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Visible Spectrophotometric Determination of Lamotrigine in Pharmaceutical Preparations

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Abstract: A simple, rapid, sensitive and reproducible visible spectrophotometric method has been developed for the determination of Lamotrigine (LMG) in bulk and in its dosage forms. The method is based on the reaction of LMG with gold (III) chloride in the pH range 2.5 – 3.5 forming Yellow color complex solution, showing absorption maxima at 400 nm. The linear plot indicates that Beer's law is obeyed in the range of 10 – 160 µg/ml of LMG. The molar absorptivity and Sandell's sensitivity are 1.3896 x 10^3 l mol-1 cm-1 and 0.0184 µg/cm2 respectively. The standard deviation of the method for ten determinations of LMG is 0.0013. The correlation coefficient (r^2) of the experimental data of the calibration plot is 0.9999. The effective range of concentration for accurate determination of LMG as ascertained from Ringbom's plot and it is 10 – 160 µg/ml. Interferences of the other ingredients and excipients were not observed. The optimum reaction conditions and other analytical parameters were evaluated. The methods were successfully applied to the determination of LMG in pharmaceutical formulations

Introduction

Lamotrigine (LMG) is chemically, 6-(2, 3-dichlorophenyl)-1, 2, 4-triazine-3,5-diamine. Its molecular formula and molecular weight are $C_9H_7N_5Cl_2$ and 256.09 respectively. Its structural formula is:

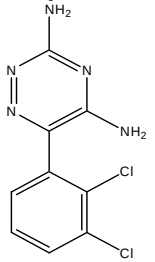

Fig 1: Chemical structure of Lamotrigine

LMG is a white to pale cream-colored powder and has a pKa of 5.7. It is very slightly soluble in water and in 0.1 M HCl. It is a novel antiepileptic drug chemically unrelated to other anticonvalsants used as an add-on therapy of seizure in children and adults [1]. LMG tablets are supplied for oral administration as 25 mg, 100 mg, 150 mg, and 200 mg tablets. Each tablet contains the labeled amount of LMG and the following inactive ingredients: lactose; magnesium stearate; microcrystalline cellulose; povidone; sodium starch glycolate. The most commonly observed adverse experiences seen in association with LMG during adjunctive therapy in adults and not seen at an equivalent frequency among placebo-treated patients were: dizziness, ataxia, somnolence, headache, diplopia, blurred vision, nausea, vomiting, and rash.

A literature survey reveals that several methods for determination of LMG and its metabolites in biological matrices have been developed including reversed-phase HPLC [2-6], gas chromatography with nitrogen phosphorus detector [7], capillary electrophoresis [8, 9], chromatography–thermospray mass spectrometry [10], immuno fluorometric assay [11] and radioimmunoassay [12]. There are some analytical methods available for the determination of LMG in bulk drug and in formulations, which include UV spectrophotometry [13, 14], HPTLC [15] and HPLC [16]. An analytical method for the detection of trace amounts of the principal synthetic route indicative impurity in LMG

including preconcentration sample extract by normal-phase HPLC which then is analysed with a reversed-phase HPLC TSP-MS has also been reported [1]. An official monograph of LMG does not exist in any of the pharmacopoeias and determination of LMG and related substances in pharmaceutical formulations has not yet been described. Therefore, it is very imperative to develop simple and suitable analytical methods for the determination of LMG in bulk and in formulations. So far no sensitive, accurate and flexible visible spectrophotometric method was reported for the determination of LMG in bulk and in pharmaceutical formulations. The author has made humble attempts in this direction and succeeded in developing new visible spectrophotometric methods based on its reaction gold (III) in the pH range 2.5 – 3.5 forming a yellow coloured complex.

Experimental

Apparatus:

All spectral and absorbance measurements were made on a Shimadzu UV-Visible digital Spectrophotometer (UV-160A) with 10mm matched quartz cells.

Materials and Methods

All chemicals used were of analytical reagent grade and double distilled water was used for preparing the reagent solutions. LMG was obtained from Dr. Reddy's labs Hyderabad. Stock solution of LMG was freshly prepared by dissolving 100mg of LMG in 100ml of methanol and then this was further diluted with methanol so as to obtain working standard solution of 100 µg/ml.

Pharmaceutical dosage form

Tablet label claim of 10 mg alfuzosin hydrochloride was procured from local Market

Preparation of Lamotrigine solution

100 mg of Lamotrigine is weighed accurately and transferred into a 100ml standard flask, dissolved and made up to the mark with methanol. This solution is diluted as required.

Preparation of Gold (III) solution

1gm of chloroauric acid (Johnson Mathews, materials technology, U.K.) is dissolved in distilled water after adding few drops dilute HCl. The solution is made upto the mark in 100 ml volumetric flask. The gold content of the solution is determined by rhodamine B method. The working solutions are prepared by diluting the stock solution.

Method:

Determination of Lamotrigine

To explore the possibility of employing the colour reaction for the determination of Lamotrigine, the absorbance of the experimental solution containing different amounts of LMG, keeping the Au (III) concentration constant is measured in the wavelength range 350 – 700 nm.

Determination of gold (III)

To explore the possibility of employing the colour reaction for the determination of gold (III) in trace level, the absorbance of the experimental solutions containing different amounts of gold (III), keeping the ATH concentration in excess, is measured in the wavelength range 400 – 700 nm.

Assay of Pharmaceutical dosage form of Lamotrigine

A know aliquot of pharmaceutical sample solution of lamotrigine is added to a 10 ml volumetric flask containing 5ml of buffer solution of pH 3.0 and 0.5 ml of gold(III) (5.0×10^{-3}M) solution 1.5 ml of 2% SDS solution. The contents are made up to the mark with distilled water. After heating for 60 minutes at 65^0C and cooling the solution to room temperature, the absorbance of the resulting solution is measured at 380 nm against the buffer blank. The amount of lamotrigine is computed from the predetermined calibration plot at 380 nm. The present method for the determination lamotrigine is applied for its determination in a pharmaceutical sample.

Results and Discussion

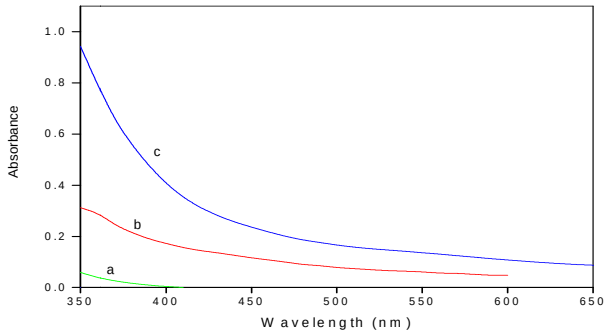

Fig. 2 Absorption spectra of LMG – Au (III) system

a. Au(III) Vs buffer blank
b. LMG – Au(III) Vs buffer blank (Au(III) excess)

c. LMG – Au(III) Vs buffer blank (LMG excess)
[Au(III)] = 5.0 x 10^{-3}M; [LMG] = 3.85 x 10^{-3}M

The absorption spectra of the gold(III) solution and lamotrigine in buffer solution of pH 3.0 and that of the experimental solution containing solutions of the gold(III), lamotrigine and the buffer (pH 3.0) against the buffer blank are recorded in the wavelength range 350 – 700 nm. The spectra are presented in fig. 2. The spectra show that the complex has an absorption maximum at 380 nm. Neither gold (III) nor lamotrigine have absorbance at 380 nm. Hence, analytical studies are made at 380 nm. However, in presence of excess lamotrigine the complex shows maximum absorbance at 400 nm.

Effect of pH on the absorbance of experimental solution

The optimum pH required for the maximum colour development is established from the results obtained in the experiment carried out. A plot is drawn between absorbance and pH. It is represented in fig 3. The fig. 3 indicates that the absorbance is maximum and constant in the pH range 2.5 to 3.5. Hence, pH 3.0 is selected for further studies as minimum interference due to associated ions and excipients is observed at this pH.

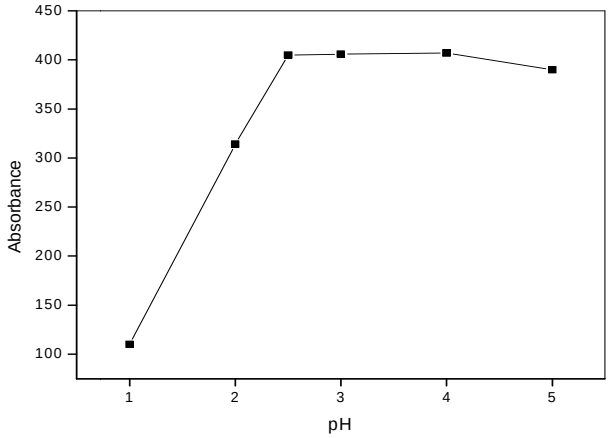

Fig.3 Effect of pH on absorbance of LMG – Au(III) system
[Au(III)] = 5.0 x 10^{-3}M; LMG = 3.85 x 10^{-3}M

Adherence of the system to Beer's law

To explore the possibility of employing the colour reaction for the determination of lamotrigine, the absorbance of the experimental solution containing different amounts of LMG, keeping the Au (III) concentration constant is measured. A plot of absorbance Vs amount of lamotrigine is presented in fig. 3 the straight line plot obtained obeys the equation A = 0.0053C - 0.0002.The linear plot indicates that Beer's law is obeyed in the range of 10 – 160 µg/ml of lamotrigine. The molar absorptivity and Sandell's sensitivity are 1.3896 x 10^3 l mol^{-1} cm^{-1} and 0.0184 µg/cm^2 respectively. The standard deviation of the method for ten determinations of 20 µg/ml of lamotrigine is 0.0013. The correlation coefficient (γ) of the experimental data of the calibration plot is 0.9999. The effective range of concentration for accurate determination of lamotrigine as ascertained from Ringbom's plot and it is 10 – 140 µg/ml.

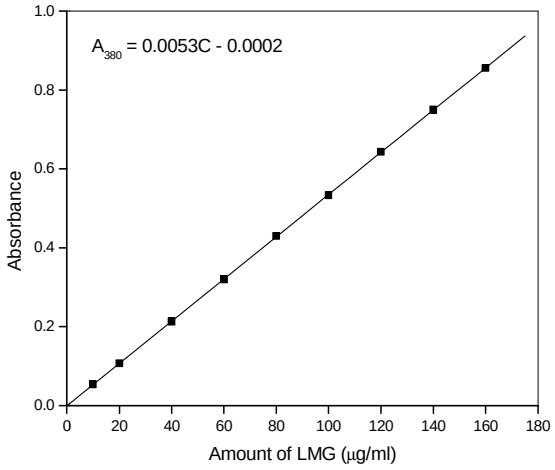

Fig. 4 Absorbance Vs Amount of LMG (µg/ml)
[Au(III)] = 5.0 x 10^{-3}M ; pH = 3.0; λ = 380 nm

Order of addition of constituent solutions on the absorbance of the experimental solution

The effect of the order of addition of the various constituent solutions (the buffer solution, gold (III) solution and lamotrigine solution) is studied by measuring the absorbance of the experimental solution at 380 nm. The results revealed that the absorbance remains unchanged irrespective of the order of mixing of various constituent solutions. Hence, it is not necessary to follow a particular order of addition of the constituents of the experimental solution.

Effect of temperature on the absorbance of the experimental solution

To determine the temperature at which the experimental solution attains maximum absorbance quickly was determined and the results are presented in table 1.

Table 1. Effect of temperature on the absorbance of the experimental solution

[lamotrigine] = 3.85×10^{-3}M ; pH = 3.0

[gold(III)] = 5.0×10^{-3}M ; λ = 380 nm

Temperature (^{0}C)	Absorbance
40	0.103
50	0.303
60	0.381
65	0.407
70	0.406
75	0.404

The results in table 1 indicate that the absorbance attains maximum value at 65^0 C. Hence, the absorbance is measured after heating the experimental solution at 65^0 C for 60 minutes and cooling it to room temperature.

Effect of excipients

Various amounts of excipients that are generally associated with the lamotrigine in its pharmaceutical formulations are added to a fixed amount of lamotrigine (20 µg/ml) solution and the absorbance measurements are carried out under optimal conditions. The concentration (µg/ml) at which various ions do not cause an error of more than ± 4% in the absorbance is taken as the tolerance limit and the results are given in table 2.

Table 2. Tolerance limit of excipients

Amount of LMG = 20 µg/ml; pH = 3.0

Excipient	Tolerance limit (µg/ml)
Fructose	1326
Glucose	951
Sucrose	1446
Lactose	1796
Gelatin	1911
Starch	1503
Sodium Alginate	1398
Boric acid	1997
Magnesium stearate	1664

The data in table 2 indicate that the excipients that are associated with lamotrigine do not interfere even in large quantities in the determination of lamotrigine making the method highly selective and direct.

Assay of lamotrigine

The present method for the determination lamotrigine is applied for its determination in a pharmaceutical sample. The results of assay were shown in table 3.

Table 3. Assay of lamotrigine in pharmaceutical formulation

Sample (manufacturer formulation)	Label claim (mg)	Amount found * (mg)	Error (%)
Lamictal, Glaxo smithkline	25.00	25.66	-1.36

*** Average of seven determinations**

Conclusions

Lamotrigine reacts with gold (III) to form stable yellow coloured 1: 1 complex at pH 3.0. Spectro photometric method was developed based on this reaction. The method was found to be sensitive for the assay of both lamotrigine and gold (III). The tolerance limit of the excipients and the foreign ions in derivative methods is found to be generally 10 – 20% greater than that of the zero order method. The present spectro photometric method was direct, simple and highly selective for the determination of gold (III) or lamotrigine. Further, the method can easily be employed by ordinary clinical laboratories as the methods can be carried out using a simple colorimeter.

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