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*Original article*

# Sensitive, selective and extraction-free spectrophotometric determination of lamotrigine in pharmaceuticals using two sulphonthalein dyes

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## Abstract:

Two direct, simple, sensitive and rapid extraction-free spectrophotometric methods have been developed for the determination of lamotrigine (LMT) in pure form and in its dosage forms. The methods are based on formation of ion-pair complex between the drug and two sulphonthalein acidic dyes namely, bromothymol blue (method A) and bromocresol green (method B), followed by the measurement of absorbance at 410 nm in both the methods. Conformity to Beer's law enabled the assay of the drug in the range 1-12 μg $mL^{-1}$ in both the methods with apparent molar absorptivities of $2.06 \times 10^4$ and $1.787 \times 10^4$ L $mol^{-1}cm^{-1}$ for method A and method B, respectively. The Sandell sensitivity values, limits of detection (LOD) and quantification (LOQ) values have also been reported for both the methods. The stoichiometry of the ion-pair complexes as evaluated by Job's continuous variations method was 1:1 and the conditional stability constant (log $K_f$) were calculated to be 7.05 and 8.24 for method A and method B, respectively. The accuracy and precision of the methods were evaluated on intra-day and inter-day basis; the relative error (%RE) was < 2.0% and the relative standard deviation (RSD) was < 1.60%. The methods were successfully applied to the determination of drug in tablets without interference by the common coformulated substances. Statistical comparison of the results with the reference method showed good agreement and indicated no significant difference in accuracy and precision.

**Keywords:** Lamotrigine; Extraction free spectrophotometry; Sulphonthalein dye; Ion-pair; Pharmaceuticals

## Introduction

Lamotrigine (LMT), [6-(2,3-dichlorophenyl)-1,2, 4-triazine-3,5-diamine], is an anticonvulsant drug and has been used as antiepileptic to treat epilepsy and bipolar disorder as monotherapy and as an adjunct with other antiepileptics for treatment of partial and generalized toxic-chronic seizures. It is also used to treat neurological lesions and as a tranquilizer [1,2]. Its chemical structure is given in Fig. 1.

LMT is not official in any pharmacopoeia. Chromatographic techniques have been widely employed for the determination of LMT in body fluids. Published methods for the determination of LMT in biological samples include high-performance liquid chromatography (HPLC) [3-10], high-performance thin layer chromatography (HPTLC) [11] and gas-chromatography (GC) [12] and for assay in pharmaceuticals include titrimetry [13], planar chromatography [14], TLC and HPLC [15], HPLC and GC [16] and capillary electrophoresis [17,18] have been reported. Two immunoassay techniques [19,20] have also been developed for determination of lamotrigine in biological samples. One uv-spectrophotometric method [21] was used for determination of LMT in tablets, where the tablet extract in 0.1 M NaOH was measured at 305 nm. Youssef and Taha [15], Rajendraprasad *et al.* [22,23] and Vinay *et al.* [24] have reported the application of visible spectrophotometry for the determination of LMT using chloranilic acid as charge transfer complexing agent, bromophenol blue, bromocresol green and bromocresol purple, as ion-pair reagents. The reported charge transfer complexation method [15] is less sensitive with a linear range 10-200 µg $mL^{-1}$ and the molar absorptivity of 1.28 x $10^3$ l $mol^{-1}$ $cm^{-1}$. Though the method is claimed to be selective, any N-containing basic moiety would definitely interfere with the assay. Whereas the other reported methods [22-24] are ion-pair extractive techniques followed by the extraction of complex into organic phase and measurement of absorbance of the ion-pair complex.

In the authors' laboratory, LMT has been determined by following ion-pair extractive spectrophotometric technique [22-24] using three different sulphonthalen dyes. Though the reported methods seem to be highly sensitive, involve tedious extraction procedure which is one of the demerits of the methods.

Many of the other reported methods [14-18] are sensitive and selective but they are time consuming, require expensive instrumental setup, and some require preliminary sample treatment. Adsorptive stripping voltammetric method [25] is highly complicated and is reported to be less precise (RSD, ~10%). Considering these drawbacks, there was a need to develop more advantageous spectrophotometric method for its determination in bulk powder and commercial dosage forms.

Ion-pair extractive spectrophotometry is commonly used for the assay of pharmaceuticals [26-28] due to its sensitivity and selectivity and has received considerable attention for the quantitative determination of many pharmaceutical compounds including the present drug [22-24, 29-31] and different alkaloids [32,33]. In these cases, an ion-pair is formed between a basic compound and an anionic dye (e.g., bromophenol blue, bromocresol purple, methyl orange, etc.). At a specific pH, the ion-pair, which is immiscible with water, is extracted into an organic solvent and the concentration of the drug is determined spectrophotometrically. Though, ion-pair extractive spectrophotometry has several advantages, it has some difficulties and inaccuracies

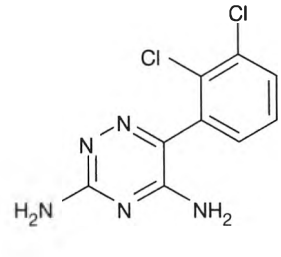



**Figure 1** Chemical structure of lamotrigine (LMT)

arising from incomplete extraction or the formation of emulsions between the organic solvent and the basic compound containing solution. In response to the problem resulting from extraction of the ion-pair, few articles were published for the analysis of pharmaceutical compounds through ion-pair formation without involving extraction [34-37].

In this paper, we describe the application of acidic dyes to the spectrophotometric determination of LMT without the application of buffers. The ion-pair formed between the drug and two sulphonthalein dyes, namely, bromothymol blue (BTB) and bromocresol green (BCG) requires no extraction and is measured directly in dichloromethane. The proposed methods were applied successfully for the determination of LMT either pure or in dosage forms with good accuracy and precision. Also, the methods were demonstrated to be both robust and rugged, and found to be free from interference by coformulated substances when applied to dosage forms.

## Experimental

### *Apparatus*

A Systronics model 106 digital spectrophotometer with 1 cm path length quartz cells was used for absorbance measurements.

### *Chemicals and reagents*

Chemicals used were of analytical reagent grade. Dichloromethane was (spectroscopic grade, Sp. gr. 1.32) purchased from Merck. Stock solutions of bromothymol blue and bromocresol green (both-Qualigens fine chemicals, Mumbai, India) each 0.2% were prepared in dichloromethane. Pure LMT (pharmaceutical grade, 99.88%) sample was kindly provided by Cipla India Ltd, Mumbai, India, as a gift and used as received. Commercial dosage forms used: lamosyn 100 and lamosyn 25 (both from Sun Pharmaceuticals Ltd, Mumbai, India) and Lametec 50-DT (Cipla India Ltd, Mumbai, India)-all tablets were purchased from local commercial sources. A stock standard solution of LMT (100 μg $mL^{-1}$) was prepared by dissolving weighed amount of pure drug in dichloromethane. Stock solution was further diluted to 20 μg $mL^{-1}$ with the same solvent.

### *General procedures*

Into a series of 5 mL calibration flasks, aliquots (0.25-3.0 mL) of lamotrigine standard solution (20 μg $mL^{-1}$) equivalent to 1-12 μg $mL^{-1}$ LMT were accurately transferred, and to each flask 2 mL of 0.2% dye solution (BTB-in method A or BCG-in method B) was added and the mixture was diluted to 5 mL with dichloromethane. After 5 minutes, the absorbance of

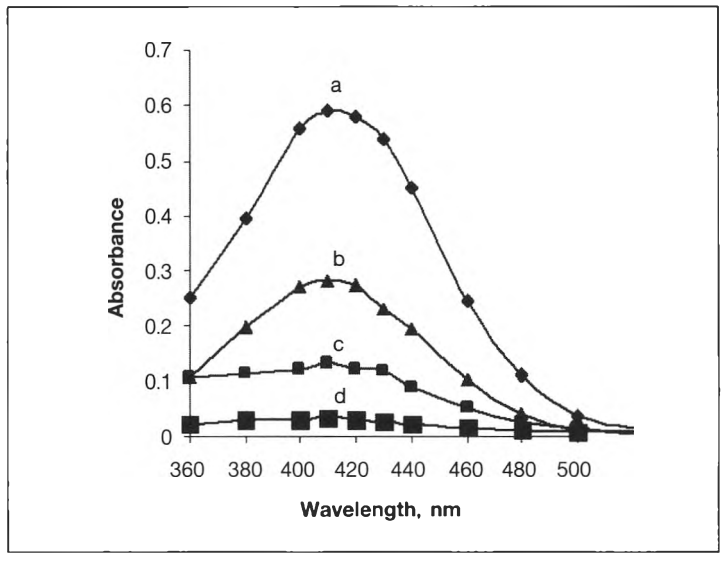



**Figure 2** Absorption spectra of (a) LMT-BCG complex (8.0 μg $mL^{-1}$ LMT), (b) LMT-BTB complex (3.0 μg $mL^{-1}$ LMT), (c) blank for LMT-BCG complex (8.0 μg $mL^{-1}$ LMT) and (d) blank for LMT-BTB complex (3.0 μg $mL^{-1}$ LMT)

the yellow coloured ion-pair complex was measured at 410 nm against the reference blank similarly prepared.

Standard graph was prepared by plotting the absorbance vs drug concentration, and the concentration of the unknown was read from the calibration graph or computed from the respective regression equation derived using the absorbance-concentration data.

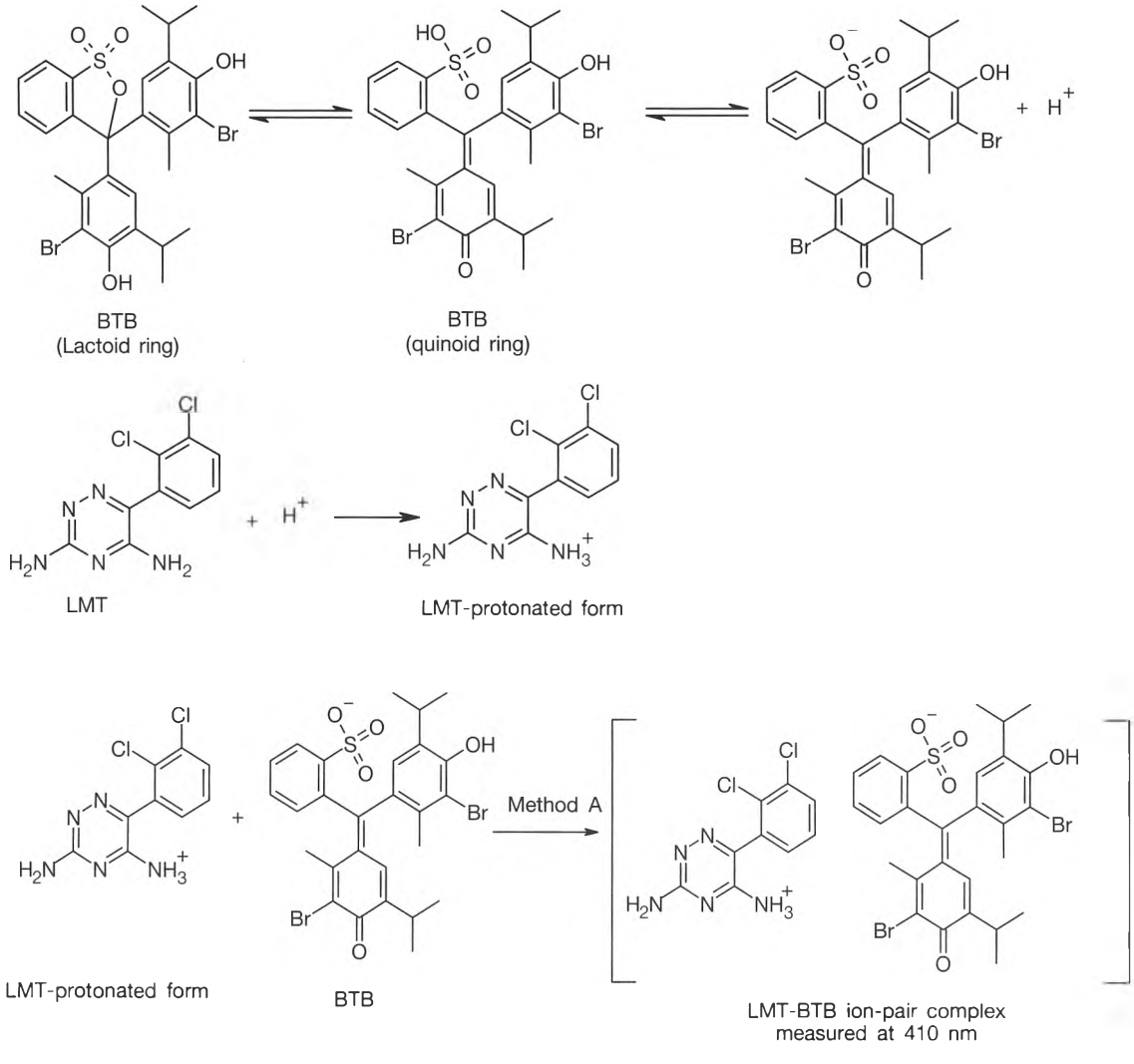
***Procedure for commercial dosage forms***

Weighed amounts of tablet powder equivalent to 10 mg of LMT were transferred into a 100 mL volumetric flask. The content was shaken well with about 50 mL of dichloromethane for 20 min and diluted to the mark with the same solvent. It was filtered using Whatman No. 42 filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot was diluted to get a working concentration of 20 μg $mL^{-1}$ and used for assay by applying procedures described earlier.

## Results and Discussion

***Absorption spectra***

Chemically, the structure of LMT features its basic nature. This structure suggests the possibility of utilizing an anionic dye as chromogenic reagent. In dichloromethane, LMT is not an absorbing species in the visible region. The dye employed has insignificant absorbance (Fig. 2). In contrast, when a solution of BTB/BCG in dichloromethane is added to the drug solution, an intense yellow coloured product is produced immediately. This is due to an opening of lactoid ring and subsequent formation of quinoid group [38]. It




**Scheme 1** Reaction mechanism for the formation of LMT-BTB ion-pair complex

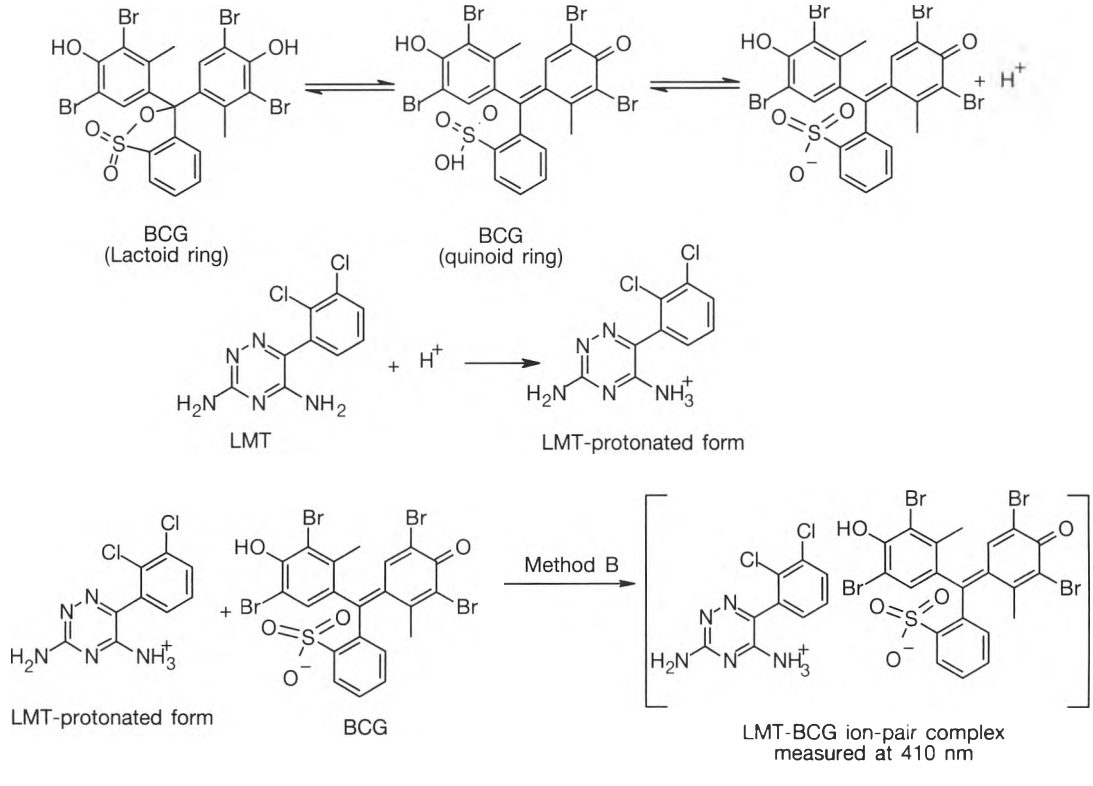
is supposed that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally, protonated LMT forms ion-pair with the dye. The possible reaction mechanisms are shown in Scheme 1 and Scheme 2. Anionic dye such as BTB or BCG forms ion pair complex with positively charged drugs. Each drug-dye ion-pair complex molecule, with two oppositely charged ions, behaves as a single unit held together by an electrostatic force of attraction (Schemes 1 and 2).

***Optimization of variables and method development***

The experimental conditions were studied separately by measuring the absorbance of the final solution resulting from the reaction mixtures containing a fixed concentration of LMT and various amounts of the dyes. It was found that 2 mL of 0.2% dye solution was sufficient to produce maximum and reproducible absorbance (Fig. 3) at 410 nm. The reaction time or standing time after the addition of dye was also examined. It was found that 5 min standing time was sufficient for the complete formation of ion-pair complex. The absorbance of the formed ion-pair complex (in both methods) was observed to be stable for at least 12 h at room temperature.

***Investigation of composition of ion-pair complex***

The composition of the ion-pair complex was established by Job's method of continuous variations [39] using equimolar concentrations of the drug and the dye (1.953 x $10^{-4}$ M in method A and 6.248 x $10^{-5}$ M in method B). The results indicated that 1:1 (drug:dye) ion-pair is formed through the electrostatic attraction between the positive protonated drug and the anion of dye. Six solutions containing LMT and the dye in various molar ratios, with a total volume of 2.5 mL and 5.0 mL in method A and method B, respectively were prepared. The volume was made up to 5 mL in method A using dichloromethane.




**Scheme 2** Reaction mechanism for the formation of LMT-BCG ion-pair complex

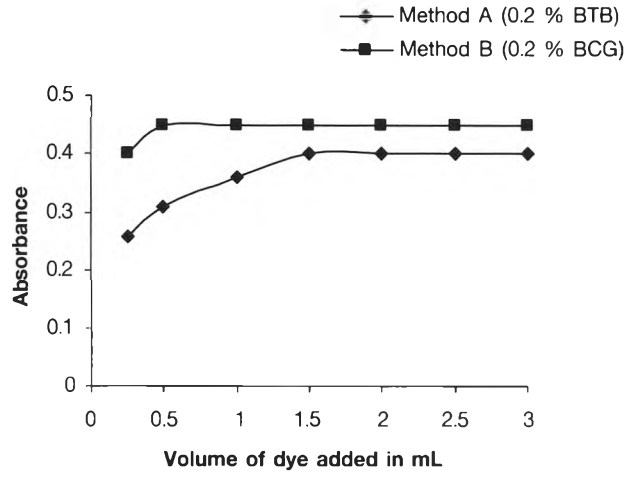



**Figure 3** Effect of dye on the formation of ion-pair complex in method A (5.0 μg $mL^{-1}$ LMT) and method B (6.5 μg $mL^{-1}$ LMT)

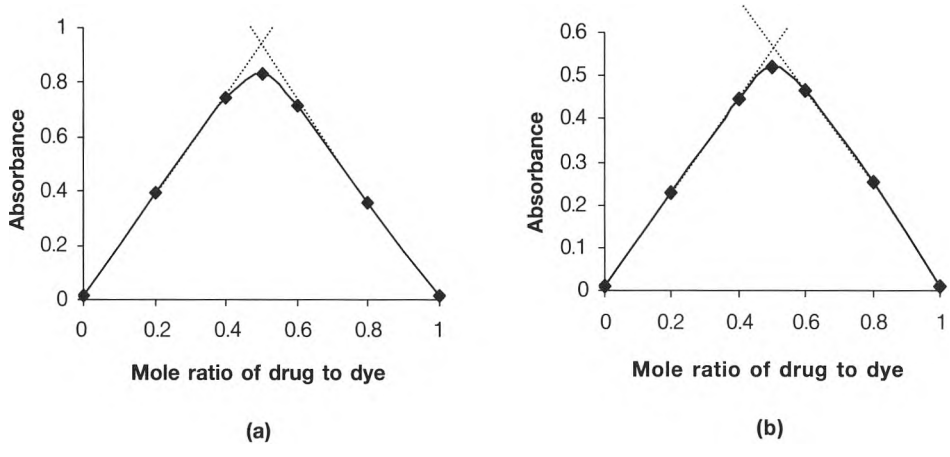



**Figure 4** Job's method of continuous variation of : (a) LMT-BTB (drug/dye: 1.953 x $10^{-4}$ M) and (b) LMT-BCG (drug/dye: 6.248 x $10^{-5}$ M)

The absorbance of all solutions was subsequently measured at 410 nm. The graph of the results obtained (Fig. 4) gave a maximum at a molar ratio of Xmax = 0.5 in both the methods which indicated the formation of a 1:1 LMT:BTB/BCG complex. The conditional stability constant ($K_f$) of the ion-association complex was calculated from the continuous variation data using the following equation [40]:

$$K_f = \frac{A / A_m}{[1 - A / A_m]^{n+2} C_M (n)^n} \quad (1)$$

where Am and A are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. $C_M$ is the mole concentration of drug at the maximum absorbance and n is the stoichiometry which BTB/BCG ion associates with drug. The log $K_f$ values were found to be 7.05 and 8.24 for method A and method B, respectively.

### *Method validation*

**Linearity, sensitivity, limits of detection and quantification**

A linear correlation was found between absorbance at $\lambda_{max}$ and concentration of LMT in the ranges given in Table 1. The graphs are described by the regression equation: Y = a + bX, where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in μg $mL^{-1}$. Regression analysis of the

Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system and the values are presented in Table 1. A plot of *log* absorbance and *log* concentration, yielded straight lines with slope equal to 0.996 and 1.009 for method A and method B, respectively, further establishing the linear relation between the two variables. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values [41] of all the three methods are also given in Table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [42] using the formulae: LOD = 3.3 S/b and LOQ = 10 S/b, (where S is the standard deviation of blank absorbance values, and b is the slope of the calibration plot) are also presented in Table 1. The high values of ε and low values of Sandell sensitivity and LOD indicate the high sensitivity of the proposed methods.

**Precision and accuracy**

The assays described under "general procedures" were repeated seven times within the day to determine the repeatability (intra-day precision) and five times on different days to determine the intermediate precision (inter-day precision) of the methods. These assays were performed for three levels of analyte. The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were ≤ 1.56% (intra-day) and ≤ 1.60% (inter-day) indicating high precision of the methods. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and taken concentrations for LMT. Bias, bias % = (concentration found-known concentration) x 100/ known concentration, was calculated at each concentration and these results are also presented in Table 2. Percent relative error (%RE) values of ≤ 2.05% demonstrate the high accuracy of the proposed methods.

**Selectivity**

A systematic study was performed to determine the effect of matrix by analyzing the placebo blank and synthetic mixture containing LMT. A placebo blank of the composition: starch (10 mg), acacia (15 mg), hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg) and sodium

**Table 1** Sensitivity and regression parameters

| Parameter | Method A | Method B |
|---|---|---|
| $\lambda_{max}$, nm | 410 | 410 |
| Color stability | > 12 h | > 12 h |
| Linear range, μg $mL^{-1}$ | 1-12 | 1-12 |
| Molar absorptivity (ε), L $mol^{-1}$ $cm^{-1}$ | 2.06 x $10^4$ | 1.787 x $10^4$ |
| Sandell sensitivity*, μg $cm^{-2}$ | 0.0124 | 0.0143 |
| Limit of detection (LOD), μg $mL^{-1}$ | 0.17 | 0.16 |
| Limit of quantification (LOQ), μg $mL^{-1}$ | 0.50 | 0.48 |
| Regression equation, Y** | | |
| Intercept (a) | 0.0012 | -0.0179 |
| Slope (b) | 0.0805 | 0.0738 |
| Standard deviation of a ($S_a$) | 0.0998 | 0.0998 |
| Standard deviation of b ($S_b$) | 0.0084 | 0.0084 |
| Variance ($S_a^2$) | 0.0099 | 0.0099 |
| Regression coefficient (r) | 0.9994 | 0.9990 |

*Limit of determination as the weight in μg per mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 $cm^2$ and l = 1 cm.

**Y=a+bX, Where Y is the absorbance, X is concentration in μg $mL^{-1}$, a is intercept, b is slope

**Table 2** Evaluation of intra-day and inter-day accuracy and precision

| Method | LMT taken, μg mL$^{-1}$ | Intra-day accuracy and precision (n=7) | | | Inter-day accuracy and precision (n=5) | | |
|---|---|---|---|---|---|---|---|
| | | LMT found ± CL, μg mL$^{-1}$ | %RE | %RSD | LMT found ± CL, μg mL$^{-1}$ | %RE | %RSD |
| A | 4.0 | 4.08 ± 0.03 | 2.00 | 0.73 | 3.98 ± 0.05 | 0.50 | 1.02 |
| | 8.0 | 7.93 ± 0.08 | 0.88 | 1.03 | 8.01 ± 0.12 | 0.13 | 1.23 |
| | 12.0 | 12.1 ± 0.14 | 0.83 | 1.26 | 11.96 ± 0.20 | 0.33 | 1.33 |
| B | 2.0 | 1.98 ± 0.03 | 1.00 | 1.56 | 2.03 ± 0.04 | 1.50 | 1.60 |
| | 6.0 | 5.93 ± 0.08 | 1.20 | 1.44 | 6.04 ± 0.10 | 0.67 | 1.23 |
| | 10.0 | 10.11 ± 0.06 | 1.10 | 0.68 | 9.99 ± 0.17 | 0.10 | 1.35 |

%RE = Percent relative error, %RSD = relative standard deviation and CL = confidence limits were calculated from: CL = ± tS/$\sqrt{n}$ (The tabulated value of t is 2.45 and 2.77 for six and four degrees of freedom respectively, at the 95% confidence level; S = standard deviation and n = number of measurements)

**Table 3** Method robustness and ruggedness expressed as intermediate precision (%RSD)

| Method | LMT taken, μg mL$^{-1}$ | Robustness | | Ruggedness | |
|---|---|---|---|---|---|
| | | Parameters altered* | | | |
| | | Volume of dye (%RSD) | Reaction time (%RSD) | Inter-instruments (%RSD) (n=4) | Inter-analysts (%RSD) (n=4) |
| A | 6.0 | 1.02 | 1.23 | 0.88 | 1.65 |
| B | 8.0 | 0.96 | 1.53 | 0.99 | 1.58 |

*The volumes of dye were 1.75 and 2.0 mL; and the reaction times were 3, 5 and 7 min.

alginate (10 mg) was made and its solution was prepared as previously described, and then subjected to analysis. The absorbance of the placebo solution in each case was almost equal to the absorbance of the blank which revealed no interference. To assess the role of the inactive ingredients on the assay of LMT, a synthetic mixture was separately prepared by adding 10 mg of LMT to the placebo mentioned above. The drug was extracted and solution prepared as described under the general procedure for tablets. The solutions after appropriate dilution were analysed following the recommended procedures. The absorbance resulting from 8 μg mL$^{-1}$ (in both the methods) was nearly the same as those obtained for pure LMT solutions of identical concentrations. This unequivocally demonstrated the non-interference of the inactive ingredients in the assay of LMT. Further, the slopes of the calibration plots prepared from the synthetic mixture solutions were about the same as those prepared from pure drug solutions.

**Robustness and ruggedness**

The robustness of the methods was evaluated by making small incremental changes in the volume of dye and contact time, and the effect of the changes was studied on the absorbance of the colored systems. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as %RSD (≤ 1.53%). Method ruggedness was demonstrated having the analysis done by four analysts, and also by a single analyst performing analysis on four different instruments in the same laboratory. Intermediate precision values (%RSD) in both instances were in the range 0.88-1.65% indicating acceptable ruggedness. The results are presented in Table 3.

**Table 4** Results of analysis of tablets by the proposed methods and statistical comparison of the results with the reference method

| Tablet brand name[Ψ] | Nominal amount, (mg/tablet) | Found* (Percent of label claim ± SD) | | |
|---|---|---|---|---|
| | | Reference method | Proposed methods | |
| | | | Method A | Method B |
| Lamosyn-100[a] | 100 | 98.58 ± 0.64 | 99.14 ± 1.12<br>t = 1.01<br>F = 3.06 | 97.94 ± 0.76<br>t = 1.44<br>F = 1.41 |
| Lamosyn-25[a] | 25 | 101.30 ± 0.52 | 102.10 ± 1.24<br>t = 1.44<br>F = 5.69 | 100.80 ± 1.05<br>t = 1.01<br>F = 4.08 |
| Lametec-50 DT[b] | 50 | 99.36 ± 0.76 | 100.40 ± 1.57<br>t = 1.41<br>F = 4.27 | 98.78 ± 1.46<br>t = 0.83<br>F = 3.69 |

*Mean value of 5 determinations.

Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77 and tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39

[Ψ]Marketed by : [a]Sun pharmaceuticals. [b]Cipla India Ltd. Mumbai

**Table 5** Results of recovery study *via* standard-addition method

| Tablet studied | Method A | | | | Method B | | | |
|---|---|---|---|---|---|---|---|---|
| | LMT in tablet, ($\mu g\ mL^{-1}$) | Pure LMT added, ($\mu g\ mL^{-1}$) | Total found, ($\mu g\ mL^{-1}$) | Pure LMT recovered (Percent ± SD*) | LMT in tablet, $\mu g\ mL^{-1}$ | Pure LMT added, $\mu g\ mL^{-1}$ | Total found, $\mu g\ mL^{-1}$ | Pure LMT recovered (Percent ± SD*) |
| Lamosyn -100 | 3.97 | 2.0 | 5.98 | 100.30 ± 1.16 | 3.92 | 2.0 | 5.87 | 97.56 ± 0.72 |
| | 3.97 | 4.0 | 7.91 | 98.56 ± 0.98 | 3.92 | 4.0 | 7.98 | 101.50 ± 1.34 |
| | 3.97 | 6.0 | 10.07 | 101.60 ± 1.24 | 3.92 | 6.0 | 10.11 | 103.10 ± 1.11 |

*Mean value of three determinations

### *Application*

The proposed methods were applied for the quantification of LMT in commercial tablets. The results were compared with these obtained using a published method [21]. The method consisted of the measurement of the absorbance of the tablet extract in 0.1 M NaOH at 305 nm. Statistical analysis of the results did not detect any significant difference between the performance of the proposed methods and reference method with respect to accuracy and precision as revealed by the Student's t-value and variance ratio F-value [43]. The results of assay are given in Table 4.

### *Recovery study*

To further assess the accuracy of the methods, recovery experiments were performed by 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 LMT at three different 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 percentage

values ranged between 97.56 and 103.10% with standard deviation in the range 0.72-1.24%. Closeness of the results to 100% showed the fairly good accuracy of the methods. The results are shown in Table 5.

## Conclusion

Two spectrophotometric methods for the determination of lamotrigine in bulk drug and in pharmaceutical dosage forms were developed and validated for accuracy, precision, linearity, robustness and ruggedness. The methods employ normal conditions than those previously reported, and rely on well-characterized ion-pair formation reactions. Besides, the methods have the advantages of simplicity without involving heating or extraction step and high sensitivity. The proposed methods are rapid, simple and in addition, offer advantages in determining LMT, (in pharmaceutical preparations), when extraction difficulties arise with other spectrophotometric methods. Hence, the proposed methods could be adopted for quality control in pharmaceutical industries.

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