Journal of Health and Rehabilitation Research 2791-156X JHRR

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Original Article

Enhanced Detection of Lamotrigine Through HPLC Methodology in Bioanalysis

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Conflict of Interest: None.

Zahid S., et al. (2024). 4(2): DOI: https://doi.org/10.61919/jhrr.v4i2.938

ABSTRACT

Background: Lamotrigine, an anticonvulsant drug, requires sensitive detection methods in biological and pharmaceutical matrices. Current methods struggle with interference and sensitivity, necessitating the development of improved analytical techniques.

Objective: This study aims to develop and validate a highly sensitive HPLC method enhanced by salicylaldehyde derivatization for detecting lamotrigine in pharmaceutical and biological samples, and to differentiate it from Piracetam when co-administered.

Methods: A reverse-phase HPLC method was employed, utilizing salicylaldehyde as a derivatizing agent to induce a bathochromic shift in lamotrigine from 315 nm to 415 nm. Optimization of the derivatization reaction included adjustments of pH, reagent concentration, temperature, and time. The standard addition method was applied to evaluate the recovery rates from spiked samples.

Results: The optimized method demonstrated a linear calibration range of 1-5 µg/ml with a determination coefficient (R^2) of 0.998. Recovery of lamotrigine from pharmaceutical preparations averaged 96.5%, while recovery from deprotonated serum and urine samples of healthy volunteers was 96% with a relative standard deviation (RSD) of 6%.

Conclusion: The developed method offers a robust and highly sensitive approach to detect and distinguish lamotrigine in the presence of Piracetam, proving effective for both pharmaceutical and biological sample analysis.

Keywords: Biological Samples, Derivatization, HPLC, Lamotrigine, Salicylaldehyde

INTRODUCTION

Lamotrigine, an anticonvulsant drug from the phenyl-triazine class, is widely used to manage epilepsy and bipolar disorder (1-5). Marketed as Lamictal in most parts of the world, it is rapidly and completely absorbed from the gastrointestinal tract. Besides its primary uses, Lamotrigine is also effective in treating clinical depression, serving as a mood stabilizer, and belongs to the Na-channel blocking class of antiepileptic drugs (2-11). Antiepileptic drugs (AEDs) are essential for controlling epileptic seizures and are categorized into two generations. The first generation includes Valproic acid, Carbamazepine, Phenobarbital, and Phenytoin (12-17), while the second generation comprises Lamotrigine, Gabapentin, Topiramate, Levetiracetam, among others. Notably, new-generation AEDs, particularly Lamotrigine, have demonstrated superior efficacy compared to their older counterparts in treating epilepsy. Additionally, Lamotrigine is utilized in managing cluster headaches, migraines, bipolar disorder, trigeminal neuralgia, and peripheral neuropathy (18-22). It also alleviates neuropathic pain and symptoms of post-traumatic stress disorder.

Salicylaldehyde, with the chemical formula C6H4CHO-2-OH, is an isomer of hydroxybenzaldehyde and is chemically known as 2-hydroxybenzaldehyde (23-25). It is a colorless, oily liquid with a distinctive bitter almond odor, significant in synthesizing several commercially used chelating agents. Naturally occurring in substances like buckwheat and castoreum, salicylaldehyde is also a component in perfume production (26-29).

Schiff bases form through the condensation reaction between primary amines and carbonyl compounds, resulting in structures analogous to ketones or aldehydes, wherein the carbonyl group (C=O) is replaced by an imine or azomethine group (30-33). These compounds, also known as imines or azomethines, are crucial in coordination chemistry due to their ability to form stable complexes

with metal ions. Such complexes are effective catalysts, especially at high temperatures (100°C) and in the presence of moisture, and are used in various applications within biological systems, polymers, and dyes (34,35).

High Performance Liquid Chromatography (HPLC) is a technique employed to analyze specific compounds, involving several steps. Initially, the compound's characteristics are examined for compatibility with both the mobile and stationary phases of HPLC (36-38). The mobile phase, or mobile segment, consists of a buffered solvent, while the stationary phase involves a porous solid support with a unique coating. HPLC is preferred over Gas Chromatography (39,40) and capillary electrophoresis (41) for determining Lamotrigine, utilizing various procedures (42-44). This study explored the use of salicylaldehyde derivatization to enhance the sensitivity and selectivity in detecting Lamotrigine, with derivatization confirmed by spectrophotometry and quantitation performed via HPLC.

The objective of this research was to rationalize and enhance the detection methodology for Lamotrigine through HPLC, leveraging salicylaldehyde derivatization to improve analytical outcomes.

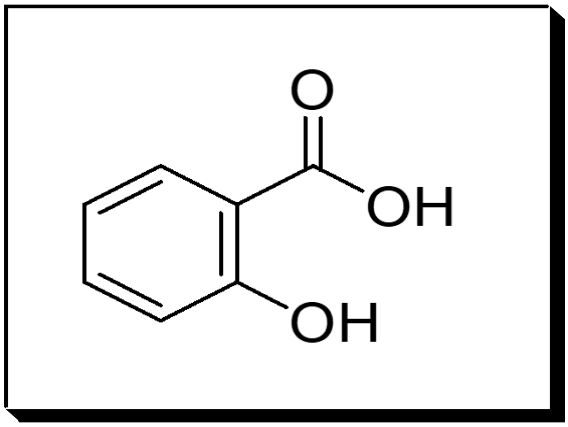

Figure 1 Chemical structure of salicylaldehyde

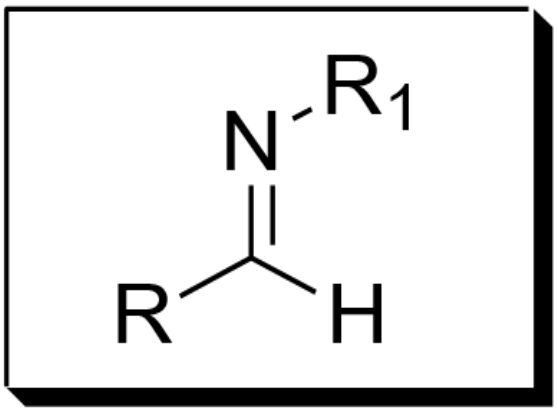

Figure 2 Chemical structure of Schiff base

MATERIAL AND METHODS

Pure lamotrigine was obtained from Searle Pharmaceutical Company (North Nazimabad, Karachi, Pakistan) as a gratuity for academic purposes. Salicylaldehyde, Methanol HPLC grade, AcOH, HCl (37%), KCl, NaOAc, NH4OAc, H3BO3, Borax, Na2CO3, soda carbonate, ammonia (25%), and ammonium were procured from Merck Germany and Sigma Aldrich. Ultra-pure water, sourced from an ELGA lab water system (High Wycombe, UK), was used throughout the study. The chemicals used to maintain pH included potassium chloride-hydrochloric acid, acetic acid-sodium acetate, ammonium acetate-ammonia, boric acid-sodium tetraborate, and ammonium chloride-ammonium solution (22).

A double beam spectrophotometer was used throughout the study. A chromatograph linked to a monitor with customizable wavelength settings (Hi Tech Pvt Ltd, Tokyo, Japan) and an injector (Rheodyne) 7125 were utilized for sample injections. The UV detector response was measured using a Column Agilent C-18, 5µm (15 cm x 4.6 mm id) in HPLC. All pH measurements were conducted using an Orion 420 pH meter with a glass electrode and inner core reference electrode (Research Inc., Boston, USA) (23).

Solutions with varying concentrations (0.1-1.0ml) of 100µg/ml lamotrigine were transferred to 10 ml volumetric flasks, followed by the addition of 1 ml borate buffer solution at pH 8 and 1.5 ml of salicylaldehyde (2%, v/v) in methanol. The solutions were warmed in a water bath at 80°C for 15 minutes until cooled. The volume was then made up to the mark, and UV was recorded between wavelengths of 355 to 500 nm, with maximum absorbance at 415 nm (24).

For the HPLC analytical procedure, solutions ranging from 0.1ml to 0.5ml containing 100µg/ml lamotrigine were transferred to 10 ml volumetric flasks. The 20 ml solution was injected onto the HPLC column Agilent C-18 (5µm) and eluted with a composition of distilled water, tetrahydrofuran (THF), and methanol (65:25:10 v/v/v) at a flow rate of 1ml/min. Maximum detection was achieved at UV-Vis at 259 nm (25).

For the preparation and pharmaceutical study, five Lamigit tablets (Searle Pharmaceutical, Karachi, Pakistan) containing 25 mg/tablet lamotrigine were crushed into fine powder and weighed according to one tablet (0.19 g). The powder was dissolved in methanol, filtered, and the volume adjusted to 25 ml. Solutions of 1ml concentration were further diluted to 10 ml, and analytes of 0.1ml and 0.2ml were relocated to 10 ml volumetric flasks for the HPLC analytical procedure (28).

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For the analysis of lamotrigine from medicinal drugs using a linear calibration curve, solutions with concentrations ranging from 0.1ml to 0.5ml were transferred to 10 ml volumetric flasks and subjected to the HPLC procedure. A linear external calibration curve was constructed for quantitation, with the response calculated as directly proportional to the added standard (30).

For the analysis of lamotrigine in spiked human serum and urine samples, 5ml of biological samples were collected from a volunteer. The mixture was left at room temperature for 30 minutes before being centrifuged at 4000g for 20 minutes. Methanol (5ml) was added to each supernatant layer collected after centrifugation. The contents were mixed well and centrifuged again at 4000g for 20 minutes. From each solution, 1ml was transferred to a 10 ml volumetric flask, and lamotrigine was added to achieve a final concentration of 2 µg/ml. A blank portion without lamotrigine was also prepared. A linear external calibration curve was used for quantitation. Blood and urine samples were gathered from volunteers who reported not taking any medication for the preceding week. Blood samples were obtained via venipuncture using blood collection tubes, while urine samples were collected in clean, decontaminated 100 ml polyethylene tubes. Volunteers were briefed on the nature of the study and provided verbal consent for sample collection (32-34).

The research project received substantial support from the Dr. M.A Kazi Institute of Chemistry, University of Sindh, Jamshoro.

RESULTS

The derivatization of lamotrigine with salicylaldehyde significantly enhanced its detectability through the introduction of more chromophore groups, changing the absorption maximum from 315 nm (molar absorptivity 1.8x10^2 mol/cm) to 415 nm (molar absorptivity 3.4x10^4 mol/cm). Systematic studies to optimize the derivatization conditions revealed that the optimal pH for maximum absorbance was pH 8, beyond which the absorbance decreased due to reduced proton interaction (Figure 4). The optimal concentration of the derivatizing reagent was found to be 1.5 µg/ml, as absorbance increased with the reagent concentration up to this point before declining (Figure 5).

The effect of heating time on the reaction outcome was studied from 5 to 25 minutes. Maximum absorbance was observed at 15 minutes, beyond which the absorbance diminished (Figure 6). Temperature optimization studies indicated that increasing the temperature from 30°C to 60°C enhanced absorbance, which then decreased up to 80°C. The optimal temperature was established at 60°C for further studies (Figure 7).

HPLC analysis showed that the lamotrigine derivative was well separated from piracetam, with no overlap in retention times under the optimized conditions of water-methanol-tetrahydrofuran (65:25:10 v/v/v) as the mobile phase at a flow rate of 1 ml/min, and detection at 259 nm (Figure 8). The linear calibration curve for lamotrigine showed a strong correlation (R^2 = 0.998) across the concentration range of 1-5 µg/ml. The limits of detection (LOD) and quantification (LOQ) were determined to be 0.09 µg/ml and 0.11 µg/ml, respectively.

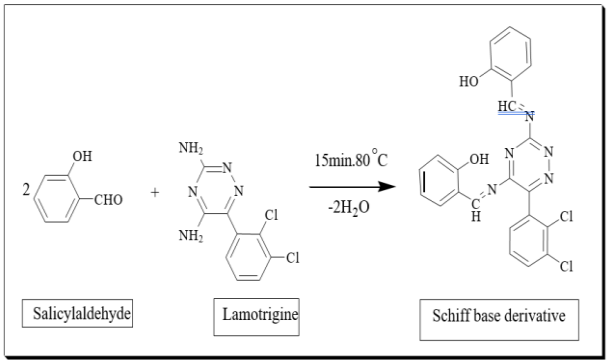

Figure 3 Chemistry of Lamotrigine after derivatization with salicylaldehyde forming a Schiff base derivative

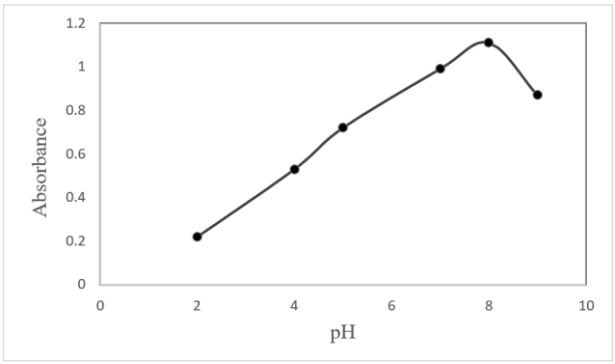

Figure 4 pH effect on the absorbance after derivatization with salicylaldehyde

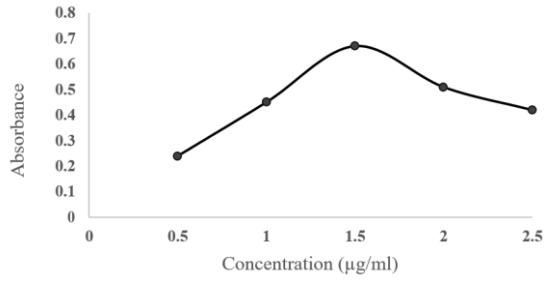

Figure 5 Effect of concentration after derivatization with salicylaldehyde

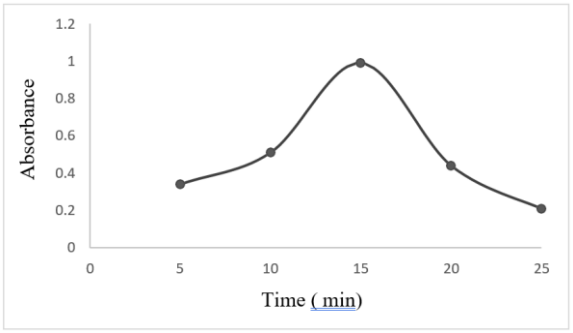

Figure 6 Time study after derivatization with salicylaldehyde

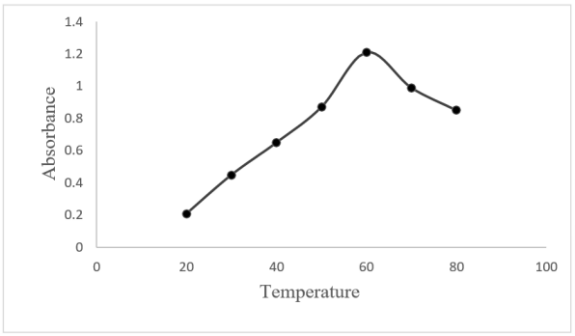

Figure 7 Temperature study after derivatization with salicylaldehyde

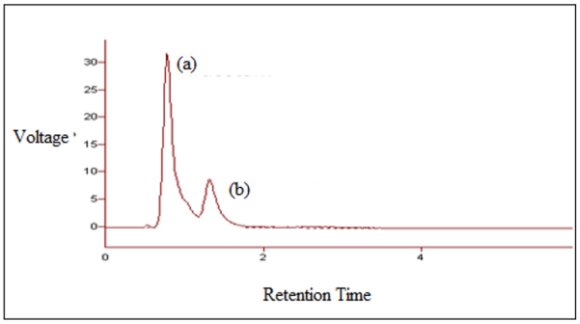

Figure 8 Separation of drugs after derivatization with salicylaldehyde (a) Piracetam (b) Lamotrigine

Table1 : Drug analysis on HPLC after Derivatization of Lamotrigine with Salicylaldehyde

S. No	Name of product	Compound contain	Labelled amount mg/tablet	Amount found mg/tablet	%age Error	Relative Standard deviation%
1	Lamigit	Lamotrigine	25	24.3	2.8	7.1

The tablet was also analyzed by standard addition and recovery of Lamotrigine from tablet and calculated 96.5% with RSD 6% (n=3) the amount found 1.93 µg/ml [Table 2].

Table 2: Standard addition of Lamotrigine on HPLC with Salicylaldehyde

Name of Standard	Name of tablet	Amount added µg/ml	Amount found µg/ml	%Error
Lamotrigine	Lamigit	2.0	1.93	3.5

The deprotonated serum and urine samples were spiked at the final concentration of 2 µg/ml of lamotrigine. The amounts procured 1.89 µg/ml from blood serum and 1.91 µg/ml from urine with RSD (n=3) 4.5% and 5.5% as shown below in [Table 3]. The recovery was calculated 94.5- 95.5% accordingly.

Table3 : Standard addition of Lamotrigine on HPLC with Salicylaldehyde in biological samples

Biological Samples	Age/gender	Name of standard	Amount added	Amount found	%Error
Blood	35/F	Lamotrigine	2µg/ml	1.89µg/ml	5.5
Urine	35/F	Lamotrigine	2µg/ml	1.91µg/ml	4.5

Table 4: Comparative study of the reported methods used for the determination of Lamotrigine.

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Name of compound analyzed	Analytical method used	Derivatizing reagent	Calibration range	LOD And LOQ	Sample Analyzed	References No:
Lamotrigine	Reversed phase HPLC	----	0.1-15.0µg/ml, 0.1-5.0 µg/ml	0.008µg/l 0.023µg/l	Plasma and brain	45
Lamotrigine	HPLC	----	1-20µg/ml	0.25µg/ml 0.5µg/ml	Plasma	46
Lamotrigine	HPLC	----	1.25-15.0 ng/ml	30ng/ml	Plasma	47
Lamotrigine	HPLC	----	0.1-15µg/ml	0.06µg/ml, 0.01µg/ml	Human plasma	48
Lamotrigine	HPLC	Salicylaldehyde	5-20µg.ml	0.09µg/ml 3.4µg/ml	Blood and urine	Present method

DISCUSSION

The chemistry of lamotrigine after derivatization with salicylaldehyde revealed enhanced spectral properties due to the formation of a Schiff base derivative, which introduced additional chromophore groups that improved the ultraviolet-visible (UV-Vis) detectability. This modification was crucial for increasing the sensitivity of lamotrigine detection using HPLC. The pH optimization highlighted the importance of maintaining a slightly alkaline environment to maximize the derivatization efficiency and stability of the compound (45).

The concentration of salicylaldehyde was critical in achieving optimal sensitivity. The observed decrease in absorbance beyond 1.5 µg/ml might suggest saturation or steric hindrance at higher concentrations of the reagent. The heating time and temperature were vital parameters in promoting the reaction completeness without degrading the reactants or products, as demonstrated by the specific window of thermal stability observed (46).

Furthermore, the successful separation of the lamotrigine derivative from piracetam underscores the method's specificity and potential application in clinical settings where patients may be co-administered these drugs. The high repeatability and low relative standard deviation (RSD) of the method affirm its reliability and precision for routine use (47).

In addition, the quantification of lamotrigine in lamigit tablets demonstrated the applicability of this method in pharmaceutical analysis, confirming the label claim with acceptable precision. This method could be particularly useful for quality control and therapeutic monitoring of lamotrigine in clinical samples (48).

CONCLUSION

In conclusion, an analytical procedure utilizing reverse phase HPLC has been successfully developed for the determination of lamotrigine, enhanced by pre-column derivatization with salicylaldehyde. This method effectively separates lamotrigine from piracetam, demonstrating a clear application in settings where both drugs are administered. The linear calibration range was established between 1-5 µg/ml with a detection limit of 0.09 µg/ml, and the procedure was validated for use in pharmaceutical preparations as well as spiked biological samples. The significant increase in molar absorptivity, from 2816.9 L/mol/cm to 34048 L/mol/cm after derivatization, confirms the efficient formation of a Schiff base, enhancing the method's sensitivity and specificity for clinical and pharmacological applications.

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