ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
https://www.icf.ro/rrch/

Rev. Roum. Chim.,
2026, *71*(7–8), 455–461
DOI: 10.33224/rrch.2026.71.7-8.02

Dedicated to Professor Mihaela Hillebrand on the occasion of her 85th anniversary

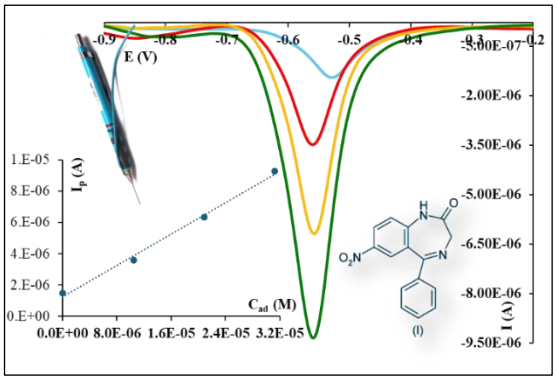
RAPID VOLTAMMETRIC QUANTIFICATION OF NITRAZEPAM AT SINGLE-USE GRAPHITE ELECTRODES

Adelaida Sorana TRIFU,[a,b] Mihaela-Carmen CHEREGI,[a], Emilia-Elena IORGULESCU,[a] Iulia Gabriela DAVID[a,*] and Simona TRIFU[c]

[a] Department of Analytical Chemistry and Physical Chemistry, Faculty of Chemistry, University of Bucharest, Panduri Av. 90–92, District 5, 050663, Bucharest Roumania
[b] Department of General Medicine, University of Medicine and Pharmacy "Carol Davila", 37 Dionisie Lupu Str., District 2, 020021 Bucharest, Roumania
[c] Department of Neurosciences, University of Medicine and Pharmacy "Carol Davila", 37 Dionisie Lupu Str., District 2, 020021 Bucharest, Roumania

Received February 27, 2026

The benzodiazepine drug nitrazepam (NZP) is used to treat psychical disorders, but also for illicit purposes. Therefore, inexpensive and reliable methods are needed for screening this drug in different samples. Thus, this article presents the voltammetric behavior of NZP at pencil graphite electrode (PGE), a cheap disposable sensor. Cyclic voltammetry studies revealed pH dependent signals related to the reduction of 4,5-azomethine and -NO_2 groups, as well as a pair of peaks related to a quasi-reversible process involving –NO/–NHOH moieties. The peak corresponding to the reduction of the nitro group was used for the quantitative determination of NZP by differential pulse voltammetry (DPV). The DPV method at PGE presented a linearity concentration range of 2.45×10^{-6} – 9.75×10^{-5} M NZP and a detection limit of 1.13×10^{-6} M NZP. The developed method was successfully applied for the NZP quantification from pharmaceuticals and spiked soft drinks.



INTRODUCTION

Benzodiazepines (BZD) are a class of drugs that have potent anxiolytic, hypnotic, antiepileptic, and muscle relaxant effects.[1] Furthermore, due to their sedative and relaxant properties, they can also be used to treat panic attacks[2] and to induce anaesthesia during various clinical procedures. BZDs are considered to have low toxicity when administered in clinical doses, but can be harmful when used in high doses.[3]

One of the representative agents of the BZD class, nitrazepam (NZP), 1,3-dihydro-7-nitro-5-phenyl-2H-1,4-benzodiazepine-2-one, is widely used as a hypnotic,[4] tranquilizer, and anticonvulsant, acting on the central nervous system.[5] It has a long duration of action, being used as an effective treatment for insomnia and anxiety, prolonging sleep time and

* Corresponding author: i_g_david@yahoo.com

reducing the time it takes to fall asleep, but several side effects can occur, such as liver damage and teratogenicity.[6] The development of tolerance or dependence and withdrawal symptoms are rare, and there are very few known interactions with other drugs. An increased response to beneficial effects, but also to adverse reactions, has been shown in elderly patients.[4]

Over the years, BZD use has spread among the drug-dependent population and has become evident in cases of drug-facilitated assault and road traffic accidents. BZD intoxication represents a health hazard due to their ability to create addiction. Improving analytical methods to rapidly detect the presence of these drugs in biological samples is essential in toxicology and forensic medicine, for identifying exposure or intoxication.[7] Although chromatography is the method of choice for the analysis of complex mixtures of structurally related compounds, as it was reported for the simultaneous determination of 14 BZD residues in aquaculture water and sediments,[8] voltammetry has also been successfully applied for the quantification of various contaminants of emerging concerns, including BZDs. Compared to chromatographic techniques, voltammetry involves lower consumption of reagents and materials, shorter analysis times and minimal pretreatment.[9] In addition, using modified sensors, like a Pt electrode modified with graphitic carbon nitride, low limits of detection (2.00×10^{-8} M NZP) can be achieved.[10] However, unmodified electrodes can also be used for the rapid screening of analytes, being more eco-friendly and sustainable. The low-cost disposable pencil graphite electrode (PGE), whose electroactive surface consists of the composite material found in commonly available pencil graphite leads, belongs to this category of electrochemical sensors.[11]

Therefore, this work describes for the first time the voltammetric behavior of NZP at the disposable PGE using cyclic voltammetry (CV), as well as the simple and rapid analyte quantification in pharmaceutical preparations and spiked beverages using differential pulse voltametry (DPV).

RESULTS AND DISCUSSION

Selection of the optimum working electrode

The electrochemical behaviour of various analytes is influenced by the working electrode material, due to several factors, such as the different types and strengths of analyte-electrode interactions, the electrode surface area, the conductivity and electrocatalytic effects of the electrode material. Therefore, usually, the first step in the development of a voltammetric method for the quantitative analysis of an analyte is the selection of the appropriate working electrode. In this respect, CVs were recorded for NZP using various carbon-based electrodes (Fig. 1). It can be observed that, during the first scan, the CV curves of NZP exhibited two cathodic peaks (*c1* and *c0* located in the ranges –0.600 to –0.700 V and –1.150 to –1.200 V, respectively) and either two anodic signals (*a1* and *a2* observed at approximately –0.560 V and 0.060 V, respectively) or a single one (*a1* at 0.200 V) for the PGE and glassy carbon electrode (GCE), respectively. Starting with the second scan, an additional reduction peak (*c2*) appeared at approximatively –0.120 V at PGE and –0.240 V at GCE. According to the literature,[12–14] peaks *c0* and *c1* are attributed to the reduction of the 4,5-azomethine group of the benzodiazepine ring and the -NO_2 group, respectively. To the best of our knowledge the signal *a1* was never mentioned in the literature and it may be possible that it is due to the PGE, while *a2* was attributed to the oxidation of the –NHOH moiety produced during the forward scan as result of the –NO_2 group reduction. The peaks *a2* and *c2* form a pair corresponding to a quasi-reversible process involving the –NHOH/–NO groups.

As it can be observed from Fig. 1, all NZP voltammetric signals recorded at PGE were more intense than those obtained at GCE. One reason is the larger surface area of the PGE (0.1589 cm^2) compared to that of the GCE (0.0706 cm^2). To characterize the electrode material, the electrodes sensitivities (S, $A \times mol^{-1} \times cm^{-2}$) for peak *a2* and the main reduction signal *c1* were calculated using DPV, since it is more sensitive than CV and it is the technique of choice for quantitative analysis. S were evaluated for GCE and PGE with different hardness (HB, B and 2B). The sensitivities of peak *c1* and peak *a2* varied in the order: HB_PGE (1.100) > 2B_PGE (1.040) > B_PGE (0.940) > GCE (0.308) and 2B_PGE (0.357) > HB_PGE (0.179) > B_PGE (0.147) > GCE (0.053), respectively. These data pointed out that the highest sensitivity was obtained for the reduction peak recorded at HB_ PGE. Therefore, this type of electrode was employed in the further steps of the investigation.

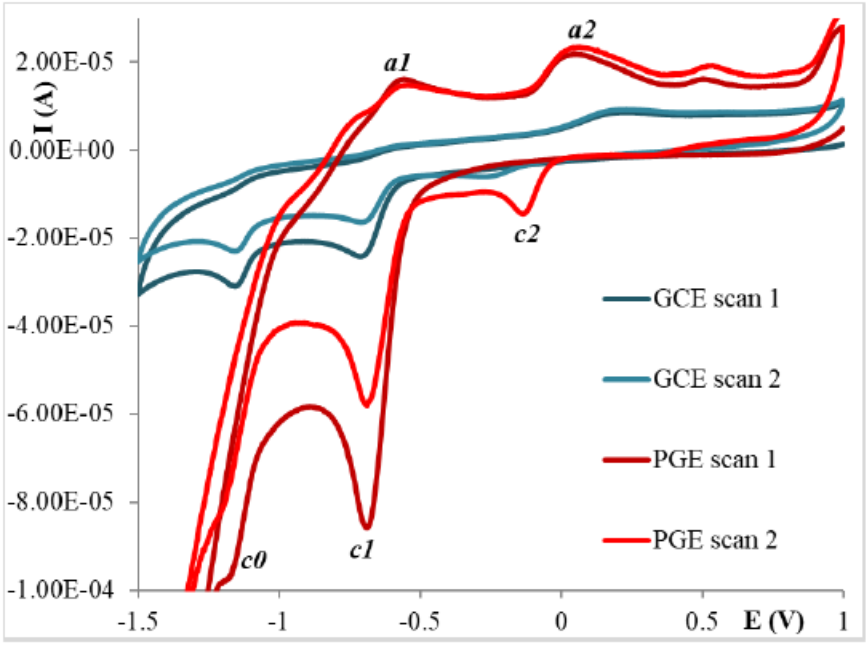


Fig. 1 – CV curves recorded at GCE and HB type PGE for 1.75×10^{-3} M NZP in phosphate buffer solution (PBS) pH 7.00 (v = 0.100 V/s).

The sensitivity and/or selectivity of an electrode could be improved by modifying its electroactive surface, the simplest way being the electrochemical pretreatment; this leads to an increase in the effective surface area and the formation of oxygen containing groups capable of interacting specifically with the analyte molecules and preconcentrating them at the electrode surface.[11] In the case of NZP electroanalysis, PGE electroactivation, carried out either by applying a potential of 2.000 V for 60 s or by cycling the potential 10 times between –0.500 and 2.000 V in various media (0.10 M HCl, PBS pH 7.00, 0.10 M NaOH), could not be utilized, as all electroactivated electrodes exhibited high background currents in the tested supporting electrolytes (PBS pH 7.00, 0.10 M KCl, 0.10 M KNO_3, Britton Robinson buffer (BRB) pH 7.00), within the potential range where NZP displayed peaks, in both the anodic and cathodic regions.

Selection of the optimum supporting electrolyte

Electrode processes of organic species with various functional groups usually also involve protons; consequently, their electrochemical behaviour depends on the pH of the supporting electrolyte. The effect of pH on the voltammetric response of NZP at the HB_PGE was investigated using CV, with BRB solutions of different pH values serving as the supporting electrolyte. To observe all NZP cathodic peaks, the second scan was used for evaluation. As can be observed from Fig. 2a, at all tested pH values, the anodic branch of the voltammograms exhibited poorly defined signals, that were difficult to measure, while all three NZP cathodic peaks shifted towards more negative potentials if the solution pH increased, confirming the participation of protons in the reduction processes. The slope values of the E_p (V) = f(pH) dependencies (Fig. 2b), close to the theoretical value of 0.05916 p/n V/pH unit, where p and n represent the number of protons and electrons, respectively, participating in the electrode reaction, indicated that each of the three NZP reduction processes involved the same number of protons and electrons.

It can be seen from Fig. 2a that the best defined and most intense peak was *c1*. Its highest intensity was reached at pH 7.24 and pH 7.96. Therefore, the influence of different supporting electrolytes, with pH values in this range (PBS pH 7.50, BRB pH 7.96, PBS pH 8.02 and BRB pH 8.36), on the intensity of the DPV peak c1 was investigated. The most intense signal was obtained using PBS pH 7.50 as supporting electrolyte; consequently, it was used in subsequent investigations.

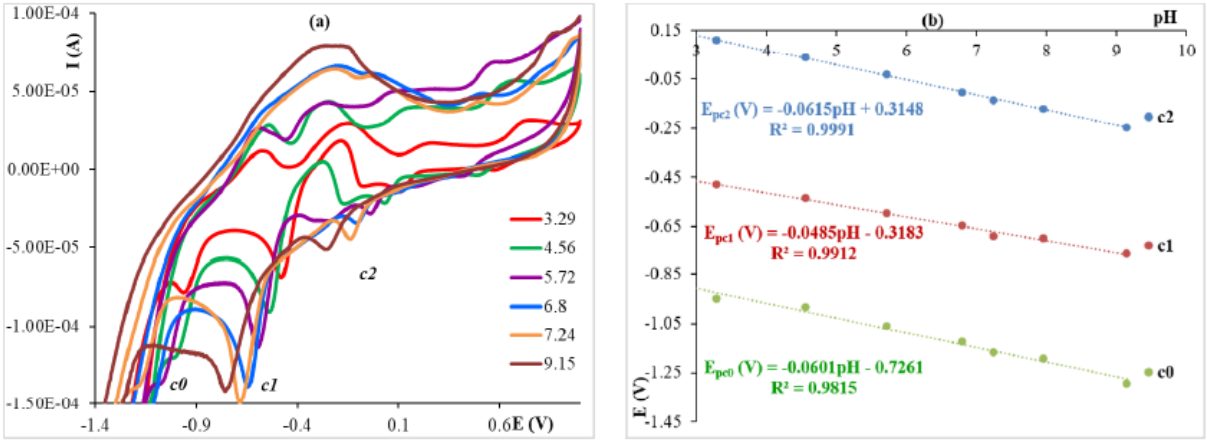


Fig. 2 – a) Selected CV curves recorded at HB_ PGE for 2.10×10^{-4} M NZP in BRB solutions with various pH values (v = 0.100 V/s); b) the E_p (V) = f(pH) dependencies of NZP reduction peaks.

The influence of scan rate

To bring insight into NZP electrode processes, the influence of the scan rate on the analyte voltametric peaks was investigated. CV curves recorded at HB_PGE at various scan rates showed that NZP voltametric peaks increased with increasing scan rate values (Fig. 3).

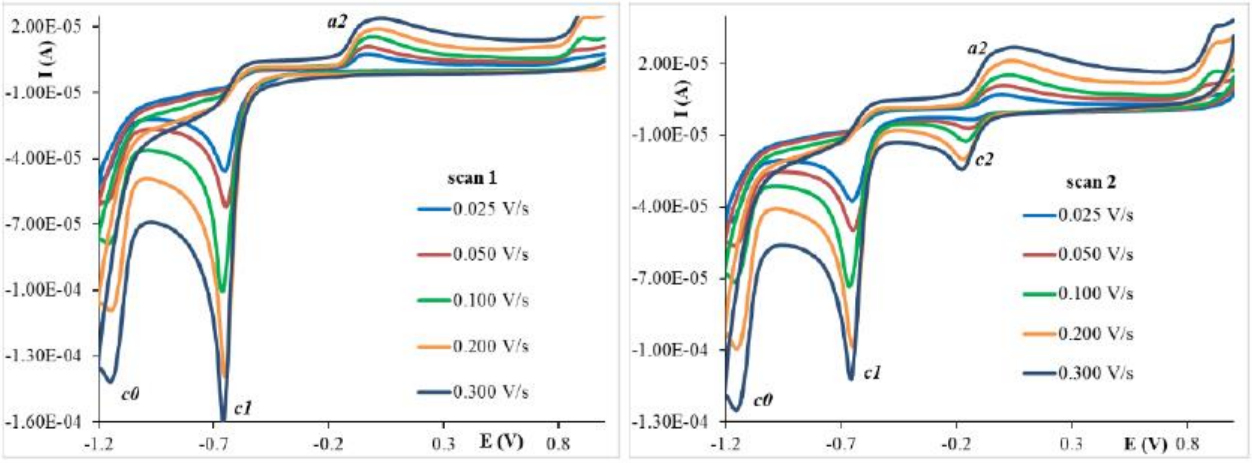


Fig. 3 – Selected CV curves recorded at HB_PGE for 3.50×10^{-4} M NZP in PBS pH 7.50 at different scan rates.

The various dependencies of the peak current (I_p, A) on the potential scan rate (v, V/s) indicated that peak *c1* was due to a mixed, diffusion-adsorption, controlled process (I_p (A) = 2.5×10^{-3} v (V/s) + 3×10^{-5} (R^2 = 0.9769) and I_p (A) = 2×10^{-4} $v^{1/2}$ $(V/s)^{1/2}$ – 2×10^{-5} (R^2 = 0.9901)), while the processes generating peaks pair *a2/c2* were limited only by the analyte diffusion towards the electrode surface (I_p (A) = f (v (V/s)) – non-linear; I_{pa2} (A) = 2×10^{-5} $v^{1/2}$ $(V/s)^{1/2}$ + 1×10^{-6} (R^2 = 0.9682) and I_{pc2} (A) = 3×10^{-5} $v^{1/2}$ $(V/s)^{1/2}$ – 6×10^{-6} (R^2 = 0.9920), respectively).

NZP quantitative determination

As previously shown, peak *c1* was the most intense of the NPZ voltammetric signals and was therefore used for the quantitative determination of the drug. DPV curves recorded at HB_PGE for various NZP concentrations emphasized that the intensity of peak *c1* increased with the analyte concentration, presenting a linear I_p (A) = f(C (M)) dependence (I_p (A) = 0.314×C (M) – 9.5×10^{-7}) in the concentration range 2.45×10^{-6} – 9.75×10^{-5} M NZP. The detection (LoD) and quantification limits of the DPV at PGE method, calculated as 3.3σ/b and 10σ/b, respectively, where σ is the residual standard deviation and b is the slope of the calibration graph, were 1.13×10^{-6} M NZP and 3.76×10^{-6} M NZP, respectively. The performance characteristics of the developed method were compared with the electrochemical ones reported in the literature over the last 10 years (Table 1).

Table 1

Performance characteristics of electrochemical methods and electrodes developed for NZP quantification

Electrode	Technique	Linear concentration range (M)	LoD (M)	Sample	Ref.
GCE activated by CV	SS-HLLME / DPV anodic PBS pH 7.00	$1.07 \times 10^{-10} – 7.11 \times 10^{-10}$ $7.11 \times 10^{-10} – 1.60 \times 10^{-8}$	3.20×10^{-12}	Urine	12
GCE / AuNPs/rGO	DPV anodic PBS pH 7.40	$5.00 \times 10^{-7} – 4.00 \times 10^{-4}$	1.66×10^{-7}	Serum, Tablets	13
GCE/GO-HNT	CV cathodic PBS pH 7.00	$1.60 \times 10^{-7} – 1.50 \times 10^{-4}$	7.90×10^{-7}	Pepsi, vodka	14
eLLI/macroITIES eLLI/microITIES	ITV 0.01 M NaCl, 0.01 M HCl, pH 2.00	$1.00 \times 10^{-5} – 3.0 \times 10^{-5}$ $2.00 \times 10^{-6} – 3.00 \times 10^{-5}$	2.15×10^{-6} (+) 1.86×10^{-6} (–) 4.20×10^{-7} (+) 3.80×10^{-7} (–)	Spiked urine, blood, pharmaceuticals	15
CPE-Z/PVC-NZP-TPB	Potentiometry	$1.00 \times 10^{-7} – 1.00 \times 10^{-2}$	4.70×10^{-8}	pure form, synthetic tablet, spiked human plasma	16
PGE	DPV cathodic PBS pH 7.50	$2.45 \times 10^{-6} – 9.75 \times 10^{-5}$	1.13×10^{-6}	Tablets Coca Cola	This work

SS-HLLME: switchable solvent based homogeneous liquid–liquid microextraction; AuNPs/rGO: Au nanoparticles reduced graphene oxide nanocomposite; GO-HNT: graphene oxide functionalized halloysite nanotubes; eLLI: electrified liquid-liquid interface; ITIES-interface between two electrolyte solutions; ITV: ion transfer voltammetry. (+) – positive signals defined as the cation transfer from the aqueous to the organic phase; (–) – negative signals defined as the cation transfer from the organic to the aqueous phase; CPE-Z/PVC-NZP-TPB – zeolite modified carbon paste electrode covered with a PVC membrane containing NZP-tetraphenyl borate

The repeatability of the cathodic voltammetric response of NZP was estimated at the lowest concentration (2.45×10^{-6} M), an intermediate concentration (8.75×10^{-6} M), and a concentration (3.90×10^{-5} M) near the upper limit of the linearity concentration range. It was expressed as the percentage relative standard deviation (RSD%). 10 and 5 measurements were performed at 2.45×10^{-6} M NZP and at the other two concentrations, respectively, each time using a new PGE. The results obtained (7.44%, 3.74% and 1.79%, respectively) were within the limits indicated by the Association of Official Agricultural Chemists (AOAC)[17], which are 11.00%, 7.30% and 5.30%, respectively, indicating a good precision of the method.

Analytical applications

The practical applicability of the DPV at HB_PGE method developed for NZP quantification was demonstrated by determining NZP from pharmaceutical tablets and spiked Coca Cola, by using the multiple standard addition method (Fig. 4a) and the calibration curve method (Fig. 4b), respectively. The obtained recoveries ranged between 95.99–103.40% and 95.64–109.94 % for tablets and Coca Cola sample solutions, respectively (Table 2).

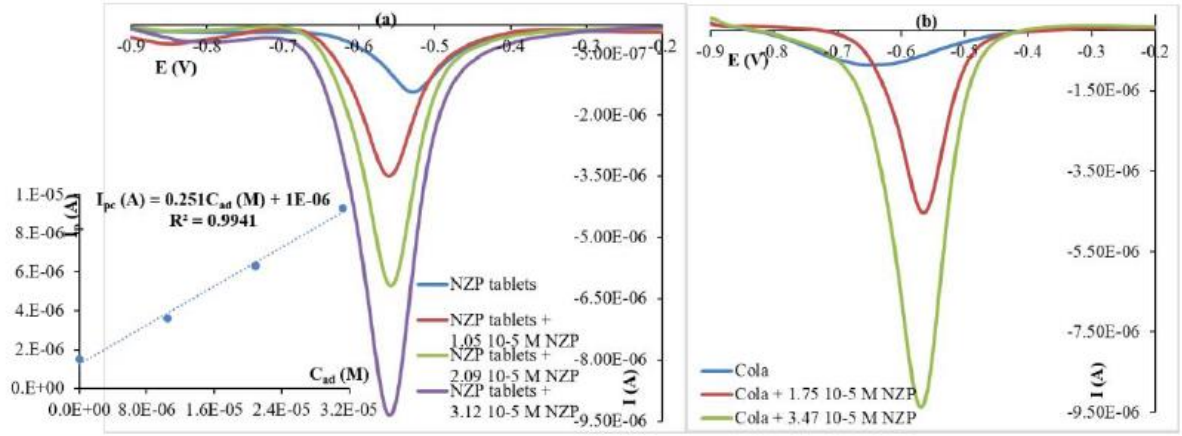


Fig. 4 – DPV curves recorded at HB_PGE for (a) NZP tablets solution without and with NZP standard additions (inset: the corresponding calibration graph) and (b) Coca Cola-PBS solution without and with added NZP. Supporting electrolyte: PBS pH 7.50.

Table 2

NZP quantification from pharmaceutical tablets and spiked Coca Cola samples obtained by DPV at HB_PGE

Tablets			Coca Cola		
NZP content (mg)			NZP concentration (M)		
Declared	Found ± SD	R% ± SD	Spiked	Found ± SD	R% ± SD
5	4.99 ± 0.11	99.76 ± 2.25	1.75×10^{-5}	$1.83 \times 10^{-5} \pm 8.12 \times 10^{-7}$	104.60 ± 4.64
			3.47×10^{-5}	$3.41 \times 10^{-5} \pm 1.03 \times 10^{-6}$	98.16 ± 2.97

EXPERIMENTAL

The voltammetric recordings were performed using a PGSTAT potentiostat/galvanostat coupled to a laptop running the GPES4.9 software and to a three-electrode voltammetric cell. The working electrode used was a PGE, the reference electrode was an Ag/AgCl/KCl electrode (3M) and a Pt wire was used as an auxiliary electrode. Before measurements carried out in the cathodic domain, oxygen was removed from the solution, by bubbling a nitrogen stream for 5 minutes. All used reagents were of analytical grade. 3.9×10^{-3} M NZP stock solution was prepared in ethanol. Working solutions were obtained by dilution with the proper supporting electrolyte.

Pharmaceuticals with a declared content of 5 mg NZP/ tablet were grounded to a fine powder and an accurately weight mass was dissolved in 10 mL ethanol under sonication. An aliquot of the suspension was filtered and subsequently diluted with the supporting electrolyte to obtain the working solution.

CO_2 from the Coca-Cola sample was eliminated by bubbling a N_2 stream.

CONCLUSIONS

This work presented for the first time the voltammetric investigation of NZP behaviour at a PGE. As expected, CV results revealed the complex electrochemical behaviour of NZP attributed to the 4,5-azomethin group within the benzodiazepine ring which underwent irreversible reduction at very negative potentials (peak *c0*) and to the nitro group, the reduction of which gave rise to peak *c1* and the *a2/c2* peak pair. *c1* was generated by the irreversible, diffusion-adsorption controlled reduction of the $–NO_2$ group to –NHOH, while *a2/c2* was associated with the quasi-reversible process involving the –NHOH/–NO groups. All processes were pH-dependent and involved an equal number of electrons and protons. DPV measurements performed under optimized conditions (HB_PGE, PBS pH 7.50) indicated that the method had an linear concentration range of over one order of magnitude and limits of detection and quantification at μM level. The precision of the method fell within the limits established by AOAC. The practical applicability of the method was demonstrated by the good recoveries obtained for NZP quantification from tablets and Coca-Cola samples. Thus, the developed DPV method, using an inexpensive, disposable and readily commercially available PGE, is a simple and cost-effective method that can be used for rapid NZP screening in various matrices.

Finally, it should be noted that few mercury-free electrochemical sensors for NZP detection have been reported in the literature; although some of these offer slightly lower LODs, the PGE has the advantage of being made from an eco-friendly material and being easy to prepare, requiring no additional reagents or processing steps. Thus, the PGE can be considered an environmentally friendly device that could be miniaturized and used for a sustainable, rapid screening of NZP in beverages or for the quality control of pharmaceutical preparations.

REFERENCES

1. D. J. Greenblatt, *J. Clin. Psychiatry*, **1992**, *53*, 7–13.
2. A. Khoshroo, L. Hosseinzadeh, A. Sobhani-Nasab, M. Rahimi-Nasrabadi and H. Ehrlich, *J. Electroanal. Chem.*, **2018**, *823*, 61–66.
3. A. Herrera-Chacón, F. Torabi, F. Faridbod, J. B. Ghasemi, A. González-Calabuig and M. Del Valle, *Sensors*, **2019**, *19*, 5002.
4. L. Kangas and D. D. Breimer, *Clin. Pharmacokinet.*, **1981**, *6*, 346–366.
5. M. Bakavol and, M. Kaykhaii, *J. Pharm. Biomed. Anal.*, **2003**, *31*, 1185–1189.
6. K. Konishi, T. Fukami, S. Gotoh and M. Nakajima, *Biochem. Pharmacol.*, **2017**, *140*, 150–160.
7. I. Álvarez-Freire, P. Brunetti, P. Cabarcos-Fernández, A. Fernández-Liste, M. J. Tabernero-Duque and A. M. Bermejo-Barrera, *J. Pharm. Biomed. Anal.*, **2018**, *159*, 45–52.
8. H. Guo, J. Chen, G. Jiang, Y. Mei, Z. Gong, M. Liu, J. Li and J. Gan, *Molecules*, **2025**, *30*, 775.
9. C. N. Nunes, L. E. Pauluk, V. E. dos Anjos, M. C. Lopes and S. P. Quinaia, *Anal. Bioanal. Chem.*, **2015**, *407*, 6171–6179.

10. M. Imran, S. Ahmed, E. A. Al-Harthi, M. E. Khan, M. M. Alam, F. Haouala, A. A. Chaudhary and A. Asghar, *Phys. Scr.*, **2023**, *98*, 075003.
11. I. G. David, M. Buleandra, D. E. Popa, M. C. Cheregi, V. David, E. E. Iorgulescu and G. O. Tartareanu, *Processes*, **2022**, 10, 472.
12. S. Shahraki, H. Ahmar and M. Nejati-Yazdinejad, *Microchem. J.,* **2018**, *142*, 229–235.
13. L. Fritea, F. Bănică, T. O. Costea, L. Moldovan, C. Iovan and S. Cavalu, *J. Electroanal. Chem.* **2018**, *830–831*, 63–71.
14. G. Kaur, V. Prakash, S. Gupta, M. K. Chaudhary, S. K. Mehta and S. Sharma, S. *FlatChem*, **2025**, *49*, 1007
15. P. Stelmaszczyk, K. Kwaczyński, K. Rudnicki, S. Skrzypek, R. Wietecha-Posłuszny and L. Poltorak, *Microchim. Acta,* **2023**, *190*, 182.
16. M. M. Azab, M. Rizk, O. M. Mohamed and A. S. Farag, *Electroanalysis* **2024**, 36, e202400063.94.
17. AOAC International. Guidelines for Standard Method Performance Requirements. Available online: https://www.aoac.org/wp-content/uploads/2019/08/app_f.pdf (accessed 12.12.2025).