

Extractive Visible Spectrophotometric Determination of Lamotrigine in Pure and Pharmaceutical Formulations

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Abstract: Two simple, sensitive, accurate and economical spectrophotometric methods (A & B) for the determination of lamotrigine (LMT) in pure and pharmaceutical formulations (tablets) are described in the present work. Method A is based on the formation of coloured ion-association complex between lamotrigine and tropaeolineo-oo (TPooo) with absorption maximum at 485 nm and obeys Beer's law in the concentration range of 5-25 µg/mL. Formation of ion-association complex between lamotrigine and alizarine Red S (ARS) to yield a coloured chromogen exhibiting absorption maximum at 440 nm (Method B) obeying Beer's law in the concentration range of 5-25 µg/mL forms the basis of Method B. The two methods have been validated statistically through recovery studies. The results are compared with those obtained using UV spectrophotometric method (Reference method) in chloroform at 260 nm.

Keywords: Lamotrigine (LMT), Tropaeolineo-oo (TPooo), Alizarine Red S (ARS)

Introduction

Lamotrigine (LMT), chemically known as [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine], is a phenyltriazine derivative (Figure 1) used to treat children with severe epilepsy¹⁻³. Several analytical methods have been reported for the determination of LMT in pharmaceuticals or in biological fluids including reverse-phase HPLC⁴⁻¹¹, GC with nitrogen phosphorous detector¹², capillary electrophoresis¹³⁻¹⁴, adsorptive stripping voltammetry^{15,16} and spectrophotometry¹⁷⁻²¹.

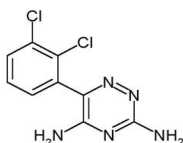


Figure 1. Structure of lamotrigine

The aim of the present work was to develop simple, accurate, precise and inexpensive visible spectrophotometric methods for the determination of lamotrigine in pure and pharmaceutical formulations.

Experimental

All spectral measurements were done using an Elico, UV - Visible spectrophotometer (SL-159) with 1.0 cm matched quartz cells.

Reagents

All the chemicals used were of analytical reagent grade and used as received. Double distilled water was used in the preparation of all solutions. All the solutions were prepared afresh daily.

Method A

TPooo solution (Fluka; 0.2%)

This solution was prepared by dissolving 200 mg of tropaeoline ooo in 100 mL of distilled water.

HCl(E. Merck, 0.1M)

This solution was prepared by diluting 8.6 mL of concentrated hydrochloric acid to 1000 mL with distilled water and standardized.

Chloroform(Qualigens)

AR grade of chloroform was used

Method B

ARS solution (Fluka;0.2%)

Prepared by dissolving 200 mg of ARS dissolved in 100 mL distilled water.

HCl (E. Merck, 0.1 M)

Prepared by diluting 8.6 mL of concentrated hydrochloric acid to 1000 mL with distilled water and standardized.

Chloroform(Qualigens)

AR grade of chloroform was used

Preparation of standard drug solutions

100 mg of lamotrigine (Hetero Drugs Limit, Hyderabad, India as a gift sample) was weighed accurately and transferred into a separate 100 mL volumetric flask and dissolved in 10 mL ethanol. To this, distilled water was added up to the mark to get the stock solution concentration of 1.0 mg/mL and this stock solution was diluted step wise with distilled water to get the working standard solutions of concentration of 100 µg/mL for method A and B.

Preparation of tablets formulations

Twenty tablets were weighed accurately and ground into a fine powder. An amount of powder containing 10 mg of LMT was accurately weighed and transferred into a 100 mL calibrated flask and 10 mL ethanol was added. The content was shaken for about 30 min; the volume was diluted to the mark with water and mixed well and filtered using a Whatman no.41 filter paper. The filtrate containing LMT at a concentration 100 µg/mL was subjected to analysis by the procedure described above.

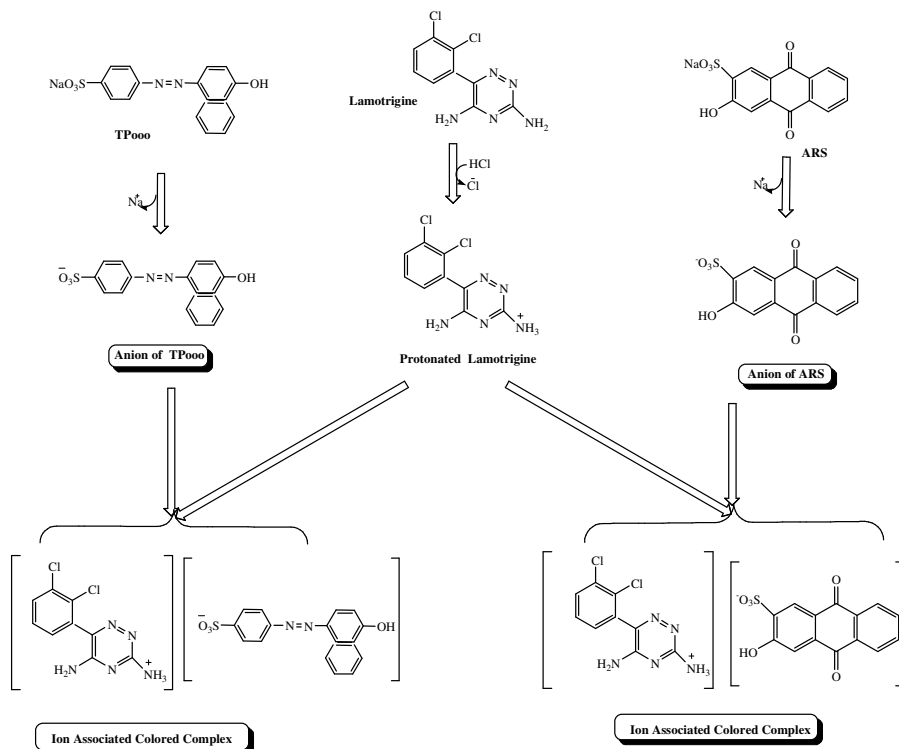
Proposed procedures

Method A & B

Into a series of 100 mL separating funnels, aliquots of standard lamotrigine solution of 0.5-2.5 mL containing 100 $\mu\text{g/mL}$ for the methods A & B, 6.0 mL, 0.1 M HCl and 2.0 mL of 2% dye solution ((TPoo) for method A and (ARS) for method B) were added successively and the total volume of aqueous phase in each separating funnel was made to 15.0 mL with distilled water. To each separating funnel 10 mL of chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at λ_{max} 485 nm with TPoo, 440nm for ARS against reagent chloroform blank.

Results and Discussion

In method A, the positively charged aliphatic primary nitrogen of lamotrigine molecule in acid medium was expected to attract the negatively charged part of the acidic dye TPoo and form an ion pair held together through electrostatic attraction forming orange red colored exhibiting absorption maximum λ_{max} at 485 nm. In method B the same ion-association reaction was obtained for lamotrigine with ARS dye forming a yellow coloured ion association complex which exhibited absorption λ_{max} at 440 nm (Scheme 1)



Scheme 1

The reaction conditions for the proposed methods were established by variation of one parameter at a time. For both the methods (A & B), use of 0.5-2.5 mL of drug solution, 2 mL of dye solution and 10 mL chloroform solvent were considered optimal.

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table.1. The regression analysis using the method of linear least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and the results are summarized in Table 1. The limits of detection (LOD) and quantification (LOQ) calculated according to ICH guidelines are also presented in Table 1. The present relative Standard deviation and percent range of error (%RSD) calculated from the six measurements $\frac{3}{4}$ of the upper Beer's law limits of lamotrigine are given in Table 1. These results showed that the proposed methods have reasonable precision. Comparison of the results obtained with the proposed and UV methods for dosage forms (Table.2) confirm the suitability of these methods for pharmaceutical dosage forms. The results of recovery experiments analyzed by the proposed methods are summarized in Table.2. The other active in gradients and excipients usually present in pharmaceutical dosage forms did not interfere.

Table 1. Optical and regression characteristics, precision and accuracy of the proposed methods for LMT

Parameter	Method A	Method B
λ_{\max} , nm	485	440
Beer's law limits, $\mu\text{g/mL}$	5 – 25	5 - 25
Molar absorptivity, $1 \text{ mol}^{-1} \cdot \text{cm}^{-1}$	4.126×10^3	3.412×10^3
Sandell's sensitivity ($\mu\text{g} \cdot \text{cm}^{-2} / 0.001$ absorbance unit)	0.08554	0.2994
Optimum photometric range, $\mu\text{g/mL}$	5 – 20	5 - 20
Regression equation ($Y=a+bc$)		
slope (b)	0.04053	0.01088
Standard deviation on slope (S_b)	2.070×10^{-2}	4.586×10^{-3}
Intercept (a)	2.499×10^{-4}	4.999×10^{-3}
Standard deviation on intercept (S_a)	1.373×10^{-2}	1.5211×10^{-2}
Standard error on estimation (S_e)	1.309×10^{-2}	1.450×10^{-2}
Correlation coefficient (r)	0.9998	0.9997
Relative standard deviation, %*	1.226	1.6015
% Range of error (confidence limits)		
0.05 level	1.410	1.841
0.01 level	2.210	2.887
% error in Bulk samples**	0.282	-0.29

*Average of six determinations considered. **Average of three determinations

Table 2. Estimation of lamotrigine in pharmaceutical formulations

Sample	Labelled amount, mg	Amount obtained, mg		UV method	%Recovery of Proposed methods**	
		Proposed methods*			Method A	Method B
		Method A	Method B			
Tablet - 1	100	99.98	101.2	99.97	100.0	101.2
Tablet - 2	200	199.97	199.75	199.99	99.98	99.87

*Average of six determinations. ** Mean and standard deviation of six determinations

Conclusion

The proposed methods are accurate, precise, simple, sensitive and rapid and can be applied successfully for the determination of lamotrigine in pure and its pharmaceutical formulations in a routine manner without interference and with good sensitivities.

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