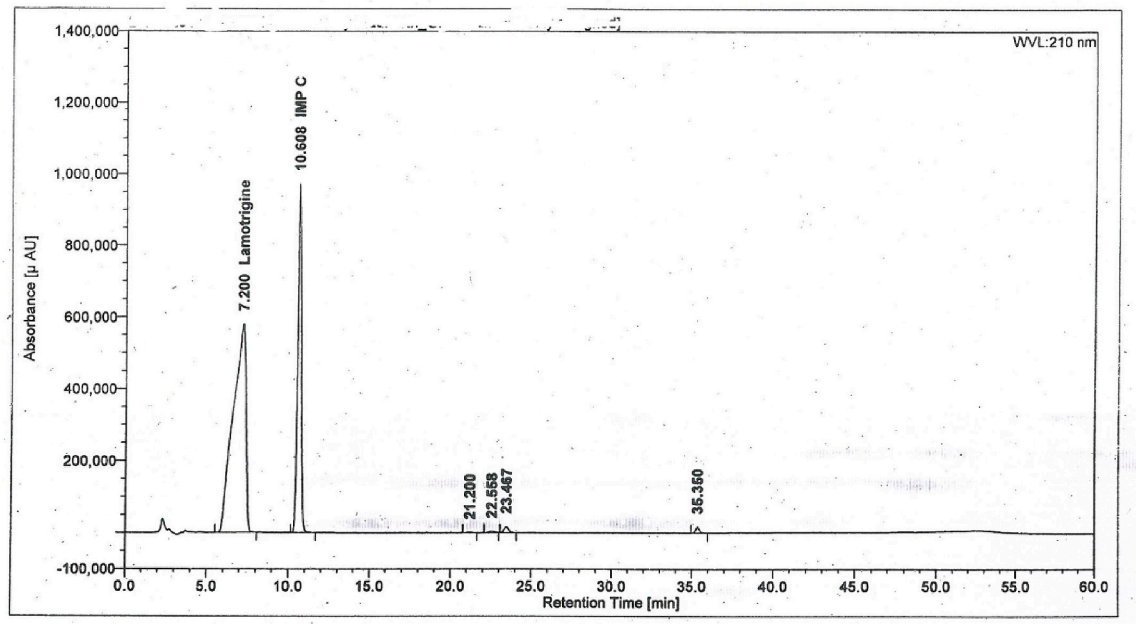
Systematic method validation is performed as per ICH guidelines.

Table 1
Optimized gradient program.

Time(min)	% Mobile phase A	% Mobile phase B	Comment
0–20	78	22	Isocratic
20–40	78–30	22–70	Linear gradient
40–55	30–78	70–22	Linear gradient
55–60	78	22	Re-equilibration



a

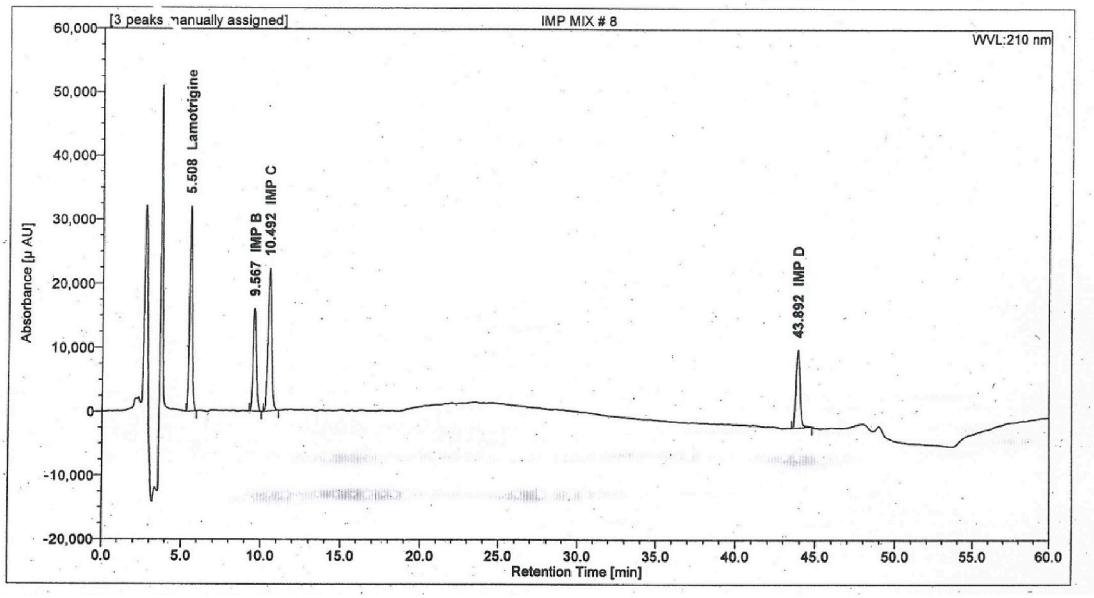


b

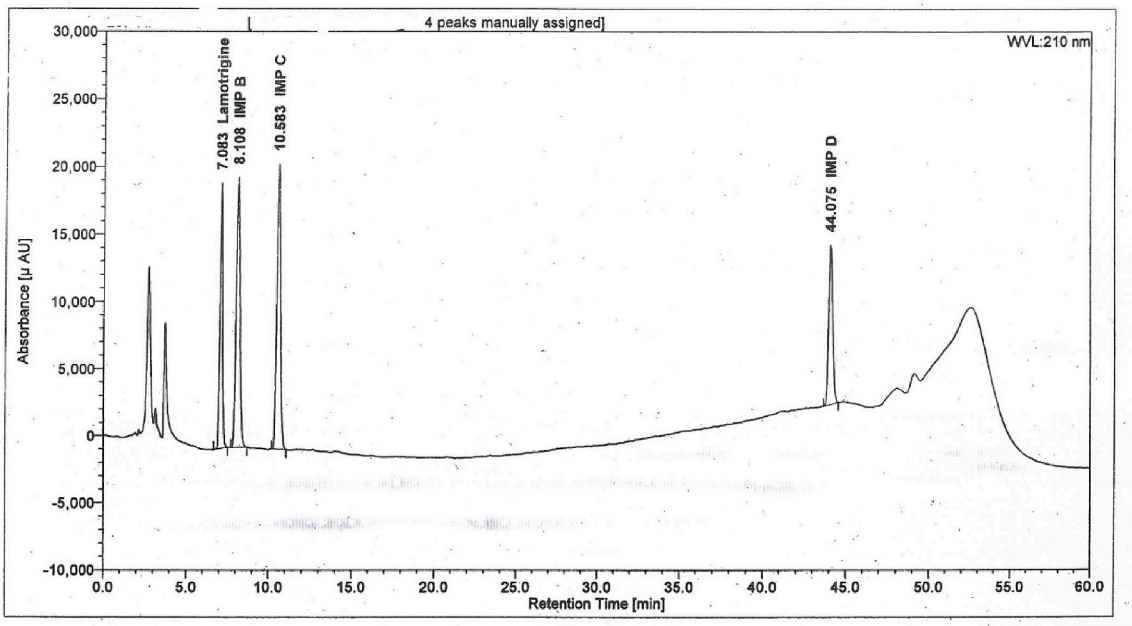
Fig. 1. a: Trial 1: RP-18 Xterra, 250 × 4.6 mm, 5µ column. b: Trial 2: Inertsil ODS C18 250 × 4.6 mm, 5 µ column. c: Trial 3: Eclipse-XDB, C18 250 × 4.6 mm, 5µ column. d: Trial 4: Eclipse-XDB, C18 250 × 4.6 mm, 5 µ column. e: Trial 5: Eclipse-XDB, C18 250 × 4.6 mm, 5 µ column. f: Trial 6: Hypersil BDS, C18 250mmx4.6 mm, 5 µ column. g: Chromatogram of Reference standard C on Hypersil BDS, C18 250 × 4.6 mm, 5 µ column.

3.1.1. System suitability

Injected standard solution in the six replicates. Disregard peak due to blank and placebo with an area less than 0.01% of Lamotrigine peak. To ascertain resolution and reproducibility of proposed chromatographic system for estimation of Lamotrigine and its related substances, system suitability parameters like tailing factor (TF), resolution (R) and column efficiency (NTP) were studied. The RSD for area in six standard solution injections should not more than 5.0% sample solution spiked with impurities (Reference



c



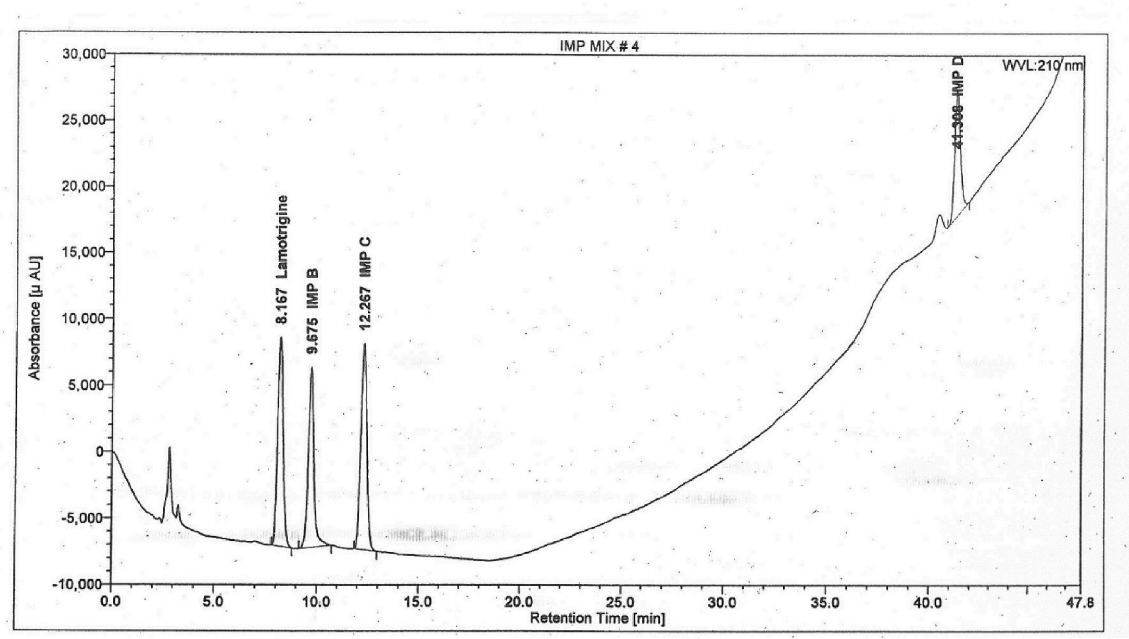
d

Fig. 1. (*continued*).

solution–C) was used for analysis. The 10 µL of system suitability solution was analyzed. The corresponding chromatograms were recorded at 220 nm (Table 2).

3.1.2. Linearity and range

Linearity solutions of Lamotrigine and Lamotrigine related comp. C impurity standards were prepared by quantitative dilution of the stock dilution to obtain solutions at LOQ, 25%, 50%, 80%, 100%, 120%, 150%, & 250% of the specification limit of Lamotrigine & Lamotrigine related comp. C impurity. This corresponds to a concentration of 0.20 ppm to 2.50 ppm for Lamotrigine and Lamotrigine



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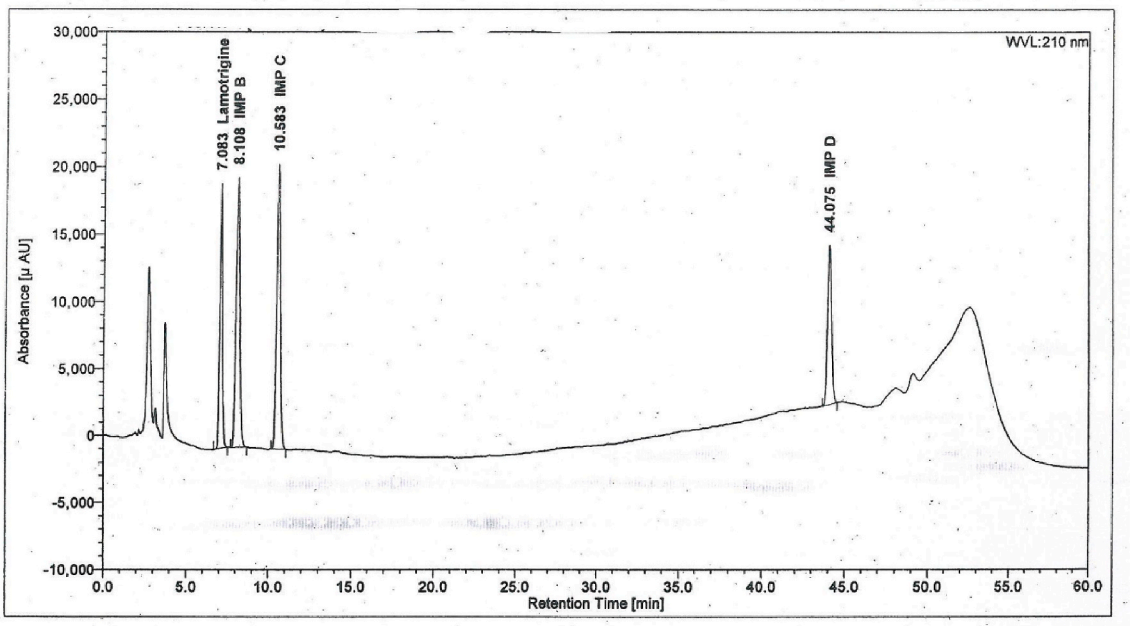
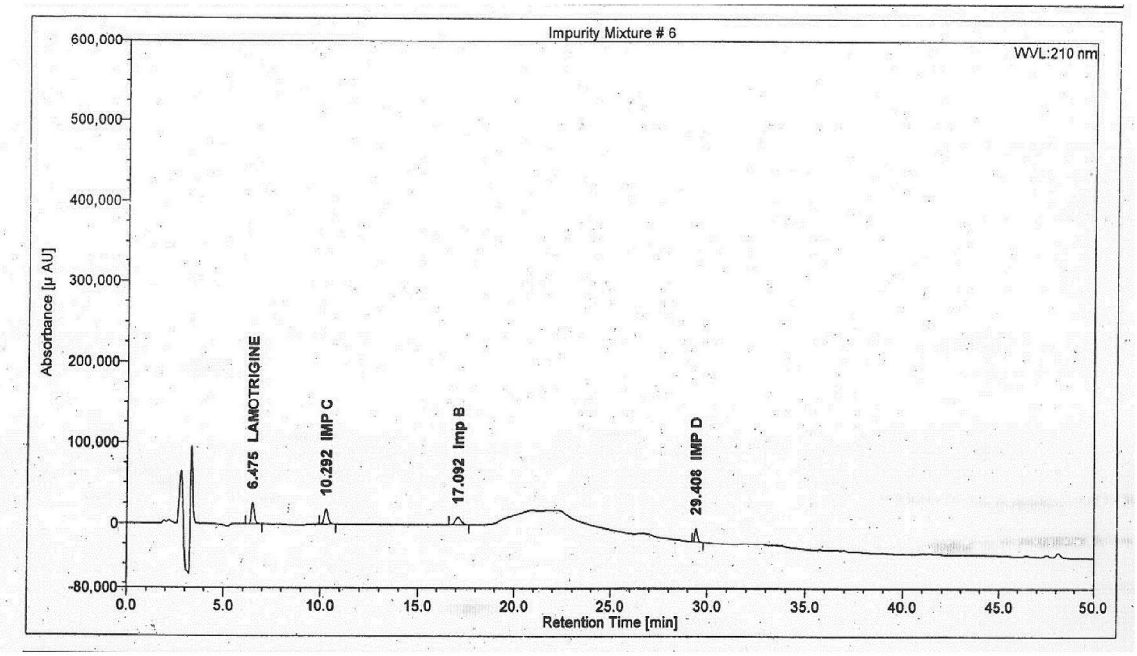


f

Fig. 1. (*continued*).

Table 2
System suitability parameters.

Imp Name	RT	RRT	RRF	Imp Type	TF	NTP
2,3 Dichlorobenzoic acid	13.71	0.38	–	Process	1.20	9266
3-amino-6(2,3-dichlorophenyl)-1,2,4-Triazin-5(4H)-one	18.69	0.83	0.93	Degradant	1.10	10267
N-(5-amino-6-(2,3-Dichlorophenyl)-1,2,4-triazin-3-yl)-2,3-dichlorobenzamide	40.26	3.99	–	Process	1.19	14625



g

Fig. 1. (*continued*).

related comp. C impurity.

The peak area Vs corrected concentration (ppm) graph was plotted for Lamotrigine & Lamotrigine related comp. C impurity. The concentration, corrected concentration & peak area values, slope, intercept, %Y intercept and correlation coefficient were determined and presented (Tables 3–4).

3.1.3. Accuracy

In order to obtain recovery at LOQ, 50%, 100%, 150%, and 250% of defined range, stock solutions of Lamotrigine related comp. C and Lamotrigine were spiked in the mixed placebo powder in triplicate preparations. For each recovery solution, the % recovery of lamotrigine was found to be 98.3%, 103.7%, 101.5%, 103.9% and 100.3% and % recovery of lamotrigine related comp. C was found to be 94.0%, 96.4%, 98.8%, 98.4% and 97.0%.

3.1.4. Precision- method precision and intermediate precision

The method precision study was performed on six sample preparation. The mean was 0.20% and 0.21%, % RSD was 3.16% and 1.96% for lamotrigine extended release tablet 25 mg and 300 mg respectively. Similarly, intermediate precision study was performed with different analytical scientist on a different day, using a different HPLC and column. The mean was found to be 0.21% and % RSD was 3.56% for both lamotrigine extended release tablet 25 mg and 300 mg (Table 5).

3.1.5. Stability of solution

The reference and test solution (spiked with Lamotrigine related compound C) were prepared according to the method and stored at controlled temperature about 25 °C. The standard and sample solution at initial, day1, day2 and day3 were injected. For related substances the % RSD of solution stability should not be more than 5.0%. The % impurities in the sample solution were found to be about 0.21% to 0.23% at each time interval. % relative standard deviation of the stored sample and standard solution on day 3 was found to be 2.53% and 2.72% respectively. Therefore, the standard and sample solution is stable up to 3 days at room temperature.

3.1.6. Specificity- interference study

Preparation of blank, placebo, lamotrigine related compound B, lamotrigine related compound C, lamotrigine related compound D, standard and sample solutions were injected into the HPLC system. For blank and placebo solution no peak was observed. For the lamotrigine related compound B, lamotrigine related compound C, lamotrigine related compound D the retention time was found to be 4.48min, 9.29min and 47.84min respectively also peak purity passing in the range of 999 to 1000. The standard and sample solution retention time was about 11.4min; peak purity passing in the range of 999 to 1000.

3.1.7. Forced degradation study

Twenty tablets were selected randomly, accurately weighed and calculated the average weight. About 845 mg of powdered sample

Table 3
Linearity.

Linearity of Lamotrigine			
Level	Conc.(ppm)	Corrected conc.(ppm)	Area
LOQ	0.20	0.20	8153
25%	0.25	0.25	8987
50%	0.50	0.51	18579
80%	0.80	0.81	29708
100%	1.00	1.01	36009
120%	1.20	1.21	44839
150%	1.50	1.50	54728
250%	2.50	2.51	91509
Slope			**36345.42068**
Intercept			**218.57932**
Correlation Coefficient			**0.99983**
Residual Sum Of Squares			**1837516.50775**
%Y- Intercept			**0.61**
Linearity of IMP C			
Level	**Conc.(ppm)**	**Corrected conc.(ppm)**	**Area**
LOQ	0.20	0.20	6694
25%	0.25	0.25	8559
50%	0.50	0.51	17499
80%	0.80	0.81	27828
100%	1.00	1.02	33536
120%	1.20	1.22	40636
150%	1.50	1.50	50749
250%	2.50	2.52	85072
Slope			**33671.91941**
Intercept			**23.43590**
Correlation Coefficient			**0.99985**
Residual Sum Of Squares			**1388206.19885**
%Y-Intercept			**0.07**

Table 4
Range at Loq and 250% level.

Injection No.	Range at LOQ Level		Range at 250% Level	
	Lamotrigine	Lamotrigine Related comp C	Lamotrigine	Lamotrigine Related comp C
1.	7396	6113	92326	85726
2.	7295	6485	92045	85633
3.	7235	7449	92449	85717
4.	7591	6543	92899	85415
5.	7069	6672	92212	85061
6.	7292	6620	90816	842325
Mean	**7313**	**6647**	**92125**	**85313**
% RSD	**2.37**	**6.62**	**0.76**	**0.64**

is taken for each FD study.

3.1.7.1. Acid degradation. About 845 mg of sample powder was transferred to a 200 mL VF added about 40 mL of methanol and sonicated for 30min with intermittent shaking. Added 40 mL 0.1 N HCl and 5 mL of 5 M HCl solution and kept at 80 °C for 5hrs on water bath further for neutralization purpose 5 mL of 5 M NaOH solution was added. The solution was equilibrated at RT and volume made up to the mark with 0.1 N HCl mixed and filtered through 0.45μ nylon filter discarding first 1 mL of the filtrate. The sample was analyzed on HPLC as per analytical method. The % single maximum impurity and % total degraded impurities were found to be 0.099% and 0.173% respectively, the peak purity match was found to be 999.

3.1.7.2. Base degradation. About 845 mg of sample powder was transferred to a 200 mL VF added about 40 mL of methanol and sonicated for 30min with intermittent shaking. Added 40 mL 0.1 N HCl and 5 mL of 5 M NaOH solution and kept at 80 °C for 5hrs on water bath. Further for neutralization purpose 5 mL of 5 M HCl solution was added. The solution was equilibrated at RT and volume made up to the mark with 0.1 N HCl mixed and filtered through 0.45μ nylon filter discarding first 1 mL of the filtrate. The sample was analyzed on HPLC as per analytical method.The % single maximum impurity and % total degraded impurities were found to be 0.195% and 0.577% respectively, the peak purity match was found to be 1000.

Table 5
Method precision and intermediate precision.

Strength	Lamotrigine extended release tablet 25 mg				Lamotrigine extended release tablet 300 mg			
	Spl. wt. (mg)	Lamotrigine Related comp C		% Total impurity	Spl. wt. (mg)	Lamotrigine Related comp C		% Total impurity
		Peak Area	% Impurity			Peak Area	% Impurity	
Method Precision								
Sample −1	847.36	32525	0.19	0.19	212.27	34412	0.20	0.20
Sample −2	846.84	33311	0.20	0.20	211.96	34590	0.21	0.21
Sample −3	847.31	33320	0.20	0.20	212.24	35038	0.21	0.21
Sample −4	847.73	33461	0.20	0.20	212.02	35627	0.21	0.21
Sample −5	846.52	33825	0.20	0.20	210.92	35604	0.21	0.21
Sample −6	848.00	35057	0.21	0.21	211.98	34528	0.21	0.21
Mean			**0.20**	**0.20**	**Mean**		**0.21**	**0.21**
% RSD			**3.16**	**3.16**	**% RSD**		**1.96**	**1.96**
Intermediate Precision								
Sample −1	846.94	33943	0.21	0.21	211.57	34966	0.20	0.20
Sample −2	845.95	33154	0.20	0.20	209.40	35418	0.21	0.21
Sample −3	846.55	35569	0.22	0.22	209.36	35609	0.21	0.21
Sample −4	845.88	34910	0.21	0.21	210.70	35678	0.21	0.21
Sample −5	846.06	35037	0.21	0.21	210.48	35539	0.21	0.21
Sample −6	847.85	36619	0.22	0.22	210.01	35858	0.21	0.21
Mean			**0.21**	**0.21**	**Mean**		**0.21**	**0.21**
% RSD			**3.56**	**3.56**	**% RSD**		**3.56**	**3.56**

3.1.7.3. Oxidative degradation. About 845 mg of sample powder was transferred to a 200 mL of VF added about 40 mL of methanol and sonicated for 30min with intermittent shaking. Added 40 mL 0.1 N HCl and 5 mL of 3% solution of H_2O_2 and kept on waterbath at 80 °C for 5hr. The preparation was equilibrated at RT, diluted to volume with 0.1 N HCl, mixed and filtered through 0.45μ nylon filter discarding first 1 mL of the filtrate. The sample was analyzed on HPLC as per analytical method. The % single maximum impurity and % total degraded impurities were found to be 0.101% and 0.194% respectively, the peak purity match was found to be 1000.

3.1.7.4. Thermal degradation. About 845 mg of sample powder was heated at 80 °C for 24hrs in an oven and cool at RT. It is transferred to a 200 mL VF added about 40 mL of methanol, sonicated for 30min with recurrent shaking. The solution was equilibrated to room temperature and diluted to volume with 0.1 N HCl, mixed and filtered through 0.45μ nylon filter by discarding first 1 mL of the filtrate. The subsequent filtrate was used. The sample was analyzed on HPLC as per analytical method. The % single maximum impurity and % total degraded impurities were found to be 0.098% and 0.175% respectively, the peak purity match was found to be 1000.

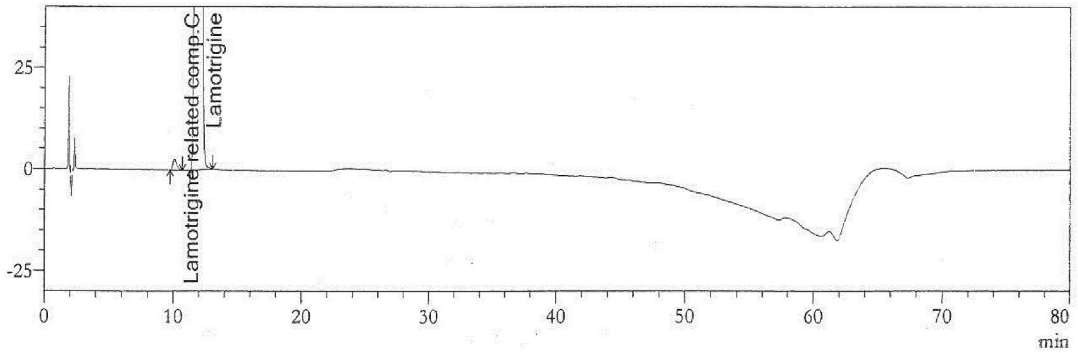
3.1.7.5. Photolytic degradation

3.1.7.5.1. Control sample. Sample powder covered with aluminium foil was exposed in the photostability chamber. Accurately weighed, sonicated for 30min with recurrent shaking then the sample was equilibrated to RT and diluted to volume with 0.1 N HCl and mixed. The solution was filtered by discarding first 2 mL of the filtrate through 0.45μ nylon filter.

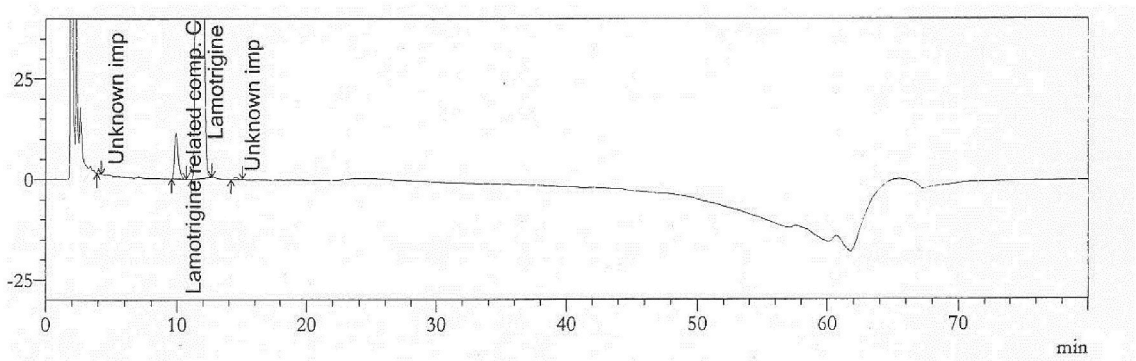
3.1.7.6. Exposed sample. The sample powder was exposed to the photo stability chamber. The sample accurately weighed, transferred to a 200 mL VF; added 40 mL of methanol and sonicated for 30min with intermittent shaking. The solution was equilibrated at RT and volume made up to the mark with 0.1 N HCl and mixed. The solution was filtered by discarding first 2 mL of the filtrate through 0.45μ nylon filter. The sample was analyzed on HPLC as per analytical method. The % single maximum impurity and % total degraded impurities were found to be 0.116% and 0.346% respectively, the peak purity match was found to be 999.

Table 6
Forced degradation.

Sr. No.	Degradation Condition	Degrading agents or condition and exposure period	% Single Maximum Impurity	% Total degraded Impurities	Peak Purity match	Peak purity Result
1	Thermal	80 °C for 24 h in an oven	0.098	0.175	1000	Pass
2	Photolytic	1.2 million lux hours; 200 W h/m^2 for 7 days	0.116	0.346	999	Pass
3	Humidity	40 °C/75%RH for 24 h	0.112	0.269	1000	Pass
4	Acid	Treated with 5 mL of 5 M HCl solution and kept at 80 °C for 5Hrs on water bath.	0.099	0.173	999	Pass
5	Base	Treated with 5 mL of 5 M NaOH solution and kept at 80 °C for 5Hrs on water bath.	0.195	0.577	1000	Pass
6	Peroxide	Treated with 5 ml of 3% H_2O_2 solution and kept at 80^0c for 5 Hrs on water bath.	0.101	0.194	1000	Pass



a



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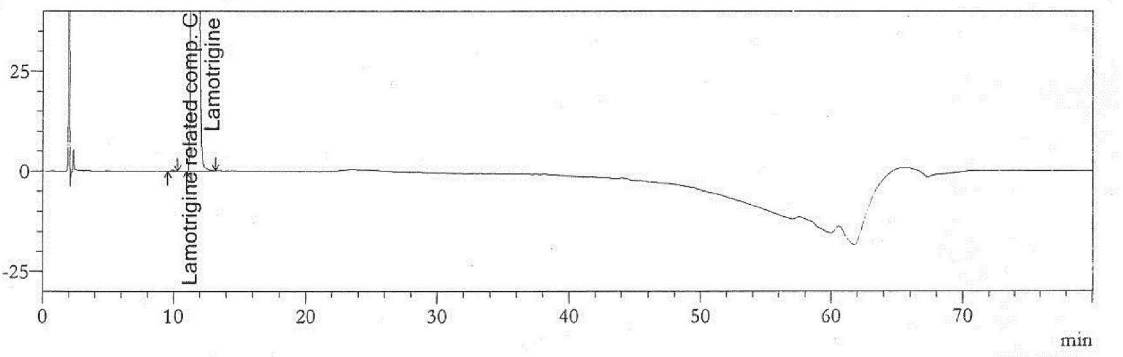


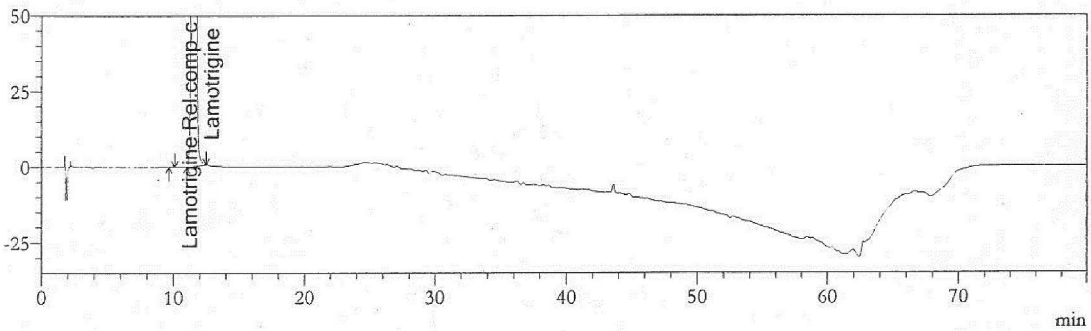
c

Fig. 2. a: Chromatogram showing acid stressed treated sample. b: Chromatogram showing base treated sample. c: Chromatogram showing oxidation. d: Chromatogram showing thermal treated sample. e: Chromatogram showing photolytic control sample. f: Chromatogram showing photolytic exposed sample. g: Chromatogram showing humidity treated sample.

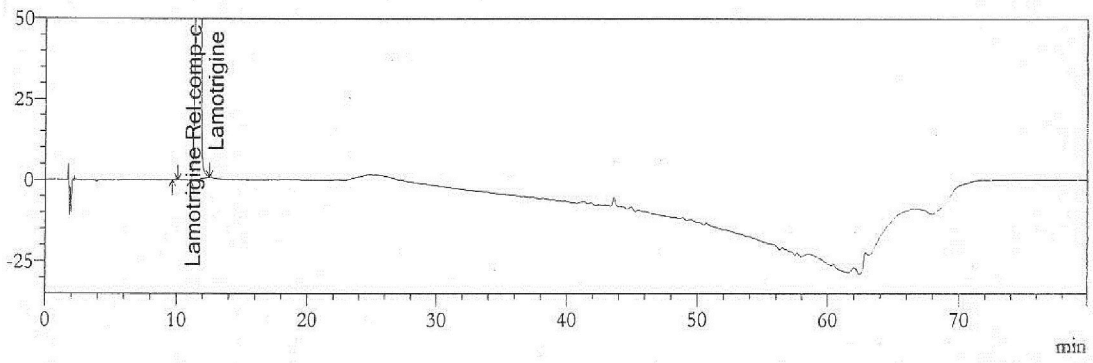
3.1.7.7. Humidity degradation. Sample powder was kept at 40 °C/75%RH for 24hrs. Sample was accurately weighed and transferred to a 200 mL VF added about 40L of methanol and sonicated for 30mins with intermittent shaking. The solution was equilibrated at RT and volume made up to the mark with diluent; filtered through 0.45µ nylon filter discarding first 1 mL of the filtrate was used. Analysis performed as per the described analytical method. The % single maximum impurity and % total degraded impurities were found to be 0.112% and 0.269% respectively, the peak purity match was found to be 1000. The obtained results are presented in (Table 6, Fig. 2a–g).

3.1.8. Filter study

Sample solution (spiked with Lamotrigine related comp C) preparation done as per the specified method. The sample solution was prepared, centrifuged, and filtered through 0.45 µm nylon filter. The absolute difference between the filtered and centrifuged sample



d



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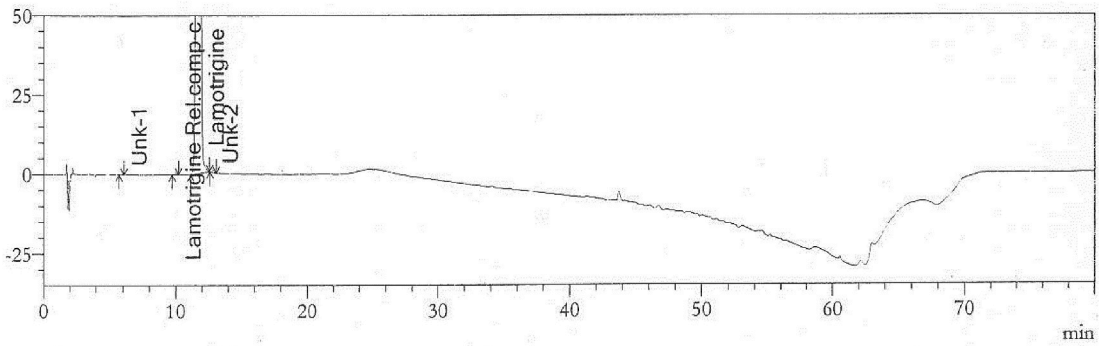
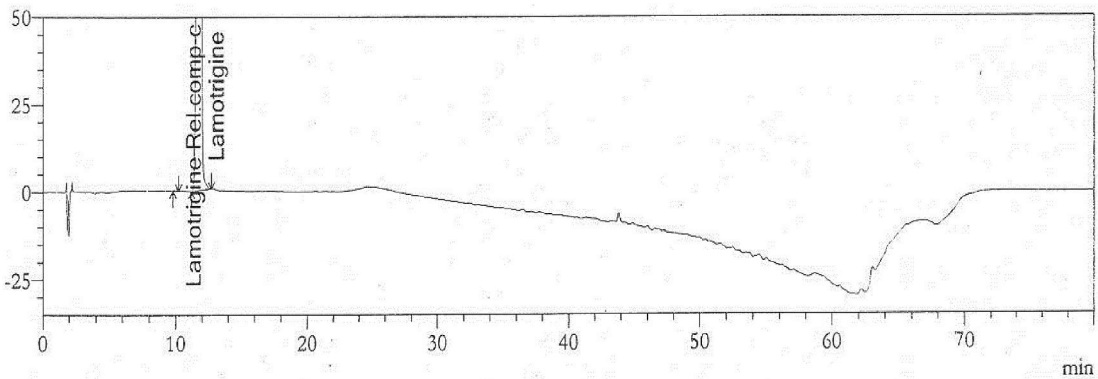


f

Fig. 2. (*continued*).



g

Fig. 2. (*continued*).

solution for the single maximum impurity above LOQ Level and the % total impurities should not be more than 2.0%. Therefore, 0.45 μm nylon filters were found to be suitable. The absolute differences in the impurity with the centrifuged and filtered solution were found to be about 0.01 and the % impurity was found to be 0.21% to 0.22%.

3.1.9. LOD and LOQ

To estimate LOD and LOQ, a solution series were prepared by diluting the Lamotrigine related comp C stock solution and Lamotrigine to obtain solution at 10%, 15%, 20%, 25%, 30% and 40% of the specification limit ranging from 0.10 ppm to 0.40 ppm for Lamotrigine related comp C and 0.10 ppm to 0.40 ppm for Lamotrigine.

Each solution injected and the chromatographs were recorded to obtain corrected concentrations. A graph of analyte area vs. corrected concentration (ppm) was prepared for Lamotrigine related comp C and Lamotrigine. The slope and residual standard deviation were calculated. Six replicates were injected to determine the LOQ level area reproducibility. The limit of detection was actual 0.034 ppm whereas proposed was 0.034 ppm. The limit of quantitation was actual 0.104 ppm whereas proposed was 0.200 ppm.

3.1.10. Robustness

The reliability of an analytical procedure can be determined by its ability can remain to execute even though small deliberate changes are made to the method's parameters.

4. The analyte peak and its impurity responses were recorded by simultaneous measurements of parameters comprising of change in wavelength by ±3 nm that is 217 nm and 223 nm, change in flow rate by ±0.1 mL/min that is 1.4 mL/min and 1.6 mL/min, change in column oven temperature by ±5 °C that is 25 °C and 35 °C, change in mobile phase composition by ±5% and change in mobile phase buffer pH by ± 0.2 that is 7.8 and 8.2 were performed. The Robustness study proved that the analytical method developed was found to be precise and selective.

4. Conclusion

For system suitability (SS), system precision (SP), method precision (MP) and intermediate precision (IP) studies; The system suitability parameters like tailing factor (TF) was NMT 2.0, resolution (R) not less than 1.5 and column efficiency (NTP) not less than 2000 were studied. The RSD for area in five or six replicate standard solution injection were not more than 5.0% Sample solution spiked with impurities (Reference Solution –C) was used for analysis; all the system suitability parameters meet the requirements as per the test method.

Linearity and Accuracy study results were found to be linear in the range of 0.2 ppm to 2.5 ppm for both Lamotrigine and Lamotrigine related comp C and correlation coefficient was found to be 0.999. The accuracy performed at LOQ to 250% level and recovery results were found to be in the range of 95% to 105%.

Specificity and Forced degradation proves that the analytical method developed was found to be sensitive and do not have any interference at the retention time of analyte peak or its impurities. The peak purity passes for analyte peaks in standard and sample solution. The analytical method which was determined to be selective based on the aforementioned observations.

Solution stability was found to be about 3 days. Robustness was performed by altering the method parameters and the results were passing the system suitability criteria.

Lamotrigine related comp. C was increased in acidic condition, hence it can be concluded that IMP C was found to be degradant impurity. Lamotrigine was found to be stable in acid, base, thermal, humidity and photolytic degradation. The analytical method performs well at separating drug from their processing impurities and degradation products. Lamotrigine extended release tablet related substances should be determined using the suggested analytical method is simple, reliable, selective, specific, and accurate. It also illustrates the evaluation of the degradation pattern; as a result, it can be utilized for the stability studies, routine analysis, and QC testing

Author contribution statement

Priyanka Gondhale- Karpe: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the results; Contributed reagents, materials, analysis; Wrote the paper.

Sonali Manwatkar: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the results; Contributed reagents, materials, analysis.

Data availability statement

Data included in article/supp. material/referenced in article.

Ethical approval

Not applicable

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e15732.

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