odic stripping voltammetry using a carbon paste electrode modified with ion-exchange resin Amberlite IR120. Ecl Quim 2006;31:45-52.
[12] Ferrari RA, Oliveira VS, Scabio A. Biodiesel from soybean: characterization and consumption in an energy generator. Quim Nova 2005;28:19-23.
[13] Silva FC, Brandão KSR. Optimization of methyl and ethyl biodiesel from babassu. Proceedings of the First Congress of the Brazilian Biodiesel Technology Network, v. 2, p.119-125. Brasília, Brazil, 2006.
[14] IUPAC - Standard methods for the analysis of oils, fats and derivatives. 7th ed. London: Blackwell Scientific Publications; 1987.

ECLÉTICA química
WWW.SCIELO.BR/EQ
VOLUME 35, NÚMERO 1, 2010
Artigo/Article

SENSITIVE SPECTROPHOTOMETRIC DETERMINATION OF LAMOTRIGINE IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS USING BROMOCRESOL GREEN

N. Rajendraprasad, K. Basavaiah and K. B. Vinay*

Department of Chemistry, University of Mysore, Manasagangothri, Mysore-570 006, India
**Corresponding e-mail: basavaiahk@yahoo.co.in. Mobile: +91-9448939105*
Fax: 0091-821-2421263, 2516133.

Abstract: Two new, simple, rapid and reproducible spectrophotometric methods have been developed for the determination of lamotrigine (LMT) both in pure form and in its tablets. The first method (method A) is based on the formation of a colored ion-pair complex (1:1 drug/dye) of LMT with bromocresol green (BCG) at pH 5.02±0.01 and extraction of the complex into dichloromethane followed by the measurement of the yellow ion-pair complex at 410 nm. In the second (method B), the drug-dye ion-pair complex was dissolved in ethanolic potassium hydroxide and the resulting base form of the dye was measured at 620 nm. Beer's law was obeyed in the concentration range of 1.5-15 µg mL^{-1} and 0.5-5.0 µg mL^{-1} for method A and method B, respectively, and the corresponding molar absorptivity values are 1.6932 x 10^4 and 3.748 x 10^4 L $mol^{-1}cm^{-1}$. The Sandell sensitivity values are 0.0151 and 0.0068 µg cm^{-2} for method A and method B, respectively. The stoichiometry of the ion-pair complex formed between the dug and dye (1:1) was determined by Job's continuous variations method and the stability constant of the complex was also calculated. The proposed methods were applied successfully for the determination of drug in commercial tablets.

Keywords: lamotrigine; spectrophotometry; ion-pair complex; bromocresol green; pharmaceuticals

Introduction

Lamotrigine, (LMT), [6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine], is an anticonvulsant drug. As an antiepileptic, it has been used successfully 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].

Lamotrigine is not official in any pharmacopoeia. The analysis of LMT in biological samples is abundantly described in the literature. Chromatographic techniques have been widely employed since they are powerful separation techniques. The methods based on the high-performance liquid chromatography (HPLC) [3-10], high-performance thin layer chromatography (HPTLC) [11] and gas-chromatography (GC) [12] have been described. There is an extensive literature on the determination of lamotrigine in pharmaceuticals include planar chromatography [13], TLC and HPLC [14], HPLC and GC [15], capillary electrophoresis [16, 17]. The immunoassay techniques [18, 19] have been developed for

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 acid. Stock solution was diluted with the same acid to get a working solution of 30 µg mL^{-1}.

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 market and were used in the investigation.

Recommended Procedures

Method A

Into a series of 125 ml separating funnels, aliquots of lamotrigine standard solution (30 µg mL^{-1}) equivalent to 1.5 – 15.0 µg mL^{-1} LMT were accurately transferred and the total volume was brought to 5 ml by adding 0.1 M H_2SO_4 to each funnel. To each funnel were added 20 ml of water, 4 ml of 1 M NaOAc and 5 ml buffer of pH 5.02 followed by 5 ml dye (0.4 %), and the content was mixed well. The funnels were shaken vigorously with 10 ml of dichloromethane (added from microburette) for 30 seconds, and then allowed to stand for clear separation of the two phases. The separated organic layer was passed over anhydrous sodium sulphate and absorbance of the yellow ion-pair complex was measured at 410 nm against a reagent blank similarly prepared.

Method B

Varying aliquots of LMT-BCG ion-pair complex (10 µg mL^{-1} in LMT; prepared by following the procedure described in method A) equivalent to 0.5 – 5.0 µg mL^{-1} with respect to LMT were transferred into a series of 10 mL standard flasks and the total volume was brought to 5 mL by adding dichloromethane. To each flask, 1 mL of 1 % alcoholic KOH was added, the content mixed and kept aside for 5 min. Finally, the volume was made upto mark with ethanol and the absorbance measured at 620 nm against the reagent blank.

In both the methods, the standard calibration curve was prepared or regression equation derived to calculate the amount of analyte drug in unknown samples.

Procedure for the Dosage Forms

Twenty tablets were weighed and ground into a fine powder using a pestle and mortar. An accurately weighed portion of the powder equivalent to 30 mg LMT was transferred 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. The volume was made upto mark with 0.1 M H_2SO_4. After 5 min, the solution was filtered through Whatman No 42 filter paper. First 10 ml portion of the filter was discarded and a suitable aliquot was diluted to get a working concentration of 30 µg mL^{-1} and used for assay by method A. The ion-pair complex of this tablet LMT-BCG (10 µg mL^{-1}) was prepared for assay by applying the procedure described in method B.

Results and Discussion

Spectral Characteristics

Absorption spectrum of the yellow colored LMT-BCG ion-pair complex is shown in Fig. 1 which has a maximum absorbance (λ_{max}) at 410 nm. This drug dye ion-pair complex was broken in ethanolic base to yield the base form of the blue dye which had maximum absorbance at 620 nm.

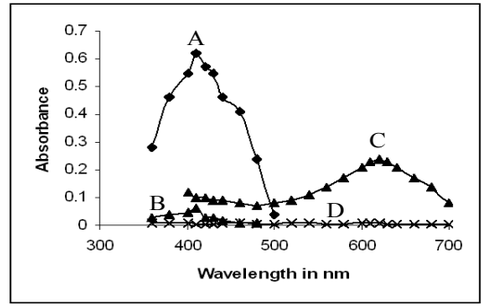
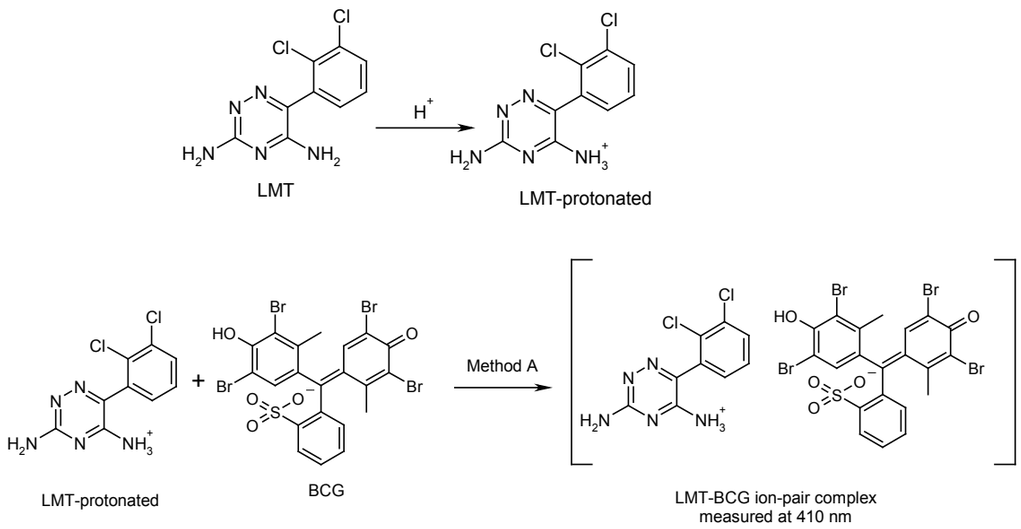


Fig. 1. Absorption spectra of A: ion-pair complex (9.5 µg mL^{-1} LMT); B: blank in method A; C: base form of dye (1.5 µg mL^{-1} LMT) and D: blank in method B. In both the cases, the blanks had negligible absorbance.

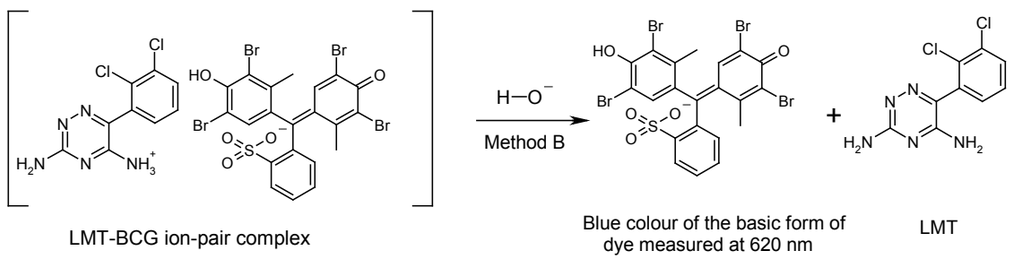
Reaction Mechanisms

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. Optimum condition was fixed by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance either at 410 nm in method A or at 620 nm in method B.

Method A

Effect of pH

In order to establish the optimum pH range, 2 ml of LMT solution in 0.1 M H_2SO_4 and 3 ml 0.1 M H_2SO_4 were taken and diluted with 5, 10, 15, 20 and 25 ml of water. Five ml of the dye solution was added after the addition of 4 ml of 1 M NaOAc and 5 ml buffer of pH 5.02. The effective pH value of above resulting aqueous solutions was 5.02±0.01. Fig. 2 shows that at 20 ml of water, the absorbance of the ion-pair complex was maximum and the respective blank had shown negligible absorbance. The pH of the aqueous phase was constant at 5.02±0.01 for water volumes ranging from 5-25 ml, at a volume of 20 ml water, blank had a lower absorbance. At water volumes greater than 20 ml, the sensitivity was less. It was also found that a ratio of ~4:1 of aqueous to organic phases was required for the efficient extraction of the colored species. Hence, an aqueous phase of 39 ml which includes 20 ml of H_2O was used in all subsequent work.

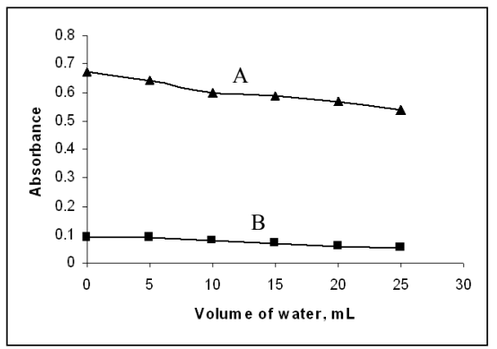


Fig. 2. Effect of water on the absorbance of A: ion-pair complex (9.0 µg mL^{-1} LMT) and B: blank.

Effect of sodium acetate, BCG and buffer solution

Various amounts of NaOAc were added to the acidic solution of lamotrigine to bring the pH to the optimum value and measurements were carried out as recommended. Maximum absorbance for sample and negligible absorbance for blank was observed when the volume of NaOAc was 4 mL (1 M) in a total volume of 39 ml. The effect of BCG 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 8.0 ml of 0.4 %BCG solution. Hence, 5 ml of 0.4 % BCG solution was fixed in a total volume of 39 ml of aqueous phase.

Various amounts of buffer solution were used in the investigation to establish its effect on absorbance. There was almost no influence on the absorbance up to 10 ml, but an amount less than 4 ml resulted in unsatisfactory separation of the organic phase during the extraction. So, 5 ml of buffer was used through out the investigation.

Reaction time

After the addition of dye, the effect of standing time was studied in the time range 5-30 min before extraction. After a contact time of 5 min, measured absorbance of the complex after extraction into dichloromethane, showed almost constant absorbance values from 5-30 min. So a contact time of 5 min was adequate to form the complex.

Effect of shaking time

Shaking times ranging from 30 to 60 sec produced no change in absorbance, by maintaining all other parameters constant. So a 30 sec shaking time was fixed.

Selecting of the Extracting Solvents

The effect of the extracting solvent on the ion-pair complex was examined. Dichloromethane was preferred to other solvents (carbon tetrachlo-

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)

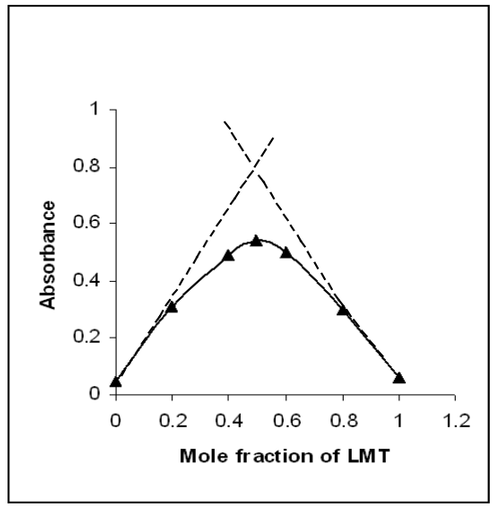


Fig. 3. Job's method of continuous variations plot for ion-pair complex of LMT-BCG in dichloromethane at 410 nm.

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]:

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

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 maximum absorbance at 620 nm. The stability of the resulted blue coloured dye was stable for more than 20 h at laboratory temperatute (30±2^0 C).

Method Validation

Linearity, sensitivity, limits of detection and quantification

Calibration graphs were constructed from ten points covering the concentration ranges 1.5-15 µg mL^{-1} and 0.5-5.0 µg mL^{-1} for method A and method B, respectively. Regression analysis of the Beer's law data indicated a linear relationship between absorbance and concentration,

Table 1. Sensitivity and regression parameters

Parameter	Method A	Method B
λ_{max}, nm	4[10]	6[20]
Color stabili¸y	> 24 h	> 20 h
Linear range, µg mL^{-1}	1.5-15	0.5-5.0
Molar absorptivity(ε) ¸l mol^{-1} cm¸1	1.6932 x 10^4	3.748 x 10^4
Sandell sensitivity*, µg cm^{-2}	0.0151	0.0068
Limit of detection (LOD) ¸µg ml^{-1}	0.43	0.19
Limit of quantification (LOQ), µg ml^{-1}	1.30	0.59
Regression equation, Y**		
Intercept (a)	-0.0007	0.0007
Slope (b)	0.0661	0.1463
Standard deviation of a (S_a)	0.0998	0.0998
± $tS_a/\sqrt{n}$	0.124	0.124
Standard deviation of b (S_b)	0.0073	0.022
± $tS_b/\sqrt{n}$	0.0091	0.027
Variance (S_a2)	0.0096	0.0096
Regression coefficient (r)	0.9993	0.9996

*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.

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 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 12 µg mL^{-1} (method A) and 4.0 µg mL^{-1} (method B) 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 volume of H_2O/ethanolic KOH 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 3. Method robustness and ruggedness expressed as intermediate precision (% RSD)

Method	LMT taken, µg mL^{-1}	Robustness		Ruggedness	
		Parameters altered			
		Volume of H2O/ Ethanolic KOH*	Reaction/Breaking time$^{\Psi}$	Inter-analysts (%RSD), (n=4)	Inter-instruments (%RSD), (n=4)
A	6.0	1.02	1.23	0.88	1.65
B	3.0	0.96	1.53	0.99	1.58

*In method A, the volume of H_2O was 17, 20 and 23 mL, and in method B 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 breaking times were 3, 5 and 7 min.

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 [20]. 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 [32]. The results of assay are given in Table 4.

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	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).
ψMarketed 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.

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

Tablet studied	Method A				Method B			
	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, µg mL^{-1}	Pure LMT added, µg mL^{-1}	Total found, µg mL-1	Pure LMT recovered (Percent±SD*)
Lamosyn-100	4.91	2.5	7.44	101.2±0.96	1.98	1.0	2.98	100.2±1.06
	4.91	5.0	9.87	99.22±0.85	1.98	2.0	4.04	103.2±1.25
	4.91	7.5	12.73	104.3±1.25	1.98	3.0	4.96	99.23±0.98

*Mean value of three determinations.

Conclusions

In the present study, the maximum color development of LMT-BCG ion-pair complex (method A) and basic dye (method B) was instantaneous. No heating or standing was needed. These methods do not involve procedural steps, do not take more operator time and expertise like HPLC and other methods. In terms of simplicity, rapidity, sensitivity and expense, the methods could be considered superior in comparison with the previously reported methods, especially with those based on chromatography [3-15]. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The method is unaffected by slight variations in experimental conditions such as time, reagent concentration or temperature. The methods are highly sensitive compared to the only spectrophotometric method [14]. Since there was no interference from the tablet excipients, the methods are highly selective in comparison with the chloranilic acid and method based on charge-transfer complexation method [14].

The proposed methods gave results with good accuracy to permit determination of low concentration even down to 0.5 µg mL^{-1}. The wide applicability of the described procedure for routine quality control is well established by the assay of LMT in pure form, as well as in pharmaceutical preparations

Aknowledgement

Authors thank M/S. Cipla India Ltd, for gifting pure lamotrigine, two of the authors (NRP & KBV) are gratefully thanks the University of Mysore, Mysore, permission and facilities and on of the authors (NRP) gratefully acknowledges the UGC, New Delhi, for providing Meritorious Research Fellowship.

References

[1] A.G. Gilman, J.G. Hardman, L.E. Limbird, “Goodman and Gilman’s the Pharmacological Basis of Therapeutics.” 10th ed. McGraw Hill, New York, U.S.A. (2001) pp. 539.
[2] C. Sean, Sweetman, Martindale: The Complete Drug Reference 34th ed. Pharmaceutical Press, London. (2005) pp. 363.
[3] M. Cociglio, R. Alric, O. Bouvier, J. Chromatogr. Biomed. Appl. 110: 1-2, J.Chromatogr. 572 (1991) 269.
[4] G.L. Lensmeyer, B.E. Gidal, D.A. Wiebe, Ther. Drug Monit. 19 (1997) 292.
[5] P. Angelis-Stoforidis, D.J. Morgan, T J. O’Brien, F.J.E. Vajda, J. Chromatogr. B, Biomed. Appl. 727 (1999) 113.
[6] D. Croci, A. Salmaggi, U.de Grazia, G. Bernardi, Ther. Drug Monit. 23 (2001) 665.
[7] M.M. Castel-Branco, A.M. Almeida, A.C. Falcao, T.A. Macedo, M.M. Caramona F.G.Lopez, J. Chromatogr. B, Biomed. Appl. 755 (2001) 119.
[8] C.L. Cheng, C.H. Chouand, O.Y.P. Hu,. J. Chromatogr. B, Anal. Technol. Biomed. Life Sci. 817 (2005) 199.
[9] K.M. Matar, P.J. Nicholls, S.A. Bawazir, M.I. Al-Hassan, A. Tekle, J. Pharm. Biomed. Anal. 17 (1998) 525.
[10] D. Londero, P. Lo Greco, J. Chromatogr. B. Biomed. Sci. Appl. 691 (1997) 1 ISSN 1387-2273 139.
[11] K.M. Patil, S.L. Bodhankar, J. Chromatogr. B, 823 (2005) 152.
[12] J. Hallbach, H. Vogel, W.G. Guder, Eur. J. Clin. Chem. Clin. Biochem. 35 (1997) 755.

[13] E. Dreassi, G.P. Corbini, M. Corti, Ulivelli, R. Rocchi,. J. AOAC. Intl. 79: 6, ISSN 1060-3271 (1996) 1277.
[14] N.F. Youssef, E.A. Taha,..Chem. Pharm. Bull. 55 (2007) 4 541.
[15] G-S. Elizabeth, S. Giannoutsos, D.R. Lower, M.A. Virji, M.D. Krasowski, J. Chromatogr. Sci. 45 (2007) 9 616.
[16] Z.K. Shihabi, K.S. Oles, J. Chromatogr. B, Biomed Appl. 683 (1996) 119.
[17] R. Theurillat, M. Kuhn, W. Thormann, J. Chromatogr. A. 979 (2002) 353.
[18] R.A. Biddlecombe, K.L. Dean, C.D. Smith, S.C. Jeal,. J. Pharm. Biomed. Anal. 8 (1990) 691.
[19] J.M. Sailstad. J.W.A. Findlay, Ther. Drug. Monit., 13 (1991) 433.
[20] R.S. Talekar, A.S.Dhake, D.B. Sonaje, V.K. Mourya, Ind. J. Pharm. Sci. 62 (2000) 51.
[21] D-R. Olga, M.E Calvo, M. Acros-Martinez, J.. Sensors. 8 (2008) 4201; DOI:10.3390/s8074201
[22] N. Rahman, S.N. Hejaz-Azmi, J. Pharm. Biomed. Anal. 24 (2000) 33.
[23] N. Silva, E.E.S. Schapoval, J. Pharm. Biomed. Anal. 29 (2002) 749.
[24] T. Perez-Ruiz, C. Martinez-Lozano, V. Tomás, A. Sanz, E. Sauquillo, J. Pharm. Biomed. Anal. 26 (2001) 609.
[25] K. Basavaiah, V.S. Charan, IL Farmaco. 57 (2002) 9.
[26] H. Maghsoudi, A. Fawzi, J. Pharm. Sci. 67 (1978) 32.
[27] H. Farsam, H.H. Yahya-saeb, A. Fawzy, Int. J. Pharm. 7 (1981) 343.
[28] A.S. Douglas, M.W. Donald. "Principels of Instrumental Analysis," Holt, Rinhart. and Winston, New York, 1971.pp. 104.
[29] N. Erk. Anal. Lett. 36 (2003) 1183.
[30] H. Zavis, D. Ludvik, K. Milan, S. Ladislaw, V. Frantisck, Handbook of Organic Reagents in Inorganic Analysis. Translated by Stanislav, K, Dr. 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.1976. pp.364.
[31] 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.
[32] J. Inczedy, T. Lengyel, A.M. Ure,. IUPAC Compendium of Analytical Nomenclature : 1998. Definitive Rules, Blackwell Science Inc., Boston.

ECLÉTICA química
WWW.SCIELO.BR/EQ
VOLUME 35, NÚMERO 1, 2010
Artigo/Article

COMPLEXES OF 4-CHLOROPHENOXYACETATES OF ND(III), GD(III) AND HO(III)

W.Ferenc[1], M.Bernat[1], J.Sarzyński[2] and H.Głuchowska[1]*

[1]Faculty of Chemistry, Maria Curie-Sklodowska University, PL 20-031 Lublin, Poland
[2]Faculty of Physics, Maria Curie-Sklodowska University, PL 20-031 Lublin, Poland
[*] Author for correspondence: e-mail: wetafer@poczta.umcs.lublin.pl

Abstract: The complexes of 4-chlorophenoxyacetates of Nd(III), Gd(III) and Ho(III) have been synthesized as polycrystalline hydrated solids, and characterized by elemental analysis, spectroscopy, magnetic studies and also by X-ray diffraction and thermogravimetric measurements. The analysed complexes have the following colours: violet for Nd(III), white for Gd(III) and cream for Ho(III) compounds. The carboxylate groups bind as bidentate chelating (Ho) or bridging ligands (Nd, Gd). On heating to 1173K in air the complexes decompose in several steps. At first, they dehydrate in one step to form anhydrous salts, that next decompose to the oxides of respective metals. The gaseous products of their thermal decomposition in nitrogen were also determined and the magnetic susceptibilites were measured over the temperature range of 76-303K and the magnetic moments were calculated. The results show that 4-chlorophenoxyacetates of Nd(III), Gd(III) and Ho(III) are high-spin complexes with weak ligand fields. The solubility value in water at 293K for analysed 4-chlorophenoxyacetates is in the order of 10^{-4}mol/dm^3.

Keywords: 4-chlorophenoxyacetates, thermal stability, magnetic properties of Nd(III), Ho(III) and Gd(III).

Introduction

The carboxylates play an important role in inorganic and bioinorganic chemistry. Many metal cations are a component of several vitamins and drugs [1,2]. The carboxylates of d- and 4f- ion elements may be used as electric materials in the modern branches of techniques and technology. They may have also applications as precursors in superconducting ceramic and magnetic field productions and may be used as catalysts, pigments, solvents, food preservatives and plastics productions.

Metal carboxylates are applied for the productions of high degree purity of metal oxides and polycarboxylic acids are used for supermolecular compound synthesis, which in many cases, form with metal ions the molecular polymers containing in their structures, pores and channels owning to them they appear catalytic and adsorption properties. Therefore they may be used for the adsorption of inorganic gases such as: argon, nitrogen and hydrocarbons or small molecules of another inorganic compounds. Polycarboxylic acids may also form the molecules with two- and three–dimentional structures, yielding special magnetic and luminescence properties which let them be used in optical and electronic industries [3-10].

4-Chlorophenoxyacetic acid is a white solid hardly soluble in water ($K=7,9 \cdot 10^{-4}$) and easily soluble in ether and ethanol [10,11]. It is used as growth hormone for plants [12]. In some papers the details of its molecular structure was presented [13-15]. Literature survey informs that its complexes with Li(I), Cu(II), Co(II), Mn(II) and