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Development and Validation a Method for Estimation of Lamotrigine using RP-HPLC

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KEYWORDS

Anticonvulsant drug, Lamotrigine, RP-HPLC method, Stability indicating, Forced degradation study.

ABSTRACT:

A stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of lamotrigine in bulk and tablet formulations. The method utilized a Thermo C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of 10 mM potassium dihydrogen phosphate (pH 3.5, adjusted with orthophosphoric acid) and acetonitrile (15:85 v/v) at a flow rate of 1.0 mL/min. Detection was performed at 224 nm. The method was validated as per ICH Q2(R1) guidelines, demonstrating linearity (5–25 µg/mL, r^2 = 0.999), accuracy (98.28– 99.34% recovery), precision (%RSD < 2), and robustness. Forced degradation studies under acidic, alkaline, oxidative, and thermal conditions confirmed the method's stability-indicating capability, with degradation ranging from 3.35% to 16.68%. The method was successfully applied for assaying lamotrigine in commercial tablets (99.40% assay, %RSD = 0.125), proving its suitability for routine quality control in pharmaceutical analysis.

1. Introduction

Lamotrigine, an antiepileptic drug also used in bipolar disorder management, requires precise analytical methods for its estimation in pharmaceutical formulations due to its narrow therapeutic index. Reverse-phase high-performance liquid chromatography (RP-HPLC) is widely adopted for its robustness and ability to separate lamotrigine from degradation products. This study aims to develop and validate a stability-indicating RP-HPLC method for lamotrigine quantification in accordance with ICH Q2(R1) guidelines. The method optimizes chromatographic conditions, conducts forced degradation studies, and validates key parameters such as linearity, accuracy, precision, and robustness to ensure reliable analysis in quality control settings. Lamotrigine, an important antiepileptic drug that is also employed in the treatment of bipolar disorder, calls for accurate analytical procedures for its determination in pharmaceutical dosage forms and biological samples in view of its very narrow therapeutic ratio. Inaccurate drug concentrations

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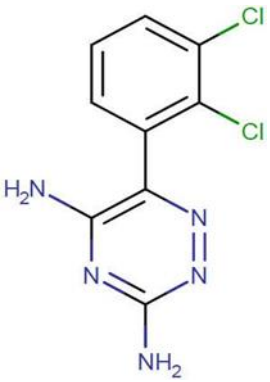
may result in failure of therapy or toxicity, which necessitates precise, sensitive, and discriminative analytical procedures. Reverse-phase high-performance liquid chromatography (RP-HPLC) has found widespread application for its ruggedness, reproducibility, and ability to resolve lamotrigine from possible degradation products. This research is aimed at the development and validation of a stability-indicating RP-HPLC technique to ensure that lamotrigine is correctly quantified in the presence of its degradative impurities by complying with regulatory conditions like ICH Q2(R1), USP, and FDA regulations. Method development entails optimized chromatographic parameters through systematic optimization, such as the use of a C18 column, composition of mobile phase (commonly a buffer with organic solvents such as methanol or acetonitrile), flow rate, and detection wavelength tailored to lamotrigine's UV absorbance. The objective is to obtain sharp, symmetrical peaks with well-defined separation between lamotrigine and its degradative products. Forced degradation experiments are carried out under acidic, basic, oxidative, thermal, and photolytic stress conditions to assess method specificity. The studies determine the major degradation pathways and ensure the method can differentiate between intact lamotrigine and its impurities, making it a genuine stability-indicating method. Validation conforms to ICH guidelines, evaluating parameters like accuracy (through recovery studies), precision (repeatability and intermediate precision), linearity (calibration curve within a defined range), sensitivity (limits of detection and quantification), and robustness (response to intentional changes in method conditions).

1.1. Drug Profile

Lamotrigine is a phenyltriazine-class antiepileptic drug used primarily in managing epilepsy and bipolar disorder. In epilepsy, it is prescribed for partial seizures, generalized tonic-clonic seizures (both primary and secondary), and seizures linked to Lennox-Gastaut syndrome. Additionally, lamotrigine serves as a mood stabilizer and is notable for being the first drug, after lithium, to receive FDA approval for the long-term treatment of bipolar I disorder. Marketed under the brand name *Lamictal* in the United States, lamotrigine is available in oral tablet form. It is structurally distinct from other anticonvulsants and is associated with a lower risk of side effects, with no routine need for blood level monitoring. Beyond its approved uses, some research supports its role in treating neuropathic pain and off-label conditions such as borderline personality disorder. Although its precise mechanism of action remains unclear, lamotrigine is believed to exert multiple cellular effects that may explain its wide therapeutic applications.



Structure of Lamotrigine

Mol.Weight:256.091

ChemicalFormula:$C_9H_7Cl_2N_5$

IUPACName:6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine

2. Materials and Methods

2.1 Chemicals and Reagents

Lamotrigine(gift sample, SIRT),methanol(AR and HPLC grade), acetonitrile(HPLC grade), and water (HPLC grade) were sourced from Merck Ltd., India. Potassium dihydrogen phosphate (KH_2PO_4) and orthophosphoric acid (OPA) were used for mobile phase preparation. The marketed formulation, Lamictal (25 mg, GlaxoSmithKline Pharmaceuticals Ltd.), was analyzed.

2.2 Instrumentation

The HPLC system comprised a Waters quaternary pump, manual injector, UVVisible detector, and a Thermo C18 column (250 × 4.6 mm, 5 µm) with a Lichrocart guard cartridge. Data were processed using Data Ace software. A Labindia 3000+ spectrophotometer with 1 cm quartz cells was used for UV-visible analysis.

2.3 Chromatographic Conditions

The mobile phase consisted of 10 mM KH_2PO_4 (pH 3.5, adjusted with OPA) and acetonitrile (15:85 v/v), filtered through a 0.45 µm membrane and degassed. The flow rate was 1.0 mL/min, with detection at 224 nm. The injection

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volume was 20 µL, and the column was maintained at ambient temperature.

2.4 Preparation of Solutions

A standard stock solution of lamotrigine(1000µg/mL) was prepared in methanol. Working standard solutions (5–25 µg/mL) were obtained by serial dilution. For tablet analysis, powderequivalentto10mglamotriginewasdissolvedinmethanol, sonicated, filtered, and diluted to 10 µg/mL.

2.5 Method Validation

Validation was performed as per ICH Q2(R1) guidelines, evaluating: -

Linearity: Calibration curves were constructed using five concentrations (5–25 µg/mL).

Accuracy: Recovery studies were conducted at 80%, 100%, and 120% levels. **Precision**: Repeatability and intermediate precision (day-to-day and analyst-to analyst) were assessed.

Robustness: Effects of deliberate variations in pH, flow rate, and mobile phase composition were studied.

Limits of Detection and Quantification (LOD/LOQ): Calculated using the standard deviation of the response and slope of the calibration curve. –

System Suitability: Parameters included retention time (RT), theoretical plates, and tailing factor. –

Forced Degradation: Conducted under acidic (0.1 N HCl, 80°C, 8 h), alkaline (0.1 M NaOH, 80°C, 8 h), oxidative (3% H_2O_2, 24 h), and thermal (50°C, 4 weeks) conditions.

2.6 Tablet Assay

Twenty tablets were weighed, powdered, and an amount equivalent to 10 mg lamotrigine was processed to obtain a 10 µg/mL solution for analysis. The assay was performed in triplicate.

3. Results

3.1 Method Development

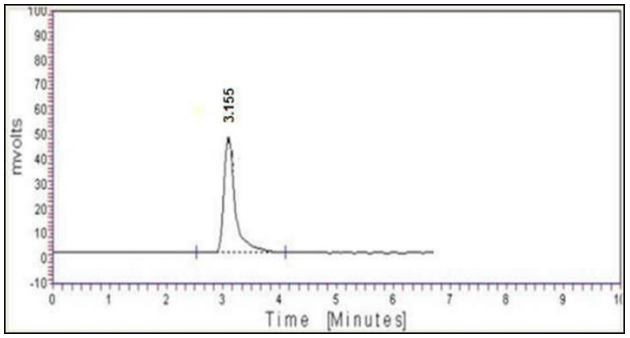
The optimal mobile phase (10 mM KH_2PO_4:acetonitrile, 15:85 v/v, pH 3.5) provided a retention time of 3.155 ± 0.009 min, with a tailing factor of 1.2267 ± 0.0681 and 3255.33 ± 10.017 theoretical plates (Table 1).

Table 1: System Suitability Parameters for Lamotrigine

Parameter	Mean ± SD
Retention Time (min)	3.153 ± 0.0091
Area Under Curve (AUC)	689.369 ± 3.4152
Theoretical Plates	3255.33 ± 10.017
Tailing Factor	1.2267 ± 0.0681

Linearity and calibration graph

(a)



(b)

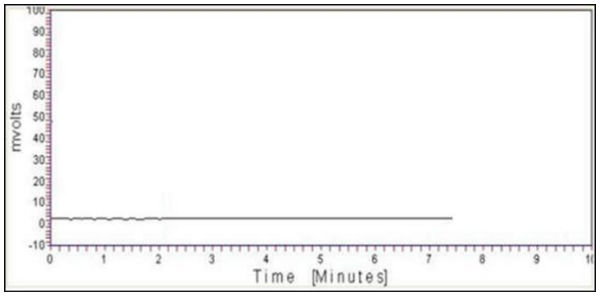


Figure 7.3: (a) Chromatogram of Blank (b) Chromatogram of Standard

3.2 Validation Results

3.2.1 Linearity: The method was linear over 5–25 µg/mL, with a regression equation of y = 69.24x + 0.893 and r^2 = 0.999 (Table 2).

3.2.2 Accuracy: Recovery ranged from 98.28% to 99.34% (%RSD < 0.672) across 80%, 100%, and 120% levels (Table 3).

3.2.3 Precision: Repeatability (%RSD = 0.103), day-to-day (%RSD = 0.090), and analystto-analyst (%RSD = 0.089) precision were within acceptable limits (Table 4).

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3.2.4 Robustness: The method remained unaffected by minor variations, with %RSD = 0.123 (Table 4).

3.2.5 LOD and LOQ: Values were 0.15 µg/mL and 0.50 µg/mL, respectively (Table 5).

3.2.6 Tablet Assay: The assay of Lamictal tablets showed 99.40% of the labeled claim (%RSD = 0.125) (Table 6).

Table 2: Linearity of Lamotrigine

Concentration (µg/mL)	Mean AUC	Response Ratio	%RSD
5	346.857	69.371	1.799
10	689.369	68.937	0.495
15	1050.759	70.051	0.416
20	1380.494	69.025	0.359
25	1730.991	69.240	0.318

Table 3: Recovery Study of Lamotrigine

Recovery Level (%)	% Mean Recovery	SD	%RSD
80	99.34	0.330	0.332
100	98.85	0.664	0.672
120	98.28	0.631	0.642

Table 4: Precision and Robustness of Lamotrigine

Parameter	% Mean ± SD	%RSD
Repeatability	98.709 ± 0.102	0.103
Day-to-Day Precision	98.867 ± 0.089	0.090
Analyst-to-Analyst Precision	98.883 ± 0.088	0.089
Robustness	98.683 ± 0.121	0.123

3.3 Forced Degradation Studies

Forced degradation studies revealed degradation of 14.35% (acidic), 16.68% (alkaline), 6.68% (oxidative), and 3.35% (thermal), confirming the method's ability to separate lamotrigine from its degradation products (Table 7).

The developed RP-HPLC method is simple, rapid, and reproducible, with a short retention time (3.155 min) and high sensitivity (LOD = 0.15 µg/mL, LOQ = 0.50 µg/mL).

Table 5: LOD and LOQ of Lamotrigine

Name	LOD (µg/mL)	LOQ (µg/mL)
Lamotrigine	0.15	0.50

Table 6: Assay of Tablet Formulation

Parameter	Lamotrigine
Label Claim (mg)	25
% Found (mg)	24.85
% Assay	99.40
%RSD	0.125

The linearity ($r^2 = 0.999$) and recovery (98.28–99.34%) confirm its accuracy, while low %RSD values (< 2) indicate precision and robustness. Forced degradation studies demonstrated the method's stability-indicating nature, effectively separating lamotrigine from its degradation products. Compared to existing methods [**??**], this method offers improved simplicity and applicability for routine analysis. The successful assay of commercial tablets (99.40%) validates its utility in pharmaceutical quality control. The proposed RP-HPLC method is a reliable, stability-indicating approach for lamotrigine quantification in bulk and tablet formulations. Its compliance with ICH Q2(R1) guidelines, coupled with high accuracy, precision, and robustness, makes it suitable for routine quality control and stability studies in the pharmaceutical industry.

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Table 7: Results of Forced Degradation Studies

Stress Condition	Drug Recovered (%)	Drug Decomposed (%)
Standard Drug	99.99	0
Acidic Hydrolysis	85.65	14.35
Alkaline Hydrolysis	83.32	16.68
Oxidative Degradation	93.32	6.68
Thermal Degradation	96.65	3.35

4. Discussion

An RP-HPLC method was established for the quantification of Lamotrigine in a combined formulation. The analysis was carried out isocratically using a mobile phase consisting of 10 mM KH_2PO_4 and acetonitrile in the ratio of 15:85 (v/v), with the pH adjusted to 3.5 using orthophosphoric acid (OPA). Separation was achieved on a Thermo C18 column (4.6 × 250 mm, 5 µm particle size), and detection was performed at 224 nm. Prior to use, the mobile phase was filtered through a 0.45 µm membrane filter to eliminate particulate matter and degassed to remove air bubbles. The flow rate was maintained at 1.0 mL/min throughout the analysis. The developed method was subsequently validated using various analytical parameters to ensure its reliability and reproducibility.

5. Conclusion

This work successfully established a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) procedure for estimating lamotrigine in commercial products. The procedure was developed by extensive literature search and optimization of experiments, supported by the validity of statistical parameters of sampling. The suggested method is easy, fast, precise, and reproducible, fulfilling the research aim of good estimation of drugs in pharmaceutical products. The RP-HPLC approach was carried out using a Waters liquid chromatographic system with a manual injector, a quaternary pump to provide uniform flow and pressure, and a UV-visible detector with Data Ace software for instrument control and data analysis. The approach used 10 mM KH2PO4 and acetonitrile (15:85 ratio, pH 3.5 adjusted with orthophosphoric acid) as the mobile phase, filtered through a 0.45 µm filter and degassed. A Thermo C18 column (4.6 x 250 mm, particle size 5 µm) was the stationary phase, with chromatograms monitored at 224 nm and a flow rate of 1.0 ml/min. The approach proved to be linear in the range of concentration 5–25 µg/ml with the correlation coefficient 0.998. Validation, carried out according to ICH Q2(R1) guidelines, comprised accuracy, precision, linearity, robustness, and sensitivity. 80%, 100%, and 120% level recovery studies ranged from 98.34 ± 0.330% to 98.85 ± 0.664%. Precision was established through repeatability, intermediate precision, and reproducibility. Robustness was checked through intentional variations in the solvent mixture and proved to be consistent. The method developed is new (no reported method for this product), quick (low retention time), easy, precise (%RSD and standard deviation < 2), and accurate. It is applicable to routine determination of lamotrigine in bulk drug and tablet formulations, meeting the aims of research of ease, quickness, and reproducibility.

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