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Sensitive spectrophotometric determination of lamotrigine in bulk drug and pharmaceutical formulations using bromocresol green
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the determination of this drug in biological samples. UV-spectrophotometric method [20] 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 [14] have reported the application of the technique for the determination of LMT using chloranilic acid as a chromogen. The reported method 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.

Many of the other reported methods [13-17] are sensitive and selective but they are time consuming, require expensive instrumental setup, and some require preliminary sample treatment. Adsorptive stripping voltammetric method [21] 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. Although many analytical methods were reported to analyze LMT in biological samples and pharmaceutical samples, none of these methods was suitable for routine analysis of LMT in pharmaceutical preparation. Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs and, therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds [22-25] and different alkaloids [26, 27].

We, therefore, developed two higly sensitive, selective, reproducible and economical spectrophotometric methods for the determination of LMT in bulk powder and in tablets by exploiting its basic nature and its ability to form ion-pair complex with an anionic dye bromocresol green. The first method (method A) is based on the formation of an ion-pair complex between drug and dye at pH 5.02±0.01 followed by extraction of the complex into dichloromethane (DCM), and the yellow drug-dye complex was measured at 410 nm. In the second method (method B), the drug-dye ion-pair was broken in ethanolic alkali and the blue color of base form of the dye was measured at 620 nm. The method B is a highly sensitive approach for determination of LMT in bulk drug and in tablets.

Experimental

Apparatus

A Systronic model 106 digital spectrophotometer equipped with 1 cm quartz cells was used for absorbance measurements. A digital pH meter Model Elico L1 120 was used for pH measurements.

Reagents

All chemicals used were of analytical grade. Solvents used were of the spectroscopic grade. Distilled water was used through out the investigation.

Sulphuric acid (0.1 M): Concentrated acid (S.D. Fine Chem, Mumbai, India, sp. gr. 1.84) was appropriately diluted with water to get 0.1 M acid.

Bromocresol green (0.4%): Dissolved 400 mg of the dye (S.D.Fine Chem Ltd, Mumbai, India) in 10 ml of ethanol and diluted to 100 ml with water.

Sodium acetate (1 M): Prepared by dissolving 13.61 g of the pure sodium acetate (Merck Specialities Pvt Ltd, Mumbai, India) in 100 ml water.

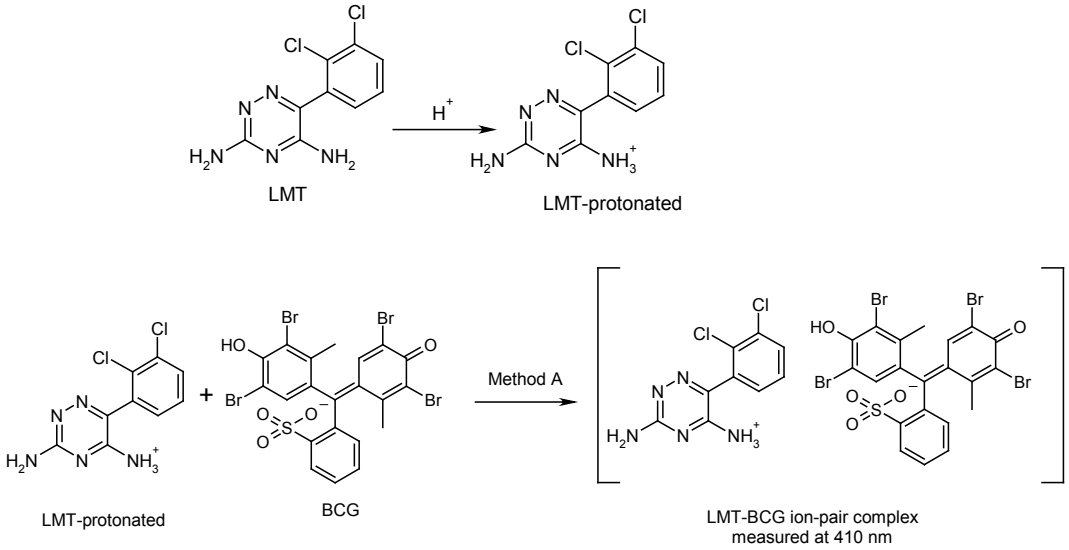
Buffer solution (pH 5.02): Mixed 50 ml of 1 M sodium acetate and 15 ml of 1 M hydrochloric acid (Merck Specialities Pvt Ltd, Mumbai, India, Sp, gr. 1.18) and volume was made upto 250 ml, and pH was adjusted to 5.02 by using dilute NaOAc/HCl solution.

Ethanolic KOH (1%): One gram of the pure KOH (S.D.Fine Chem Ltd, Mumbai, India) was dissolved in and diluted to 100 ml with ethanol.

Standard drug solution (30 µg mL^{-1}): LMT (pharmaceutical grade, 99.88 % pure) was procured from Cipla India Ltd, Mumbai, India, as a gift and was used as received. A stock standard solution of lamotrigine (300 µg mL^{-1}) was first prepared by dissolving 30 mg LMT in 0.1 M H_2SO_4 and diluting to 100 ml in calibrated flask with the same

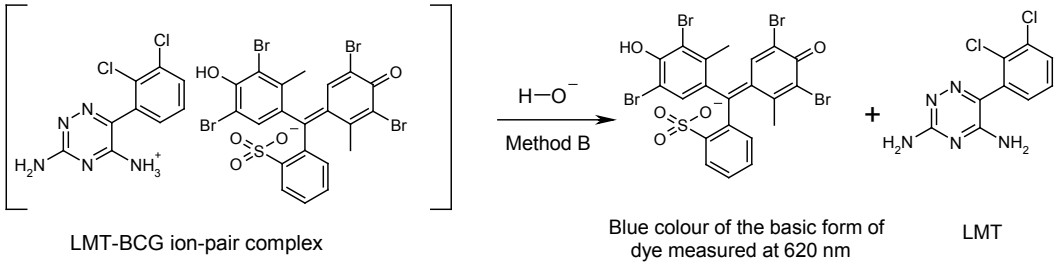
Reaction Mechanisms

Anionic dyes such as BCG forms ion-pair complex with positively charged drugs. The drug-dye stoichiometric ratio as calculated by the Job's continuous variations method [28] is found to be 1:1. Each drug-dye complex molecule, with two oppositely charged ions, behaves as a single unit held together by an electrostatic force of attraction (Scheme 1).



Scheme 1. Reaction pathway for method A.

In alcoholic alkaline medium, this ion-pair complex gets disturbed and it breaks to form a blue colored basic dye and the drug. The mechanism of this breaking is shown in scheme 2.



Scheme 2. Reaction pathway for method B.

Optimisation of Variables and Method Development

A number of preliminary experiments established optimum conditions necessary for rapid and quantitative formation of colored ion-pair complex to achieve the maximum stability and sensitivity.

ride, benzene, cyclohexane, hexane, chloroform, 1, 2-dichloroethane and ether) because of its slightly higher efficiency on color intensity, selective extraction of the LMT-BCG complex from the aqueous phase and obtained highest absorbance with dichloromethane.

Effect of number of extractions

Under optimum conditions, the drug-dye complex in the aqueous phase was extracted with three 10 ml portions of DCM and absorbance was measured each time. After the second extraction, The absorbance of the organic layer was negligibly small. Hence, a single extraction with 10 ml DCM was selected for the extraction because of complete recovery of the complex.

Equilibration time and stability of the coloured complexes

The organic and aqueous phases were clearly separated in less than 1 min. The drug-dye ion-pair complex was stable for more than 15 h at laboratory temperatute (30±2^0 C).

Effect of order of addition of reactants

The sequence of order of addition of the reactants prior to extraction had small change in the absorbance values. So the order of addition of reactants should be in the described manner.

Composition of Ion-pair Complexes

The composition of the ion-pair complex was established by Job's method of continuous variations [28] using equimolar concentrations of the drug and the dye (1.955 x 10^{-4} M). 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 BCG in various molar ratios, with a total volume of 5 ml, in addition to 20 ml H_2O, 4 ml of 1 M NaOAc and 5 ml buffer solution were prepared. The extraction was performed using 10 ml of dichloromethane and the absorbance was subsequently measured at 410 nm. The graph of the results obtained (Fig. 3)

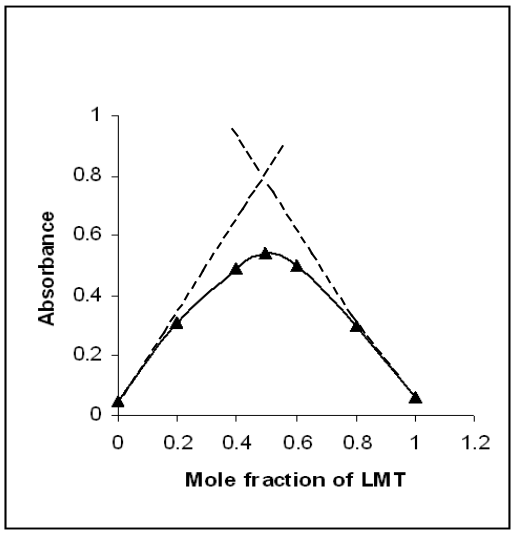


Fig. 3. Job's method of continuous variations plot for ion-pair complex of LMT-BCG in dichloromethane at 410 nm.

gave a maximum at a molar ratio of Xmax = 0.5 which indicated the formatiuon of a 1:1 LMT:BCG complex. The conditional stability constant (K_f) of the ion-association complex was calculated from the continuous variation data using the following equation [29]:

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

where A and Am 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 BCG ion associates with drug. The log K_f value was found to be 5.60.

Method B

Studies on the effect of alkali concentration required to break the complex into its components revealed that 1 mL of 1 % alcoholic KOH with a standing time of 5 min was sufficient to yield ma-

which is corroborated by high values (close to unity) of the correlation coefficients. 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 calculated molar absorptivity and Sandell sensitivity [30] values are summarized in Table 1. The limits of detection (LOD) and quantification (LOQ), calculated according to the ICH guidelines [31] 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 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 results of this study are summarized in Table 2.

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	6.0	5.90±0.08	1.67	1.52	5.96±0.14	0.67	1.82
	9.0	8.92±0.11	0.89	1.36	8.99±0.11	0.11	1.02
	12.0	12.01±0.22	0.08	1.96	11.98±0.30	0.17	2.01
B	1.0	1.01±0.01	1.00	0.884	1.02±0.02	2.00	1.16
	3.0	3.04±0.02	1.33	0.603	3.02±0.04	0.67	$0._9$2
	5.0	5.01±0.02	0.20	0.450	4.98±0.05	0.40	$0._8$0

%RE. Percent relative error, %RSD. relative standard deviation and CL. Confidence limits were calculated from: CL = ± tS/√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).

The percentage relative standard deviation (%RSD) values were < 2 % (intra-day) and ≤ 2.01 % (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) x 100 / known concentration]. Bias was calculated at each concentration and these results are also presented in Table 2. Percent relative error (%RE) values ≤ 2.0 % 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 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 10 mg of LMT to the placebo mentio-

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

Tablet brand name[w]	Nominal amount, (mg/tablet)	Found* (Percent of label claim ± SD)		
		Reference method	Method A	Method B
Lamosyn-100[a]	100	98.56±0.76	98.14±1.12 t=0.71 F=2.17	99.04±1.06 t=0.83 F=1.95
Lamosyn-25[a]	25	101.3±0.62	100.6±0.86 t=1.49 F=1.92	101.1±0.90 t=0.42 F=2.11
Lametec-50 DT[b]	50	102.5±0.86	101.2±1.05 t=2.15 F=1.49	101.8±0.72 t=1.40 F=1.43

*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).
[w]Marketed by : [a]Sun pharmaceuticals.
[b]Cipla India Ltd, Mumbai.

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 99.22 and 104.3 % with standard deviation in the range 0.85 – 1.25 %. Closeness of the results to 100 % showed the fairly good accuracy of the methods. The results are shown in Table 5.

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