*Bulgarian Chemical Communications, Volume 46, Number 4 (pp. 764– 770) 2014*

# Voltammetric behavior of lercanidipine and anodic adsorptive stripping voltammetric method for assay in pharmaceutical dosage forms and biological fluids

F. Öztürk[1,*], D. Koyuncu Zeybek[2], E. Kilic[3]

[1]*Namık Kemal University, Faculty of Arts and Science, Department of Chemistry, Tekirdağ, Turkey*
[2]*Dumlupınar University, Faculty of Arts and Science, Department of Chemistry, Kütahya, Turkey*
[3] *Ankara University, Faculty of Science, Department of Chemistry, Ankara, Turkey*

Received September 24, 2013; Revised December 4, 2013

Electrochemistry of lercanidipine (LCN) was investigated and its optimal conditions were evaluated based on the irreversible and diffusion-controlled electrochemical oxidation signal (+ 0.93 V) of a carbon paste electrode (CPE) *versus* Ag/AgCl in Britton Robinson buffer (BR) and ethanol mixture of pH 4.5. Electrochemical determination was performed by square wave anodic adsorptive stripping voltammetry (SWAAdSV). Linear range was found to be between $3.3\times10^{-7}$ mol $L^{-1}$ and $4.5\times10^{-5}$ mol $L^{-1}$ in two different regions; preconcentration potential and time were found to be 0.0 V and 150 s, respectively. In this method, the limit of quantification (LOQ) was found to be $2.0\times10^{-8}$ mol $L^{-1}$ (0.012 mg $L^{-1}$). The method was applied to determine the content of LCN in a commercial pharmaceutical preparation, spiked human serum and spiked human urine. The method was found to be highly accurate and precise, having a relative standard deviation of less than 10% for all applications.

**Keywords:** Carbon paste electrode, human urine, human serum, lercanidipine, pharmaceuticals.

## INTRODUCTION

LCN (Fig.1), 2-[(3,3-diphenylpropyl) methyl-amine]-1,1-dimethylethylmethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylic ester is an antihypertensive drug which belongs to the dihydropyridine derivatives known as calcium antagonists. Similar to other calcium antagonist drugs, LCN blocks the influx of calcium ions through L–type calcium channels in cell membranes and reduces the blood pressure [1, 2].

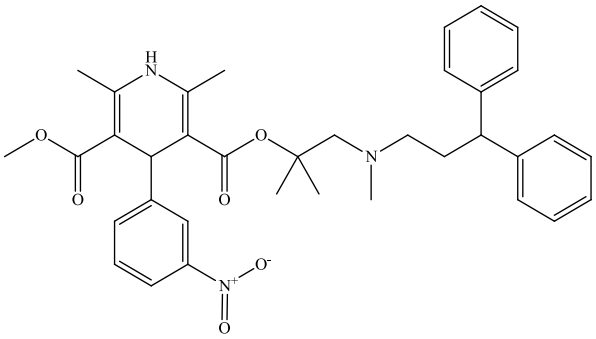


**Fig. 1.** Chemical structure of LCN

LCN and other 1,4-dihydropyridines have been determined in pharmaceutical samples by spectrophotometric [3], liquid chromatographic/ tandem mass spectrometric [4], capillary electrophoresis [5, 6], high performance liquid chromatography with UV detection, high performance liquid chromatography with electrochemical detection [7], high performance liquid chromatography with amperometric detection [8] and electrochemical methods [9-11]. Electrode modification and alternation of working electrodes is of great importance in electroanalysis. To the best of our knowledge, no study based on the oxidation of LCN at modified electrodes and CPE has been applied to pharmaceutical samples and human body fluids. Only Altun *et al*. [11], studied the electrooxidative behavior and redox properties of LCN using a boron-doped diamond electrode. CPE is becoming a popular electrode material in electrochemical studies [12, 13].

The aim of this study is to investigate the oxidation properties of LCN on a CPE and to develop an adsorptive stripping voltammetric method for its determination.

## EXPERIMENTAL

### *Apparatus and reagents*

All electrochemical studies were carried out using a BAS 100B electrochemical analyzer in a single-compartment three–electrode cell system (BAS C3 Cell Stand). For cyclic voltammetry (CV), square wave voltammetry (SWV) with and without anodic adsorptive stripping mode experiments, a CPE with 3 mm internal diameter,

* To whom all correspondence should be sent:
E-mail: fozanozturk@yahoo.com

Ag/AgCl electrode (BAS MF–2052 RE–5B, 3.0 mol $L^{-1}$ KCl) and platinum wire electrode (BAS MW–1034) were used as the working, reference and counter electrode, respectively. The bulk electrolysis was performed by a glassy carbon sieve (approximately 65 $cm^2$ area) as a working electrode, coiled platinum wire as a counter electrode (23 cm) (BAS MW–1033) and Ag/AgCl (BAS MF–2052 RE–5B, 3.0 mol $L^{-1}$ KCl) reference electrode.

All pH measurements were performed using Thermo Orion Model 720A pH-ion meter with an Orion combined glass pH electrode (912600). Ultrapure water (18.2 MΩ cm) was obtained from ELGA Purelab Classic water purification system. All data were obtained at room temperature (23±2 $^oC$).

Standard LCN (99.0 %) was supplied from Fako drug manifacturing company, Turkey and a stock LCN solution ($1.0\times10^{-3}$ mol $L^{-1}$) in ethanol was prepared. The calibration solutions were prepared by diluting the stock solution with a mixture of ethanol and Britton–Robinson buffer (BR) in a volume ratio of 20:80 as solvent–supporting electrolyte mixture. The pH values of these solutions were adjusted using 0.2 mol $L^{-1}$ NaOH solutions. All LCN solutions were protected from light and used within a day to avoid photochemical decomposition.

BR buffer was prepared and pH of the working solutions was adjusted as described in literature [10].

### *Preparation of CPE*

The carbon paste was prepared by hand mixing of graphite powder and paraffin oil in a mass ratio of 5:3 until a homogeneous paste was obtained. A portion of the paste was placed firmly into a cavity of a polyetheretherketone (PEEK) rod electrode body (with 3 mm internal diameter) and a brass wire was introduced into the opposite end of the electrode body to establish an electrical contact. Then the CPE surfaces were smoothed. The resulting CPE was used as a working electrode.

### *Preparation and Analysis of Samples*

Lercadip® tablets containing LCN (10 mg per tablet) manufactured by Fako were used as the pharmaceutical dosage form. Ten tablets were weighed, finely powdered and mixed in a mortar. The average mass per tablet was determined and then the powder equivalent to one tablet was weighed and transferred to a 100.0 mL calibrated flask containing about 50 mL of ethanol. After sonication for 30 min, the volume was filled up with ethanol. The solution was centrifuged at 1500 rpm for 30 min. For preparation of the stock solution, a 10.0 mL sample was diluted to 100.0 mL with ethanol-BR buffer mixture. It was stored at +4 $^oC$ in dark. Appropriate volumes of this solution were transferred to the electrochemical cell containing 10.0 mL solvent–supporting electrolyte mixture, then pH was adjusted to the desired value and LCN was determined by the calibration curve method.

The spiked human serum and urine samples were analyzed in the same manner. The samples obtained from healthy persons were kept frozen until analysis. After melting, an aliquot of serum (or urine) sample was appended to the electrochemical cell containing 10.0 mL of solvent–supporting electrolyte mixture and appropriate volumes (0.01 mL, 0.02 mL, 0.025 mL, and 0.03 mL) of standard LCN solutions were transferred to the cell. After the solution was deaerated for 10 min with argon, measurements were performed to determine the LCN content.

### *Voltammetric procedure*

All voltammetric experiments were performed in a cell containing 10.0 mL of LCN solution prepared in a solvent–supporting electrolyte mixture. The CPE was placed into the cell and the solution was purged with purified argon (99.99 % purity) for 10 min before the first run and for 30 s between runs. The voltammograms were recorded by anodic sweeping from 0.00 V to +1.10 V.

## RESULTS AND DISCUSSION

### *Electrochemical Behavior of LCN*

Electrochemical behavior, diffusion and adsorption properties of LCN were investigated by cyclic voltammetry (CV), square wave voltammetry (SWV), and bulk electrolysis (BE). Fig. 2 shows the voltammograms of CPE in the ethanol-BR mixture in absence (Fig. 2a) and presence (Fig. 2b) of $5\times10^{-5}$ M LCN at pH 4.5. As can be seen, there is a single irreversible oxidation peak at about +0.93 V in presence of LCN, and no peak in absence of LCN. It can be hence concluded that the oxidation peak is due to the oxidation of LCN molecules on CPE.

The effects of the scan rate (between 0.005 – 2.0 $Vs^{-1}$) on the anodic peak potential ($E_{p,a}$) and anodic peak current ($i_{p,a}$) for $5\times10^{-5}$ M LCN were investigated. The peak potential shifts to more positive values and the peak current increases with

increasing the scan rate (Fig. 3 (A)), indicating that the electrooxidation steps are not reversible

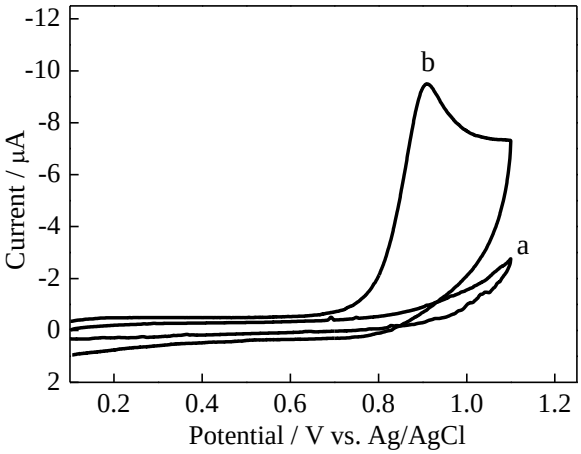
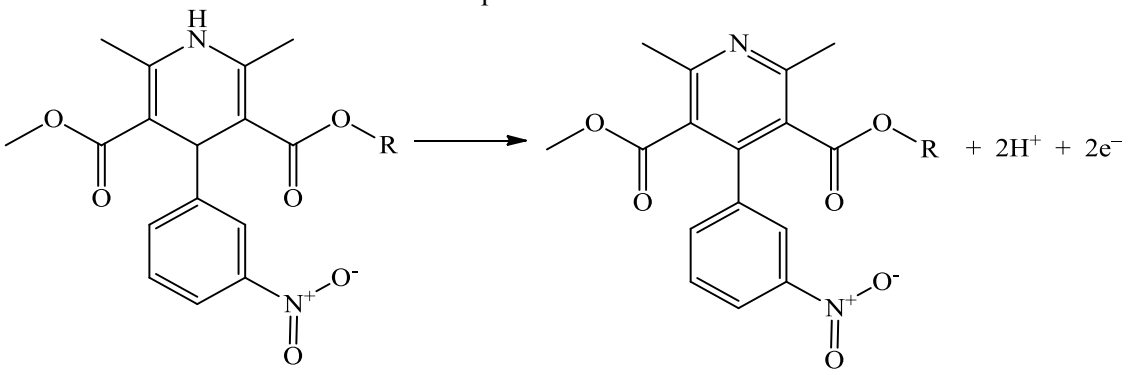



**Fig. 2.** Cyclic voltammograms of CPE in absence (a) and presence (b) of $5\times10^{-5}$ M LCN at pH 4.5 in EtOH–BR

[14] . The logarithm of the peak current *versus* the logarithm of the scan rate (Fig. 3 (B)) was plotted and a straight line with a slope of 0.56 was observed for LCN. This value of the slope is very close to the theoretical value of 0.5 for a diffusion controlled mechanism [15]. Also, the relationship between the peak current and the square root of the scan rate is shown in Fig. 3(C). The linearity of the plot indicates a diffusion controlled process at the solution/electrode interface. The fact that no pre– and post–peaks are observed in the CV at high scan rates is another indication that adsorption does not occur to a considerable extent on the electrode surface [16].

To investigate the effect of pH on the electrochemical behavior of LCN, square wave voltammograms of $5\times10^{-5}$ mol $L^{-1}$ LCN were recorded in the pH range of 2.1 – 4.5 (Fig. 4). At pH values higher than 4.5, the solubility of LCN dramatically decreases and precipitation begins; more and more ethanol is needed to overcome the solubility problem, therefore the effect of higher pH values could not be studied. As seen from the figure, the oxidation peaks shift towards less positive values with increasing pH. The shift in the peak potentials with pH may indicate that hydrogen ions are involved in the electrode reactions [17]. To calculate the number of electrons involved in the oxidation mechanism BE was carried out at 1.15 V and the results were compared with those obtained by voltammetric studies. According to the obtained results and literature data on the oxidation of molecules containing a pyridine group, it can be concluded that the R-C-N-H group of LCN is oxidized to R-C=N according to the following reaction mechanism:




*R* = *N*-isobutyl-*N*-methyl-3,3-diphenylpropan-1-amine

A similar type of mechanism was described for the oxidation of the $C_6H_5$–N–H group [18-20].

*Electroanalytical determination of LCN*

The electrochemical determination of LCN was performed with adsorptive stripping techniques to obtain the lower limit of detection. For this purpose, initially, instrumental parameters and experimental conditions such as pH, LCN concentration, deposition time, and deposition potential were optimized. To obtain a well-defined peak shape and higher peak current, the instrumental parameters were optimized for $6.5\times10^{-7}$ mol $L^{-1}$ LCN in an EtOH–BR mixture of pH 4.5 and the following parameters were employed in all experiments: frequency, *f*, 15 Hz; scan increment, $\Delta E_i$, 4 mV; and pulse amplitude, $\Delta E_a$, 25 mV.

The solution pH of is a critical factor affecting both the rate and equilibrium state of the accumulation process and the rate of the electrode reaction. The influence of pH on the SWAAdSV responses was studied at a CPE in the pH range from 2.1 to 4.5 and results are presented in the previous section. In order to obtain useful peak shape and linearity range, 4.5 was selected as the optimum pH.

The effects of deposition potential and deposition time on the SWAAdSV responses were studied for $6.5\times10^{-7}$ mol $L^{-1}$ LCN over the range

from 0.0 V to 0.7 V and from 30 s to 180 s, respectively. As can be seen from Fig. 5(A), the maximum values for the peak current are established at 0.0 V and peak current decreases with more positive potential values. The maximum peak current in the deposition step was observed for a deposition time of 150 s in the SWAAdSV (Fig 5(B)). In a further stripping assay, deposition potential and deposition time were employed as 0.0 V and 150 s, respectively.

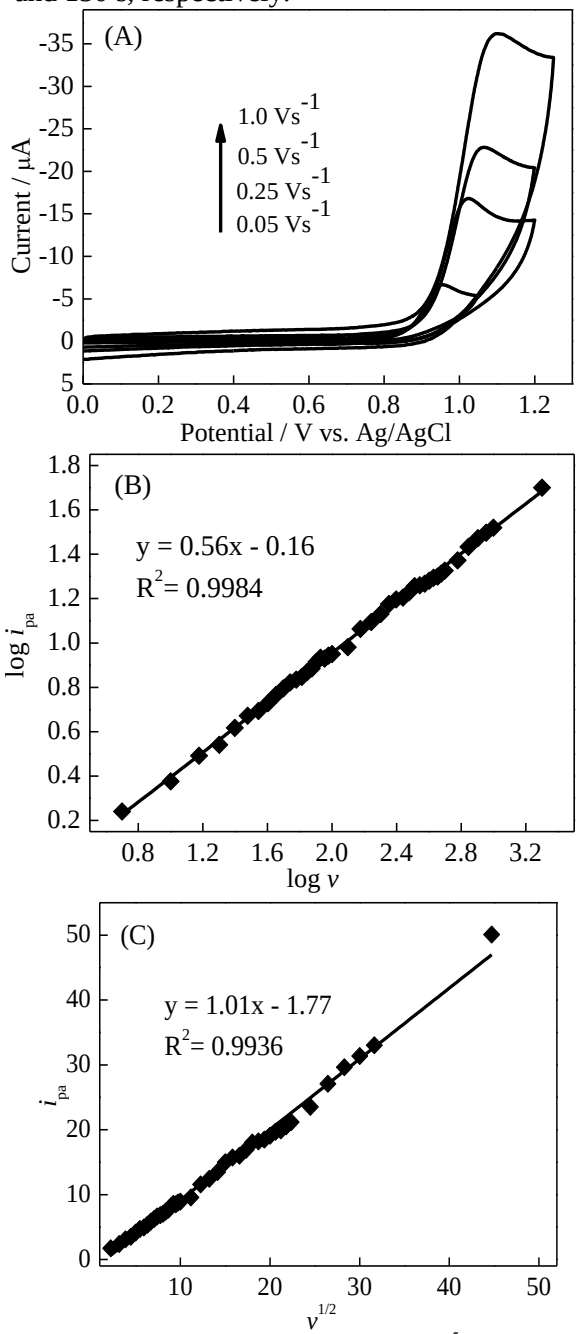



**Fig. 3.** Cyclic voltammograms of $5\times10^{-5}$ M LCN at different scan rates: (A) plot of the logarithm of the peak current *versus* the logarithm of the scan rate; (B) plot of the peak current *versus* the square root of the scan rate; (C) EtOH–BR buffer solution at pH 4.5.

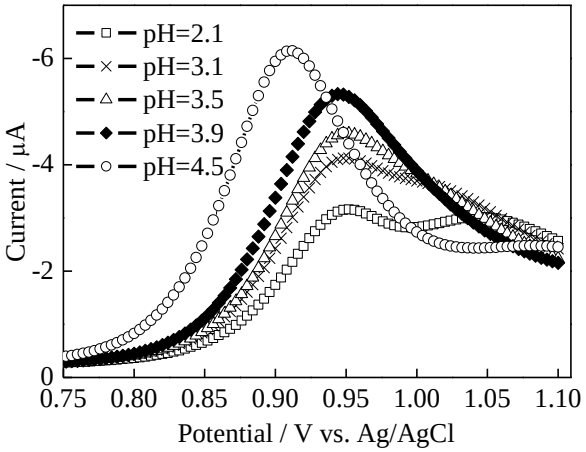



**Fig. 4.** Influence of different pH on square wave voltammograms of $5\times10^{-5}$ mol $L^{-1}$ LCN

In order to determine the linearity range of LCN in the proposed method, standard solutions containing LCN in the range from $3\times10^{-7}$ mol $L^{-1}$ to $4.5\times10^{-5}$ mol $L^{-1}$ were used. For each concentration, three replicate measurements were performed and the mean of these measurements was used in the plot of the calibration curve for the corresponding concentration.

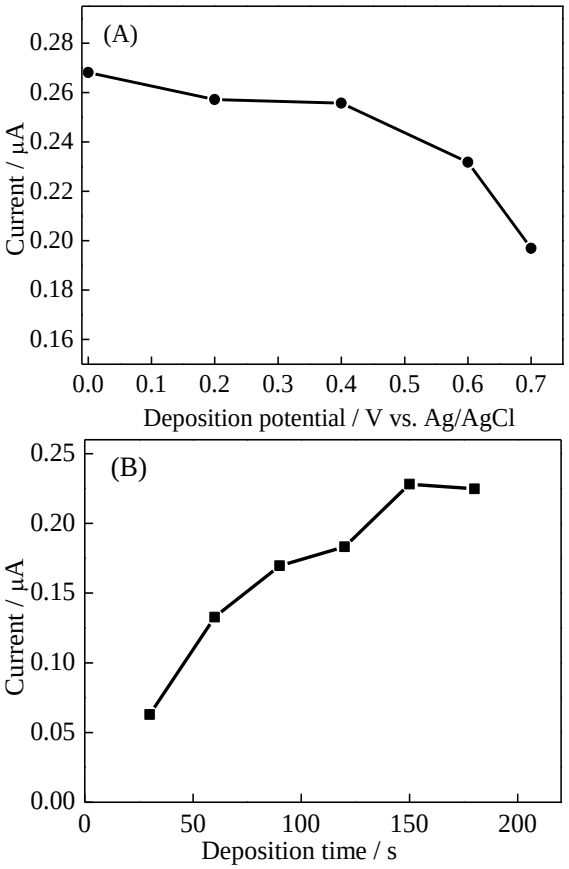



**Fig. 5.** Effect of deposition potential (A) and deposition time (B) on peak current of $6.5\times10^{-7}$ mol $L^{-1}$ LCN at pH 4.5 in SWAAdSV (deposition potential: 0.0 V, deposition time: 150 s).

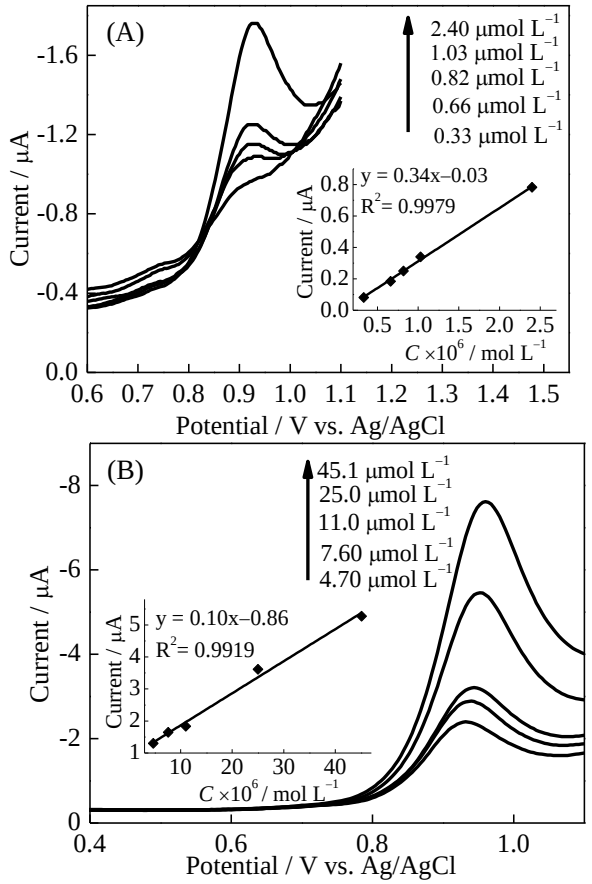



**Fig. 6**. SWAAdSVs of LCN at different concentrations (inset: calibration curve for the corresponding concentrations), first (A) and second (B) linear range

The currents of the oxidation peaks were linear with LCN concentration in two regions. The first linear range was from $3.3\times10^{-7}$ mol $L^{-1}$ to $2.4\times10^{-6}$ mol $L^{-1}$ with a regression equation of $i_{p(\mu A)} = 0.34 \times C_{LCN}$ ($\times10^6$ mol $L^{-1}$) - 0.03 ($R^2$ = 0.9979) (Fig. 6 (A)) and the second one was from $4.7\times10^{-6}$ mol $L^{-1}$ to $4.5\times10^{-5}$ mol $L^{-1}$ with a regression equation of $i_{p(\mu A)} = 0.10 \times C_{LCN}$ ($\times10^6$ molL$^{-1}$) - 0.86 ($R^2$ = 0.9919) (Fig. 6 (B)).

The characteristics of the calibration plots are summarized in Table 1.

### *Application of the Proposed Method: The Dosage Form and Biological Samples*

The determination of LCN in commercial tablets (labeled as 10 mg of LCN per tablet) was performed using the calibration curve method and the validity of the proposed SWAAdSV method was evaluated. Pretreatment procedures such as extraction or evaporation were not required for sample preparation. The amount of LCN determined using the proposed method is presented in Table 2. The applicability of the proposed method was also checked for spiked human urine and spiked human serum as described in the Experimental section. The obtained results are presented in Table 3. The accuracy of the proposed method was determined by its recovery values.

### *Validation of Method*

The validation of an analytical method aims at demonstrating that the analytical procedure is suitable for the intended use. It involves determination of accuracy, precision, repeatability, intermediate precision, reproducibility, specificity, detection limit, quantitation limit, linearity range and robustness of the method [21]. The results of the validation studies are given in the above sections. Limit of detection (LOD) and limit of quantification (LOQ) values were calculated as described in [22] and were found to be $6\times10^{-9}$ mol $L^{-1}$ and $2 \times 10^{-8}$ mol $L^{-1}$, respectively. The accuracy of the measurement by means of the described procedure was checked by calculating the recovery of a known concentration of LCN following the proposed method. Recovery values ranged from 99.51 % to 102.55 % for tablet analysis, from 100.42 % to 105.48 % for urine analysis and from 98.06 % to 100.63 % for serum analysis (Tables 2 and 3).

**Table 1.** Regression data of the calibration curve for assay of LCN by SWAAdSV

| Calibration Parameter | First Linear Region | Second Linear Region |
|---|---|---|
| Linearity Range, mol $L^{-1}$ | $(0.33 – 2.4) \times 10^{-6}$ | $(4.7– 45) \times 10^{-6}$ |
| Calibration Equation | $i_{p(\mu A)} = 0.34 \times C_{LCN}$ ($\times10^6$ mol $L^{-1}$) - 0.03 | $i_{p(\mu A)} = 0.10 \times C_{LCN}$ ($\times10^6$ mol $L^{-1}$) - 0.86 |
| Slope of Calibration Curve, ALmol$^{-1}$,(m) | 0.34 | 0.10 |
| Intercept, A | $–2.89\times10^{-8}$ | $8.70\times10^{-7}$ |
| SD (Standard Deviation) of Calibration, A | $1.92\times10^{-9}$ | $1.73\times10^{-7}$ |
| SD of Slope, ALmol$^{-1}$ | $1.20\times10^{-3}$ | $5.18\times10^{-3}$ |
| SD of Intercept, (s), A | $6.78\times10^{-10}$ | $6.12\times10^{-8}$ |
| Limit of Detection (LOD), mol $L^{-1}$ | $6.0\times10^{-9}$ | $1.83\times10^{-6}$ |
| Limit of Quantification (LOQ) mol $L^{-1}$ | $2.0\times10^{-8}$ | $6.11\times10^{-6}$ |
| Regression Coefficient, $R^2$ | 0.9979 | 0.9919 |
| Repeatability of peak current [a], (RSD, %) | 2.93 | 7.46 |
| Repeatability of peak potential [a], (RSD, %) | 0.25 | 0.25 |

[a] Calculated for 5 replicate measurements

**Table 2.** Results of proposed method for determination of LCN from the solution of lercadip® tablets

| Sample[a] | Labeled value per tablet, mg | Found values per tablet, mg | Recovery value[b], % | RSD[c],% |
|---|---|---|---|---|
| I | 10 | 10.19, 10.28, 10.30 | 102.56 ±1.46 | 0.57 |
| II | 10 | 9.65, 9.93, 10.27 | 99.50 ± 7.71 | 3.12 |

[a] Sample I is in the first linear region and II is in the second linear region
[b] Results of recovery values are given as mean ± ts/√N (at 95 % confidence level)
[c] RSD is relative standard deviation

**Table 3.** Results of the proposed method for determination of spiked standard LCN solution into various biological media

| Sample[a] | Spiked, µg | Found, µg | Recovery value[b] | RSD[c], % |
|---|---|---|---|---|
| Standard in Urine I | 101.79 | 108.32, 107.02, 106.76 | 105.48 ± 1.98 | 0.78 |
| Standard in Urine II | 152.69 | 156.43, 152.06, 151.50 | 100.42 ± 4.39 | 1.76 |
| Standard in Serum I | 101.79 | 100.89, 100.51, 98.05 | 98.06 ± 3.76 | 1.54 |
| Standard in Serum II | 203.58 | 213.47, 201.79, 199.33 | 100.63 ± 9.22 | 3.69 |

a Samples given in linear regions
[b] Results of recovery values are given as mean ± ts/√N (at 95 % confidence level)
c RSD is relative standard deviation

## CONCLUSION

In the present study, for the first time the electrooxidation behavior of LCN was studied on a CPE. It was demonstrated that LCN has one oxidation peak at the CPE at +0.93 V. The proposed method was used for determination of LCN in pharmaceutical tablets without pretreatment. The method developed in this study has a high potential to be applied in determining the content of LCN in commercial pharmaceutical preparations, spiked human serum and spiked human urine due to its high accuracy and precision (RSD < 10%).

***Acknowledgement:*** *The authors wish to thank to Prof. Nevin Erk from Ankara University, Faculty of Pharmacy, because of her kindly help to provide the standard LCN.*

## REFERENCES

1. K. J. McClellan, B. Jarvis, *Drugs*, **60**, 1123 (2000).
2. V. Barrios, C. Escobar, Á. Navarro, L. Barrios, J. Navarro-Cid, A. Calderón, *Int. J. Clin. Pract.*, **60**, 1364 (2006).
3. M. A. Abu El-Enin, D. R. El-Wasseef, D. T. El-Sherbiny, S. M. El-Ashry, *Int. J. Biomed. Sci.*, **5**, 261 (2009).
4. C. A. Mueller, A. B. B. González, W. Weinmann, *J. Mass Spectrom.*, **39**, 639 (2004).
5. T. Christians, D. Diewald, C. Wessler, Y. Otte, J. Lehmann, U. Holzgrabe, *J. Chromatogr., A*, **853**, 455 (1999).
6. T. Christians, U. Holzgrabe, *Electrophoresis*, **21**, 3609 (2000).
7. A. Álvarez-Lueje, S. Pujol, J. A. Squella, L. J. Núñez-Vergara, *J. Pharm. Biomed. Anal.*, **31**, 1 (2003).
8. A. B. Baranda, R. M. Jiménez, R. M. Alonso, *J. Chromatogr., A*, **1031**, 275 (2004).
9. A. Álvarez-Lueje, S. Pujol, L. J. Núñez-Vergara, J. A. Squella, *J. AOAC Int.*, **85**, 1247 (2002).
10. F. Öztürk, I. H. Taşdemir, D. A. Erdoğan, N. Erk, E. Kiliç, *Acta Chimica Slovenica*, **58**, 830 (2011).
11. Y. Altun, B. Uslu, S. A. Ozkan, *Anal. Lett.*, **43**, 1958 (2010).
12. M. A. T. Gilmartin, J. P. Hart, *Analyst*, **120**, 1029 (1995).
13. I. Švancara, K. Vytřas, K. Kalcher, A. Walcarius, J. Wang, *Electroanalysis*, **21**, 7 (2009).
14. R. S. Nicholson, I. Shain, *Anal. Chem.*, **36**, 706 (1964).
15. J. Wang, Study of Electrode Reactions and Interfacial Properties. In *Analytical Electrochemistry*, John Wiley & Sons, Inc.: 2006; pp 29.
16. Z. Mandić, B. Nigović, B. Šimunić, *Electrochim. Acta*, **49**, 607 (2004).
17. N. Menek, Y. Karaman, *Dyes Pigm.*, **68**, 101 (2006).
18. J. A. Squella, A. E. Iribarren, J. C. Sturm, L. J. Núñez-Vergara, *J. AOAC Int.*, **82**, 1077 (1999).
19. G. Altiokka, D. Dogrukol-Ak, M. Tunçel, H. Y. Aboul-Enein, *Arch. Pharm.*, **335**, 104 (2002).
20. R. N. Goyal, S. Bishnoi, *Bioelectrochemistry*, **79**, 234 (2010).
21. 2A. E. Bretnall, G. S. Clarke, 11 - Validation of Analytical Test Methods. In *SS&T*, Satinder, A.; Stephen, S., Eds. Academic Press: 2011; Vol. Volume 10, pp 429.
22. F. Ozturk, I. H. Tasdemir, Z. Durmus, E. Kilic, *Collect. Czech. Chem. Commun.*, **75**, 685 (2010).

# ВОЛТАМПЕРОМЕТРИЧНИ ОТНАСЯНИЯ НА ЛЕРКАНИДИПИН И АНОДНО-АДСОРБЦИОНЕН ВОЛТАМПЕРОМЕТРИЧЕН МЕТОД ЗА АНАЛИЗ НА ФАРМАЦЕВТИЧНИ ДОЗИРОВКИ И БИОЛОГИЧНИ ФЛУИДИ

Ф. Йозтюрк[1,*], Д. Коюнджу Зейбек[2], Е. Килич[3]

[1] *Департамент по химия, Факултет за изкуства и наука, Университет Намък Кемал, Текирдаг, Турция*
[2] *Департамент по химия, Факултет за изкуства и наука, Университет Думлупънар, Кютахия, Турция*
[3] *Департамент по химия, Факултет за наука, Университет в Анкара, Анкара, Турция*

Постъпила на 24 септември, 2013 г.; коригирана на 4 декември, 2014 г.

(Резюме)

Изследвани са електрохимичните отнасяния на лерканидипин (LCN) и са определени оптималните условия за необратима и дифузионно-контролирана окислителна реакция при потенциал (+ 0.93 V) при електрод от въглеродна паста (CPE) спрямоAg/AgCl - електрод в буфер на Britton Robinson (BR) и водно-етанолова смес при pH 4.5. Електрохимичните изследвания са извършени при анодно-адсорбционна волтамперометрия с правоъгълни импулси (SWAAdSV). Намерена е линейна област между $3.3\times10^{-7}$ и $4.5\times10^{-5}$ mol $L^{-1}$ в две различни области; потенциалът и времето на пред-концентриране са намерени съответно 0.0 V and 150 s. По този метод е намерена границата на чувствителност като $2.0\times10^{-8}$ mol $L^{-1}$ (0.012 mg $L^{-1}$). Методът е използван за определянето на съдържанието на LCN в търговски фармацевтични препарати човешки серум и човешка урина. Методът е много точен и чувствиетлен със стандартно отклонение под 1% във всички изследвани случаи.