Indian Journal of Chemical Technology
Vol. 17, May 2010, pp. 220-228

Micro and nanogram determination of lamotrigine in pharmaceuticals by visible spectrophotometry using bromophenol blue

N Rajendraprasad, K Basavaiah* & K B Vinay

Department of Chemistry, University of Mysore, Manasagangothri, Mysore 570 006, India
Email: basavaiahk@yahoo.co.in

Received 10 August 2009; revised 8 March 2010

Three reliable, rapid, highly sensitive and selective methods have been developed and validated for the determination of lamotrigine (LMT) in pure drug and in tablets. The first method (method A) is based on the formation of chloroform extractable ion-pair complex between LMT and bromophenol blue (BPB) at pH 1.44±0.01 with a wavelength of maximum absorption at 420 nm. In the second (method B) and third (method C) methods, the drug-dye ion pair is dissolved in either ethanolic H_2SO_4 and resulting acid form of the dye is measured at 420 nm or ethanolic KOH and the resulting base form of the dye is measured at 600 nm. All variables affecting the drug-dye complex formation and its extraction into $CHCl_3$ have been investigated and conditions optimized. Beer's law was obeyed over 2.5-25 μg mL^{-1}, 50-400 ng mL^{-1} and 10-80 ng mL^{-1}, for method A, method B and method C, respectively. The calculated molar absorptivity values are 7.26×10^3, 5.4×10^5 and 2.6×10^6 l mol^{-1} cm^{-1}, respectively, for methods A, B and C; and the corresponding Sandell sensitivities are 0.0353, 0.0005 and 0.0001 μg cm^{-2}. The limits of detection (LOD) and quantification (LOQ) have also been reported. The stoichiometry of the formed ion-pair complex was found to be 1:1.for method A, and the stability constant is also calculated. The accuracy and precision of the methods were evaluated on intra-day and inter-day basis; and the relative error (RE) and the relative standard deviation (RSD) were ≤ 2.0% and ≤ 1.4%, respectively. The proposed methods were successfully applied for the determination of LMT in bulk powder and in tablets.

Keywords: Lamotrigine, Assay, Spectrophotometry, Ion-pair complex, Bromophenol blue, Pharmaceuticals

Lamotrigine (LMT), chemically known as [6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine], is a broad spectrum antiepileptic, used as monotherapy and as an adjunct with other antiepileptics for treatment of partial and generalized toxic-clonic seizures. Its use as a tranquilizer and in the treatment neurological lesions has also been studied[1,2].

LMT is not official in any pharmacopoeia. Previous studies have reported the determination of LMT, most of them employing high-performance liquid chromatography (HPLC) with uv-detector and focusing on its quantification in biological fluids[3-12]. LMT in human plasma has been assayed using solid phase microextraction (SPME) and gas chromatography with thermionic specific detection[13] whereas in serum it has been determined by high performance thin layer chromatography[14].

However, literature on the methods for the determination of LMT in pharmaceuticals is scanty. Talekar *et al.*[15] have reported the development of a UV-spectrophotometric method for the determination of LMT in bulk drug and in tablets. A method using planar chromatography was developed for determining LMT in human plasma and tablets by Dreassi *et al.*[16]. Separation of LMT by HPTLC followed by quantification by densitometry at 312 nm has been reported by Patil *et al.*[17]. Youssef and Taha[18] have reported three methods for the assay of LMT in bulk powder, in dosage form and in the presence of its impurity, 2,3-dichlorobenzoic acid. The first method is based on the measurement of red charge-transfer complex at 520 nm formed by LMT with chloranilic acid, a π-acceptor. The second method is based on TLC separation of the cited drug followed by densitometric measurement of the intact drug at 275 nm. The third stability indicating HPLC method based on the separation of LMT from its impurity on a reversed phase C_{18} column, using a mobile phase of CH_3CN-MeOH-0.01 M potassium orthophosphate (pH 6.7±0.01) (30:20:50, *v/v/v*) and UV-detection at 275 nm has also been described.

Determination of LMT in pharmaceutical preparations[19] has also been achieved by differential pulse adsorptive stripping voltammetry using carbon screen-printed electrodes (SPE) and mercury coated carbon-screen-printed electrodes. Simultaneous

analysis of LMT, oxcarbazepine and zonisamide by HPLC with uv detection has very recently been reported by Elizabeth *et al.*[20].

Although some of the reported methods are sensitive and selective but they are time consuming, require expensive instrumental setup, and some require preliminary sample treatment. The adsorptive stripping voltammetric method[20] is highly complicated and is reported to be less precise (RSD ~10%).

The aim of the present work was to develop and validate sensitive and selective methods for the determination of LMT in different dosage forms. The developed 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. Besides, the drug-dye ion pair complex was dissociated either in acid medium and the acid form of the dye measured at 420 nm or in alkaline medium and the base form of the dye was measured at 600 nm. The latter methods provide extremely sensitive approaches for the determination of LMT in bulk drug and in tablets.

Experimental Procedure

Absorbance measurements were made with a Systronics model 106 digital spectrophotometer equipped with 1 cm matched quartz cells. An Elico 120 digital pH meter was used for pH measurements.

Reagents and Solutions

All chemicals used were of analytical reagent grade and distilled water was used throughout the investigation. Spectroscopic grade organic solvents were used. Sulphuric acid (0.1 M) was prepared by successive dilutions of appropriate volume of concentrated acid (S.D. Fine Chem, Mumbai, India, sp. gr. 1.84) in water. Bromophenol blue (0.5%) was prepared by dissolving 500 mg of the dye (Ranbaxy Fine Chem Ltd., Mumbai, India) in 10 mL of ethanol, and diluted to 100 mL with water. Ethanolic sulphuric acid (1%) was prepared by dissolving 1 mL of concentrated acid (18 M) in 100 mL of ethanol. Ethanolic potassium hydroxide (1%) was prepared by dissolving 1 g of the chemical (S.D. Fine Chem, Mumbai, India) in 100 mL of ethanol.

Phramaceutical grade lamotrigine (LMT) was procured from Cipla India Ltd, Mumbai, India, as a gift, and was used as received. A stock standard solution containing 200 µg mL^{-1} LMT was prepared by dissolving 20 mg of pure LMT in 0.1 M H_2SO_4 in a 100 mL volumetric flask and diluted to the mark with the same acid. The stock solution was appropriately diluted to 50 µg mL^{-1} with the same acid.

General procedures

Method A (Based on the measurement of drug-dye ion-pair)

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 µg mL^{-1}) LMT solution were transferred into a series of 125 mL separating funnels and the total volume was brought to 5.0 mL by adding 0.1 M H_2SO_4. Twenty mL of water and 5 mL of 0.5% BPB were added to each separating funnels and the contents were mixed well. After 5 min, 10 mL of chloroform was added by means of microburette and shaken vigorously for 30s. 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 LMT-BPB ion-pair complex

5 mL of LMT solution (50 µg mL^{-1}) was pipetted out into a 125 mL separating funnel and 20 mL of water was added followed by the addition of 5 mL of 0.5% BPB dye. The contents were mixed well. After 5 min 10 mL of $CHCl_3$ was added, and the mixture was shaken vigorously for 30 s to extract the LMT-BPB 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 (10 µg mL^{-1} in LMT) solution was diluted to 1 µg mL^{-1} and 0.2 µg mL^{-1} with $CHCl_3$ for use in method B and method C, respectively.

Method B (Based on the measurement of acid form of the dye)

Varying aliquots (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mL) of LMT-BPB complex (1.0 µg mL^{-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, 1 mL of ethanolic H_2SO_4 was added. The contents were mixed and allowed to stand for 5 min and diluted to 10 mL with ethanol. After mixing well, the absorbance was measured at 420 nm against the reagent blank.

Method C (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 LMT-BPB complex (0.2 µg mL^{-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, 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 well, the absorbance was measured at 600 nm against the reagent blank.

In all the three methods, standard graph was prepared by plotting the absorbance versus 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 tablets

Lamosyn 100 and Lamosyn 25 (both Sun Pharmaceuticals) and Lametec 50 DT (Cipla India Ltd, Mumbai, India)-all tablets, were used in the investigation. Twenty tablets were weighed and ground into a fine powder. An amount of finely ground tablet powder equivalent to 5.0 mg of LMT was added into a 100 mL calibrated flask, 50 mL of 0.1 M H_2SO_4 was added, and the flask was shaken for 20 min; and finally made upto the mark with the same 0.1 M H_2SO_4. The content was kept aside for 5 min, and filtered using Whatmann No 42 filter paper. First 10 mL portion of the filtrate was discarded and a suitable aliquot (say 3 mL) was used for assay by method A. The ion-pair complex (10 µg mL^{-1} in LMT) prepared above, was diluted to obtain 1.0 µg mL^{-1} and 0.2 µg mL^{-1} complex solutions for assay by applying the procedures described for method B and method C, respectively.

Results and Discussion

The drug lamotrigine reacted with bromophenol blue, an anionic dye, in acidic condition (pH 1.44±0.01) to form a yellow coloured ion-pair complex which was quantitatively extracted into chloroform in method A. The ion-pair complex with BPB absorbed maximally at 420 nm. The drug-dye ion-pair was found to break and dissolve in ethanolic H_2SO_4 or ethanolic KOH medium yielding the free dye in protonated form (λmax 420 nm) or deprotonated form (λmax 600 nm) under the optimum conditions described, the net pH in the acid medium was measured to be 1.69±0.05 (n=3) whereas that in alkaline medium was 13.94±0.07 (n=3). The blanks had negligible absorbance in all the three instances. Figure 1 shows the absorption spectra of BPB-LMT complex, and of the undissociated and dissociated forms of the dye. The probable reactions showing the formation of ion-pair and its breaking are shown in schemes 1 and 2.

Optimum reaction conditions necessary for rapid and quantitative formation of coloured products with maximum stability and sensitivity were established via various preliminary experiments. In method A, by keeping the concentration of LMT fixed, effects of pH, volume of aqueous phase, volume of dye, different extractive 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 method B and method C, to a fixed concentration of LMT-BPB ion-pair complex, different volumes of ethanolic H_2SO_4/ethanolic KOH were added and the resulting acid and base form of the dye was measured at 420 nm (method B) or 620 nm (method C). The effect of time on breaking the ion-pair complex was similarly studied.

Method development: Optimization of experimental variables

Method A

LMT is not soluble in water. The investigations were done by dissolving LMT in different acids like HCl, H_3PO_4, H_2SO_4 and acetic acid. The solubility of LMT and the sensitivity of the methods are better in 0.1 M H_2SO_4 than the rest of the acids. Therefore H_2SO_4 has been selected as the solvent. In order to establish the optimum pH range, 5 mL LMT 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

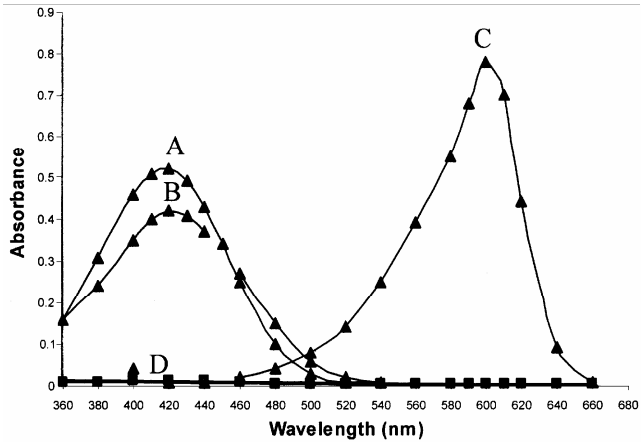
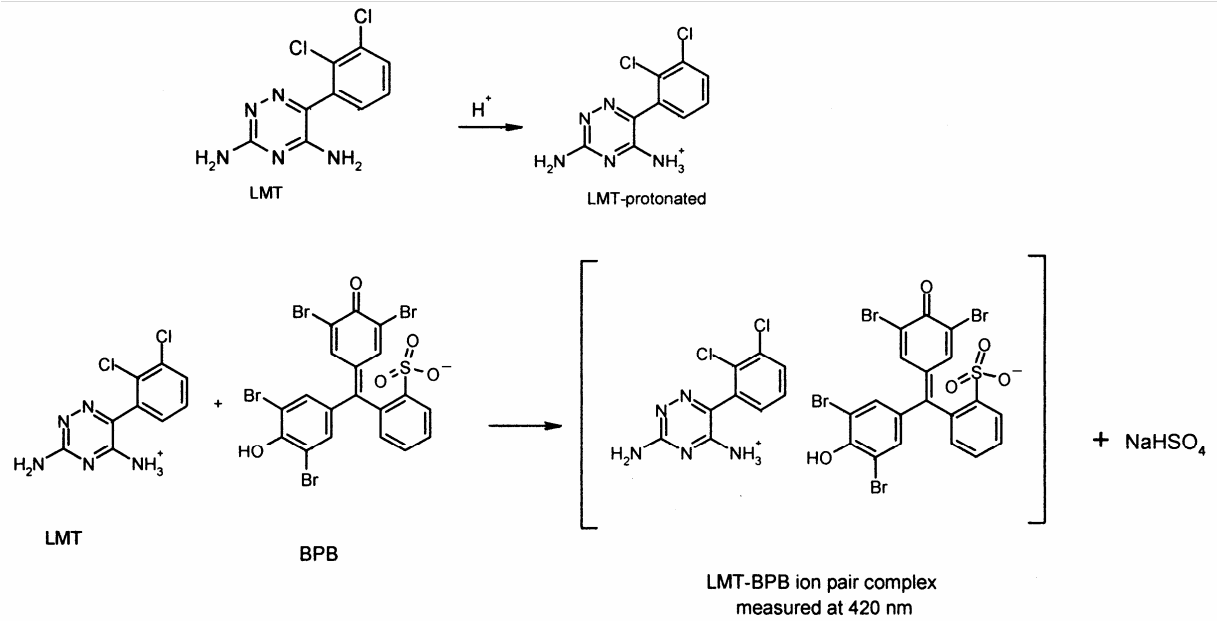
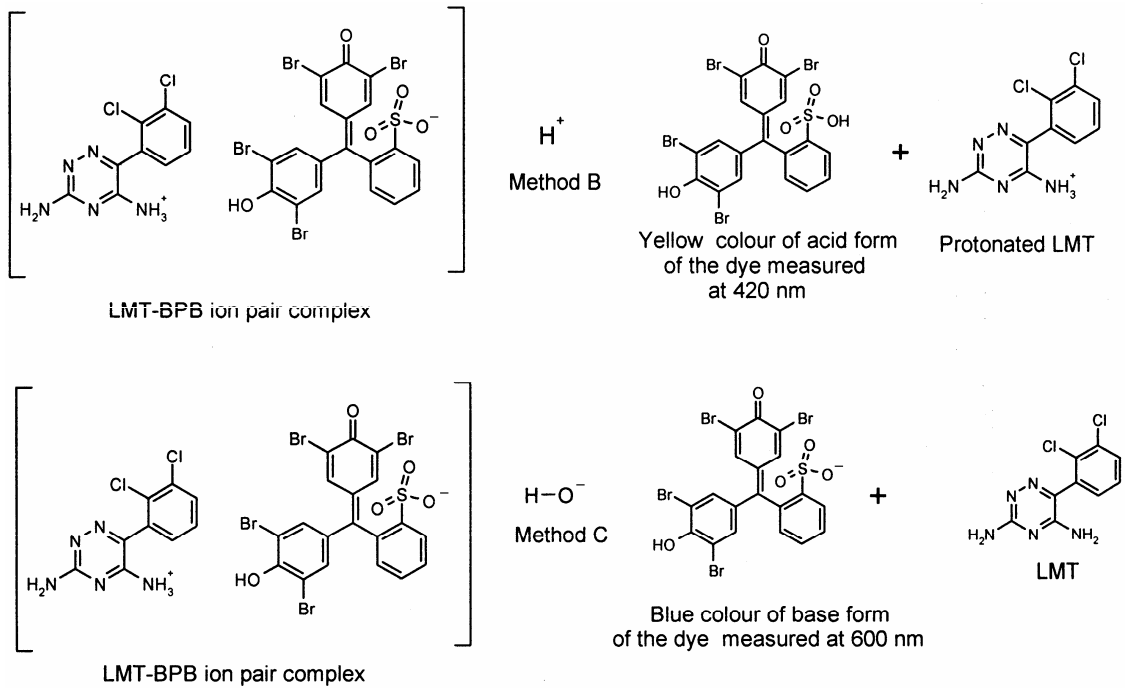


Fig. 1—Absorption spectra: A, ion-pair complex (15 µg mL^{-1} LMT); B, acid form of dye (200 ng mL^{-1} LMT); C, base form of dye (80 ng mL^{-1} LMT) and D, blank.



Scheme 1— Formation of drug-dye ion-pair.



Scheme 2— Formation of acidic and basic forms of the dye.

ranged from 1.38 to 1.50. The absorbance remained constant 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 unaffected 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 preliminary experiments dichloromethane, benzene, carbon tetrachloride, cyclohexane and hexane were tried as extraction solvents. None of

these systems showed better results than chloroform. Hence, chloroform was chosen as the solvent for extraction. The volume of aqueous phase was varied by keeping all the parameters constant, from 15 to 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.

Shaking time ranging from 30 to 60 s produced no change in absorbance, by maintaining all other parameters constant. So a 30 s shaking time was selected. Under optimum conditions, the drug-dye complex in the aqueous phase was extracted with three 10 mL portions of chloroform and absorbance was measured each 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 about 20 h at laboratory temperature (30±2ºC). The sequence of order of addition of the reactants prior to extraction had no effect on the complex formation, extraction and the absorbance values.

The complexing ratio of LMT and BPB in method A was examined by Job's method of continuous variations[21]. The concentration of the aqueous dye and the drug was 7.46×10^{-4} M. Six solutions containing LMT and BPB in various molar ratios, with a total volume of 5 mL, in addition to 20 mL H_2O were prepared. The extraction was performed using 10 mL of chloroform and the absorbance subsequently was measured at 420 nm. The plot of the results obtained (Fig. 2) gave a maximum at a molar ratio of X_{max}= 0.5 which indicated the formation of a 1:1 (LMT:BPB) complex. The conditional stability constant (K_f) of the ion-association complex was calculated from the continuous variation data using the following Eq.[22]:

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

where A and A_m are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_M is the molar concentration of drug at the maximum absorbance and n is the stoichiometry with which BPB ion associates with drug. The log K_f value was found to be 4.83.

Method B

Preliminary experiments were performed to determine the ethanolic acid concentration which would give a reasonable maximum absorbance at 420 nm and this was found to be 1 mL of 1%. Hence, different concentrations of LMT-BPB complex were treated with 1 mL of 1% alcoholic H_2SO_4, and after the contact time elapsed, the absorbance of the dye was measured and related to drug concentration.

The complex breaking time was studied by adding 1 mL of ethanolic H_2SO_4 to a fixed amount of LMT-BPB complex taken in a series of 10 mL volumetric flasks. After some interval of time ranging from 5 to 30 min the solution was made up to mark with ethanol and the absorbance measured at 420 nm. There was no change in absorbance values between the time ranges 5-30 min. Hence, 10 min was fixed as a contact time. The yellow dye was stable for more than about 24 h at laboratory temperature (30±2ºC).

Method C

A series of measurements were made to fix the amount of alkali (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 LMT-BPB complex. The absorbance values were almost constant at the alkali amount ranging from 1 to 5 mL in a total volume of 10 mL. Hence, different concentrations of LMT-BPB complex were treated with 1 mL of 1% alcoholic KOH, and after the contact time elapsed, the absorbance of the dye was measured and related to

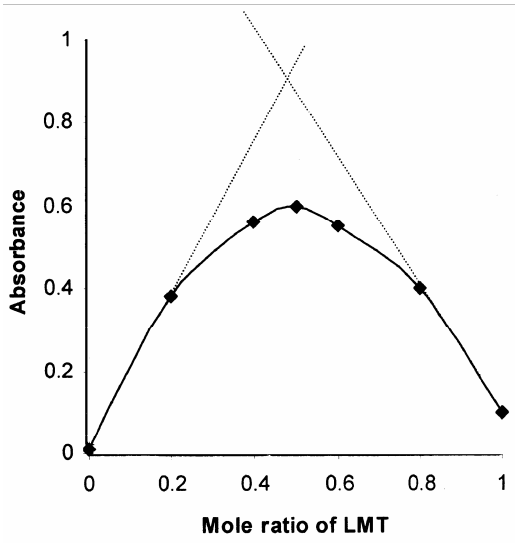


Fig. 2— Job's plot for LMT-BPB ion-pair complex.

complex concentration. The complex breaking time was studied by adding 1 mL of ethanolic KOH to a fixed amount of LMT-BPB complex. The mixture was diluted up to mark with ethanol and the absorbance measured (600 nm) at different time intervals. There was no change in absorbance values between the times ranging from 5 to 30 min. Hence, 10 min was fixed as a contact time. The blue coloured dye was stable for at least 24 h at laboratory temperature (30±2ºC).

Method validation

Linearity, sensitivity, limits of detection and quantification

Calibration graphs were constructed from ten, eight and eight points covering the concentration ranges 2.5-25 µg mL^{-1}, 50-400 ng mL^{-1} and 10-80 ng mL^{-1} for methods A, B and C, respectively. Regression analysis of the Beer's law data indicated a linear relationship between absorbance and concentration, (Table 1) which is corroborated by high values (close to unity) of the correlations coefficients. A plot of log absorbance versus log concentration in each case produced a straight line with a slope close to unity i.e., 1.03, 0.9986 and 0.9957 for methods A, B and C, respectively; further establishing the linear relationship between the two variables.

The calculated molar absorptivity and Sandell sensitivity[23] values are summarized in Table 1. The limits of detection (LOD) and quantification (LOQ), calculated according to the ICH[24] guidelines are also summarized 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 percentage relative standard deviation (%RSD) values were ≤ 1.4% (intra-day) and ≤ 1.2% (inter-day) indicating high precision of the methods. The accuracy of the methods was determined by the percent mean deviation from known concentration, bias% = [(Concentration found- known concentration) × 100/known concentration]. Bias was calculated at each concentration. The percent relative error (%RE) values ≤ 2.5% 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),

Table 1—Sensitivity and regression parameters.

Parameter	Method A	Method B	Method C
λ_{max}, nm	420	420	600
Colour stability, min.	> 20 h	> 24 h	> 24 h
Linear range	2.5-25 µg mL^{-1}	50-400 ng mL^{-1}	10-80 ng mL^{-1}
Molar absorptivity(ε), L mol^{-1} cm^{-1}	7.26 x 10^3	5.4 x 10^5	2.6 x 10^6
Sandell sensitivity*, µg cm^{-2}	0.0353	0.0005	0.0001
Limit of detection (LOD), µg mL^{-1}	0.15	0.003	0.00055
Limit of quantification (LOQ), µg mL^{-1}	0.47	0.01	0.0017
Regression equation, Y**			
Intercept (a)	-0.045	0.0071	-0.0004
Slope (b)	0.0326	2.0738	10.2024
Standard deviation of a (S_a)	0.0998	0.0998	0.0998
± $tS_a/\sqrt{n}$	0.0713	0.0830	0.0830
Standard deviation of b (S_b)	0.00631	0.3117	1.559
± $tS_b/\sqrt{n}$	0.0005	0.2600	1.3030
Variance (S_a^2)	0.010	0.010	0.010
Regression coefficient (r)	0.9992	0.9998	0.9999

*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, ± $tS_a/\sqrt{n}$ = confidence limit for intercept, ± $tS_b/\sqrt{n}$ = confidence limit for slope.

hydroxyl cellulose (10 mg), sodium citrate (10 mg), talc (20 mg), magnesium stearate (15 mg) and sodium alginate (10 mg) was made and its solution was prepared as described under tablets, 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 20 mg of LMT to the placebo mentioned above. The drug was extracted and solution prepared as described under the general procedure for tablets. The solution after appropriate dilution were analysed following the recommended procedures. The absorbance resulting from 15 μg mL^{-1} (method A), 200 ng mL^{-1} (method B) and 50 ng mL^{-1} (method C) were 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 two selected variables (volume of H_2O and reaction time in method A, volume of ethanolic H_2SO_4/ethanolic KOH and the breaking times in method B and method C) and the effect of the changes was studied on the absorbance of the coloured systems. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as% RSD. These results are summarized in Table 2.

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 in both instances were in the range 1.5-2.5% indicating acceptable ruggedness. These results are summarized in Table 2.

Application

The proposed methods are applied for the quantification of LMT in commercial tablets. The results were compared with these obtained using a published method[15]. 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[25]. The results of assay are given in Table 3.

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

Table 2—Method robustness and ruggedness expressed as intermediate precision (% RSD)

Method	LMT taken*	Robustness		Ruggedness	
		Parameters altered			
		Volume of H_2O/Ethanolic H_2SO_4/Ethanolic KOH**	Reaction/Breaking time$^{\Psi}$	Inter-analysts (%RSD), (n=4)	Inter-instruments (%RSD), (n=4)
	7.5	1.76	1.26	1.60	2.50
A	15.0	1.82	1.74	1.51	1.50
	22.5	0.97	1.32	1.98	2.30
	150	1.06	1.01	1.88	1.73
B	250	1.33	0.88	1.62	1.85
	350	1.18	0.98	1.50	1.99
	30.0	0.99	0.97	1.65	1.65
C	50.0	1.05	1.04	1.95	1.86
	70.0	0.95	1.10	1.63	1.69

*The values are in μg mL^{-1} in method A and ng mL^{-1} in method B and method C.

**In method A, the volume of H_2O was 18, 20 and 22 mL, in method B the volumes of ethanolic H_2SO_4 added were 0.8, 1.0 and 1.2 mL and in method C the volumes of ethanolic KOH added were 0.8, 1.0 and 1.20 mL.

$^{\Psi}$In method A, the reaction times were 3, 5 and 7 min and in method B and method C the breaking times were 3, 5 and 7 min.

Table 3—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	Method A	Method B	Method C
Lamosyn-100[a]	100	103.6±0.52	104.1±1.26 t=0.89 F=5.87	102.8±0.96 t=1.71 F=3.41	103.9±0.99 t=0.63 F=3.62
Lamosyn-25[b]	25	97.48±0.75	98.73±1.35 t=1.88 F=3.24	98.04±1.08 t=0.97 F=2.07	98.8±1.01 t=2.37 F=1.81
Lametec-50 DT[c]	50	98.54±0.68	99.14±1.09 t=1.07 F=2.57	100.1±1.35 t=2.43 F=3.94	99.9±1.2 t=2.29 F=3.11

[*]Mean value of 5 determinations.
Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77.
Tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39.
[ψ] Marketed by : [a]Sun pharmaceuticals, [b]Sun pharmaceuticals, [c]Cipla India Ltd, Mumbai.

Table 4—Results of recovery study by standard-addition method.

Tablet studied	Method A				Method B				Method C			
	LMT in tablet, µg mL^{-1}	Pure LMT added, µg mL^{-1}	Total found, µg mL^{-1}	Pure LMT recovered (Percent±SD[*])	LMT in tablet, ng mL^{-1}	Pure LMT added, ng mL^{-1}	Total found, ng mL^{-1}	Pure LMT recovered (Percent±SD[*])	LMT in tablet, ng mL^{-1}	Pure LMT added, ng mL^{-1}	Total found, ng mL^{-1}	Pure LMT recovered (Percent±SD[*])
Lamosyn-100	10.4	5.0	15.34	98.75±0.56	102.8	50.0	151.58	97.56±0.72	20.78	10	30.46	96.76±1.31
	10.4	10.0	20.71	103.1±1.23	102.8	100	204.10	101.3±1.35	20.78	20	41.06	101.4±0.86
	10.4	15.0	25.58	101.2±1.05	102.8	150	257.60	103.2±1.52	20.78	30	50.28	98.34±0.76

[*]Mean value of three determinations.

test was repeated three times. In all the cases, the recovery percentage values ranged between 96.76 and 103.2 with relative standard deviation in the range 0.58-1.50%. Closeness of the results to 100% showed the fairly good accuracy of the methods. This data is presented in Table 4.

Conclusion

The present work describes three validated spectrophotometric methods for the determination of LMT in pharmaceutical formulations. All the methods have been found to be selective, and two of the proposed methods (i.e. method B and method C) are extremely sensitive and applicable at ng mL^{-1} levels, a sensitivity matched only by LC with fluorescence or electrochemical detector. The methods are quite selective as the drug contains basic moiety which preferentially interacts with BPB, and the drug-dye ion-pair is extracted into the organic solvent before measurement. The methods are free from interferences from the common excipients and additives. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. The methods, therefore, should find wide use in pharmaceutical quality control laboratories for routine analysis.

Acknowledgement

Authors thank M/S. Cipla India Ltd, for gifting pure lamotrigine. One of the authors (NRP) is grateful to the University Grants Commission, New Delhi, India for providing Meritorious Research Scholarship. Two of the authors (NRP & KBV) are also thankful to the authorities of the University of Mysore, Mysore, for permission and facilities.

References

1 Gilman A G, Hardman J G & Limbird L E, *Goodman and Gilman's the Pharmacological Basis of Therapeutics*, 10th edn (McGraw Hill, New York, USA), 2001, 539.

2 Sean C, Sweetman, *Martindale, The Complete Drug Reference,* 34th edn (Pharmaceutical Press, London), 2005, 363.

3 Cociglio M, Alric R & Bouvier O, *J Chromatogr Biomed Appl,* 110 1-2; *J Chromatogr*, 572 (1991) 269.

4 Contin M, Balboni M, Callegati E, Candela C, Albani F, Riva R & Baruzzi A, *J Chromatogr B,* 828 (2005) 113.
5 Ching-Ling C, Chen-His Chou & Hu O Y-P, *J Chromatogr B,* 817 (2005) 199.
6 Matar K M, Nicholls P J, Tekle A, Bawazir S A & Al-Hassan, *J Pharm Biomed Anal*, 17 (1998) 525.
7 Londero D & Lo Greco P, *J Chromatogr B Biomed Sci Appl*, 691 (1997) 139.
8 Croci D, Salmaggi A, de Grazia U & Bernardi G, *Ther Drug Monit*, 23 (2001) 665.
9 Angelis-Stoforidis P, Morgan D J, O'Brien T J & Vajda F J E, *J Chromatogr B, Biomed Appl,* 727 (1999) 113.
10 Lensmeyer G L, Gidal B E & Wiebe D A, *Ther Drug Monit*, 19 (1997) 292.
11 Castel-Branco M M, Almeida A M, Falcao A C, Macedo T A, Caramona M M & Lopez F G, *J Chromatogr. B, Biomed Appl,* 755 (2001) 119.
12 Saracino M, Koukopoulos A, Sani G, Amore M & Raggi M A, *Ther Drug Monit*, 29 (2007) 773.
13 Queiroz M E C, Silva S M, Carvalho D & Lancas F M, *J Chromatogr Sci,* 40 (2002) 219.
14 Patil K M & Bodhankar S L, *J Chromatogr B,* 823 (2005) 152.
15 Talekar R S, Dhake A S, Sonaje D B & Mourya V K, *Indian J Pharm Sci,* 62**,** (2000) 51.
16 Dreassi E, Corbini G, Corti P, Ulivelli M & Rocchi R, *J AOAC Intl,* ISSN 1060-3271 (1996) 1277.
17 Patil K M, Aggarwal A K & Bodhankar S L, *Indian J Pharm Sci,* 66 (2004) 283.
18 Youssef N F & Taha E A, *Chem Pharm Bull,* 55 (2007) 541.
19 Olga D-R, Calvo M E & Acros-Martinez M J, *Sensors*, 8 (2008) 4201 DOI: 10.3390/s8074201.
20 Elizabeth G-S, Daria R L, Mohamed A V & Matthew D K, *Biomed Chromatogr,* 21 (2007) 225.
21 Douglas A S & Donald M W, *Principels of Instrumental Analysis* (Holt, Rinhart and Winston, New York), 1971, 104.
22 Erk N, *Anal Lett,* 36 (2003) 1183.
23 Zavis H, Ludvik D, Milan K, Ladislaw S & Frantisck V, *Handbook of Organic Reagents in Inorganic Analysis*. Translated by Stanislav K, Chalmers (The Series and Translation Editor: University of Aberdem, Ellis Horwood Limited, Chichester, A Division of John Wiley & Sons IC, New York, London, Sydney, Toronto), 364.
24 *International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use*, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London.
25 Inczedy J, Lengyel T & Ure A M, *IUPAC Compendium of Analytical Nomenclature : Definitive Rules* (Blackwell Science Inc., Boston), 1998.