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SPECTROPHOTOMETRICAL DETERMINATION OF LAMOTRIGINE DRUG IN ITS BRANDED TABLETS

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Three highly sensitive spectro photometric methods for the determination of Lamotrigine in pharmaceutical tablets and other preparation have been procured. These methods are selective. Methods 02 and 03 are extremely sensitive and are applicable in ngmL^{-1} levels too. Its method is based on the formation of chloroform extractable ion-pair complex between lamotrigine and Bromophenol blue reagent at a lower pH 1.45. with a maximum absorption wavelength at 424 nm.

In methods (02) and methods (03) the lamotrigine-Bromophenol blue ion pair is dissolved in either ethanolic sulphuric acid and resulting acid form of the dye is measured at 424 nm or in ethanolic KOH and resulting base form of the dye is measured at 600 nm. All variables that can affect the drug-dye complexation and its extraction into chloroform have been investigated and its optimum values have been achieved. Beer's law was obeyed in all these three methods. The limits of detection (LOD) and LOQ (Limits of quantification) have also been calculated. The accuracy and precision of the methods have been evaluated on intra day and inter day basis and the relative error (RE) and the relative standard deviation (RSD) have been also observed. The proposed methods can be successfully applied for the determination of lamotrigine in bulk powder or in tablets too equally.

KEYWORDS : Lamotrigine, Spectrophotometry, Bromophenol blue, LOD & LOQ.

INTRODUCTION

Lamotrigine also sometimes called Lamictal has been found effective against refractory partial seizures. It acts by blocking sodium channels and thus preventing glutamate release'. As an anticonvulsant and antiepileptic both it is in use. But its selectivity is as antiepileptic. There is some difference as anticonvulsant is an agent that blocks experimentally produced seizures in laboratory animals and an antiepileptic drug is used medically to control the

epilepsies, not all of which are convulsive in humans, such classification is accepted due to its accuracy and facilitation in diagnosis drug selection and precise discussion of seizure disorders [2, 3].

It is chemically known as [6 – (2, 3 dichlorophenyl) –1, 2, 4 triazine –3, 5 diamine]

It is a broad spectrum antiepileptic Lamotrigine is used as tranquilizer and in the treatment of neurological lesions also [4, 5]. Lamotrigine is not official in any pharma Copoeia. Normally it is determined using HPLC with UV-detector and focussing on its quantification in biological fluids [6, 9].

Lamotrigine has been assayed in human plasma using solid phase micro extraction (SPME) and gas-chromatography with thermionic specific detection [10].

In serum, it has been determined by high performance thin layer chromatography [11].

However, literature on the methods of its determination in pharmaceuticals is very few [12, 13] Agrwala [14] has seperated Lamotrigine by HPTLC followed by quantification by densitometry at 312 nm.

Youssef and Taha [15] have reported three methods for the assay of Lamotrigine in bulk powder, in dosage form and in the presence of its impurity 2, 3 dichlorobenzoic acid. Elizabeth *et al* [16] has reported a good sensitive and selective method but it is time consuming and requires expensive instrumental set up. The absorptive stripping voltametric method is highly complicated and is reported to be less precise too.

Our present work is to develop and validate sensitive and selective methods for the determination of lamotrigine in different dosage forms.

Methods are based on the formation of ion pair complex with the dye bromophenol blue and the complex is quantitatively extracted into chloroform and measured at 420 nm. Complexes were dissociated in acid/base medium separately and then its visible spectrophotometry were observed at 420 and 600 nm respectively.

Experimental

Absorbance measurement were observed with a systronics model 106 digital spectrophotometer equipped with 1 cm matched quartz cells. pH– measurements were done using systronics digital pH meter MK–VI model.

All chemicals used were of Anal–R grade and distilled water was used throughout the work. Spectrophotometric grade organic solvents were used. Sulfuric acid (0·1 M) was prepared by dilutions of appropriate volume of concentrated acid (Merck, sp.gr. - 1·84) in water. Bromophenol blue (0·5%) was prepared by dissolving 500 mg of the dye in 10ml of ethanol. (Ranbaxy Fine chem. Ltd, Mumbai, India) and then diluted to 100 ml with water.

Ethanolic sulphuric acid (01%) was prepared by dissolving 1 ml of Conc H_2SO_4 (18M) in 100 ml of ethanol. Ethanolic KOH (1%) was prepared by dissolving 1gm of chemical (CDH) in 100 ml of ethanol.

Pharmaceutical grade lamotrigine was procured from Cipla India Ltd. Mumbai as a gift and was used as such. A stock standard solution containing 200 μgml^{-1} lamotrigine was prepared by dissolving 20 mg of it in 0·1 M H_2SO_4 in a 100ml flask and diluted to the mark with the same acid. The stock solution was approximately diluted to 50 μgml^{-1} with the same acid.

Method–01 :–

Different aliquots (0·0, 0·5, 1·0, 1·5, 2·0, 2·5, 3·0, 3·5, 4·0, 4·5 and 5·0 ml) of standard (50 μgml^{-1}) lamotrigine solution were transferred into a series of 125ml separating funnels and the total volume was brought to 5·0 ml by adding 0·1 M H_2SO_4, 20ml of water and 5 ml of 0·5% bromophenol blue (BPB) were added to each separating funnels and the contents were mixed well. After 5 minute, 10 ml of chloroform was added by means of microburette and shaked well for about one minute. Then two phases were allowed to separate and the chloroform layer was collected and passed over anhydrous sodium sulphate and the absorbance measured at 420 nm against the reagent blank.

Preparation of Lamotrigine– BPB ion pair complex

5 ml of lamotrigine solution (50 μgml^{-1}) was pipetted out into a 125 ml seperating funnel and 20 ml of water was added. To it 5 ml 0.5% BPB dye was now given. It was stirred well. After 5 minute, 10 ml of $CHCl_3$ was added and whole mixture was stirred vigorously for about one minute to extract the drugdye complex into the $CHCl_3$. Then two phases were allowed to separate and the chloroform layer was passed over anhydrous sodium sulphate and collected in a 25 ml volumetric flask. This complex solution was diluted from 10 μgml^{-1} to 1 μgml^{-1} and 0·2 μgml^{-1} with $CHCl_3$ for use in method no. 02 and 03 respectively.

Method No. 02 :—

(It is based on the measurement of acid form of the dye)

Different aliquots (0·0, 0·5, 1·0, 1·5, 2·0, 2·5, 3·0, 3·5 and 4·0 ml of drugdye complex (1·0 μgml^{-1}) were taken into a series of 10 ml calibrated flasks and the total volume was adjusted to 4·0 ml with $CHCl_3$. To each flask 1ml of ehanolic H_2SO_4 was mixed and allowed to stand for 5 min and diluted to 10 ml with ethanol with vigorous shaking. Now its absorbance was measured at 420 nm against the reagent blank.

Method No. 03 :—

(It is based on the measurement of the base form of the dye.)

Different aliquots (0·0, 0·5, 1·0, 1·5, 2·0, 2·5, 3·0, 3·5 and 4·0 ml) of lamotrigine –BPB complex (0·2 μgml^{1}) were taken in a series of 10ml calibrated flasks and the total volume was adjusted to 4·0 ml with $CHCl_3$.

To each flask, 1 ml of ethanolic KOH was added. The contents were mixed and allowed to stand for 5 min. and diluted to 10 ml with ethanol. After mixing evenly, the absorbance was measured at 600 nm against the reagent blank.

In all the three methods, standard graph was prepared by plotting the absorbance Vs drug concentration and the concentration of the unknown was read from the calibration graph from the respective segression equation derived using the absorbance concentration data.

Procedure for Tablets :—

Lamosyn 100 (Sun pharma) and Lametec 50 DT (Cipla) were used for investigation. Twenty tablets were weighed and crushed into its fine powder. An amount of finely crushed powder containing 5·0 mg of limotrogine was added into a 100 ml calibrated flask.

To it 50 ml 0·1 M H_2SO_4 was added and it was shaken for 30 minutes and finally made upto the mark with 0·1 M H_2SO_4. This solution was kept a side for 10 minutes and then filtered it using whatmann paper –42. First 10 ml portion of the filtrate was discarded and a

suitable aliquot (say 3ml) was used for assay by method no. –01. The ion-pair complex (10 μgml^{-1} in lamotrogine) prepared above was diluted to obtain 1·0 μgml^{-1} and 0·2 μgml^{-1} complex solutions for assay by applying the procedures described for method –02 and method –03 respectively.

Result and discussion

In method–01, the drug lamotrigine on reaction with bromophenol blue dye in acidic medium (pH 1·44) forms a yellow coloured ion-pair complex. It was extracted into chloroform. This complex absorbed maximally at 420 nm. This drug-dye ion-pair was found to dissolve in ethanolic H_2SO_4 or in ethanolic KOH solution yielding the free dye in protonated form (λ max 420 nm) or in deprotonated form (λ max 600 nm) in method 02 under the optimum condition.

In acid medium pH measured was 1·69 ± 0·05 and in alkaline medium pH was 13·95 ± 0·06.

The blanks had negligible absorbance in all the three instances. The probable reaction showing the formation of the ion-pair complex and its dissociation are shown in schemes 1 and 2 as follows :

Scheme 02 :– Formation of acidic and basic forms of the dye.

Optimum reaction conditions for rapid and quantitative formation of coloured products with greater stability were established on the basis of preliminary experiments.

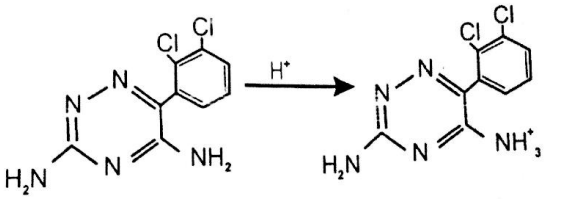
In method–01, by keeping Concentration of the drug constant, effect of chang in pH, volume of aqueous phase, volume of dye, different extractive organic solvents reaction time, shaking time and stability of the complex were studied by measuring the absorbance of the ion-pair complex at 420 nm.

In 2nd and 3rd method, to a constant concentration of ion-pair complex, different volumes of ethanolic KOH/H_2SO_4 were added and the resulting acid and base form of the dye was measured at 420 nm in 2nd method, or at 620 nm in method 03. The effect of time on breaking the ion-pair complex was similarly studied.

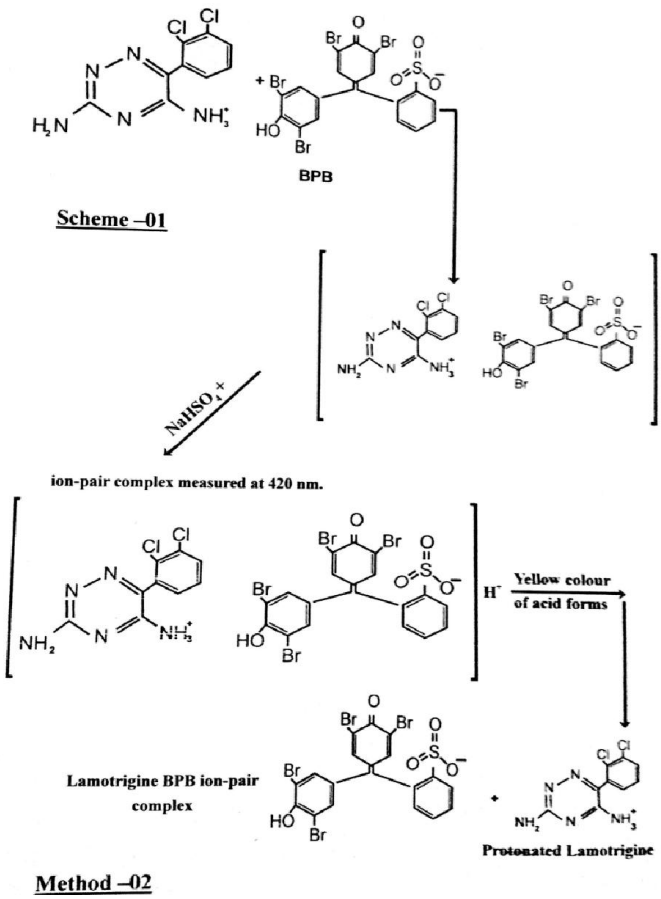
Optimization of Experimental variables :—

Method No. – 01

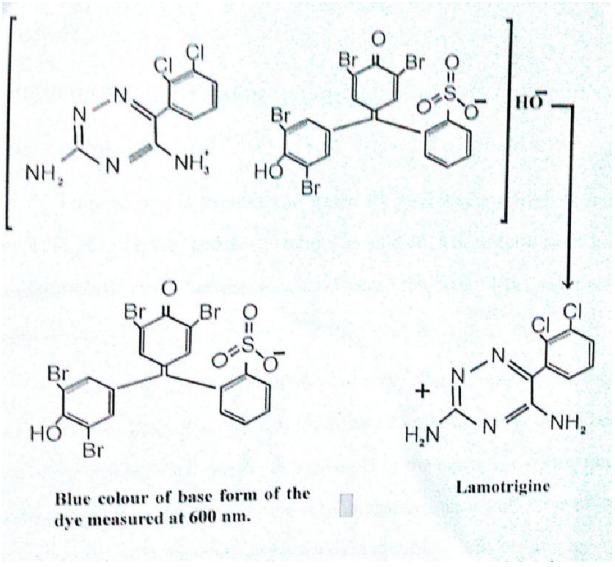
Lamotrigine is insoluble in water. Its solubility in different acids such as HCl, H_3PO_4, H_2SO_4 and acetic acid was studied. The solubility of Lamotrigine and the sensitivity of the methods was found better in 0·1 M H_2SO_4 than the rather acids.



Lamotrigine **Protonated Lamotrigine**


BPB
Scheme –01
NaHSO3 +
ion-pair complex measured at 420 nm.
H+
Yellow colour of acid forms
Lamotrigine BPB ion-pair complex
Protonated Lamotrigine

Method –02


HO−
Blue colour of base form of the dye measured at 600 nm.
Lamotrigine

In order to get the optimum pH range, 5 ml Lamotrigine solution in 0·1 M H_2SO_4 was diluted with 5, 10, 15, 20 and 25 ml of water before mixing with 5 ml of the dye solution and the effective pH of the aqueous solution ranged from 1.40 to 1.50. The absorbance remained unchanged, in the effective pH range and the absorbance of the reagent blank was negligible in this pH range.

Hence, an aqueous phase volume of 30 ml (pH – 1.4) was used in all subsequent experiments. The effect of BPB concentration was investigated by varying the volume of dye-solution and using a fixed amount of drug.

The complex formation and its extraction were uneffected in the range of 3·0 to 7·0 ml of 0·5% BPB solution. Hence 5 ml of 0·5% BPB solution was fixed in a total volume of 30 ml of aqueous phase. In the initial experiments, dichloromethane, carbon tetrachloride, n-hexane, cyclohexane were tried as extraction experiments. None of these systems showed better results than chloroform. So, chloroform was choosen as the solvent for extraction. The volume of aqueous phase was tried by keeping all the parameters constant, from 15–30 ml by adding different volumes of distilled water.

The coloured complex was extracted with 10 ml of chloroform in each case. It was found that a ratio of 3·1 of aqueous phase to organic was adequate for efficient extraction of the coloured species.

There was found no change in absorbance varying the shaking time from 30 second to 60 seconds taking all other parameters to be constant.

So, a 30 sec. shaking time was selected. Under optimum conditions, the drug-dye complex in the aqueous phase was extracted with three 10 ml portion of chloroform and absorbance was measured every time.

After the second extraction, absorbance of the organic layer was negligibly less. Hence, a single extraction with 10 ml chloroform was selected for the extraction because of complete recovery of the complex.

The organic and aqueous phases were clearly separated in less than 1 min.

The coloured complex was stable for more than ~20 hr. at laboratory temperature 35°C. The sequence of order of addition of the reactants prior to extraction had no effect the complexation, extraction and the absorbance values.

The complexing ratio of Lamotrigine drug and bromophenol blue dye in 1st method was examined by Job's method of continuous variations. The concentration of the aqueous dye and the drug was $7{\cdot}46 \times 10^{-4}$ M. Six solutions containing Lamotrigine and BPB in various molar concentration with a total volume of 5 ml in addition to 20 ml water were prepared. The extraction was performed using 10 ml $CHCl_3$ and the absorbance was measured each time at 420 nm. The plot of the results gave a maximum at a molar ratio of X_{max} = 0·5 which indicated the formation of 1 : 1 complex stability constant of the ion-association complex was calculated from the continuous variation data using following equation.

Here, A and Am = observed maximum absorbance. C_M = Molar concentration of Lamotrigine at the maximum absorbance and n = stoichiometry with which BPB ion associates with drug. K_f = conditional stability constant.

The log K_f = 4·83 (exptal)

Method No. 02 :—

1 ml of 1% ethanolic acid concentration was found to give a reasonable maximum absorbance at 420 nm. Now, different concentrations of Lamotrigine BPB complex were treated with 1 ml of 1% ethanolic H_2SO_4 and after the contact time elapsed, the absorbance of the dye was measured and related to drug concentration.

The complex dissociating time was studied by adding 1ml of ethanolic H_2SO_4 to a fixed amount of Lamotrigine-BPB complex taken in a series of 10 ml flasks. After some interval of time ranging from 5 to 30 minutes the solution was made upto mark with ethanol and the absorbance was change in absorbance values between the time range 5·30 min. So, 10 min. was fixed as a contact time. The yellow dye was stable for more than about 30 hr. at room temperature of 35°C.

Method No. 3 :–

A series of measurements was made to fix the amount of ethanolic KOH to obtain the maximum absorbance. The absorbance was measured at 600 nm by adding different volumes of 1% ethanolic KOH to series of flasks containing fixed amount of Lamotrigine – BPB addition salt.

The absorbance values were almost constant at the alkali amount ranging from 1 to 5 ml in a total volume of 10 ml. So, different concentration of drug-dye complex were treated with 1ml of 1% alcoholic KOH and after the contact time elapsed, the absorbance of the dye was measured and related to complex concentration.

The complex breaking time was studied by adding 1 ml of ethanolic KOH to a fixed amount of drug-dye complex.

The mixture was diluted upto mark with ethanol and the absorbance measured at 600nm at different time intervals. There was no change in absorbance values between the times ranging from 5 to 30 min. Hence, 10 min. was taken as a constant time. The blue coloured dye was stable for 24 hr. and some more at room temperature of 35°C.

Validity Test :—

Calibration graphs were constructed from ten and eight points covering the concentration ranges 2·5 – 25 μgmL^{-1}, 50–400 ng mL^{-1} and 10–80 ng ml^{-1} for all the three methods. Regression analysis of the Beer's law data indicated a linear relationship between absorbance and concentration written in table–1.

The calculated molar absorptivity using sandell sensitivity values and limits of detection and quantification calculated according to ICH guidelines are also summarized in table [19].

Table 1. Sensitivity and Regression Parameters

Parameter	Method-1	Method-2	Method-3
λ_{max} (nm)	420	420	600
colour stability, min	> 20 hr.	>24 hr.	>24 hr.
Linear Range	2·5-25 μgmL^{-1}	50·400 $ngmL^{-1}$	10-80 $ngmL^{-1}$
Molar absorptivity ($Lmol^{-1}cm^{-1}$)	$7{\cdot}26 \times 10^3$	$5{\cdot}4 \times 10^5$	$2{\cdot}6 \times 10^6$
Sandell sensitivity (μ gcm^{-2})	0·0352	0·005	0·0001
Limit of detection (LOD) (μg mL^{-1})	0·15	0·003	0·00055
Limit of quantification (LOQ) (μgmL^{-1})	0·46	0·01	0·0016

Regression equation y' Intercept (a)	-0·045	0·0070	-0·0003
Slope (b)	0·0320	2·0734	10·2023
Standard deviation of	0·0997	0·0997	0·0997
a (S_a) ± S_a /v_n	0·0712	0·0830	0·0830
Standard deviation of b (S_b)	0·0063	0·3116	1·558
± S_b/v_n	0·0005	0·2600	1·3030
Variance ()	0·010	0·010	0·010
Regression Co-efficient (r)	0·9992	0·9998	0·9998

$$y^1 = a + b_x;$$

where y = absorbance

x = Concentration in μgmL^{-1}

a = intercept, b = slope

± S_a/v_n = confidence limit for intercept

± S_b/v_n = Confidence limit for slope.

Recovery Study : Accuracy of the methods is ascertained by recovery experiments applying the standard addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre analysed tablet powder with pure drug at three two levels (50–100 and 150%) of the content present in the tablet powder taken and the total was found by the proposed methods. Each test was repeated three times. In all the cases the % recovery values ranged between 95·75 and 102·3 with relative standard deviation in the range 0·58–1·50%. Closeness of the results to 100% shows the fairly good accuracy of the methods values are written in table– 03.

Conclusion

Determination of Lamotrigine in branded tablet by three new methods have been proposed. Methods have been found to be selective and accurate also. These are applicable at ng mL^{-1} level. These methods don't need for use of common excipients and additives. So, these may be their additional advantage over the known method.

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References

1. Knopfel, T., Kuhn, R. and Boxer, P.A., *Annu. Rep. Med. Chem*., **29**, 13 (1994).
2. Gastaut, H. and Broughton, R., Thomas, C. (ed), In *Radouco-Anti convulsant Drugs*, Vol. **1**, International Encyclopedia of Pharmacology and Therapentics, N. Y. Pergamon, P–3 (1973).
3. Commission on classification and Terminology of the International League Against Epilepsy, Epilepsia, 22-489 (1981).
4. Gilman, A.G., Hardman, J.G. and Limbird, L.E., *Goodman and Gilman's the pharmacological basis of Therapeutics*, 10th edition (Mc. Graw Hill, N.Y. (USA), 539 (2001).
5. Sean, S., Sweetman, Mahindale, *The complete Drug Reference*, 34th edition, Pharmaceutical Press London, 363 (2005).
6. Matar, K. M., Nicholls, P.J., Tekle, A., Bawazir, S. A. and Hassan, A. L., *J. Pharm Biomed. Anal*., **17**, 525 (1998).
7. Castel Branco, M.M., Gidal, B.E. and Wiebe, D. A., *Ther Drug Monit*, **23**, 665 (2001).
8. Ching-Ling, C., Chou Chem-His and Huo, Y.P., *J. Chromatography*, **B**, **817**, 199 (2005).
9. Saracino, M., Koukopoulos, A., Sani, G., Amore, M. and Raggi, M.A., *Ther. Drug Monit*, **29**, 773 (2007).
10. Queiraj, M.E.C., Silva, S. M., Carvelho, D. and Lancas, F. M., *J. Chromatogr Sci*., **40**, 219 (2002).
11. Patil, K. M. and Bodhankar, S. L., *J. Chromatogr*, **B**, **823**, 152 (2005).
12. Talekar, R. S., Dhake, A. S., Sonaje, D. B. and Mourya, V. K., *Indian. J. Pharm. Sci*., **62**, 51 (2000).
13. Dreassi, E., Corbini, G., Corti, P. and Rocchi, R., *J. AOAC Inti*., ISSN (1060–3271), 1277 (1996).
14. Patil, K. M., Agrawal, A. K. and Bodhankar, S. L., *Indian J. Pharm. Sci*., **66**, 283 (2004).
15. Youssef, N. F. and Taha, E.A., *Chem. Pharm. Bull*., **55**, 541 (2007).
16. Elizabeth, G. S., Daria, R.L., Mohamad, A.V. and Mathew, D.K., *Biomed Chromatogr*, **21**, 225 (2007).
17. Douglas, A. S. and Donald, M.W., *Principles of Instrumental Analysis* (Holts, Rinhart and Winston N.Y.), 104 (1971).
18. Erk, N., *Anal. Lett*., **36**, 1183 (2003).
19. International conference on Harmonisation of Technical requirements for Registration of Pharmaceuticals forttuman use. ICH Harmonised Tripartite Guideline validation of analytical procedures. Text and methodology Q2 (R1), complementary Guideline on methodology dated 06·11·1996 in corporated in London Nov. (2005).

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