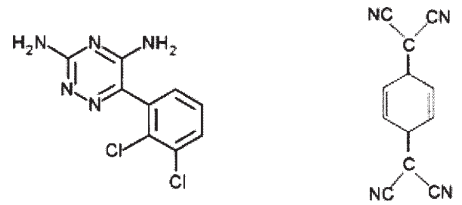
Sensitive and Simple Spectrophotometric Method for the Determination of Lamotrigine in Pure and Pharmaceutical Preparations of Charge-transfer Complex

SEVGI TATAR ULU*
Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul University, Istanbul, 34452, Turkey

A simple, rapid and sensitive spectrophotometric method has been proposed for the determination of lamotrigine in pharmaceutical preparations. The charge-transfer (CT) interactions between lamotrigine as electron donor and 7,7,8,8-tetracyanoquinodimethane (TCNQ), as π-electron acceptors have been investigated spectrophotometrically. The obtained complex was measured spectrophotometrically at 843 nm. The optimum experimental conditions have been studied carefully. Beer's law is obeyed over the concentration range of 2.0–25 μg/mL. The detection and quantification limits were 0.95 and 2.85 μg/mL, respectively. The method was validated for linearity, limit of detection, limit of quantification, precision, accuracy, recovery and robustness. Good values of precision are obtained, intra-day mean RSD are 0.82–0.98 % and the inter- day RSD are 0.79–0.87 %. The method is applicable for the assay of the investigated drug in tablet form and the results are in good agreement with those obtained by the reference HPLC method. The proposed procedures were successfully applied to the determination of lamotrigine in tablet form, with high percentage of recovery, good accuracy and precision.

Keywords: charge-transfer complexes, 7,7,8,8-tetracyanoquinodimethane, lamotrigine, pharmaceutical preparations

Lamotrigine (LA) (fig.1), an antiepileptic drug (AED) of the phenyltriazine class, is chemically unrelated to existing antiepileptic drugs. Its chemical name is 3,5-diamino-6-(2,3-dichlorophenyl)-as-triazine [1].



Lamotrigine **TCNQ**

Fig.1. Chemical structure of lamotrigine and TCNQ

LA an antiepileptic agent chemically unrelated to other anticonvulsants (phenyltriazine derivative), is a new generation antiepileptic drug which has shown to be effective against partial and secondarily generalized tonic–clonic seizures either on adjunctive treatment in patients with refractory epilepsy or when received as monotherapy [2-3].

Few methods have been reported for the determination of LA in pharmaceutical preparations including: HPLC [4] and adsorptive stripping voltammetry [5]. Several methods have been reported for determination of LA in biological fluids. HPLC [6-18], gas chromatography with nitrogen phosphorus detector [19], capillary electrophoresis [20-21] chromatography–thermospray mass spectrometry [22], immuno fluorometric assay [23] and radioimmunoassay [24] methods have been published for determination of LA in biological fluids.

However, no spectrophotometric and colorimetric methods for determination of LA in pharmaceutical preparations have been reported.

In this study, attempts were made to determine LA (*n* donors) through charge transfer complexation with (π acceptor) TCNQ. The proposed method is simple and suitable for routine determination of this drug. Also this method provide economic procedures, less time consuming and more sensitive compared with other reported methods.

Experimental

Materials and reagents

LA was supplied from Sigma (St. Louis, MO, USA) and its pharmaceutical preparation Lamictal tablets (25 mg) were purchased from a local pharmacy. TCNQ was purchased from Sigma–Aldrich. All solvents were purchased from Merck Ltd., Darmstadt, Germany. A aquaMAX™ water system (Young instrument, Korea) produced ultra pure analytical grade water.

Solutions

The stock solution of 1mg/mL LA was prepared by dissolving 100 mg LA in 100 mL methanol and stored in refrigerator. Working standard solution was prepared as required by suitable dilution of the stock solution with methanol (100 μg/mL).

0.15% (w/v) TCNQ was prepared in acetonitrile, solution was found be stable for at least 8 days at 4°C.

Instrumentation

A UV-160A ultraviolet-visible spectrophotometer (Shimadzu Tokyo Japan) was used for the absorbance measurements. UV-Visible spectra were automatically obtained by Shimadzu UV-160A system software.

Methods

General procedure

Different aliquots of each of the LA working solution in acetonitrile (0.2–2.5 mL;100 μg/mL) were transferred into

* *email: sevgitatar@yahoo.com*

separate 10 mL volumetric flasks, then 1.0 mL of TCNQ solution was added, and mixed. The solutions were allowed to stay for 10 min in a water bath at 80°C, cooled to room temperature. The reaction mixture was mixed and the volume was completed to 10 mL with acetonitrile. The immediately formed green color was measured at λ_{max}=843 nm against a blank prepared in the same manner except addition of drug.

Analysis of pharmaceutical formulations

Twenty tablets of each formulation were weighed and finely powdered. A quantity of the powder equivalent to 100 mg was transferred into a 100 mL calibrated flask, dissolved in 25 mL methanol, and sonicated for 20 min, completed to volume with the methanol (as in stock solutions), shaken well for 5 min, and filtered into a 100 mL calibrated flask and then diluted to volume with methanol. 10 mL volumes of the filtrates were then adjusted to 100 mL with methanol in calibrated flask, and procedure followed as mentioned under general procedure.

Results and discussion

Absorption spectra

TCNQ in acetonitrile reacts with the amino group of LA and give an intense green CT complex whose absorption is maximal at λ_{max}=843 nm (fig.2), most probably due to the formation of CT complex between LA acting as n-donor (D) and TCNQ as π acceptors (A):

$$D + A \rightleftharpoons \underset{\text{complex}}{(D\text{–}A)} \overset{\text{polar solvent}}{\rightleftharpoons} \underset{\text{radical ions}}{D^{\bullet+} + A^{\bullet-}}$$

The dissociation of the complex was promoted by the high ionizing power of acetonitrile solvent [25]. Acetonitrile was considered an ideal solvent as it afforded maximum sensitivity, due to its high dielectric constant that promotes maximum yield of radical anions in addition to its high solvating power of the reagents [26].

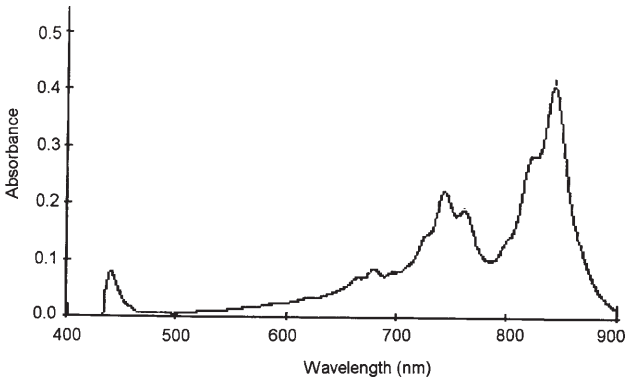


Fig.2. Absorption spectra of LA-TCNQ complex (10 μg/mL)

Effect of solvent

The effect of different solvents, namely, acetonitrile, acetone, ethanol and water on the color development was studied. Experiment indicated that, acetonitrile was proved to be the most suitable diluting solvent because it afforded an excellent solvating power for TCNQ reagent and gave high absorbance.

Effect of TCNQ concentration

The amount of TCNQ solution is tested for 1.00mL standard solution of drug. Experiment indicated that 1.0 mL TCNQ solution is enough for each drug, the final concentration of TCNQ is 7.30×10^{-3} mol/L.

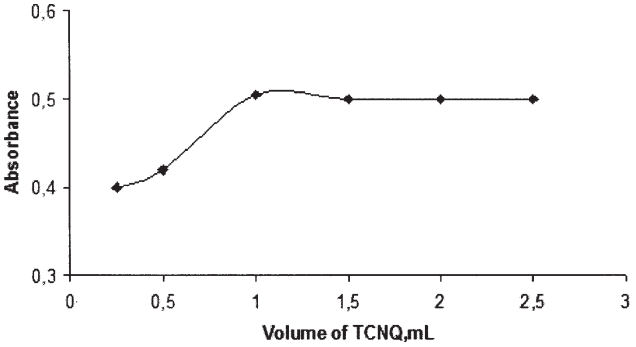


Fig. 3. Effect of TCNQ concentration (7.30×10^{-3} mol/L)

Effect of time and temperature

Sample solutions containing LA and blank were treated identically with the reagents within different time and temperature ranging from 25 to 80°C. The results obtained indicated that, complete color development was attained immediately at room temperature 80°C, after 10min (fig. 4).

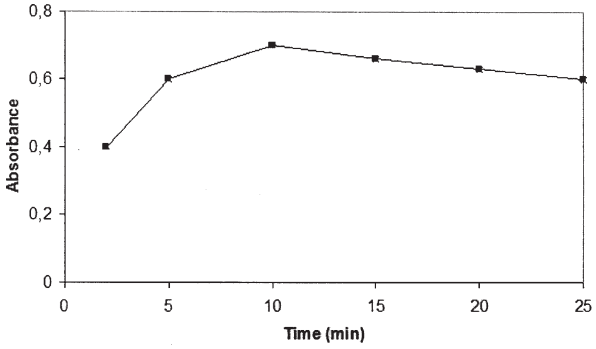


Fig.4. Effect of time on the color intensity at 80°C

Method validation

Linearity

Under the optimum experimental conditions, a linear relationship between of absorbance and concentration of LA was obtained over the concentration range 2.0 – 25μg/mL. The calibration data were fitted by least square treatment to obtain the regression equations A=0.025C+ 0.149 (r = 0.9999) (table 1).

Table 1

OPTICAL CHARACTERISTICS, STATISTICAL DATA OF THE REGRESSION EQUATIONS AND VALIDATION PARAMETERS FOR LA (n=6)

Parameter	Proposed method
Optical characteristics	
Molar absorptivity (1 mol^{-1} cm^{-1})	1.20×10^{4}
Sandell's sensitivity (μg cm^{-2}/0.001A)	2.13×10^{-2}
Regression analysis	
Slope	0.025
Intercept	0.149
Regression coefficient (r)	0.9999
Validation parameters	
Linearity (μg/mL)	2.0–25
LOD (μg/mL)	0.95
LOQ (μg /mL)	2.85

Concentration ($\mu g\ ml^{-1}$)	*Intra-day repeatability % RSD*			*Inter-day repeatability % RSD*
	Day 1	*Day 2*	*Day 3*	
2.0	0.986	0.976	0.968	0.867
10.0	0.854	0.798	0.795	0.842
25.0	0.832	0.815	0.809	0.786

Table 2
RESULTS OF PRECISION STUDY (n=6)

Spectrophotometric method	*Concentration of drug in formulations (µg/ mL)*	*Concentration of pure drug added (µg/ mL)*	*Total concentration of drug found (µg/ mL)*	*% Analytical recovery (± RSD %)*
	10.0	5.0	14.95	99.00 ± 1.15
	10.0	10.0	20.12	101.2 ± 0.97
	10.0	15.0	24.91	99.40 ± 0.91

Table 3
RESULTS OF STANDARD ADDITION METHOD (n=6)

Table 4
ROBUSTNESS OF THE PROPOSED METHOD

Conditions	Mean ± SD
	LA-TCNQ complex (15.0 µg/mL)
Optimum	14.91 ± 0.436
TCNQ amount	
1.1 mL	14.87 ± 0.401
0.9 mL	14.79 ± 0.389
Reaction time	
11 min	14.84 ± 0.355
9 min	14.81 ± 0.328
λmax	
844nm	14.75 ± 0.435
843 nm	14.72 ± 0.468

Table 5
DETERMINATION OF LA IN PHARMACEUTICAL PREPARATIONS BY THE PROPOSED AND REFERENCE [4] METHOD

Commercial product	**Proposed method**		**Reference method [4]**	
	Amount found	**% Assay**	**Amount found**	**% Assay**
Lamictal tablets (25 mg)				
Mean (mg) ± SD	24.93 ± 0.88	99.72	24.87 ± 0.94	99.48
t	0.19			
F	1.14			

The values in parenthesis are the tabulated values of *t* and *F* at $P = 0.05$ n=6, t=2.23 F=5.05

Limit of Detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation [27]. LOD and LOQ were found to be 0.95 and 2.85 µg/mL, respectively.

Precision

The precision of the assay (intra-day and inter-day) were determined at the LA concentrations cited in table 2. The inter-day precision was assessed by analyzing six replicates of each sample as a batch in a single assay run, and the intra day precision was assessed by analyzing the same sample, as triplicate, in three separate assay runs. The relative standard deviations (RSD) were less than 1% (table 2).

Accuracy

Recovery studies of LA were performed using the method of standard addition for measuring accuracy of method. The percent recovery of the added pure drug was calculated as, % Recovery = $[(Cv - Cu)/Ca] \times 100$, where Cv is the total drug concentration measured after standard addition; Cu, drug concentration in the formulation; Ca, drug concentration added to formulation [28]. The accuracy of the method was determined using LA samples at 5.0, 10.0 and 15.0µg/mL (table 3).

Specificity

In order to evaluate the selectivity of the proposed method for analysis of pharmaceutical preparations, the effect of common excipients (calcium carbonate, hydroxypropylcellulose, magnesium aluminium silicate, magnesium steareate, saccharin sodium) present in formulations was investigated. It was found that presence of common excipients of tablets did not interfere in the determination.

Robustness

The result of the robustness of the assay method is demonstrated in table 4. Method robustness checked after deliberate alterations of TCNQ amount, reaction time and wavelength shows that the changes of the operational parameters do not lead to essential changes of the spectrophotometric system.

Tables analysis

On comparison of the results obtained by the proposed method with the reference HPLC method [4] using the *t*-

test for the accuracy and F-test for the precision assessment, the calculated values did not exceed the corresponding theoretical values (tabulated value of *t*-test and F-test is under confidence level 95% = 6.05 and 2.23 for $n = 6$ degrees of freedom and $n - 1 = 5$ respectively) indicating insignificant differences between the results and also refer to the robustness of the proposed procedures (table 5).

Conclusion

The charge-transfer complexation reaction of LA as electron donor and π electron acceptor has been developed. The obtained complex was studied by ultraviolet–visible spectrophotometry. In summary, the proposed method was simple, rapid, accurate, precise and inexpensive. The proposed method offers the advantages of accuracy and time saving as well as simplicity of reagents and apparatus. Unlike the gas chromatographic and HPLC procedures, the instrument is simple and is not of high cost [4-24]. The proposed method is suitable for routine quality control.

References

1. *** The Merck Index, 2001), 959
2. RAMBECK, B., WOLF, P., Clin. Pharmacokinet. **25**,1993, p.433.
3. BARTOLI, A., GUERRINI, R., BELMONTE, A., ALESSANDRI, M.G., GATTI, G., PERUCCA, E. Ther. Drug Monit. **19,** 1997, p.252
4. EMAMI, J., GHASSAMI, N., AHMADI, F., J.Pharm. Biomed. Anal. **40**, 2006, p. 999
5. CALVO, M. E.B., RENEDO, O. D., MARTINEZ, M. J. A., Anal. Chim.Acta. p.549, 2005, p.74
6. CONTIN, M., BALBONI, M., CALLEGATI, E., CANDELA, C., ALBANI, F., RIVA, R., BARUZZI, A., J. Chromatogr. B. **828**, 2005, p.113.
7. KULDEEP, M. P., BODHANKAR, S. L., J. Chromatogr. B. **823**, 2005, p. 152
8. PATIL, K.M., BODHANKAR, S.L., J.Pharm. Biomed. Anal. **39,** 2005, p.181.
9. CHENG, C. L., CHOU, C. H., HU, O. Y. P., J. Chromatogr. B. **817,** 2005, p.199
10. BUGAMELLI, F., SABBIONI, C., MANDRIOLI, R., KENNDLER, E., ALBANI, F., RAGGI, M. A., Anal. Chim. Acta. **472**, 2002, p.1
11. BARBOSA, N.R., MIDIO, A. F., J. Chromatogr. B. **741**, 2000, p.289
12. VIDAL, E., PASCUAL, C., POU, L., J. Chromatogr. B. **736,** 1999, p.295
13. STOFORIDIS, P.A., MORGAN, D.J., O'BRIEN, T. J., VAJDA, F. J. E., J. Chromatogr. B. **727,** 1999, p.113
14. MATAR, K. M., NICHOLLS, P. J., BAWAZIR, S. A., AL-HASSAN, M. I., TEKLE, A., J.Pharm. Biomed. Anal. **17** ,1998), p. 525
15. OERTEL, R., RICHTER, K., GRAMATTE , T., KIRCH, W., J. Chromatogr. A. **797**, 1998, p. 203
16. COOPER, J. D. H., SHEARSBY, N. J., TAYLOR, J. E., FOOK SHEUNG, C. T. C., J. Chromatogr. B. **702**, 1997, p. 227
17. LONDERO, D., GRECO, P.L., J.Pharm. Biomed. Anal. **691**, 1997, 139
18. FORSSBLAD, E., ERIKSSON, A.S., BECK, O., J.Pharm. Biomed. Anal. **14**,1996, p.755
19. WATEELLE, M., DEMEDTS, P., FRANCK, F., DE DEYN, P.P., WAUTERS, A., NEELS, H., Ther. Drug Monit. **19,** 1997, p. 460
20. THEURILLAT, R., KUHN M., THORMANN, W., J. Chromatogr. A. **979** (2002) 353–368
21. SHIHABI, Z.K., OLES, K.S., J. Chromatogr. B. **683**, 1996, p.119
22. DOIG, M.V., CLARE, R.A., J. Chromatogr. B **554,** 1991, p.181
23. SAILSTAD, J.M., FINDLAY, J.W. Ther. Drug Monit. **13**, 1991, p.433
24. BIDDLECOMBE, R.A., DEAN, K.L., SMITH, C.D., JEAL, S.C., J. Pharm. Biomed. Anal. **8**, 1990, p. 691
25. LIPTAY, W., BRIEGLEB, G., SCHINDLER, K.Z., Electrochem. **66**, 1962, p. 331
26. SALEH, G. A., ASKAL, H.F., RADWAN, M. F., OMAR, A. M., Talanta **54,** 2001, p. 1205
27. *** Validation of analytical procedures, 6 November (1996) Methodology ICH Harmonised Tripartite Guideline,Having reached Step 4 of the ICH Process at the ICH Steering Committee meeting on.
28. VENUGOPAL, K., SAHA, R.N., Il Farmaco **60,** 2005, p. 906

Manuscript received: 26.08.2010