

Scientific paper

Development of Green Differential Pulse Voltammetric Strategy for the Determination of Alfuzosin Hydrochloride at Pencil Graphite and Modified Carbon Paste Electrodes

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Abstract

A sensitive and inexpensive differential pulse voltammetric technique was applied to investigate the electrochemical behavior of alfuzosin hydrochloride at two different working electrodes: silica gel modified carbon paste and pencil graphite electrodes (PGE). The voltammetric conditions were optimized using cyclic voltammetry, showing an irreversible anodic peak in Britton-Robinson buffered medium (pH 6) at 0.86–0.90 V. The electrochemical responses were linearly correlated with alfuzosin concentrations ($R^2 > 0.999$) in the ranges of 0.6–20 and 0.3–20 μM , exhibiting higher electrocatalytic activity at PGE with a low detection limit/ detectability of 0.099 μM . In addition, this study was a successful attempt for the drug determination in tablets and spiked urine samples with green profile evaluation, employing the National Environmental Methods Index, analytical Eco-Scale score, and Green Analytical Procedure Index.

Keywords: Alfuzosin hydrochloride; carbon paste electrode; cyclic voltammetry; differential pulse voltammetry; pencil graphite electrode.

1. Introduction

Alfuzosin hydrochloride (ALF), a selective α_1 -adrenoceptor antagonist, is defined as (2RS) -N- [3-[(4-Amino- 6,7 dimethoxyquinazolin-2-yl) methylamino] propyl] tetrahydrofuran-2-carboxamide hydrochloride.¹ The action of ALF as a vasodilator may be less frequent, but it acts more selectively on the smooth muscle tone within the prostate and bladder neck, causing relaxation of these muscles. Therefore, it results in symptomatic relief of the benign prostatic hyperplasia, a common progressive disease encountered in aging men, within weeks.² It alleviates the symptoms of urinary obstruction after oral administration of sustained release ALF by reducing outflow resistance and enhancing bladder emptying.

Several analytical methods were published for ALF determination either alone or in combinations, including

micellar flow injection analysis with fluorescence detection,³ kinetic colorimetric,⁴ and UV-spectrophotometric methods.⁵ Spectrofluorimetric methods adopting utilization of ortho-phthalaldehyde⁶ and micellar matrix⁷ for ALF assay in biological fluids and stability indicating spectroscopic studies were also developed.⁸ In addition, chromatographic methods such as HPLC with UV,⁹ MS/MS,¹⁰ and fluorescence^{11, 12} detections, chiral HPLC,¹³ UPLC,¹⁴ and stability indicating HPTLC¹⁵ were developed.

Different electrochemical techniques were also established for potentiometric,¹⁶ conductometric,¹⁷ and voltammetric analysis of ALF by linear sweep and normal pulse polarography,¹⁸ Coulometric fast Fourier transform linear sweep voltammetry,¹⁹ and differential pulse^{20,21} and square-wave voltammetry.²²

To our knowledge from the literature review, few analytical studies were performed to investigate the elec-

trochemical behavior of ALF, as depicted in Figure 1. Meanwhile, most of the reported researches were directed towards the chromatographic and spectroscopic analysis of the drug, which required expensive instrumentations, high amounts of solvents, hazardous reagents, prolonged analysis time, or complicated steps for sample pretreatment. Therefore, the development of analytical methods with simpler, greener, lower-cost and faster procedures, and achieving the validation criteria such as higher sensitivity and selectivity is widely demanded for drug analysis. The electrochemical techniques such as voltammetric methods are considered a satisfactory alternative²³ among the available approaches and an appealing choice for pharmaceutical analysis²⁴. Although ALF is an electroactive compound, few voltammetric studies were reported for its electrochemical analysis, employing various working electrodes such as Hg electrode,¹⁸ bare²² and modified glassy carbon electrodes with hybrid of ionic liquid-ZrO₂ in graphene oxide,¹⁹ and multiwall carbon nanotubes sensors incorporated with the ionic liquid 1-hexylpyridinium hexafluorophosphate²⁰ and nickel oxide nanoparticles.²¹

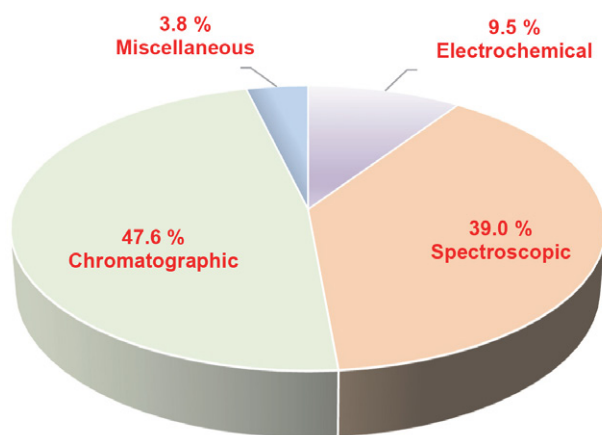


Figure 1: Classification of the analytical techniques reported for ALF determination.

However, the reported voltammetric methods showed good sensitivity and satisfactory analytical performance, the applied electrodes exhibited some drawbacks such as mercuric toxicity, and the use of expensive nanomaterials which also required complicated and time consuming procedures for preparation of these modified electrodes. Therefore, the continuous development and chemical modification of the electrochemical sensors with low-cost, easily-prepared, and eco-friendly modifiers have received an extensive interest to enhance their performance as chemical and biological sensors in electroanalysis.

The carbon paste electrode (CPE), a widely applicable electrochemical sensor, has been employed in the areas of electrochemistry and electroanalysis due to its

attractive characteristics, including simple preparation, affordable implementation, easy surface renewal, a low background current, and a wide range of potential window.^{23,25} In addition, CPE can be easily modified and simply manipulated in order to obtain a stable response with the possibility of lowering the overpotential, and to increase the sensitivity and selectivity of some electroactive species.²⁶ Thus, the feasibility of CPE modification with different modifiers were utilized in previous studies, such as sephadex modifier,²⁷ and rosaniline,²⁸ phthalo blue,²⁹ glycine,³⁰ and helianthium dye³¹ which were electropolymerized on CPE for their effective functioning, exhibiting linearity for the studied analytes over the ranges of 0.005–1, 1–3.5, 0.25–1.25, 0.06–1, and 0.06–0.15 mM, respectively.

In the present work, chemically modified graphite electrode with silica gel was employed to investigate the electrochemical behavior of ALF. Silica gel is a granular and porous form of silicon dioxide and can be easily incorporated into the carbon paste as an inexpensive and effective modifier. It possesses some attractive electrochemical properties which is extremely useful for electroanalytical purposes, such as high surface area, strong adsorption capacity, insolubility in most solvents, high thermal stability, and readily surface modification.^{32–34}

In recent years, disposable sensors composed of convenient matrices for surface renewal, such as pencil graphite electrodes (PGE), gained a large applicability to quantitative assays.^{35–37} PGE stand as an excellent versatile tool in the electroanalysis having favorable advantages over the traditional electrodes of being simple, cheap, commercially available, easily modified, low technology, and good mechanical rigidity. Moreover, the preparation of PGE for each measurement is faster and easier than the procedures required for other conventional carbon electrodes, including tedious hand mixing and polishing, and hazardous steps.³⁸

Differential pulse voltammetry (DPV) is a simple pulse voltammetric technique that is widely applicable for the determination of various pharmaceuticals.²⁴ Herein, we employ DPV to study the electrochemical oxidation of ALF at two different electrochemical sensors: silica gel modified carbon paste and pencil graphite electrodes, with a comparison of their performance.

Thus, our study aims to develop an easy, simple, and rapid DPV method for highly sensitive estimation of ALF in tablets and biological samples. As far as we know, it is the first report to employ a disposable pencil graphite electrode for the voltammetric assessment of ALF and to demonstrate the practical usefulness of two simple electrodes of low cost for the direct assay of ALF in urine samples. Moreover, the ecological impact of the proposed method was also evaluated using three metrics, namely, the National Environmental Methods Index (NEMI), analytical Eco-Scale score, and Green Analytical Procedure Index (GAPI).

2. Experimental

2. 1. Instrumentation

The electroanalytical study was performed using a SP-150 potentiostat (Bio-Logic Science Instrument, France) connected to a Lenovo computer provided with EC-Lab for windows v11.02 software. The cell potentials were measured with respect to the Ag/AgCl/3.0 M KCl reference electrode (BAS, USA) in a glass cell, comprising a working electrode and platinum wire (BAS, USA) as an auxiliary electrode.

A digital analyzer pH meter (Jenway 3510, USA) and ultrasonic bath sonicator (UCI-750, RAYPA, Spain) were also used.

2. 2. Working Electrodes

Voltammetric measurements were performed using two different electrodes:

2. 2. 1. Modified Carbon Paste Electrode

Silica gel modified carbon paste electrode (Si-gel/CPE) was prepared by thoroughly hand-mixing of 0.2 g silica gel and 0.8 g graphite powder with 0.6 mL paraffin oil by a ceramic pestle in a glass mortar to obtain a homogeneous paste. The preparation of the bare (plain or unmodified) CPE was likewise done by blending of 1.0 g graphite powder with 0.6 mL paraffin oil. The resulting paste was packed into the electrode body hole, and then the external surface of the electrode was polished on a soft paper with figure-eight motions in order to remove the excess of the paste and obtain a shiny appearance prior to using. In addition, subsequent renewal of the carbon paste surface for the each measurement should be performed, where a small portion of the paste at the electrode tip was scraped out and replaced by a new portion then repolished to generate a fresh electrode surface.

2. 2. 2. Pencil Graphite Electrode

XQ pencil leads of 2B grade with 0.9 mm diameter and 60 mm length from the local bookstore were employed for the voltammetric measurements of ALF. An insulating tape was used for wrapping PGE gently, where 25 mm of the pencil lead at one end was inserted into a home-designed brass holder for the electrical connection with the device. Meanwhile, the exposed surface of PGE at the other end was only 10 mm devoted as the sensitive part for the voltammetric assay and was gently polished before each recording using a cloth felt pad.

2. 3. Materials and Reagents

Pure sample of ALF was generously provided by Eva Pharma, Egypt (lot no. 1422R118) with purity of 100.80% according to the comparison method.¹

Bi-distilled water obtained from a Milli-Q water purification system and chemical reagents of highest purity were used in this study. Graphite powder, paraffin oil, Sephadex G-50, C18 silica gel, and chitosan were supplied from Sigma-Aldrich. Methanol, sodium hydroxide, glacial acetic acid, boric acid, phosphoric acid, and hydrochloric acid were obtained from El Nasr Pharmaceutical Chemicals CO. (Cairo, Egypt).

Britton-Robinson buffer (B-R buffer), a widely used multi-buffer system in the voltammetric studies, was employed as a non-complexing supporting electrolyte for the voltammetric measurements of ALF. The multi-acid B-R buffer system is consisted of three different buffering components of diminishing strength, so a linear pH response is obtained from pH 2.5 to pH 9.2 upon adding the alkali. Thus, it was easily prepared at the desired pH value without changing the chemical composition of the buffered components. It was prepared from a mixture of 0.04 M of each acid; boric, phosphoric and acetic acids, and the desired pH was adjusted using NaOH.

2. 4. Pharmaceutical Formulation

Prostetrol® modified release tablets (10 mg of ALF/tablet), a product of Eva Pharma with batch no. (10)190238, were purchased from the Egyptian market to be analyzed by the proposed method.

2. 5. Stock and Working Standard Solutions

A stock standard solution of ALF (1.0×10^{-2} M) was prepared using methanol for dissolving 42.59 mg of ALF into a 10 mL volumetric flask. Further dilution was carried out by transferring 0.25 mL of the prepared solution into a 25 mL volumetric flask and completing the final volume with bi-distilled water to obtain a standard solution of 1.0×10^{-4} M. The ALF working solutions were made by further dilution of the standard solution with B-R buffer solution to cover the concentration ranges of 6×10^{-7} to 2×10^{-5} M and 3×10^{-7} to 2×10^{-5} M at Si-gel/CPE and PGE, respectively.

2. 6. General Procedure

2. 6. 1. Voltammetric Procedures

The surface of the working electrode was conditioned at first by performing successive anodic cyclic voltammetry (CV) scans within the potential of 0 up to 1.6 V in an electrochemical cell containing B-R buffer solution of pH 6. After achieving stable background and response, an appropriate volume of the ALF standard solution was added to the cell, and then the solution was stirred for 1 min at an open circuit potential followed by a rest period for 30 s. The voltammograms were then recorded at a scan rate of 200 mVs^{-1} and ambient temperature.

2. 6. 2. Construction of Calibration Curves

The quantitative determination of ALF was performed using the DPV method within a potential range of 0 to 1.3 V, employing the optimum parameters of 50 mV pulses and step height, 50 ms pulses width, and 100 ms step time. Different aliquots of the ALF standard solution (1.0×10^{-4} M) were transferred by a micropipette into an electrochemical cell containing 10 mL buffer of pH 6, and then the peak currents were measured after stirring of the cell content for 1 min. The oxidation current peak (μ A) that developed at each working electrode was plotted against its corresponding drug concentration (μ M) to construct two calibration curves, covering the concentration ranges of 0.6–20 and 0.3–20 μ M at Si-gel/CPE and PGE, respectively, and being fit with two linear regression equations.

2. 6. 3. Assay of Pharmaceutical Preparations

Ten Prostetrol® tablets were finely pulverized, and an equivalent amount to 1.0×10^{-2} M ALF was accurately weighed, transferred into a 25 mL volumetric flask, and dissolved in 20 mL methanol by sonication for 30 min in an ultrasonic bath. The final volume was made up using the same solvent, and then the flask content was well mixed and allowed to settle for 15 min before filtering the supernatant.³⁹ Further dilution was done with bi-distilled water to obtain 1.0×10^{-4} M solution of ALF. Different aliquots from the prepared solution were investigated for ALF quantification in its dosage form, applying the procedures described under construction of calibration curves section and the percentage recoveries were calculated from the corresponding regression equation.

2. 6. 4. ALF Assay in Spiked Human Urine

Human urine samples, collected from a healthy donor and stored at -20 °C, were allowed to be partially thawed at room temperature to collect a clear supernatant. In order to reduce the matrix effect, the urine supernatant underwent fifty and twenty fold dilutions with the B-R buffer of pH 6 for the voltammetric measurements at Si-gel/CPE and PGE, respectively. 10 mL of the sample solution was transferred into the voltammetric cell and its voltammogram was recorded as a blank solution for subsequent measurements. Appropriate aliquots of 1.0×10^{-4} M of ALF solution were then spiked for their direct analysis in urine matrix, using the procedures described under construction of calibration curves section for the recovery studies of the spiked urine samples.

3. Results and Discussion

The electrochemical behavior of ALF was investigated at two different electrodes: CPE and PGE using CV technique, where different chemical and electrochemical

parameters were thoroughly studied for optimization its voltammetric performance. The voltammograms of the studied drug were recorded from 0 to 1.6 V in the B-R buffer solution as the supporting electrolyte. ALF exhibited a well-defined anodic peak current at around 0.90 V without a cathodic peak on the reverse scan, indicating the irreversible electro-oxidation process of ALF. The reasonable oxidation mechanism, as postulated by the previous electrochemical studies of ALF,^{20,22} might occur at the electro-active nitrogen atom in the amine group of the pyrimidine moiety, followed by deprotonation and dimerization.

3. 1. Optimization of the Experimental Conditions Using Cyclic Voltammetry

3. 1. 1. Effect of the Solution pH

The pH of the supporting electrolyte has an important impact on the voltammetric behavior of the drug. Therefore, the change in the pH range of B-R buffer from 3 to 9 was studied using CV method and the electro-oxidation peak potential and peak current of 10 μ M ALF were assessed. Upon increasing the pH values, the anodic peak potential shifted in the less positive direction due to the proton dependent electro-oxidation mechanism of ALF on the working electrode and followed linear regression equation 1 (Figure 2B):

$$E_{pa} = -0.0561\text{pH} + 1.3287 \quad (R^2 = 0.9900) \quad (1)$$

where: E_{pa} is the anodic peak potential of 10 μ M ALF as a function of the applied pH of B-R buffer.

In addition, the slope of 0.0561 V/pH is close to the theoretical value of 0.059 V /pH of the Nernst equation, indicating that equal number of electrons and protons participated in the process of drug oxidation. Moreover, the anodic peak current of ALF enhanced in less acidic medium, reaching the optimum at pH 6. Meanwhile, badly-defined oxidation peaks and a gradual decrease in the peak height were observed with the further increase in the pH values. This might be attributed to the dissociation constant of the studied drug, where the pKa values of ALF are 2.26 and 5.56.⁴⁰ Therefore, the pH of the solution could affect the existing form of ALF as the drug would exist in its protonated (water soluble) form in the acidic medium, while at higher pH values, it would be deprotonated to its barely soluble form. Thus, the decrease in the peak currents was observed at higher pH values ($>$ pKa values of ALF). As can be seen in Figure 2, B-R buffer of pH 6 was fit for all subsequent measurements of ALF.

3. 1. 2. Effect of Surfactants as Electrolyte Additives

The impact of surfactant addition to the supporting electrolyte was investigated in the present study, as sur-

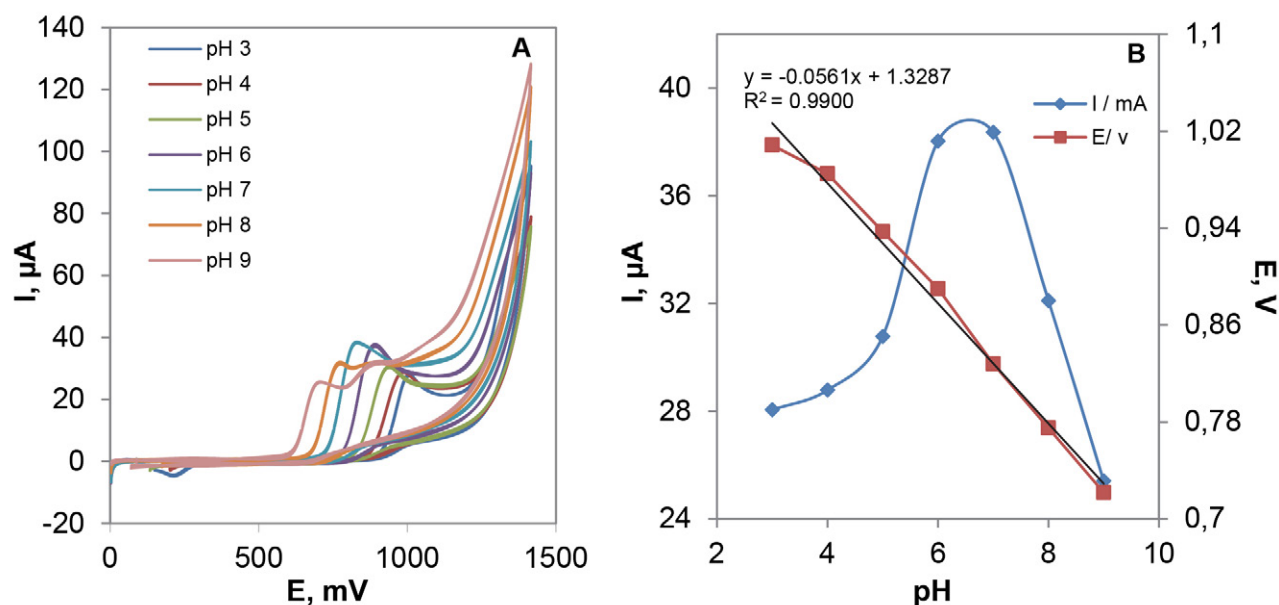


Figure 2: (A) Cyclic voltammograms of 10 μM ALF recorded at different pH values (3–9) of B-R buffer at 200 mV/s scan rate; (B) Dependence of the anodic peak potential and peak current on the pH values.

factants are widely used in electroanalysis for enhancing the electrochemical performance of some analytes. Therefore, the oxidation behavior of ALF was studied in the presence of different types of surfactants, including SDS, CTAB, and Brij-35 as anionic, cationic, and nonionic surfactants, respectively. The aqueous electrolytic solution of B-R buffer showed the best response as can be seen in Figure S1, meanwhile no distinct effect was noticed upon adding the surfactants to the analyte in the B-R buffer solution of pH6.

3. 1. 3. Effect of the Stirring Time

The anodic peak current of ALF in dependence of the stirring time was studied at different time intervals from 0 to 3 min using CV, where the voltammograms were recorded after stirring the solution at 400 rpm, followed by 30 s quiescent time at an open circuit potential. As shown in Figure S2, the oxidation peak current improved after the solution stirring for 1 min, then became stable up to 3 min.

3. 1. 4. Effect of the Carbon Paste Composition

The incorporation of different modifiers such as silica gel, chitosan, sephadex and iron nanoparticles into the carbon paste composition were investigated to attain highly responsive sensor. The oxidation peak current of the studied drug enhanced upon modifying CPE with silica gel which exhibited a higher electrocatalytic activity and sensitivity towards ALF oxidation, Figure S3. The effect of silica gel content was also checked by recording the voltammetric responses of CPE containing various

proportions of silica gel. The addition of silica gel in the ratio of 20% w/w of the paste total mass showed an efficient synergistic effect for improving the oxidation peak current of ALF, Figure S4.

3. 1. 5. Effect of the Pencil Graphite Types

Different types and diameters of disposable PGE were examined using CV for B-R solution containing 5 μM ALF. Pencil leads are usually marked with either H (hardness), B (blackness), or HB letters which mainly differ in their composition ratio of graphite and clay. PGE of the soft B type containing more graphite and with larger diameter are appropriate for quantitative assay as this type provides an easy electronic transfer which generates higher signals.³⁸ PGE from different commercial manufacturers such as HB, rotring, faber castle, and XQ were also investigated, where electroactive species may show different voltammetric behavior on PGE produced from different manufacturers with the same hardness due to different interactions of the analyte with common components of PGE.³⁸ 2B XQ pencil lead of 0.9 mm diameter was the optimum which gave the best electrochemical response with relatively low noise, Figure S5.

3. 1. 6 Optimization of the Scan Rate

Studying the scan rate effect gave valuable information about the electrochemical behavior of the studied drug at the working electrode, whether the electro-oxidation process would be under diffusion or adsorption con-

trolled mechanism. The variation in the peak current (i_p) and peak potential (E_p) of 10 μM ALF in B-R buffer solution (pH 6) at Si-gel/CPE was examined by the CV mode at different scan rates. Upon increasing the scan rate from 25 up to 300 mV/s, the anodic peak current grew gradually and the oxidation peak potential shifted slightly towards more positive direction, indicating the irreversible oxidation process. The scan rate of 200 mV/s was selected for the successive voltammetric measurements, exhibiting a well-shaped peak with relatively narrow width and high sensitivity, Figure 3 A.

A direct relationship was found between the oxidation peak current of ALF and square root of the scan rate

($v^{1/2}$) over the scan rate range of 25–200 mV/s⁻¹, based on $I(\mu\text{A}) = 2.7885 v^{1/2} (\text{mV/s}) + 2.0843$ ($R^2 = 0.9816$). Thus, the electrochemical oxidation of ALF was controlled by a diffusion process, Figure 3 B.

Moreover, plotting the logarithm of the peak current versus logarithm of the scan rate exhibited a straight line following equation 2 (Figure 3 C):

$$\log I_p (\mu\text{A}) = 0.4932 \log v (\text{mV/s}) + 0.4922, R^2 = 0.9799 \quad (2)$$

where I_p represents the anodic peak current and v is the scan rate.

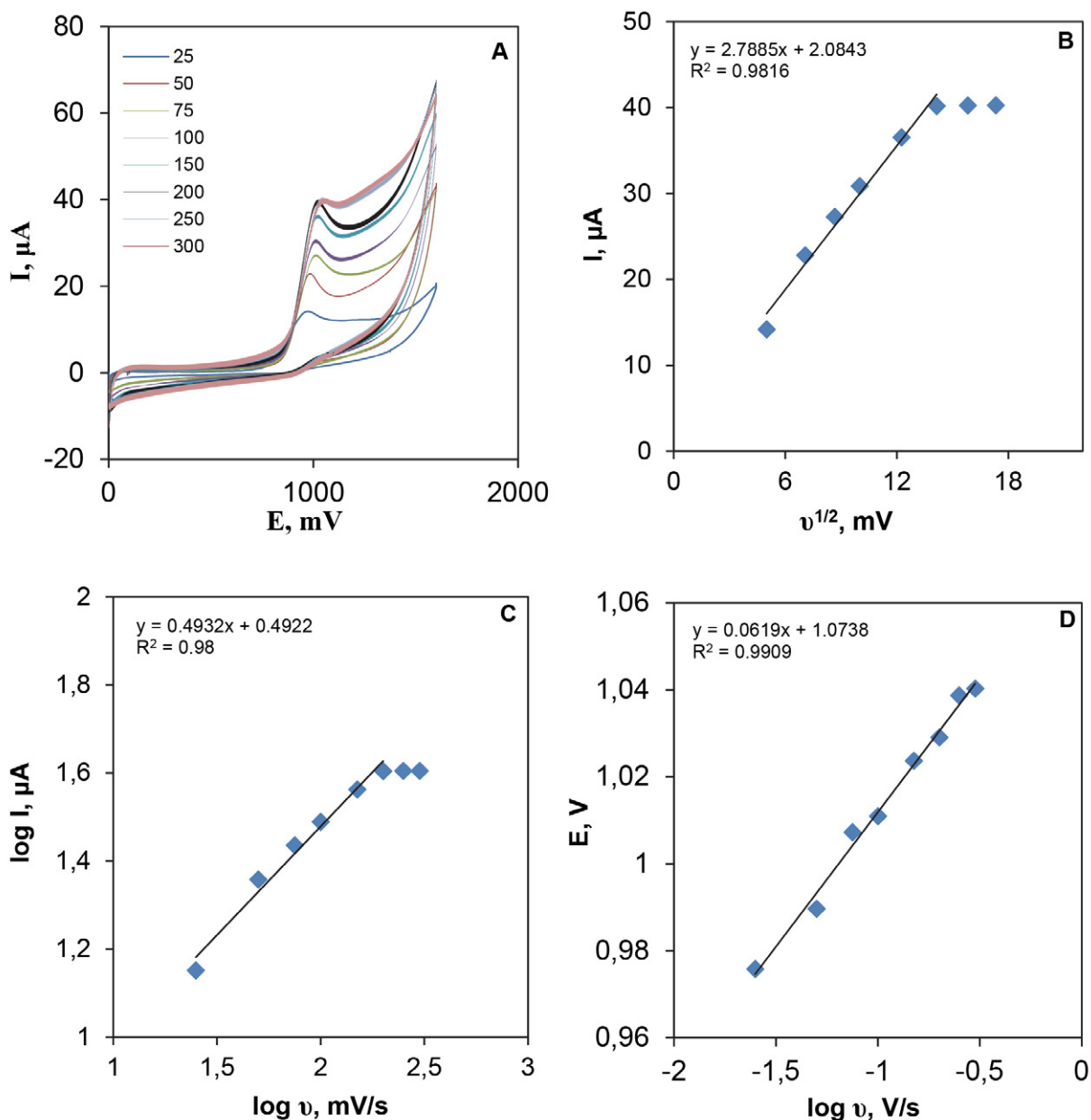


Figure 3: (A) Cyclic voltammograms of 10 μM ALF in B-R buffer of pH 6 at different scan rates; (B) Linear plot of the peak current versus square root of the scan rate; (C) Dependence of the logarithm of the peak current on logarithm of the scan rate; (D) Linear plot of the peak potential as function of logarithm of the scan rate.

The obtained slope value of 0.4932 was close to the theoretical value of 0.5 for the diffusion-controlled electrode process.⁴¹ Furthermore, the relationship between the peak potential and logarithm of the scan rate was found to be linear according to equation 3 (Figure 3 D):

$$E_{pa}(V) = 0.0619 \log v (V/s) + 1.0738, R^2 = 0.9909 \quad (3)$$

where E_{pa} represents the anodic peak potential and v is the scan rate.

In accordance with Laviron's equation given for an irreversible electrochemical process, the number of electrons transferred at the surface of the electrode due to ALF oxidation was calculated as follows:⁴²

$$E_p = E^\circ + \frac{2.303RT}{\alpha nF} \left(\log \frac{RTk^\circ}{\alpha nF} \right) + \frac{2.303RT}{\alpha nF} \log v \quad (4)$$

Herein, the following elements in equation 4 possess their conventional meanings: the electron transfer coefficient (α), the number of electrons transferred (n), temperature ($T = 298 \text{ K}$), gas constant ($R = 8.314 \text{ J K mol}^{-1}$), Faraday constant ($F = 96485 \text{ C mol}^{-1}$), and the voltammetric scan rate (v). k° denotes the standard heterogeneous rate constant of the surface reaction, E° represents the formal redox potential, and E_p is the anodic peak potential.

The E° value can be obtained from the intercept of E_p against v on the ordinate by extrapolating the line to the vertical axis at $v = 0$. From the linear relationship between E_{pa} versus $\log v$, the value of αn can be deduced from the slope and found to be 0.0619. The supposed value of α equals 0.5 for the irreversible electrochemical process,⁴³ thus the n value was calculated to be 1.911 (approximately 2.0). This value referred to the participation of two electrons and protons in the electro-oxidation reaction of ALF which coincided with previously published results.^{20,21}

3. 2. Method Validation

The developed DPV method at two different electrodes was validated according ICH guidelines⁴⁴ with regard to the following parameters:

3. 2. 1. Linearity and Ranges

Under the optimum conditions, the linearity of ALF was investigated in the concentration ranges of 6×10^{-7} – $2 \times 10^{-5} \text{ M}$ at Si-gel/CPE and 3×10^{-7} – $2 \times 10^{-5} \text{ M}$ at PGE using DPV, Figure 4. Statistical data analysis was performed by plotting the peak current height (μA) as a function of concentration (μM), fitting the resulting calibration graphs into linear regression equations as follows:

$$\text{Si-gel/CPE: } I = 0.2501 C + 2.7407 (R^2 = 0.9995) \quad (5)$$

$$\text{PGE: } I = 0.6057 C + 3.3274 (R^2 = 0.9996) \quad (6)$$

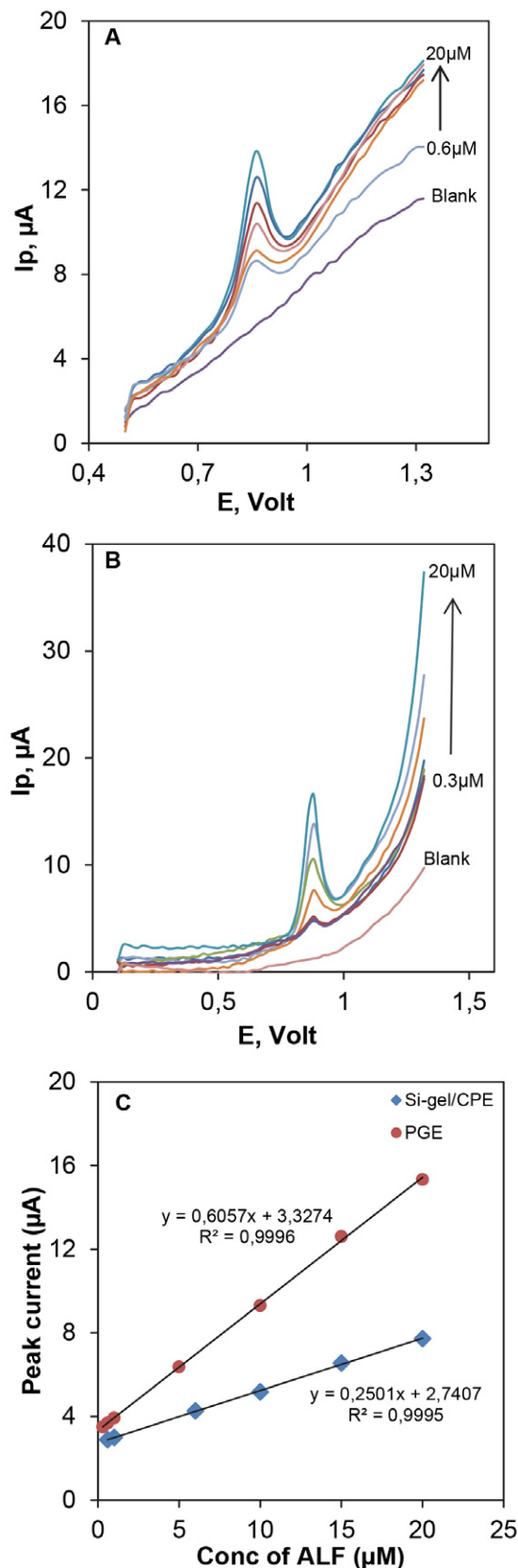


Figure 4: DPV voltammograms of (A) 0.6–20 μM of ALF at Si-gel/CPE; (B) 0.3–20 μM of ALF at PGE in B-R buffer solution of pH 6. (C) Calibration plot of the peak current versus concentration.

Table 1. Regression and analytical performance data of ALF assay by DPV method at two different electrodes.

Parameters	Si-gel/CPE	PGE
Linearity range (μM)	0.6–20	0.3–20
Intercept	2.7407	3.3274
Slope	0.2501	0.6057
Correlation Coefficient(R^2)	0.9995	0.9996
LOD (μM)	0.17	0.099
LOQ (μM)	0.53	0.299
Accuracy		
(mean recovery% $\pm$ Er%)	100.85 $\pm$ 0.85	98.97–1.03
	97.57–2.43	99.74–0.26
	98.99–1.01	100.94 $\pm$ 0.94
Precision (RSD%)		
Repeatability ^[a]	1.756	1.399
Intermediate precision ^[b]	1.794	1.788

^[a] The intra-day and ^[b] inter-day RSD ($n=9$) of 1, 10, and 20 μM ALF at Si-gel/CPE and 1, 10, and 15 μM ALF at PGE within the same day and in three successive days.

The obtained data with high correlation coefficients were indicative of good linearity of the proposed method at the two applied electrodes, Table 1.

3. 2. 2. Sensitivity

The limits of detection (LOD) and quantification (LOQ) were calculated according to the ICH guidelines:⁴⁴ $\text{LOD} = 3.3 \sigma/s$ and $\text{LOQ} = 10 \sigma/s$, where, σ is the standard deviation of the responses of four replicated blanks obtained at the same potential applied for the sample and s is the slope of the calibration graph. The obtained values of LOD and LOQ indicated the sensitivity of the proposed method at the applied electrodes, as shown in Table 1.

3. 2. 3. Accuracy and Precision

The accuracy of the proposed method was examined using nine samples of three different concentrations of the pure drug selected to cover the low, medium and high ranges of the calibration graph. The mean percentage recoveries of these ALF concentrations were calculated, as shown in Table 1.

The precision was also evaluated through triplicate determinations of three different concentrations of pure drug within the same day (intra-day) and on three different days (inter-day). The obtained results in Table 1 exhibited acceptable values of relative standard deviation (RSD%) and percentage relative error (Er%).

3.2.4. Specificity

The specificity of the proposed method was confirmed by ALF assay in its tablet formulation and urine samples. The selectivity of DPV at the applied electrodes was also tested in the presence of commonly used exci-

ents in tablets, such as silica, lactose, PVP, talc, and magnesium stearate. The excipients were added to ALF at the same concentration (10^{-5} M) and analyzed under optimum conditions.

Table 2 exhibits sufficiently good recoveries with no interference, while lactose showed a slight decrease in the electrochemical response of ALF.

Table 2: Voltammetric analysis of ALF in the presence of some common excipients by DPV at two different electrodes.

Excipients Added ^[a] (1×10^{-5} M)	Recovery % ^[b]	
	Si-gel/CPE (1×10^{-5} M)	PGE (1×10^{-5} M)
Silica	103.40	101.88
Lactose	95.00	94.23
Povidone k30	102.87	97.67
Talc	100.10	100.40
Magnesium stearate	101.18	101.71

^[a] Drug: excipients in the ratio of 1: 1 M.

^[b] Average of three determinations.

3. 3. Statistical Analysis

The results of DPV at both electrodes were statistically examined using the Student's t-test and variance ratio F-test at the 95% confidence level. The obtained results were compared with those of the official potentiometric method for the ALF assay¹ and showed no significant differences, Table 3.

Table 3. Statistical analysis of the results obtained by DPV at two different electrodes and the pharmacopeial method for ALF assay.

	Si-gel/CPE	PGE	Comparison method [1] ^[a]
Mean $\pm$ SD	100.10 $\pm$ 1.58	99.39 $\pm$ 1.40	100.80 $\pm$ 1.42
Variance	2.508	1.970	2.016
n	6	7	3
Student-t-test	0.642 (2.365) ^[b]	1.549 (2.306) ^[b]	–
F-test	1.244 (5.790) ^[b]	1.023 (5.140) ^[b]	–

^[a] Potentiometric official method for ALF assay.

^[b] The corresponding theoretical values for t and F tests at $p = 0.05$.

3. 4. Analytical Applications

The employed electrodes showed satisfactory results for the ALF assay in Prostetrol[®] tablets and spiked urine samples using DPV. The recovery values of the target analyte and the standard deviations (SD) proved the suitability of the proposed method for fast routine analysis of ALF in its tablets and human urine samples (Table 4).

Thus, the proposed DPV method exhibited simpler, time saving, greener, and good practical applicability for

ALF analysis in real samples with acceptable percentage recoveries. As compared to the voltammetric performance of ALF at Si-gel/CPE, PGE showed a better response with a smaller background current, good conductivity, sharp oxidation peaks, and higher sensitivity (Figure 4) due to the presence of sp^2 hybridized carbon of graphite bound with clay in the pencil lead composition. Herein, clay, an aggregate of minerals and colloidal substances, contributed to the good sensing performance of PGE because of its highly attractive characteristics such as chemical and mechanical stability, strong sorption properties revealed in high ionic exchange capabilities, and porosity which exhibits beneficial ionic conductivity and electrocatalytic activity.⁴⁵ Table 1 ascertains the good electrocatalytic activity of PGE towards the ALF oxidation, where the sensitivity obtained at PGE was two times higher than that at Si-gel/CPE with a lower LOD of 0.099 μM .

3. 5. Greenness profile of the Proposed Method

The assessment of the analytical method procedures from the green perspective has recently attracted the authors' concern in the field of green analytical chemistry (GAC). Electroanalytical techniques mostly comply with the GAC principles as they are free of hazardous chemicals and organic solvents, and do not produce large volumes of analytical waste compared to the classical chromatographic methods.

To deepen this view, the greenness profile of the developed method was established using three assessment tools: NEMI, Analytical Eco-Scale, and GAPI methods. The applied metrics introduced more easier and visible information on the environmental impact of the applied analytical procedures.

NEMI labeling is considered as one of the oldest tools for qualitative greenness assessment of the analytical

procedures. The NEMI pictogram is symbolized by a circle divided into four fields that reflect four different criteria of the described analytical methodology: persistence, bioaccumulation potential, and toxicity (PBT), hazardous chemicals, corrosiveness, and waste. Each field is filled with green when its required criterion is met by the developed method.⁴⁶ Adopting these criteria, the pictogram of the applied method showed green-colored quadrants due to satisfaction with their requirements as shown in Table 5, except for the hazardous quadrant due to methanol usage, regardless of the minute amount used per sample.

From a glance at the NEMI symbol, general information about environmental impact of the analytical procedure can be easily read with no significance to energy consumption or the quantity of chemicals.

Thus, the analytical Eco-Scale approach was employed for the greenness assessment in a more quantitative way, where a numerical score was given for the developed method. The analytical Eco-Scale score was calculated by subtraction of the total penalty points from the basis of 100 points (ideal green analysis). The penalty points of the analytical procedure were assigned to four main categories: the amount and type of chemicals, energy consumption, occupational hazard, and amount of generated waste, and the way for its treatment. The score was ranked on a scale, where the method greenness was excellent if the score is higher than 75, acceptable if it is higher than 50, and inadequate if it is less than 50.⁴⁷ The DPV method scored 80 at Si-gel/CPE and 82 at PGE (higher than 75), so it ranked as an excellent green method. As can be seen in Table 5, the analytical Eco-Scale scores were calculated in detail for assessing its greenness profile more clearly than that obtained by NEMI.

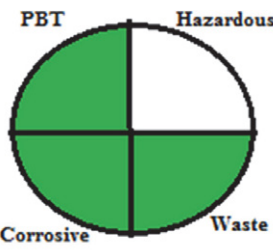
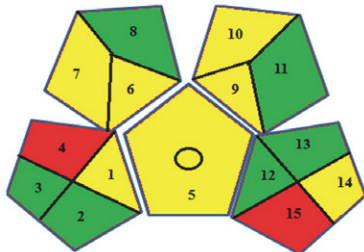
A recent assessment tool known as GAPI was also employed, showing a specific symbol with five pentagrams segmented into 15 zones to encompass five main categories: sample handling, general method type, sample prepa-

Table 4. Analytical application of the DPV method for ALF determination in tablets formulation and spiked urine samples at two different electrodes.

Parameters	Si-gel/CPE						PGE					
	Prostetrol [®] modified release tablets			Urine samples			Prostetrol [®] modified release tablets			Urine samples		
	Taken μM	Found μM	Recover $y^{[a]}\%$	Taken μM	Found μM	Recover $y^{[a]}\%$	Taken μM	Found μM	Recover $y^{[a]}\%$	Taken μM	Found μM	Recover $y^{[a]}\%$
	5	4.02	101.92	3	3.08	102.57	0.6	0.59	98.43	5	5.10	102.01
	7	4.49	99.96	5	5.12	102.36	5	4.89	97.74	7	7.19	102.66
	13	6.05	101.72	10	10.10	100.95	7	6.97	99.61	10	10.02	100.24
	16	6.82	101.97	15	15.15	101.03	10	10.04	100.43	12	11.80	98.85
	20	7.84	102.00				12	11.65	97.10			
Mean $\pm$ SD	101.51 $\pm$ 0.88			101.73 $\pm$ 0.85			98.66 $\pm$ 1.36			100.94 $\pm$ 1.73		
N	5			4			5			4		
SE	0.392			0.427			0.607			0.866		
V	0.767			0.729			1.842			2.997		

^[a] Average of three determinations.

Table 5. Greenness assessment of the proposed method by the Analytical Eco-Scale, NEMI, and GAPI approaches.

Analytical Eco-Scale score parameters	Penalty points (PPs)		NEMI pictograms
	Si-gel/CPE	PGE	
I-Reagents/ word sign /no of pictograms			
Bi-distilled water/ – / 0		0	
Methanol/ danger / 2		4	
1M NaOH/ danger / 1		2	
Silica gel / – / 0	0	–	
Paraffin oil/ danger / 1	2	–	
Graphite/ – / 0		0	
0.04 M acetic acid / danger / 1		2	
0.04 M orthophosphoric acid/ danger / 1		2	
0.04 M boric acid/ danger / 1		2	
	$\Sigma = 14$	$\Sigma = 12$	
II-Instruments a-Energy			
Potentiostat		0	
pH meter		0	
Vortex mixer		0	
Sonicator		0	
b-Occupational hazards		3	
c-Waste		3	
	$\Sigma = 6$		
Total PPs	20	18	
Analytical eco-scale score = 100- total PPs	80	82	
	Excellent green analysis		

* Red zones depict high ecological impact; yellow zones represent lower impact; and green zones represent more safe effect to the environment.

ration, reagents/solvents used and instrumentation. Thus, the GAPI approach evaluates 15 criteria covering every step in the whole analytical procedure using a color code: green, yellow, and red signifying low, medium, and high adherence to GAC standards, respectively. Color codes given to each segment were specified according to the detailed information described in a report by Wasylka⁴⁸. As shown in Table 5, the environmental impact of employing two different electrodes in the proposed method for ALF assay was the same in all evaluation parameters represented in one GAPI pictogram. The visual inspection of the first eight segments of the pictogram refers to few simple steps required for the sample preparation in every analytical procedure, including the use of green solvents and reagents without macro-extraction or derivatization steps since the red zone represents special storage conditions of urine samples. Meanwhile, the segments from 9 to 11 reflects the use of lower amount of less hazardous solvents and reagents. In addition, the lower energy consumption of our instrumentation with lower waste production per sample were represented on the last pentagram that also exhibits one red zone due to no treatment applied for the generated waste. Therefore, the resulting GAPI pictogram of the DPV method showed (6) green (7) yellow, and (2) red shaded zones that reflects its lower impact on the environment.

Overall, our simple study is shed light on the merits of the applied voltammetric method as a green and safe

practice for ALF quantification that can be used in routine work and quality control purposes in pharmaceutical industries.

3. 6. Perspectives

Development of the voltammetric techniques is a growing trend in the electroanalytical field to attain more affordable, greener, easily used, and highly sensitive electrochemical methods. Recently, the chemical modification of electrochemical sensors and the use of disposable and multiplexed electrodes received a great deal of attention to attain stable and rapid responses with high sensitivity, accurate selectivity, and reliability. Thus, incorporation of different modifiers and fabrication of new applicable sensors have been utilized to construct electrodes with high conductivity, great catalytic activity, lower toxicity, and effortless synthesis. Combination of the nanotechnology with electrochemical techniques have been applied, such as the application of multi-walled carbon nanotubes and metal nanoparticles for enhancing the electrocatalytic activity of the sensors. As a result, the continuous development of the sensor technology based on the electrochemical technique have been applied to gas sensors, environmental monitoring sensors, biosensors, *etc.*, which can be employed in different aspects such as forensic medicine and evidence science. In addition, further modifications have been widely proposed to be reasonably integrated

with portable voltammetric analyzers for on-site analysis. Moreover, the growing interest in green analytical chemistry also requires a fresh perspective for the appropriate modification and selection of the electrochemical sensors. Thus, development and exploration of electrochemical sensors with specific selectivity, good conductivity, and economic feasibility for an eco- and user-friendly voltammetric assay is an urgent task.

4. Conclusion

In the proposed study, a differential pulse voltammetric method was developed for rapid determination of ALF at two different electrodes with an evaluation of their green-profile. Carbon paste electrode modified with silica gel and pencil graphite electrode proved to be high efficient and sensitive; nevertheless, they are simpler and more economic than those employed in the previously reported methods. The electrochemical behavior of ALF was studied in B-R buffer solution and exhibited well-defined and irreversible anodic peaks with an optimal current density at pH 6. The electrochemical activity of ALF towards PGE was superior to that at Si-gel/CPE which demonstrated as a two fold increase in the sensitivity with no need for its surface modification. The analytical procedures were fully validated and successfully applied for ALF assay in tablets formulation and human urine samples with satisfactory recoveries. This is the first report to apply a simple disposable PGE for fast green analysis and perform a direct voltammetric assay of ALF in urine sample which required no hazardous chemicals, extraction or tedious separation steps.

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Povzetek

Uporabljena je bila občutljiva in poceni diferencialna pulzna voltametrična tehnika za raziskovanje elektrokemičnega obnašanja alfuzosin hidroklorida na dveh različnih delovnih elektrodah: ogljikova pasta, modificirana s silikagelom, in svinčnikova grafitna elektroda (PGE). Voltametrični pogoji so bili optimizirani s ciklično voltametrijjo, ki je pokazala ireverzibilni anodni vrh v Britton-Robinsonovem pufrskem mediju (pH 6) pri 0,86–0,90 V. Elektrokemični odzivi so bili linearno odvisni od koncentracije alfuzosina ($R^2 > 0,999$) v razponu od 0,6–20 in 0,3–20 μM , ki kaže večjo elektrokatalitsko aktivnost pri PGE z nizko mejo zaznave 0,099 μM . Poleg tega je bila ta študija uspešen poskus določanja zdravila v tabletah in vzorcih urina z dodatkom učinkovine z vrednotenjem zelenega profila z uporabo Nacionalnega indeksa okoljskih metod, analitične ocene Eco-Scale in Indeksa zelenih analitičnih postopkov.



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