



DEVELOPMENT AND VALIDATION OF INDIRECT SPECTROPHOTOMETRIC METHODS FOR LAMOTRIGINE IN PURE AND THE TABLET DOSAGE FORMS

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abstract: Three simple, sensitive, accurate and indirect visible spectrophotometric methods have been developed for the estimation of lamotrigine (LMT) in both pure and in pharmaceutical preparations. They are based on the oxidation of lamotrigine with a known excess of cerium(IV) in acid medium. The unreacted cerium(IV) oxidize leuco crystal violet to crystal violet colored dye (Method A), leuco malachite green to bluish-green malachite green dye (Method B), leuco xylene cyanol FF to xylene cyanol FF bluish dye (Method C) and exhibiting absorption maxima (λ_{max}) at 580, 610 and 610 nm, respectively. In these methods, the amount of cerium(IV) reacted corresponds to the amount of LMT. The results of the three methods have been validated statistically and by recovery studies. The results obtained in the proposed methods were in good agreement with the label claim.

key words: oxidation; indirect visible spectrophotometry; lamotrigine; pharmaceutical preparation.

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1. Introduction

Lamotrigine (LMT) is chemically known as [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine], is a phenyltriazine derivative (Figure.1) that structurally differs from all other antiepileptic drugs shows a broad spectrum of clinical efficacy and is effective in treating a wide spectrum of childhood epilepsies and it is being prescribed to a greater extent to treat children with severe epilepsy [1-3].

Several analytical methods have been utilized for the detection and determination of LMT in pharmaceuticals or in biological fluids including; reversed-phase HPLC [4-11], GC with nitrogen phosphorous detector [12], capillary electrophoresis [13-14], adsorptive stripping voltammetry [15,16] and spectrophotometry [17-21]. Different manipulation steps are involved in some of these methods (except spectrophotometry), which are not simple for the routine analysis of pharmaceutical formulations.

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The reported chromatographic techniques [4-12] require expensive experimental set-up, not affordable in every laboratory for routine analysis. The stripping voltammetric technique requires sophisticated instrumentation, involvement of scrupulous experimental condition and is time consuming. The reported spectrophotometric methods [17-21] are sensitive and selective but time consuming. Thus, there is a need to develop sensitive, accurate and cost-effective methods for its determination.

The aim of the present study is to develop a simple and sensitive validated spectrophotometric methods for the analysis of LMT in pure form and in pharmaceutical samples using cerium(IV), which in recent years has widely been used for the analysis of pharmaceuticals [22-26]. These developed methods (A-C) are indirect procedures, involving the oxidation of LMT with a known excess of cerium(IV) in acidic media, and the unreacted cerium(IV) oxidizes leuco forms of the dyes to respective colored dyes. The absorbance of the formed color dye was measured at specified wavelengths in the methods A-C.

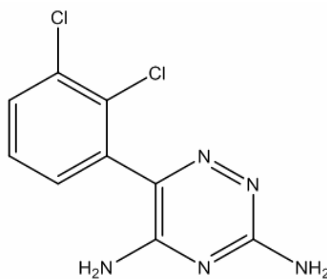


Fig. 1 Structure of Lamotrigine.

2. Experimental

2.1. Apparatus

All absorbance measurements were performed using a Systronics Model 166 digital spectrophotometer provided with 1-cm matched quartz cells. An Elico 120 digital pH meter was used for pH measurements.

2.2. Reagents and Standards

All chemicals and reagents used were of analytical reagent grade and distilled water was used throughout the investigation.

2.2.1. Standard LMT solution

Pharmaceutical grade LMT was procured from Cipla India Ltd., Mumbai, India, as a gift and was used as received. A stock standard solution equivalent to 100 µg/mL of LMT was prepared by dissolving 10 mg of the pure drug in 1 mL ethanol and diluted to the volume in a 100 mL calibrated flask using distilled water.

2.2.2. Standard cerium(IV) solution (1000 µg/mL). It was prepared by dissolving 0.3916 g ammonium ceric nitrate (BDH, Anal R) in 100 mL water containing 0.5 mL of conc.

HNO₃. A working standard solution was prepared by a suitable dilution of a standard solution as and when required.

2.2.3. *Leuco crystal violet (LCV, 0.025%)*. It was prepared by adding 250 mg of LCV (Sigma-Aldrich, Steinheim, Germany), 200 mL of water and 3 mL of 85% phosphoric acid to 1000 mL volumetric flask and shaken gently until the dye dissolved. The contents of the flask were then diluted to the mark with distilled water. The reagent was stable for more than 2 months.

2.2.4. *Leuco malachite green (LMG, 0.05%)*: It was prepared by adding 5 mg of LMG (Sigma - Aldrich, Steinheim, Germany), 20 mL of water and 0.5 mL of 85% phosphoric acid to 100 mL calibrated flask and shaken gently until the dye dissolved (phosphoric acid was added to dissolve the dye completely and to keep the solution stable for a longer time). The contents of the flask were diluted to 100 mL with water. The reagent was stable for more than 2 months.

2.2.5. *Leuco xylene cyanol FF (LXCFF, 0.1%)*. It was prepared by dissolving 100 mg of Xylene cyanol FF in 2 mL of water containing 0.7-0.8 g of zinc dust and 2 mL of 1 M acetic acid, stirred well and kept aside for 20 min, and then the resulting solution was diluted to 100 mL with water (filter if necessary).

2.2.6. *Sulfuric acid*. 0.05 M and 0.5 M.

2.2.7. *Acetate buffer (pH-4.0)*. It was prepared by dissolving 13.6 g of sodium acetate trihydrate in 80 mL of water. The solution pH was adjusted to 4.0 with acetic acid, and the mixture was diluted to 100 mL with water.

2.3. Procedures

2.3.1. Method A

Different aliquots (0.0, 0.5, 1.0...4.0 mL) of standard LMT solution (100 µg/mL) were accurately measured and transferred into a series of 10 mL calibrated flasks. A volume of 0.6 mL of cerium(IV) solution (50 µg/mL) was added to each flask. Then, 0.5 mL each of the 0.5 M H₂SO₄ and 0.025% LCV were added. The reaction mixture is kept in a water bath (40° C) for 5 min; after being cooled to room temperature, the contents were diluted to the mark with acetate buffer of pH-4.0 and mixed well. The absorbance was measured at 580 nm against distilled water.

2.3.2. Method B

Different aliquots (0.0, 0.5, 1.0...3.0 mL) of standard LMT solution (100 µg/mL) were accurately measured and transferred into a series of 10 mL calibrated flasks, and a volume of 0.6 mL of cerium(IV) solution (50 µg/mL) was also transferred to each flask. Then, 0.5 mL each of the 0.5 M H₂SO₄ and 0.05% LMG were added. The reaction mixture was placed in a water bath (60° C) for 20 min and then cooled to room temperature. A volume of 3 mL acetate buffer was added to each flask, and then the mixture was diluted to the mark with distilled water and mixed well. The absorbance of the formed dye was measured at 610 nm against distilled water.

2.3.3. Method C

Various aliquots (0.0, 0.5, 1.0...3.0 mL) of standard LMT solution (100 µg/mL) were accurately measured by means of micro burette and transferred into a series of 10 mL

volumetric flasks. To this, 0.8 mL of cerium(IV) (50 µg/mL) and 0.5 mL each of 0.05 M H₂SO₄ and 0.1% LXCFF were added and the reaction mixture was kept in a water bath (~90°C) for 10 min; after being cooled to room temperature, the contents were diluted to the mark with acetate buffer (pH-4.0) and mixed well. The absorbance of the formed xylene cyanol FF dye was measured at 610 nm against distilled water.

In each method, the calibration graph was prepared by plotting the difference in absorbance (obtained by subtracting the absorbance of the test solution from that of the absorbance of the blank solution) against the concentration of the LMT. The blank was prepared by following the above procedure, but without the addition of LMT. The absorbance of the formed colored dye was measured at 580 nm for LCV and at 610 nm for both LMG and LXCFF against distilled water. The calibration graph or regression equation was used to determine LMT in an unknown sample.

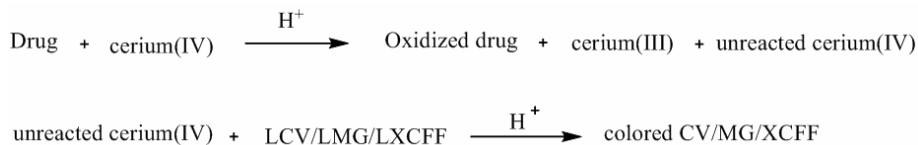
2.4. Procedure for tablets

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.

3. Results and discussion

In an acid medium, cerium(IV) quantitatively oxidizes LXCFF into xylene cyanol FF, which shows maximum absorption at 610 nm [27]. In a similar reaction, cerium(IV) oxidizes leuco malachite green (LMG) to malachite green [28]. Both methods were used to determine micro amounts of cerium(IV) spectrophotometrically. Preliminary experiment was performed to fix the linear range for cerium(IV) with the use of LCV/LMG/LXCFF in methods A, B and C, respectively. Under the optimum experimental conditions, the linear range of cerium(IV) was found to be 0.2- 3.0 µg/mL Ce(IV) for methods A and B and 0.4-4.0 µg/mL for method C. Therefore, in this work, a known but excessive amount of cerium(IV) (0.6 mL of 50 µg/mL cerium(IV) in methods A and B, and 0.8 mL of 50 µg/mL in method C) was utilized to oxidize LMT in sulfuric acid medium with a sufficient heating, and the unreacted cerium(IV) was determined by reacting it with LCV (method A), LMG (method B) and LXCFF (method C) in the same acidic conditions. The formed dye in each method was measured at specified wavelengths. The amount of cerium(IV) reacted corresponds to the amount of LMT in these methods. This has been the basis for the determination of LMT in pure and in pharmaceutical samples.

The drug LMT, when added in increasing amounts to a fixed amount of cerium(IV), LMT consumes cerium(IV) and there is a concomitant decrease in the absorbance of the colored dye on increasing the concentration of drug. The reaction pathways for all the methods are shown in Scheme 1.

**Scheme 1** Probable reaction scheme.

The optimum conditions for color development for methods A, B and C have been established by varying the parameters one at a time and keeping the other parameters fixed, and the effects of the formation of dye including temperature and other reagents concentration were investigated and incorporated in the procedures. The effect of temperature was studied for all the methods A, B and C. At room temperature, unreacted cerium(IV) is not completely oxidize LCV, LMG or LXCFF. The complete oxidation of the cited dyes by cerium(IV) required sufficient heating. For method A, 5 min of heating at a temperature of 40 °C and for method B, 20 min heating at 60 °C were maintained. In method C, 10 min of heating at 90 °C was maintained to achieve constant and maximum absorbance values. In each method, the above specified time and temperature were fixed to get full color development and constant absorbance readings. Below this temperature and heating time, constant absorbance reading was not obtained. The formed dye was stable in sodium acetate buffer media (pH 4.0) in all these methods. Constant absorbance readings were obtained in the pH 2-4. At lower acidity (pH ≤ 2), the color did not developed and at higher acidity (pH ≥ 4), the stability and sensitivity of the dye was affected. Sulfuric acid was used in these methods to oxidize the drug by cerium(IV). The formed dye in each method was stable for more than a week.

Analytical parameters such as linear range, Sandell's sensitivity, molar absorptivity, limits of detection (LOD) and quantification (LOQ) and regression equation for methods A, B and C are tabulated in Table 1.

Table 1 Analytical and regression parameters of the proposed methods.

Parameter	Method A	Method B	Method C
λ_{max} nm	580	610	610
Linear range ($\mu\text{g/mL}$)	2 - 35	3 - 25	1.5 - 24
Molar absorptivity (ϵ), ($\text{L mol}^{-1}\text{cm}^{-1}$)	2.26×10^3	3.85×10^3	2.23×10^3
Sandell sensitivity ($\mu\text{g cm}^{-2}$)	0.1133	0.0665	0.1149
Intercept (a)	-0.0043	0.0121	-0.0065
Slope (b)	0.0094	0.0131	0.0139
Correlation coefficient (r)	0.9973	0.9978	0.9996
S_a	0.0291	0.0098	0.03284
S_b	0.0009	0.00047	0.00157
LOQ ($\mu\text{g/mL}$)	1.955	2.473	0.7884
LOD ($\mu\text{g/mL}$)	0.645	0.816	0.2601

* $y = a + bx$, where x is the concentration of LMT in $\mu\text{g/mL}$ and y is the absorbance at the respective λ_{max} , S_a is the standard deviation of the intercept, S_b is the standard deviation of the slope.

3.1. Application to analysis of commercial samples

To check the validity of the proposed methods, LMT was determined in some commercial formulations. The result obtained from the determination is in close agreement between the results obtained by the proposed methods and the label claim. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed method and the literature method [20] at the 95% confidence level with respect to accuracy and precision (Table 2).

Table 2 Results of determination of LMT in tablets.

Tablet brand Name	Nominal amount mg per tablet	Found**(%of nominal amount±SD)			
		Reference method [20]	Method A	Method B	Method C
LAMITOR OD *	50 mg	103.25 ±0.28	102.60±0.59 t=2.27, F=4.27	102.82±0.53 t=1.70, F=3.52	102.99±0.33 t=1.23, F=1.52

*Marketed by: *[Torrent (Mind)]; **Mean value of five determinations

**Tabulated t and F-values at 95% confidence level are 2.77 and 6.39, respectively.

3.2. Recovery study

The accuracy and precision of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure drug at three different concentrations and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative and revealed that co-formulated substances did not interfere in the determination. The results of recovery study are compiled in Table 3.

Table 3 Results of recovery experiments *via* the standard addition technique

Tablet brand name	LMT tablet µg/mL	Pure LMT added, µg/mL	Method A		Method B		Method C	
			Total found µg/mL	LMT recovered* %±SD	Total found µg/mL	LMT recovered* %±SD	Total found µg/mL	LMT recovered* %±SD
Lamitor OD	10	5	15.04	100.85±0.21	14.98	99.76±0.23	15.06	101.15±0.43
Torrent	10	10	19.95	99.86±0.16	20.18	101.81±0.16	20.07	100.74±0.22
(Mind)	10	15	25.16	101.04±0.18	25.23	101.51±0.33	25.09	100.63±0.03

* Mean value of three measurements

4. Conclusions

Three simple, rapid, fairly accurate, precise and sensitive spectrophotometric methods were developed for the determination of lamotrigine in bulk drug and in tablets. The methods rely on the use of simple and cost effective chemicals in all the three methods and can be successfully applied for the routine estimation of lamotrigine in bulk and tablet dosage forms.

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