Research Article

Agata Skorupa*, Slawomir Michalkiewicz, Magdalena Jakubczyk

Highly sensitive determination of α-lipoic acid in pharmaceuticals on a boron-doped diamond electrode

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Abstract: A simple, highly sensitive, and selective differential pulse voltammetry method for the determination of α-lipoic acid (LA) in pharmaceutical preparations was developed and validated. The method is based on a quasi-reversible, diffusion-controlled, one-electron anodic oxidation of LA on a boron-doped diamond electrode (BDDE) in a McIlvaine (citrate-phosphate, C-PB) buffer solution at pH 3.0. For the first time, this environment was used for LA determination. A linear calibration curve was obtained within the concentration range 5.82 × 10⁻⁸ to 4.00 × 10⁻⁴ mol L⁻¹ with a correlation coefficient of 0.9999. The limits of detection was estimated to be 1.94 × 10⁻⁸ mol L⁻¹, which is one of the lowest values characteristic of voltammetric and chromatographic methods of LA determination. The proposed procedure is sensitive, accurate, and precise. Its utility was demonstrated in the determination of LA in pharmaceuticals without the need for its separation from the matrices. The results were comparable to those obtained by high performance liquid chromatography reference method and were in good accordance with the once declared by manufacturers. Thus, our method can be considered as an alternative to the dominant chromatographic determinations of α-LA in real samples.

Keywords: α-LA, oxidation, determination, BDDE, aqueous buffers

1 Introduction

Reactive oxygen species (ROS) are molecules with at least one unpaired electron. Its presence causes these compounds to be highly reactive and dangerous for living organisms. Their excessive concentration in cells is indicated as the main factor responsible for the pathogenesis of many diseases [1]. The compounds with antioxidant properties play an important role in maintaining the ROS concentration in the equilibrium state. This equilibrium is established by the action of enzymatic antioxidants: superoxide dismutase, catalase and glutathione peroxidase, and low molecular weight antioxidants of a hydrophilic nature, such as ascorbate (vitamin C), glutathione, cysteine, and hydrophobic ones: vitamin D₃, carotenoids, coenzyme Q, and tocopherols [2]. α-Lipoic acid (LA) belongs to a group of compounds that have amphiphilic properties. It is soluble in both aqueous and fatty environments. Therefore, it can simultaneously protect the lipid membranes of cells, as well as intercellular spaces into which water-soluble components penetrate. Both α-LA and its reduced form called dihydrolipoic acid (DHLA) have antioxidant properties [3,4].

LA and DHLA exhibit the features that, according to Packer et al. [5], should be fulfilled by an antioxidant used in therapy. These characteristics include the ability to react with free radicals and to chelate transition metal ions, the possible interaction with other antioxidants, the participation in gene expression, as well as easy absorption from a diet and concentration in tissues, cells, and body fluids. The therapeutic properties of LA also manifest in the treatment of diseases associated with oxidative stress. These include cardiovascular diseases [6,7], multiple sclerosis [4,8,9], and Alzheimer’s disease [10]. Recent studies show the anti-cancer properties of LA [11].
They also highlight its effective role in reducing chemotherapy-induced side effects, as well as preventing chemoresistance. LA supplementation largely prevents the effects of the administration of chemotherapeutic agents [3].

α-LA also protects against the consequences of poisoning with mercury, arsenic, lead, and other heavy metals [12–16]. LA is often used in mushroom poisoning, and in the treatment of liver diseases caused by alcohol abuse [17].

α-LA is used in the treatment of obesity, reduction of low-density lipoproteins, total cholesterol, and triglycerides [18], as well as in the treatment of patients with type 2 diabetes and diabetic neuropathy [3,17,19,20]. Research into the influence of LA on AIDS has shown that this compound interrupts the replication of this virus HIV [17,21].

The therapeutic advantages of α-LA increase interest in obtaining it external sources. In biological systems, it occurs in a bounded form as lipoyllysine (LLys) [22,23].

The widespread occurrence and significant therapeutic properties of LA make it important to control its content in biological matrices (plant and animal tissues) and in pharmaceutical preparations (medicines or dietary supplements). α-LA can be determined both as free acid and LLys [24–28]. Various analytical techniques are used for the quantitative analysis of LA. These are mainly high performance liquid chromatography (HPLC) with various detection methods: chemiluminescence [29], spectrophotometric UV-Vis [30,31], (also recommended by the Polish Pharmacopoeia [32]), fluorescence [33], electrochemical (EC) [34], and also gas chromatography with flame photometric detection [35] or mass spectrometry [36], as well as spectrophotometry [37–40], capillary electrophoresis [41,42], coulometry [43,44], or voltammetry [45–55].

Voltammetric studies on the EC behavior and determination of LA were mainly carried out on various types of electrode materials including platinum (Pt) [45], glassy carbon electrode (GCE) [46], a boron-doped diamond electrode (BDDE) [47], fluorine-doped tin oxide (FTO) [48], a pyrolytic graphite electrode modified with cobalt phthalocyanine (PG/CoPc) [49], a manganese(iv) oxide-modified screen-printed graphene electrode (MnO2/SPGE) [50], and a poly(vanillin) modified platinum electrode (p(VA)/PtE) [51], on a GCE modified with SnO2 nanoparticles and cetyltribhenylphosphonium bromide (SnO2 NP-CTPPB/GCE) [52] or with functionalized multi-walled carbon nanotubes-polyindole/TiO2 (f-MWCNTs-PIN/TiO2/GCE) [53], and also on a carbon fiber microelectrode (CF) [54,55].

The voltammetric investigations were usually performed in aqueous buffer solutions: phosphate [45,46,49,53], acetate [45,50], Britton–Robinson [47,51], or in their mixtures with alcohols [46,50]. Anhydrous acetic acid was also used as a reaction environment [54,55]. Literature data indicate that the process of LA oxidation in aqueous buffered solutions occurs in one step, is irreversible, and is controlled by diffusion [45–49,52,53]. There are disagreements about the number of electrons transferred in the electrode reaction. Some authors claim that this is a one-electron process [45–47,51] that takes place without [46,47] or with the participation of protons [45]. According to the cited authors, the oxidation product of LA is sulf oxide, and this process takes place on one of the sulfur atoms of the dithiol ring. Sulfoxide as a product of the LA oxidation process in aqueous solutions is also indicated in other works [48,49,52], where a two-electron process is postulated. The anodic oxidation of α-LA in acetic acid is a one-electron process, quasi-reversible, and diffusion-controlled. The primary oxidation products of the electrode reaction are chemically unstable and participate in irreversible homogeneous reactions resulting in the formation of non-electroactive products (E1C1 mechanism) [54].

Our research aimed to determine the EC properties of LA on a BDDE in aqueous buffered solutions. A GCE was used for comparative studies. The investigations were carried out in buffers such as Britton–Robinson, acetate, citrate, or McIlvaine at different pH values. So far, there is a lack of literature data on the use citrate-phosphate buffer for this purpose.

An attempt was also made to develop a new method for the voltammetric determination of LA on BDDE in pharmaceutical preparations.

## 2 Materials and methods

### 2.1 Chemicals

All reagents were of high grade and used as received: (±)-α-LA (LA, ≥99%, Sigma-Aldrich), sodium acetate (CH3COONa, AcNa, anhydrous, >99.0%, Fluka), sodium citrate (Na3C6H5O7 × 2H2O, ≥99.0%, Sigma-Aldrich), disodium hydrogen phosphate (Na2HPO4, anhydrous, ACS, Reag. Ph Eur, Supelco), potassium dihydrogen phosphate (KH2PO4, anhydrous, ACS, Reag. Ph Eur, Supelco), sodium hydroxide (NaOH, p.a., 98.8%, Pol-Aura), glacial acetic acid (CH3COOH, AcH, p.a. ACS, Merck), phosphoric acid (H3PO4, 85 wt% in H2O, Sigma-Aldrich), citric acid (H3C6H5O7, ≥99.5%, Sigma-Aldrich), nitric(v) acid (HNO3, p.a., 65%, Pol-Aura), ethanol (C2H5OH, EtOH, 96%, ACS, Reag. Ph Eur, Merck), and acetonitrile (AN, p.a. ACS, Merck).
Various types of medicinal samples with different formulations purchased on the Polish pharmaceutical market were analyzed: coated tablets (Thiogamma®, Wörwag Pharma GmbH & Co. KG, Germany), soft capsules (Alfapilon, BIOTON S.A., Poland), and hard capsules (Liponexin, SOLINEA, Poland). They contained 600, 350, and 300 mg of LA, respectively.

### 2.2 Apparatus

The voltammetric measurements were carried out using a computer-controlled M161 digital EC analyzer with EALab 2.1 software for processing and storing data (mtm-ankö, Poland). A 5 mL glassy EC cell with a three-electrode system was applied in the experiments. The working electrode was a BDDE of 3 mm diameter (BioLogic, France). A GCE of the same diameter (BASI, USA) was used for comparative studies.

All potentials were measured against Ag/AgCl (3 mol L\(^{-1}\) KCl) as a reference electrode (Mineral, Poland), and the auxiliary electrode was platinum wire (BASI, USA). Before each voltammetric experiment, the surface of the working electrodes was carefully prepared. BDDE was activated in 1 mol L\(^{-1}\) HNO\(_3\) by cyclic polarization in the range of potentials from −1.6 to 2.0 V (\(\nu = 0.1\) V s\(^{-1}\)) for 10 cycles. The cyclic voltammetry (CV) scans were started and finished at negative potentials. This procedure was repeated each day before starting all voltammetric measurements. The surface of the working electrode does not require additional treatment between the registrations of the voltammetric curves. Only the exchange of the studied solutions required washing electrode with water and ethanol and drying with the blotting paper. The surface of the GCE was polished with the use of 0.05 μm alumina powder (BASI, USA), rinsed, and dried.

The pH measurements were carried out using a CX-732 multifunction computer meter equipped with a pH sensor consisting of a glass indicator electrode and an Ag/AgCl reference electrode (Elmetron, Poland).

HPLC measurements were carried out with a Model 210 Varian ProStar Instrument (USA) with UV-Vis detector set at 332 nm. The analytical column C-18 (250 mm × 4.6 mm i.d.) was used. The acquisition and processing of the data were performed using Star Chromatography Workstation version 6.30.

All experiments were conducted at the constant temperature (25 ± 1°C).

### 2.3 EC measurements

The EC behavior of LA on BDDE was studied by CV and differential pulse voltammetry (DPV). CV curves were recorded with scan rates from 6.25 to 1,000 mV s\(^{-1}\) in the potential range from −0.9 to 1.3 V. To improve the electrode response, the parameters of the DPV technique were optimized: pulse amplitude, \(dE = 40\) mV, pulse width, \(\tau = 60\) ms, and scan rate, \(\nu = 20\) mV s\(^{-1}\). The DPV curves were recorded in the potential range from −0.9 V (start) to 1.5 V (end) vs Ag/AgCl. This wide potential range provides very good reproducibility of the successively recorded curves.

Due to the limited solubility of LA in water (0.127 g L\(^{-1}\)) [56], its stock solution (1.84 × 10\(^{-2}\) mol L\(^{-1}\)) and the samples assayed were prepared in ethanol.

Double-distilled water was used to make the buffer solutions. Britton–Robinson buffer solutions (B-RB) were prepared by mixing phosphoric acid, boric acid, and acetic acid (all in the concentration of 0.04 mol L\(^{-1}\)). Their pH was adjusted with 0.2 mol L\(^{-1}\) NaOH. McIlvaine buffers (citrate-phosphate, C-PB) were obtained by mixing different volumes of 0.2 mol L\(^{-1}\) disodium hydrogen phosphate and 0.1 mol L\(^{-1}\) citric acid. Citrate buffers (CB) were prepared by mixing 0.2 mol L\(^{-1}\) solutions of citric acid and sodium citrate in different amounts. The pH range of these buffer solutions was from about 2.1 to 7.5. Acetate buffers (AcB) were obtained by mixing 0.2 mol L\(^{-1}\) solutions of acetic acid and sodium acetate to obtain the desired pH in the range from 3.7 to 5.6.

The main voltammetric measurements were conducted on BDDE in an experimentally chosen solution of the citrate-phosphate buffer at pH 3.0 containing ethanol (4%, v/v).

The calibration procedure was based on DPV oxidation curves of LA in the concentration range from 2.6 × 10\(^{-7}\) to 7.4 × 10\(^{-4}\) mol L\(^{-1}\) (44 different concentrations). The solutions tested with a desired amount of LA were obtained by mixing an ethanolic stock solution (1.84 × 10\(^{-2}\) mol L\(^{-1}\)) with an appropriately defined pH buffer.

The test solutions of the medicinal samples were prepared in a 25-mL volumetric flask by diluting their ethanolic solutions with a citrate-phosphate buffer at pH 3.0 and maintaining a constant ethanol concentration of 4%, v/v. The volume of the parent solutions taken depended on the concentration of LA in these samples. It was experimentally proved that an optimum peak current for its determination should be about 0.5 μA. The prepared solutions did not need any additional steps, e.g. extraction, and were directly analyzed.
The voltammetric determination of LA in the test solutions and in the pharmaceutical preparations was performed with the use of the DPV technique and of a multiple standard addition method. The DPV technique was used because it is characterized by the high sensitivity and resolution of the signals recorded in multicomponent solutions. It also limits the participation of the adsorption phenomena accompanying the oxidation process, which ensures the repeatability of the successively recorded curves. The standard solution in C-PB (pH 3.0) contained LA in the concentration of $7.4 \times 10^{-4}$ mol L$^{-1}$ and 4% ethanol (v/v). It was added to the solutions investigated in portions of 50 µL.

All the solutions investigated were stored at a temperature of 4°C.

2.4 HPLC measurements

α-LA was also determined using the reference HPLC method according to a procedure described by Aboul-Enein and Hoenen [30] with some modification. A mixture of acetonitrile;0.05 M potassium dihydrogen phosphate, pH 2.5 (45:55, v/v), was used as the mobile phase. Flow rate was set at 1.0 mL min$^{-1}$, and the injection volume was 20 µL. The stock standard solutions of LA were prepared in acetonitrile. Calibration plot was constructed based on the LA peak areas versus concentration. This curve was used for the determination of α-LA in pharmaceuticals.

The samples of pharmaceutical were prepared in acetonitrile. Samples were filtered through a 0.45 µm membrane filter and degassed before HPLC analysis.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Investigation of the EC behavior of LA

The DPV curves were recorded in various types of buffer solutions with different pH in order to obtain an optimal environment for the investigation of the EC properties of LA as well as for its voltammetric determination. The environments tested were the aqueous buffers: Britton–Robinson buffer, McIlvaine, the citrate, and the acetate.

The parameters such as the peak current, $I_p$, the peak potential, $E_p$, and the peak width at half height, $W_{1/2}$, were compared. Well-shaped curves were obtained in all the solutions investigated, especially in a strongly acidic medium (Figure 1a). It was found that an increase in pH caused negligible shifts in the peak potentials. Additionally, their widths increased in all the solutions tested. This can make the identification of the analyte difficult in a pH range over 5 (data not shown).

It was found that, depending of the type of buffer solution, the maximum DPV peak current was achieved...
at different pH values (Figure 1). The best results were obtained with the use of the citrate-phosphate buffer at pH 3.0. A very well-shaped, narrow ($W_{1/2}$ value of about 90 mV), and excellently reproducible DPV curves with the peak potential of 0.885 V vs Ag/AgCl were recorded in this environment (Table 1). The use of the McIlvaine buffer allows for over 40 and 15% increase in the peak current of DPV curves compared to those recorded in Britton–Robinson and citrate buffer, at the same pH, respectively (Figure 1b). The solution of the C-PB at pH 3.0 was thus applied in all subsequent investigations.

The study of the EC behavior of LA on BDDE in the experimentally chosen environment was done mainly with CV. Well-shaped CV curves with very small background currents were recorded (Figure 2). The LA oxidation peak was at the potential of above 0.8 V vs Ag/AgCl.

Irrespective of pH, no cathodic peak was observed even when the direction of polarization, $E_λ$, was near the anodic peak (Figure 2a). This proves that the primary

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**Table 1:** Voltammetric parameters of the anodic oxidation of LA on BDDE and GCE in C-PB (pH 3.0)

| Electrode | $v$ (mV s$^{-1}$) | $E_p$ (V) vs Ag/AgCl | $E_{p-2}$ (V) | $I_p$ ($\mu A$ s$^{-1}$) |
|-----------|------------------|----------------------|--------------|------------------|
| BDDE      | 6.25             | 0.856                | 0.0520       | 0.46             |
|           | 50               | 0.886                | 0.0541       |                  |
|           | 100              | 0.898                | 0.0575       |                  |
| GCE       | 6.25             | 0.820                | 0.0578       | 0.44             |
|           | 50               | 0.839                | 0.0595       |                  |
|           | 100              | 0.846                | 0.0600       |                  |

$^1$peak potential, $^2$reversibility criteria (CV), $^3$slope of the relationship $\log(I_p, \mu A) = f(\log(v, V s^{-1}))$, $^4$pulse amplitude, $^5$peak width at half height (DPV).

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**Figure 2:** (a) CV curves ($v = 100$ mV s$^{-1}$) of $5 \times 10^{-4}$ mol L$^{-1}$ LA recorded on BDDE in C-PB solution (pH 3.0). Direction of electrode polarization was reversed from anodic to cathodic at potentials $E_λ$ given at the curve. Dashed line is residual current. (b) CV curves recorded in the same conditions at different scan rates (given at the curves in mV s$^{-1}$). Relationships: (c) $I_p = f(v^{1/2})$ and (d) $\log I_p = f(\log v)$. 

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product of the anodic oxidation of LA is unstable, and it transforms in the course of a successive irreversible chemical reaction into non-electroactive products. As can be seen from Figure 2b, the peak currents increase with increasing potential scan rates, and the peak potentials shift slightly in the direction of higher values (Table 1). According to the Randles–Sevcik equation [57], a linear plot of the peak current vs square root of the scan rate with the regression equation: \( I_p (\mu A) = 0.325 + 41.14 \sqrt{v} \) (Vs\(^{-1}\)) and with a correlation coefficient of 0.9998 (Figure 2c) was obtained for LA. This means that the electrode process is controlled by mass transport. This assumption is confirmed by the slope of 0.46 of the plot \( \log(I_p) (\mu A) = 1.58 + 0.46 \log(v) (V s^{-1}) (r = 0.9999) \), which is very close to the theoretical value of 0.5 (Figure 2d) for a diffusion-controlled process [58,59].

The reversibility of the anodic oxidation process of LA was checked using a criterium based on the difference between the peak potential and the potential corresponding to 1/2 of the peak current \((E_p - E_{p/2})\). The obtained values are close to the theoretical one \((0.0564/n V) [57]\) but increase slightly with the scan rate (Table 1), which indicates a quasi-reversible character of the electrode process with the exchange of one electron. The slight changes in the peak potential with increasing scan rates (Figure 2b) confirm that the anodic oxidation of LA is not fully reversible.

The quasi-reversible nature of the electrode process, which takes place with the exchange of one electron, is confirmed additionally by the application of the parameter called the peak width of the DPV curves at half-height, which is described by the equation: \( W_{1/2} = 3.52 RT/nF \) [57]. The theoretical value of \( W_{1/2} \) should be 0.0904/n V at 25°C. The obtained value of 0.0900 V (Table 1) is very close to the theoretical one. It should be noted that in contrast to other investigated solutions, known from the literature [45–49,52,53], in which the anodic oxidation of LA is described as irreversible, the quasi-reversible nature of this electrode process on BDDE in the C-PB (pH 3.0) can influence the increase of sensitivity of LA determinations.

The results obtained and the literature data [48,49,52] indicate that the anodic oxidation of LA on BDDE in the C-PB solutions, pH 3.0, occurs on one of its sulfur atoms and leads to the formation of an unstable cation radical. The significant changes in the peak potential with pH were not observed, which indicates that no protons are involved in the anodic oxidation. Next, primary oxidation product undergoes an irreversible chemical reaction with the formation of the non-electroactive final product. The overall process can be thus described as \( E_{aq} + C_4 \). The final product is most probably LA S-oxide.

The similar behavior of LA in C-PB was observed on the GCE. Figure 3 presents the comparison of the course of CV curves recorded on BDDE and on GCE. The oxidation process of the analyte occurs in the same potential range. However, much higher background currents, and slightly smaller diffusion currents, are recorded on GCE. In addition, the reversibility criterium and \( W_{1/2} \) of the CV and DPV curves, respectively, indicate that the anodic oxidation of LA on GCE occurs more irreversible (Table 1). BDDE is thus more useful for the identification and quantification of LA. Additionally, this electrode is only slightly more expensive than other carbon electrodes but surpasses them in many EC properties, i.e. very low and stable background current, stability of response in different media, high resistance to deactivation by surface fouling, and the wide potential window in aqueous solutions. This environmentally friendly electrode can thus be applied to the analysis of various biologically electroactive compounds at very high positive and negative potentials [60–62].

### 3.2 Calibration curve for LA determination

Voltammetric measurements on BDDE in experimentally chosen environment C-PB (pH 3.0) were performed by the DPV technique with the use of the optimized parameters described in the Experimental section. The DPV curves were recorded in solutions containing different amounts of LA. It was found that an increasing LA concentration caused an increase in the peak current of the DPV curves. No changes in their position on potential axis were observed (Figure 4). The increase of the current at the
end of the potential window with increasing concentration of LA can be explained by the overlapping signals derived from the successive stage of LA oxidation and from the oxidation of other components of the solution (e.g. citric acid).

The peak potential of 0.885 V vs Ag/AgCl can thus be used for the identification of LA. The observed linear dependence between the peak current and the concentration in the range of $5.82 \times 10^{-8}$ to $4.00 \times 10^{-4}$ mol L$^{-1}$ (Inset B in Figure 4) is described with the following equation: $I_p (\mu A) = -0.0152 + 18.23c$ (mmol L$^{-1}$) with $r = 0.9998$ ($n = 40$). Thus, the determination of LA seems to be possible in this solution. The limits of detection (LOD) and quantification (LOQ) were calculated using the following equations: $LOD = 3.3\sigma/a_1$, $LOQ = 3 \times LOD$, where $\sigma$ is the standard deviation of the blank and $a_1$ denotes the slope of the calibration plot [63]. The results obtained indicate that the determination of LA in the proposed medium is characterized by a wide linearity range and low LOD and LOQ values (Table 2).

Table 3 presents the comparison of the essential analytical parameter, characteristic of the developed voltammetric method of LA determination in pharmaceuticals, with the literature data for different procedures. Our method is characterized by a very low value of LOD which is only higher than obtained on a PG/CoPc [49], comparable to that for the HPLC-BDDE [34], and lower for the HPLC-UV-Vis [30,31], and for other voltammetric methods. Linearity range, however, is wider or comparable to procedures presented in Table 3. These results confirm the high sensitivity and utility of the developed method. One of the reasons for improving the sensitivity and diminish the LOD value is the quasi-reversible character of the electrode process of LA (higher oxidation currents compared to irreversible processes dominant in other environments) [45–49,52,53].

### 3.3 Repeatability and reproducibility

The precision of the developed method was checked by the repetitive recording of the DPV curves ($n = 10$) in a solutions containing LA in the concentrations of $2.5 \times 10^{-6}$ and $1 \times 10^{-4}$ mol L$^{-1}$. The repeatability of the peak current was excellent, and RSD did not exceed 0.9, and 0.7%, respectively. The reproducibility study was evaluated by measuring this peak current in the same solutions over a period of 5 days. RSD value obtained did not exceed 1.5 and 1.2%, respectively. The peak potential characteristic for LA was stable in the linear range of the calibration plot.

### 3.4 Recovery studies

The accuracy and reliability of the developed voltammetric method were checked in control determinations. A solution with a specified amount of LA ($2.58 \times 10^{-5}$ mol L$^{-1}$, 5.32 mg L$^{-1}$) was prepared for this purpose. 2.0 mL of the test solution was placed in a measuring cell, and the DPV curves were recorded before and after the successive additions of the standard solution in the concentration of $7.4 \times 10^{-4}$ mol L$^{-1}$. The calibration curves were constructed based on the peak current after subtracting the background current. The determination was repeated five times, and the results were statistically examined. The experimentally obtained amount of LA (Table 4) only slightly differs from the one introduced...
Table 3: Comparison of the linearity range (LR) and limit of detection (LOD) for α-LA determination in pharmaceuticals obtained in this work with voltammetric and chromatographic methods

| Method          | LR (mol L⁻¹)         | LOD (mol L⁻¹) | Reference |
|-----------------|----------------------|---------------|-----------|
| HPLC-UV-Vis     | 4.85 × 10⁻⁵ to 2.42 × 10⁻³ | 2.13 × 10⁻⁵   | [30]      |
| HPLC-UV-Vis     | 2.42 × 10⁻⁴ to 8.48 × 10⁻⁴ | 2.42 × 10⁻⁶   | [31]      |
| HPLC-BDDE       | 4.85 × 10⁻⁸ to 7.27 × 10⁻⁴ | 1.45 × 10⁻⁸   | [34]      |
| DPV (PG/CoPc)   | 4.85 × 10⁻⁷ to 1.89 × 10⁻⁵ | 3.39 × 10⁻⁹   | [49]      |
| DPV (GCE)       | 2.42 × 10⁻⁶ to 7.27 × 10⁻⁸ | 1.79 × 10⁻⁶   | [66]      |
| SWV (FTO)       | 4.99 × 10⁻⁶ to 2.00 × 10⁻⁴ | 3.68 × 10⁻⁶   | [48]      |
| DPV (CF)        | 9.69 × 10⁻⁷ to 5.97 × 10⁻⁴ | 3.88 × 10⁻⁷   | [55]      |
| DPV (BDDE)      | 5.82 × 10⁻⁸ to 4.00 × 10⁻⁸ | 1.94 × 10⁻⁸   | This work |

1Symbols have been explained in the introduction.

To the test solution (R = 100.7%). The developed method can thus be considered as accurate. The RSD value of 0.7% also proves its high precision.

3.5 Interferences study and LA signal identification in pharmaceuticals

The effect of some possible interferents, which can be present in pharmaceutical samples, on the LA oxidation peak current was investigated. They were added in a 10-fold excess to a solution containing 4 × 10⁻⁵ mol L⁻¹ LA. The obtained responses indicate that the compounds tested: glucose, vitamins B1, B2, B6, chloride ions do not oxidize in the potential range characteristic of LA and thus do not interfere with the analytical signal of LA.

The influence of the excess of these substances on the peak current is not greater than 1.3%. Only the signal derived from ascorbic acid (the broad signal with E_p of about 1.25 V vs Ag/AgCl) interferes with that of LA, causing an increase of about 30% in the peak current. Therefore, its existence in high excess can disturb the quantification of LA. This interferent was not a component of the preparations investigated.

The first stage of the studies conducted in the solutions containing real samples of the pharmaceuticals was the identification of the voltammetric signals characteristic of LA (Figure 5). The DPV peak, corresponding to the oxidation of α-LA, is observed at the potential of about 0.9 V vs Ag/AgCl. It is very close to that obtained for LA standard. This indicates that the components of the matrix accompanying the analyte do not influence the position and height of the analytical signal. The identification of LA and its determination in real samples can thus be possible without the need for the application of any separation procedures.

3.6 Determination of LA in pharmaceutical products

The developed analytical procedure was applied to the determination of LA in pharmaceuticals. The solutions containing these samples were prepared as described in

Table 4: Results of LA determination in control and in pharmaceuticals by DPV on BDDE with HPLC reference method

| Sample          | DPV | HPLC | t-test | F-test |
|-----------------|-----|------|--------|--------|
|                 | Amount (mg/tablet) | 2R (%) | 3RSD (%) (n = 5) | Amount (mg/tablet) | 2R (%) | 3RSD (%) (n = 5) | 4(2.31) | 4(6.39) |
|                 | Declared | 1Found |            |Declared | 1Found |            |        |         |
| Control (mg L⁻¹) | 5.32 | 5.36 ± 0.05 | 100.7 | 0.7 | — | — | — | — |
| Alfalipon       | 350 | 346.6 ± 13.4 | 99.1 | 3.0 | 334.1 ± 16.2 | 95.5 | 3.9 | 0.74 | 1.5 |
| Thiogamma®      | 600 | 598.7 ± 7.9 | 99.8 | 1.1 | 571.8 ± 22.7 | 95.3 | 3.2 | 0.75 | 8.3 |
| Liponexin       | 300 | 301.6 ± 2.5 | 100.5 | 0.6 | 303.1 ± 10.0 | 101.0 | 2.7 | 0.18 | 16.7 |

1x = x_{av} ± t_{0.95} S_{av} for n = 5 and t_{0.95} = 2.776 (tabulated), S_{av} – denote standard deviation of mean. 2Recovery, R = (x_{av}/concentration involved) × 100%. 3Relative standard deviation. 4Values in parenthesis are tabulated t and F at P = 0.05, n = 5.
the Experimental section. The analytical procedure for
the quantification of LA was the same as for the recovery
studies. Figure 6 presents the DPV curves obtained for LA
in a solution containing a pharmaceutical Thiogamma®
taken as an example and the calibration plot for five
determinations. The peak current increases with the
addition of the standard solution of LA. It should be
noted that excellent repeatability was observed for the
successively recorded curves (Figure 6a – all signals
were repeated three-times). The experimentally obtained
concentrations of LA in the solutions tested were then
converted to the analyte content of one tablet of the
appropriate preparation and were statistically examined.
The results of the determinations are presented in Table 4.

The developed procedure gives results very close to those
declared by the manufacturers. The recovery and relative
standard deviation values are in the range of 99.1–100.5%
and 0.6–3.0%, respectively. Therefore, the method can
be considered as reliable and accurate.

The HPLC was used as the comparative method. The results were statistically examined and presented in Table 4. It can be seen that the precision of the results obtained by HPLC (RSD values were not lower than 2.7%) is worse than these of DPV. A comparison of the results using the F-test indicates that precision of these methods is comparable only for Alfalipon formulation. The accuracy of both techniques (see R values, and comparison of calculated and tabulated r-values, Table 4) is comparable. Because the differences in the amounts labeled and measured means (R) are higher for the reference method, the voltammetric procedure can be recognized as accurate and more credible. It should be noted that the matrix effect reduction for complex samples like pharmaceuticals is the important advantage of DPV method.

4 Conclusion

The anodic oxidation of α-LA on a BDDE in citrate-phosphate buffer solutions at pH 3.0 was applied to develop
the DPV method of its quantification in pharmaceutical preparations. For the first time, McIlvaine buffer was
used for LA determination. In this experimentally chosen environment, the exchange of electron is quasi-reversible
and diffusion-controlled. The increased reversibility of

![Figure 5: DPV curves recorded on BDDE in solutions of C-PB (pH 3.0) containing pharmaceuticals investigated or LA standard.](image)

![Figure 6: (a) DPV curves recorded in solution containing Thiogamma® (8.4 mg L⁻¹) and after additions of LA standard solution (c = 7.4 × 10⁻⁴ mol L⁻¹, the volumes in µL are given at the curves). (b) Calibration curve in the standard addition method (five determinations).](image)
this process in comparison with the literature data results an increase in sensitivity of the determinations and a decrease in LOD value \((1.94 \times 10^{-8} \text{ mol L}^{-1})\), which is one of the lowest obtained by other voltammetric and chromatographic methods.

The developed procedure was successfully applied to the determination of LA in pharmaceutical formulations with satisfactory recovery ranging from 99.1 to 100.5%. It can be considered reliable, highly sensitive, precise, and accurate. The advantage of this method is a stable and excellent reproducible response of BDDE for LA without any undesirable influence of interferents commonly accompanying this analyte, the simplicity of sample preparation, which is limited only to its dissolution in the applied medium and thus allows to shorten the time of analysis. The use of aqueous buffer solutions and the small volumes of the samples also cause that developed method is in accordance with the principles of the green chemistry. The listed advantages in combination with no expensive equipment and reagents and significantly fewer organic solvents consumption make our procedure much less expensive compared to the chromatographic methods commonly used in pharmaceutical analysis.

A presented procedure can be used routinely in laboratories of pharmaceutical industries being an alternative to the dominant chromatographic procedures, HPLC in particular.

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