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Spectrophotometric determination of lamotrigine in pharmaceutical preparations and urine by charge-transfer complexation

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Received May 17, 2008, accepted May 30, 2008

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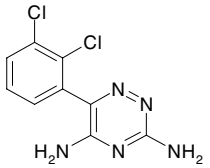
Pharmazie 63: 791–795 (2008) *doi: 10.1691/ph.2008.8616*

Rapid and sensitive spectrophotometric methods are developed for the determination of lamotrigine (LTG) in pharmaceutical dosage forms and urine samples, based on the formation of the charge-transfer (CT) complexes between LTG as an n-donor and the acceptors: bromocresol green (BCG), bromocresol purple (BCP), and chlorophenol red (CPR). These complexes are studied spectrophotometrically in chloroform solution in order to obtain some information about their stoichiometry and stability of complexation. The analytical parameters and their effects on the extraction of drug from urine samples are investigated. The reactions were extremely rapid at room temperature, and the absorbance values remained unchanged after 24 h for all reactions. Beer's law was obeyed in the concentration ranges 0.15–19.8, 0.15–19.8 and 0.05–34.1 $\mu g \cdot ml^{-1}$ for CPR, BCP and BCG, respectively. The proposed methods were applied successfully for the determination of LTG in pharmaceutical formulations, and human urine samples in the presence of other antiepileptic drugs such as carbamazepine, oxcarbazepine and phenobarbital, with good accuracy and precision.

1. Introduction

Lamotrigine (LTG) (3, 5-diamino-6-(2, 3-dichlorophenyl)-1, 2, 4-triazine) is an antiepileptic agent chemically unrelated to other anticonvulsants, that is increasingly used in the management of partial and generalized epilepsies (Stoforidis et al. 1999). It acts by inhibiting pre-synaptic voltage-sensitive sodium channels and excitatory neurotransmitter firing of action potentials characteristic of epileptic release (principally glutamate), and inhibits repetitive foci. It has been effective against refractory partial seizures, as well as generalized tonic-clonic seizures and other generalized seizures (Vidal et al. 1999). Lamotrigine is metabolised predominantly by glucuronidation. The major inactive urinary metabolites isolated are 2-*N*-glucuronide (76%) and a 5-*N*-glucuronide (10%). The aromatic ring is deactivated by the presence of chlorine atoms inhibiting aromatic oxide formation. Various analytical methods to estimate lamotrigine in dosage forms and in biological fluids have been reported in the literature. These methods include stripping voltammetry (Calvo et al. 2005), HPTLC (Patil and Bodhankar 2005), HPLC (Emami et al. 2006), GC (Queiroz et al. 2002), GC-MS (Hallbach et al. 1997), radioimmunoassay (Biddlecombe et al. 1990), electrospray ionization-mass spectrometry (ESI-MS) (Zheng et al. 2004), and capillary electrophoresis (Theurillat et al. 2002). A literature review revealed that HPLC is currently considered the technique of choice for the separation and determination of lamotrigine (Emami et al. 2006). An official monograph of lamotrigine does not exist in any pharmacopoeia, and the determination of lamotrigine and related substances in pharmaceutical formulations has not been yet described (Emami et al. 2006). Therefore, it is important to develop a simple and suitable analytical method for the measurement of lamotrigine and related compounds in bulk and in formulations.

Many drugs are determined spectrophotometrically, based on the formation of colored charge transfer complexes between either π or σ electron acceptors, and drugs as either n or σ electron donors (Chakraborty et al. 2001; Mostafa et al. 2002; Boraei 2002; Saha and Mukherjee 2004; Roy et al. 2005a, 2006a; Pal et al. 2005; El-Mossalamy 2004; Duymus 2006; Darwish 2005; Arslan et al. 2007), or the formation of colored compounds with a number of organic acid dyes (Rahman and Hejaz-Azmi 2000; Silva and Schapoval 2002; Abdine et al. 2002; El-Yazbi et al. 1999; Liu 2002; Amin et al. 2007; Nour El-Dien et al. 2006; Rahman et al. 2004; Tatar Ulu 2007; Abdellatef 2007). The charge transfer complexation arises from the partial transfer of an electron from a donating molecule having a sufficiently low ionization potential to an accepting one having a sufficiently high electron affinity and as a result,



formation of intensely coloured charge transfer complexes in which absorb radiation in the visible region (Foster 1993).

This paper proposes simple and sensitive colorimetric procedures for the determination of lamotrigine in pharmaceutical formulations and in human urine samples in the presence of other antiepileptic drugs such as carbamazepine, oxcarbazepine and phenobarbital.

2. Investigations, results and discussion

Figure 1 shows the electronic absorption spectra of the drug complexes obtained between lamotrigine and BCG at different LTG/BCG mole ratios. Lamotrigine forms ion-pair complexes with acid dyes such as BCG, BCP and CPR in chloroform. The maximum absorption wavelengths of the ion-pair complexes, BCG-LTG, BCP-LTG, and CPR-LTG are located at 420, 415, and 415 nm, respectively.

2.1. Stoichiometry, reaction time and stability

In order to establish the molar ratios between lamotrigine and the acid dyes used, the mole ratio method was applied. In this method, *in situ* titration of 3 ml acid dyes of BCG (3.05×10^{-5} mol · l^{-1}), BCP (2.94×10^{-5} mol · l^{-1}) and CPR (5.19×10^{-5} mol · l^{-1}) solutions was carried out with concentrated drug solution. The absorbance of each solution was measured at λ_{max} against the blank solvent. The results shown in Fig. 3 indicate that the interaction between LTG and each of the ion-pair reagents occurs on an equimolar basis (1 : 1). The reaction time was determined by following the absorbance of the developed color at different time intervals at ambient temperature (25 °C). Complete color development was attained instantaneously after mixing lamotrigine with each acid dye. The yellow colors were stable for lamotrigine-dye without any change in color intensity and maximum absorbance at room temperature. The molar absorptivity and stability constant of the resulting 1 : 1 adducts in chloroform solution were evaluated from computer fitting of the mole ratio data to a proper equation, the results of which displayed in Table 1. The stability sequence of 1 : 1 complexes for LTG and the dyes in chloroform solution is BCG > BCP > CPR (Table 1).

To take full advantage of the procedure for determination of lamotrigine from urine, reaction conditions must be op-

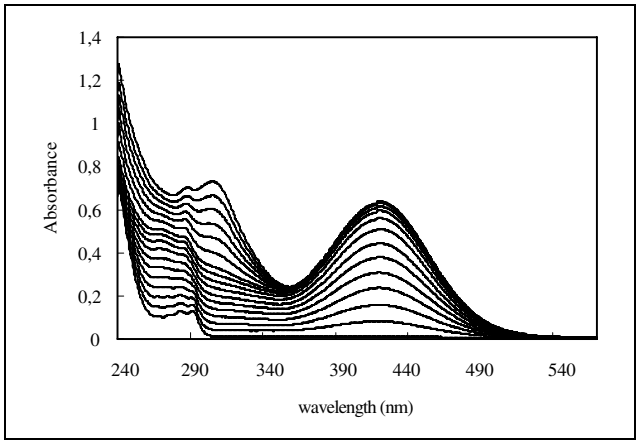


Fig. 1: Charge transfer absorption spectra of mixture containing lamotrigine ($0–6\times10^{-5}$mol · l^{-1}) and BCG ($0–3.05\times10^{-5}$), against the chloroform as a reference

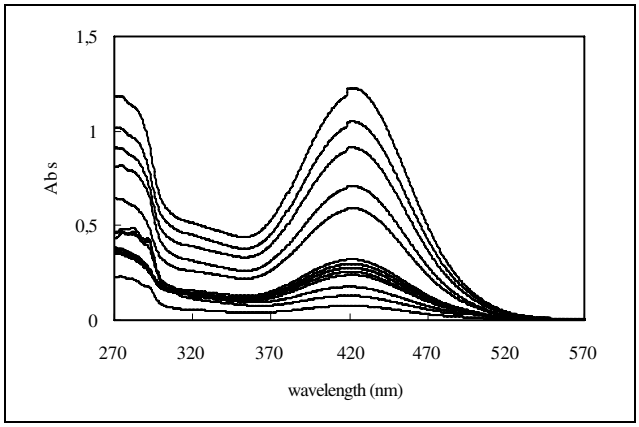


Fig. 2: Absorption spectra obtained after extraction of LTG from spiked urine containing $0–9\times10^{-5}$ mol · l^{-1}) and measured with BCG

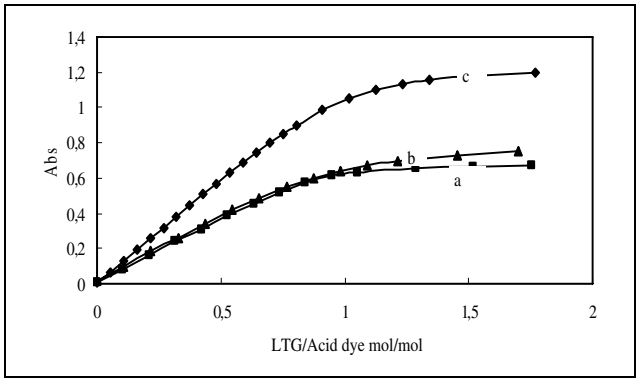


Fig. 3: Mole ratio plots for CT complexes of LTG with different acceptors at the fixed concentration, (a) BCG (3.05×10^{-5}), (b) BCP (2.94×10^{-5}) and CPR (5.19×10^{-5}) and increasing concentration of LTG

Table 1: Spectral properties charge transfer complexes, absorption maxima, molar extinction coefficient, stoichiometry, formation constant values, and analytical parameters for the determination of lamotrigine in pure form

Parameters	BCG	BCP	CPR
λ_{CT} (nm)	420	415	415
ε_{max} (L mol^{-1} cm^{-1})	2.30×10^4	2.73×10^4	2.43×10^4
Stochiometery	1 : 1	1 : 1	1 : 1
K (l/mol)	1.84×10^6	7.01×10^5	4.60×10^5
Linear range (μg/ml)	0.05–34.10	0.15–19.80	0.147–19.80
Limit of detection (μg/ml)	0.01	0.030	0.020
Limit of quantification (μg/ml)	0.03	0.11	0.08
Sandell sensitivity (μg · ml^{-1} per 0.001 absorbance)	1.1×10^{-2}	9.35×10^{-3}	9.24×10^{-3}
Slope	0.084	0.093	0.0894
Intercept	0.064	0.100	0.0602
Correlation coefficient (R^2)	0.997	0.994	0.998

timized. Various experimental parameters that affect the extraction of lamotrigine from urine were studied in order to obtain an optimized system. These parameters were optimized by setting all parameters to be constant, and then optimizing one at a time.

2.2. Effect of pH, ionic strength and number of extraction on extraction from urine

The effect of pH on the extraction of lamotrigine from urine was studied in the range of pH 6–13 using NaOH. A maximum extraction was clearly detected between pH 7 and 8. Thus, the pH was adjusted to 7 using phosphate buffer.

The addition of a certain amount of a salt can decrease the solubility of the analytes in the aqueous samples and enhance the distribution constant of compounds between the aqueous phase and the organic solvent. The resulting salting-out effect was assessed by the addition of NaCl to the aqueous solution in a range of 0–4 mol · l^{-1}. As expected, the extraction efficiency increases with increased concentration of NaCl; a single extraction using 2 mol · l^{-1} NaCl was found to be sufficient.

2.3. Analytical data

Under the experimental conditions described, standard calibration curves for lamotrigine with BCG, BCP and CPR were constructed. The analytical results obtained from this investigation are summarized in Tables 1 and 2. The calibration data were fitted by least square treatment and the regression equations and a linear relationship was found between absorbance and concentration in the ranges given in Table 1 and 2 for pure form and urine, respectively. In each case, the correlation coefficient was found to be greater than 0.99, indicating good linearity in the calibration graphs.

The limit of detection (LOD) and limit of quantitation (LOQ) (Miller and Miller 1993) for both pure form and urine matrix were determined based on the standard deviation among (n = 10) responses and the slope of the regression equation of a curve constructed at lower concentration levels (see Tables 1 and 2).

Table 2: Analytical parameters for determination of lamotrigine in urine matrix

Figure of merit	BCG	BCP	CPR
Linear range (μg/ml)	0.76–38.40	0.77–23.04	0.51–25.60
Limit of detection (μg/ml)	0.60	0.61	0.32
Limit of quantitation (μg/l)	1.99	2.04	1.06
Regression equation			
Slope	0.075	0.082	0.101
Intercept	0.139	0.207	0.088
Correlation coefficient (R^2)	0.997	0.994	0.997

In order to determine the precision and accuracy of the methods, solutions containing a known amount of drug were prepared and analysed in 10 replicates. The analytical results obtained from these investigations are summarised in Table 3. The relative standard deviation (RSD) can be considered to be very satisfactory.

2.4. Analytical application

The proposed method was applied to the determination of lamotrigine in tablet form and a urine sample to evaluate its analytical applicability. The measurements were carried out according to the procedure described in sections 2.4 and 2.5 using BCG, BCP and CPR. The tablet and urine LTG contents were determined following the procedures described for the calibration graphs. The summarized results are presented in Table 3.

To further confirm the selectivity of the assay, we conducted an interference study with the most common antiepileptics administered concomitantly with lamotrigine. The drugs studied spiked in urine to give the following concentrations: oxcarbazepine (10 μg · ml^{-1}), phenobarbital (9.3 μg · ml^{-1}), carbamazepine (9.5 μg · ml^{-1}) and lamotrigine (10 μg · ml^{-1}). Following the extraction procedure and measurement with acid dyes, the results showed that these drugs do not interfere in the determination of lamotrigine using the proposed method (Fig. 4).

Table 3: Results obtained for measurements of LTG concentration in pure form, tablet and urine by using different dyes

Sample	Parameters	Dyes		
		BCG	BCP	CPR
Pure form	Real concentration (μg/ml)	13.3	13.0	12.0
	Amount found (μg/ml)	13.6 ± 0.26	12.8 ± 0.53	11.8 ± 0.14
	R.S.D% (n = 10)	0.3	0.1	0.1
	Relative error (%)	2.2	−1.5	−1.6
Tablet	Real concentration (μg/ml)	11.4	11.3	11.3
	Amount found (μg/ml)	11.9 ± 0.29	10.8 ± 0.53	11.4 ± 0.14
	R.S.D% (n = 7)	0.1	0.2	0.1
	Relative error (%)	3.9	−4.2	0.9
Urine	Real concentration (μg/ml)	10.2	10.2	10.2
	Amount found (μg/ml)	10.4 ± 1.3	10.5 ± 0.8	10.0 ± 1.0
	R.S.D% (n = 10)	2.8	2.8	1.2
	Relative error (%)	2.0	2.7	−2.0
Urine[1]	Real concentration (μg/ml)	10.2	10.2	10.2
	Amount found (μg/ml)	9.8 ± 1.4	9.8 ± 1.0	9.9 ± 1.1
	R.S.D% (n = 8)	2.1	3	1.5
	Relative error (%)	−4.1	−4.0	−3.8

[1] Measurement of LTG (10.2 μg · ml^{-1}) in prescence of oxcarbazepine (10 μg · ml^{-1}), phenobarbital (9.5 μg · ml^{-1}) and carbamazepine (9.5 μg · ml^{-1}).

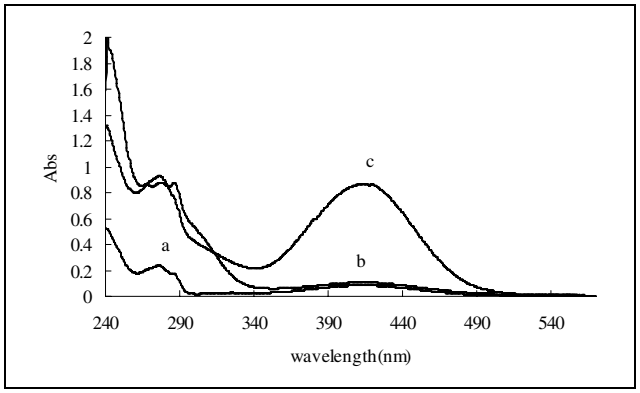


Fig. 4: Effect of interference: Absorption spectra obtained after extraction of LTG (4×10^{-5} mol · l^{-1}) from urine sample containing oxcarbazepine (4×10^{-5} mol · l^{-1}), phenobarbital (4×10^{-5} mol · l^{-1}) and carbamazepine (4×10^{-5}mol · l^{-1}) measured with BCP; (a) urine as blank, (b) urine spiked with other antiepileptic drugs and (c) LTG in the presence of other antiepileptic drugs

3. Experimental

3.1. Apparatus

All absorption spectra were made using a Perkin Elmer (model lambda 25) spectrophotometer with a scanning speed of 960 nm/min and 10 mm qurartz cells. pH measurements were made with a Metrohm Model 744 pH-meter.

3.2. Materials and reagents

All solvents used were of analytical reagent grade. Ethanol, chloroform, bromocresol green (BCG), bromocresol purple (BCP), chlorophenol red (CPR) and other chemicals were purchased from Merck. Pure grade carbamazepine, oxcarbazepine, phenobarbital and lamotrigine were obtained as a gift from the Center of Quality Control of Drugs, Tehran, Iran. The following available commercial preparations were analyzed: carbamazepine (Ramopharmin Co. Iran), labeled to contain 200 mg per tablet; phenobarbital (Amin Co. Iran), labeled to contain 60 mg per tablet; and lamotrigine and oxcarbazepine (Sobhan Co. Iran), labeled to contain 100 and 300 mg per tablet, respectively.

Stock solutions of BCG, BCP and CPR as acceptor dyes 5×10^{-3}, 5×10^{-3} and 3×10^{-4} mol · l^{-1} were prepared freshly in chloroform. The standard solutions of lamotrigine 1.6×10^{-3} mol · l^{-1} were prepared by dissolving an accurately weighed quantity of pure drug in an appropriate volume of chloroform with 15 min sonication, then storing the solution in the dark at 4 °C. Additional dilute solutions were prepared daily by accurate dilution just before use. The solutions were stable for several weeks.

Phosphate buffer was prepared by mixing an appropriate volume of sodium dihydrogen phosphate (0.02 mol · l^{-1}) and disodium hydrogen phosphate (0.1 mol · l^{-1}).

3.3. General Procedure

UV-Vis spectrum of a mixture of acid dyes and lamotrigine with different mole ratios in chloroform was recorded in the range of 240–540 nm against chloroform. A sample of the spectrum is shown in Fig. 1. The absorbances of the yellow colored complexes were measured at 420 nm for BCG and 415 nm for both BCP and CPR against the blank solvent at ambient temperature (25 °C), and calibration plots were drawn to calculate the amount of drug in unknown analyte samples.

3.4. Procedure for the tablet

The content of ten tablets of lamotrigine was weighed and ground into a fine powder. A quantity of the powder equivalent to 12.8 mg of the drug was dissolved in 35 ml chloroform and sonicated for 45 min, then filtered through a Whatman no. 41 filter paper. The filtrate was diluted to 50 ml with chloroform, and an aliquot of the filtrate was diluted to prepare working sample solutions. The nominal content of the tablets was Calculate from the calibration graph.

3.5. Procedure for human urine

An accurately weighed amount of standard lamotrigine (25.6 mg) was transferred into a 10 ml calibrated flask, dissolved in 7 ml ethanol and sonicated for 15 min, then diluted to 10 ml with ethanol (solution A). A fresh urine sample was collected from a healthy volunteer who was (roughly) 25 years old. A 5 ml urine sample was spiked with varying amounts of solution A to produce a solution containing 2.01–408 μg · ml^{-1} of lamotrigine, then transferred into a 20 ml volumetric flask. Then 2.34 g of NaCl salt, 14.2 ml of phosphate buffer and ethanol were added to obtain a solution consisting of urine – ethanol – buffer (25 : 4 : 71 v/v/v), which was pH adjusted to 7. This solution (15 ml) was then transferred into a sample tube, and 15 ml of chloroform was added. The contents were stirred on a magnetic stirrer for exactly 10 min. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulfate. Then 3 ml of chloroformic solution were added to an appropriate volume of acceptor reagent solution. The absorbance of the resulting solution was measured in the range of 270–570 nm against chloroform (Fig. 2), and the calibration graphs were drawn.

Acknowledgements: The authors would like to acknowledge the financial support of Tarbiat modares University and the K. N. Toosi University of Technology.

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