Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 85 (7) 923–937 (2020)
JSCS–5349
UDC 615.212tramadol+612.461:546.27:544.6.076.32–036:543.552
Original scientific paper

Determination of tramadol in pharmaceutical forms and urine samples using a boron-doped diamond electrode

ERTUĞRUL KESKIN[1]*, SHABNAM ALLAHVERDIYEVA[2], ESMA ŞEYHO[3] and YAVUZ YARDIM[3]**

[1]*Department of Analytical Chemistry, Faculty of Pharmacy Adıyaman University, 02040 Adıyaman, Turkey,* [2]*Department of Biochemistry, Faculty of Science Van Yuzuncu Yil University, 65080 Van, Turkey and* [3]*Department of Analytical Chemistry, Faculty of Pharmacy Van Yuzuncu Yil University, 65080 Van, Turkey*

(Received 6 September, revised 8 November, accepted 16 December 2019)

Abstract: The present work describes the electroanalytical investigation and a novel voltammetric method for the cheap, fast and simple quantification of tramadol (TRH) using a boron-doped diamond electrode. TRH displayed one well-defined, irreversible and adsorption-controlled oxidation peak at about 1.58 V (*vs.* Ag/AgCl) in Britton–Robinson buffer (BR, 0.1 mol L^{-1}, pH 3.0) using the cyclic voltammetry technique. The voltammetric responses of the oxidation peaks are dependent on pH and their sensitivity was significantly enhanced in the presence of surfactant media (sodium dodecyl sulphate, SDS). Under the optimized experiment conditions, employing the square-wave stripping mode, it was found that there was an excellent correlation between oxidation peak current and the TRH concentration in the range 0.25 to 50.0 μg mL^{-1} (8.34×10^{-7}–1.67×10^{-4} mol L^{-1}), with a detection limit of 0.072 μg mL^{-1} (2.40×10^{-7} mol L^{-1}) in 0.1 mol L^{-1} BR buffer (pH 3.0) solution comprising 8×10^{-4} mol L^{-1} SDS at 1.52 V (after 30 s accumulation at open-circuit conditions). The developed approach could be used for the quantification of TRH in pharmaceutical formulations and spiked human urine samples with acceptable recoveries.

Keywords: tramadol; boron-doped diamond electrode; pharmaceutical formulation; urine samples; sodium dodecyl sulphate.

INTRODUCTION

The main goal of pain management is to reduce trauma and to ameliorate the quality of lives of the patients. Opioid class narcotics are the class of drugs commonly used for increasing patient comfort. Tramadol, (IUPAC name: (1*R*,2*R*)-2-

*,** Corresponding authors. E-mail: (*)keskinertugrul@gmail.com; (**)yavuzyardim2002@yahoo.com
https://doi.org/10.2298/JSC190906138K

Available on line at www.shd.org.rs/JSCS/
(CC) 2020 SCS.

-[(dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexan-1-ol hydrochloride, here abbreviated as TRH (Fig. 1), is one of the atypical opioid class drugs that serve for this purpose.[1] TRH is also a synthetic analogue of opioid class codeine that is especially applied in the medication of chronic and acute pains as a centrally acting analgesic.[2] Although the pain relieving dose varies depending on the response of the patient and the intensity of the pain, the therapeutic concentration of TRH was reported to be 100–300 ng L^{-1} level in the plasma.[3] It relieves pain with a double-acting action mechanism both by acting as an opioid receptor and by inhibiting the reuptake of serotonin and norepinephrine.[4] This opioid can also be used in combination with other analgesics and antipyretics in the treatment of advanced cancer. TRH is immediately absorbed following oral administration and reaches a bioavailability level of 65–70 % after the first pass metabolism.[5] Approximately 30 % of the parent drug is excreted through the urine without any change in the body and the remaining 70 % through the kidney converted into metabolites.[6] Therefore, its determination in biological samples, such as blood, urine, and serum, is of great importance because the results of analyses provide information about long-term abuse and can also be used for forensic purposes.[2–6]

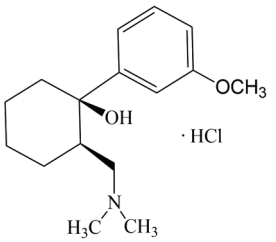

Fig. 1. Molecular structure of tramadol.

To date, a number of studies have been performed including high performance liquid chromatography,[7,8] spectrophotometry,[9] electrochemiluminescence,[10] gas chromatography[11] and electrochemical methods[12–14] for the quantitative analysis of TRH from drugs, biological fluids and environmental water samples. Electrochemical methods, one of these methods, provide not only benefits, such as speed, precision, selectivity and cost compliance, for chemical analysis, but also provide useful information about the oxidation–reduction mechanism of the corresponding electroactive species. Therefore, it is one of the most frequently used methods in the analysis of electroactive compounds in drugs, environmental samples and foods.[15]

Electrochemical studies developed for TRH analysis from different samples are presented later on in Table I. Most of these studies were realised either by attaching specific chemicals to a glassy carbon electrode or by filling different composite materials to a carbon paste electrode. After these processes, some modified electrodes reached very satisfactory detection limit levels.

(CC) 2020 SCS.

Boron-doped diamond (BDD) electrodes are a prestigious carbon material that gains a new horizon to electrochemical analysis and they enable the analysis of many electroactive species that cannot be performed by traditional solid electrodes. The electrode material to be used in electrochemical analyses is expected to produce a stable electrode response, have a large potential window, enabled to work in even the most aggressive environments, respond quickly, have a low baseline current, and be economical. BDD is one of the outstanding electrode materials that fulfil all these expectations.[16] As a result of these properties, the BDD electrode has increased both the extent and quantity of electrochemical analyses.[17]

Surfactants increase the solubility of electroactive species by forming a micelle structure in solution and allow the electroactive species to adsorb more easily to the electrode surface. As a result, quantitative analysis of the related compound can be achieved with better sensitivity.[18] To the best of our knowledge, no study on the electrochemical behaviour and quantitative analysis of TRH using unmodified BDD electrodes could hitherto be found in the literature except for two papers which report the simultaneous electroanalytical determination of acetaminophen and TRH using a BDD electrode.[19,20] The objective of the present work was to examine the electrochemical properties of TRH on an unmodified BDD substrate in the presence of sodium dodecyl sulphate (anionic surfactant, SDS) and then to develop a fast, simple and environment-friendly voltammetric technique for the quantification of TRH by unmodified BDD in connection with the square-wave voltammetric technique. Based on the results obtained, the practical applicability of the developed technique was demonstrated using commercial pharmaceuticals and urine samples.

EXPERIMENTAL

Chemicals and solutions

The reference standard of TRH (Reagent Plus®, ≥99 %) was purchased from Sigma–Aldrich and used without further purification. Purified water from a Millipore Milli-Q system (Millipore, resistivity ≥ 18.2 MΩ cm) and analytical-grade reagents were used for the preparation of the Britton–Robinson buffer (BR, 0.1 mol L^{-1}, pH 2–8), phosphate buffer (0.1 mol L^{-1}, pH 2.5 and 7.4), acetate buffer (0.1 mol L^{-1}, pH 4.7), $HClO_4$ (0.1 mol L^{-1}) and HNO_3 (0.1 mol L^{-1}) solutions. A stock solution (1.0 mg mL^{-1}) of TRH was prepared in water. It was stored in a refrigerator at 4 °C when not in use and protected from exposure to direct daylight during use in the laboratory. The solutions of TRH used in calibration studies and sample analysis were prepared by diluting the stock solution to the appropriate volume with the supporting electrolyte.

Apparatus and measurements

The electrochemical analysis was performed with a μAutolab type III potentiostat/galvanostat (Metrohm Autolab B.V, the Netherlands), which was managed by GPES 4.9 software. The raw signals of the square wave voltammograms generated by the electrochemical instrument were recorded after correction processing by the moving average method (0.01 V

(CC) 2020 SCS.

peak widths) and smoothing processing by the Savicky and Golay algorithm in this software. All voltammetric experiments were conducted in a three-electrode system in a glass electrochemical cell (volume of 10 mL) maintained at ambient temperature. A platinum wire and Ag/AgCl (3 mol L^{-1} NaCl, Model RE-1, BAS, USA) were used as the counter and reference electrodes, respectively. All potential are referred on Ag/AgCl scale. The BDD electrode was obtained from Windsor Scientific Ltd. (UK). The BDD film electrode (boron content 1000 ppm), with poly-crystalline structure deposited on a polyether ether ketone tube with a 0.5 mm thickness and diameter of 3 mm declared by the provider, was employed as the working electrode. At the beginning of every experiment day, an anodic potential of 1.8 V for 180 s followed by a cathodic potential of –1.8 V for 180 s was applied to the BDD electrode in order to form oxygen- and hydrogen-terminated on its surface in 0.5 M H_2SO_4. Before each voltammetric experiment, the electrode was softly rubbed with a polishing pad (for less than 1 min) and then rinsed with deionised water. A pH meter model WTW inoLab720 equipped with a combined glass electrode was used to measure all pH values.

The cyclic voltammetry (CV) method was used first in order to determine the electrochemical behaviour of TRH on bare BDD electrode in preliminary studies followed by the square wave adsorptive stripping voltammetry (SW-AdSV) method for testing the analytical performance and practicability of the method.

The employed procedure for SW-AdSV analysis of TRH was as follows: the previously treated BDD electrode was immersed in a stirred (at 500 rpm) sample solution for a certain period, at the chosen accumulation potential in order to accomplish TRH pre-concentration. After a rest period of 5 s, anodic scans were implemented in the range of 0.4 to 1.8 V using the SW waveform to settle the solution and decrease the background current.

Prior to analytical applications, the best device signals were obtained at 50 Hz frequency, 50 mV pulse amplitude, and 14 mV step potential values among the SWV variables. Consecutive measurements were performed by applying the above procedure to the working electrode recursively. All measurements were performed at room temperature (25±5 °C) and in triplicate.

Preparation of the samples

TRH injection solution (Tradolex®, Mentapharma Co., Turkey) containing 50 mg TRH per mL^{-1} was used for drug sample analysis. 2 mL of this injection solution was transferred to a calibrated amber glass flask and the volume was made up to 1 L with deionised water. Known amounts of this solution were smoothly added to a 10 mL volume electrochemical cell containing 8×10^{-4} mol L^{-1} SDS and 0.1 mol L^{-1} BR buffer solutions (pH 3.0). Then, the unknown sample analysis was calculated by the corresponding regression equation in the calibration graph obtained for the standard TRH solutions.

Drug-free human urine samples were obtained from a healthy 19-year-old male donor the day before the experiment. After adding 9 mL of urine sample to 1 mL of TRH stock solution (1 mg mL^{-1}) in a test tube, the resulting mixture was vortexed for one minute. The appropriate volume of this mixture was then transferred into the voltammetric cell containing the selected supporting electrolyte.

RESULTS AND DISCUSSION

Cyclic voltammetric behaviour of TRH on the BDD electrode

The electrochemical behaviour of TRH was first examined using the CV technique in 0.1 mol L^{-1} BR buffer solution (pH 3.0) within the potential range

(CC) 2020 SCS.

from 0.8 to 1.8 V at a scan rate of 100 mV s^{-1} without an accumulation step. In addition, the CV of the solution containing only the supporting electrolyte (without TRH) was recorded for comparison purposes (Fig. 2A, dashed line). As can be seen from Fig. 2A, a well-shaped oxidation peak was observed at approximately 1.58 V in the first cycle, whereas no reduction peak was seen in the reverse scan. Consequently, it could be concluded that the oxidation reaction of TRH on the BDD electrode is irreversible.[21] The decrease in the anodic peak current in the 2nd and 3rd scans of consecutive CV scans could be interpreted as the adsorption of TRH and/or the oxidation products on the BDD electrode. To clarify this situation, the effect of scan rate on the current response of 100 μg mL^{-1} TRH was investigated by CV in BR buffer pH 3.0 using the BDD electrode (Fig. 2B). There was a slight shift of the oxidation peak potentials of TRH towards more positive values as the scan rate was increased. The linear relation between the oxidation peak current (I_p) and scan rate (ν) was obtained in the range of 50–500 mV s^{-1} (n = 7). The equation is noted below:

$$I_p / \mu A = 0.0083(\nu / \text{mV s}^{-1}) + 2.2429 \ (r = 0.996) \quad (1)$$

In addition, the plot of log I_p *versus* log ν was also linear according to the following equation:

$$\log (I_p / \mu A) = 0.5946 \log (\nu / \text{mV s}^{-1}) + 0.2862 \ (r = 0.993) \quad (2)$$

These results strongly indicated that the TRH oxidation reaction at the BDD electrode is an adsorption controlled process.

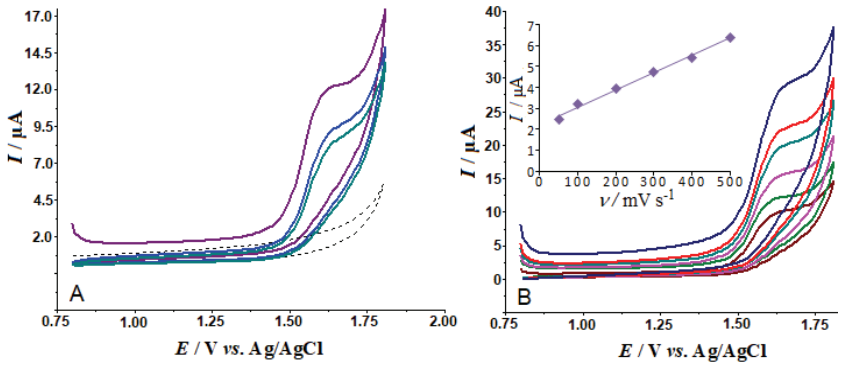

Fig. 2. The repetitive cyclic voltammograms at scan rate of 100 mV s^{-1} (A), and the cyclic voltammograms at different scan rates (50, 100, 200, 300, 400 and 500 mV s^{-1}, B) of 100 μg mL^{-1} TRH in BR buffer solution (pH 3.0). Dashed line represents the background current. The inset depicts a plot of peak current *vs.* scan rate (ν).

Effect of supporting electrolyte and pH

The influence of the pH on the oxidation peak current response of TRH was investigated by SW-AdSV on the BDD electrode using different supporting elec-

(CC) 2020 SCS.

trolytes at various pH values in order to obtain the best voltammetric response for analytical purposes. In Fig. 3A, the baseline corrected SW-AdSVs are depicted within the pH range 2.0–8.0 in BR buffer by performing SW-AdSV measurements on 20 μg mL^{-1} TRH solution, with an open-circuit accumulation at 30 s, with the potential window from 0.4 to 1.8 V. As could be seen in this figure, the SW-AdSs recorded at BDD exhibit one oxidation peak in the working potential range, except for pH 8.0. At pH 8.0, a small additional anodic peak was noticed at about 1.71 V. The shift of the oxidation peak potential of TRH to lower values on increasing the pH from 2.0 to 8.0 is a clear indication that the oxidation process of TRH on the BDD electrode is accompanied by a protonation reaction. The dependences of peak potential, E_p, of TRH on solution pH were investigated in the range pH 2.0–8.0. It was found to be linear in the pH range of 2.0–8.0 and could be described by Eq. (3):

$$E_p / V = -0.0215pH + 1.6231 \ (r = 0.996) \quad (3)$$

The slope was found to be 0.0215 V per pH unit, which indicated that the numbers of electrons and protons participating in the electrode reaction are unequal.[22]

The SW-AdS voltammograms of TRH in various supporting electrolytes are shown in Fig. 3B. Using 0.1 mol L^{-1} $HClO_4$, HNO_3, PBS pH 2.5, ABS pH 4.7 and PBS pH 7.4, oxidation peak potentials of 1.57 (5.41 μA), 1.57 (4.47 μA), 1.57 (4.19 μA), 1.52 (4.77 μA) and 1.45 V (3.38 μA) were obtained, respectively. Meanwhile, in the case of phosphate buffer at pH 7.4, one more oxidation peak potential was detected at 1.69 V (0.98 μA).

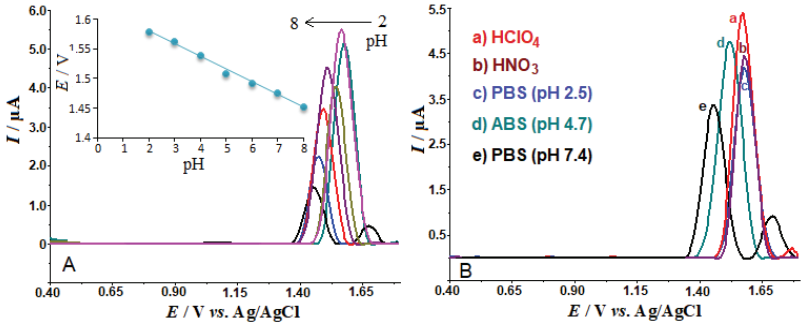

Fig. 3. The stripping voltammograms of 20 μg mL^{-1} TRH in BR buffer solution pH 2.0–8.0 (A), and in various supporting electrolytes (B). Inset depicts the plot of E_p *vs.* pH. Electrode BDD; accumulation time 30 s at open-circuit conditions. SWV parameters: frequency, 50 Hz; step potential, 8 mV; pulse amplitude, 30 mV.

As can be seen from Fig. 3A and B, the maximum and best-shaped signal of 20 μg mL^{-1} TRH on BDD electrode was obtained with BR buffer pH 3.0. Thus, 0.1 mol L^{-1} BR buffer (pH 3.0) was used for the further studies.

(CC) 2020 SCS.

Effect of accumulation time and accumulation potential0

Considering the apparent adsorptive character of TRH on the BDD electrode, it could be predicted that the accumulation time and potential would have an effect on the oxidation signal of TRH. To verify this prediction, the effects of accumulation time (t_{acc}) and accumulation potential (E_{acc}, data not shown) were investigated under optimized experimental circumstance for 10 μg mL^{-1} TRH. The effect of t_{acc} on the oxidation signal of TRH was examined in the range of 0–240 s by applying open circuit potential to the electrochemical cell. No significant increase in the oxidation peak was observed with applied accumulation times of more than 30 s. For this reason, 30 s was chosen as the shortest time for optimum t_{acc} and doubtlessly the practical use of the electrode. Although the usage of accumulation slightly increased the oxidation peak currents (t_{acc}= 30 s, I_p = 2.18 μA), compared to the SW voltammograms obtained without accumulation (t_{acc}= 0 s, I_p=1.37 μA), its use contributed to increased sensitivity and regeneration of the diffusion layer between measurements. The dependence of the stripping peak current on the E_{acc} was evaluated either at open-circuit conditions or over the potential range 0.1 to 1.2 V. No important effects were determined under the studied potential values. Thus, a t_{acc} of 30 s and E_{acc} at open-circuit accumulation were found reasonable for the further analytical investigations.

Effect of SWV parameters

The SWV parameters such as frequency (*f*), pulse amplitude (ΔE_{sw}), and step potential (ΔE_s) were optimized in order to obtain the highest value of the oxidation peak current, the maximum selectivity and improved reproducibility. While one parameter was varied, all others were kept fixed. The *f* value was evaluated in the range from 25 to 125 Hz (with the ΔE_s and ΔE_{sw} fixed at 8 and 30 mV, respectively). The higher the *f* value, the higher was the obtained oxidation peak current. However, for values larger than 50 Hz, a considerable widening of the peak width was observed. Evaluation of this phenomenon in SW voltammetric responses represents a loss in analytical selectivity. Thus, *f* = 50 Hz was chosen for all subsequent experiments. The influence of the ΔE_{sw} (other parameters: ΔE_s = 8 mV, *f* = 50 Hz) on the oxidation peak current intensity was also examined in the range from 30 to 70 mV. A linear increase was observed between the oxidation peak current values and the ΔE_{sw} in the investigated range. However, at higher values of 50 mV, an increase in ΔE_{sw} resulted in a considerable widening in the SW voltammograms. The ΔE_s was varied between 6–16 mV with a fixed parameters of *f* = 50 Hz and ΔE_{sw} = 50 mV. The recorded voltammetric signal increased gradually until a value of 14 mV, after which it slightly increased. This effect was also accompanied by peak broadening. Thus, ΔE_s = 14

(CC) 2020 SCS.

mV was chosen. For further SW-AdS voltammetric measurements, the optimal values were *f*, 50 Hz; ΔE_{sw}, 50 mV and ΔE_s, 14 mV.

Effect of anionic surfactant

Finally, the influence of SDS, anionic surfactant, was also evaluated in order to improve the sensitivity of the electrochemical process on the oxidation signals of TRH. This effect was examined by keeping the TRH concentration constant at 7.5 μg mL^{-1} in the electrochemical cell containing BR buffer (pH 3.0) and chan ging of SDS concentrations in the range from 10^{-4} to 10^{-3} mol L^{-1}. When compared the voltammetric behaviour of TRH in the absence and presence of SDS (Fig. 4), a slight negatively shift was observed in the peak potentials of the electrolyte solution containing SDS, but the increase in SDS concentration did not change its position. On the other hand, an important signal enhancement was observed in cooperation with SDS. The stripping peak currents increased with SDS concentration up to 8×10^{-4} mol L^{-1}. Above this concentration, a very small change was observed (Fig. 4 inset). Therefore, a concentration of SDS of 8×10^{-4} mol L^{-1} was chosen for the remaining analytical investigation. In this case, TRH signals were approximately 2.5 times higher compared to that for the solution without surfactant.

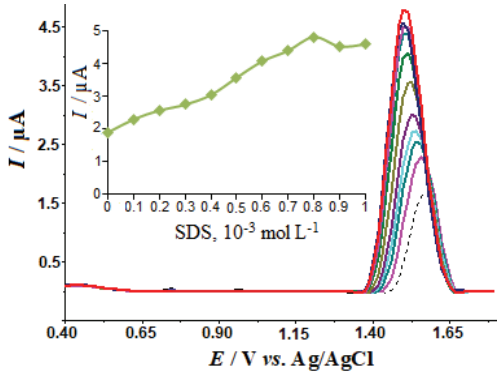

Fig. 4. The stripping voltammograms of 7.5 μg mL^{-1} TRH in 0.1 mol L^{-1} BR buffer solution (pH 3.0) in the presence of different SDS concentrations (10^{-4}–10^{-3} mol L^{-1}). Dashed line represents the voltammogram without SDS. Inset: plot of I_p *vs.* C_{SDS}. Electrode, BDD; accumulation time 30 s under open-circuit condition. SWV parameters: frequency, 50 Hz; step potential, 14 mV; pulse amplitude, 50 mV.

Analytical performance evaluation

After optimization of the working conditions (instrumental parameters and chemical conditions), the analytical performance was evaluated by examining the oxidation peak current as a function of concentrations of TRH. Construction of the analytical curve was obtained for TRH on the BDD electrode. In this context, known amounts of TRH stock solution were added sequentially to the voltammetric cell and the current responses obtained from SW-AdS for each addition were evaluated. The SW-AdS voltammograms were recorded by additions of TRH over the 0.25 to 50.0 μg mL^{-1} (8.34×10^{-7}–1.67×10^{-4} mol L^{-1}) concentration range and the respective analytical curve is shown in Fig. 5, inset.

(CC) 2020 SCS.

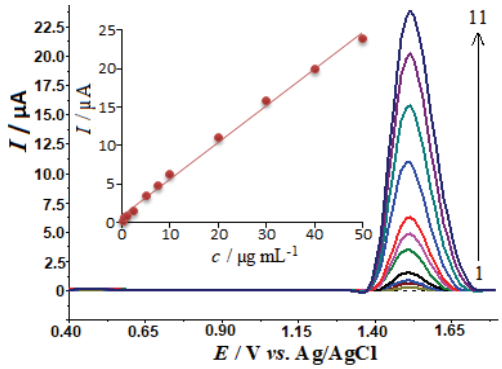

Fig. 5. The stripping voltammograms for TRH levels of: 1) 0.25, 2) 0.50, 3) 1.0, 4) 2.5, 5) 5.0, 6) 7.5, 7) 10, 8) 20, 9) 30, 10) 40 and 11) 50 μg mL^{-1} in 0.1 mol L^{-1} BR buffer solution (pH 3.0) in the presence of 8×10^{-4} mol L^{-1} SDS. Inset depicts the corresponding calibration plot for the quantification of TRH. Other operating conditions as indicated in Fig. 4.

A linear calibration graph was obtained by plotting oxidation peak currents against TRH concentrations in the specified range, expressed by Eq. (4):

$$I_p / \mu A = 0.479(c / \mu g\ mL^{-1}) + 0.811\ (r = 0.998, n = 11) \quad (4)$$

where I_p represents the oxidation peak current, C TRH concentration, r correlation coefficient and n the number of experiments.

From the value obtained from the analytical curve, the limit of detection (*LOD*) and limit of quantification (*LOQ*) were found to be 0.072 μg mL^{-1} (2.40×10^{-7} mol L^{-1}) and 0.24 μg mL^{-1} (8.01×10^{-7} mol L^{-1}), respectively. The *LOD* and *LOQ* were calculated as three and ten times the standard deviation of the lowest concentration (in the linear range) divided by the slope of the calibration curve, respectively.

A comparison of the analytical performance of the BDD electrode created in this study with those in other previously reported electroanalytical is depicted in Table I. As could be seen from this table, the BDD electrode presented a more sensitive electroanalytical response than a modified carbon paste electrode (M--CPE),[12] a coated wire electrode (CWE),[23] a carbon nanoparticles glassy carbon electrode (CNPs/GCE)[24] and multiwalled carbon nanotube glassy carbon (MWCNT/ /GCE)[25] electrodes. However, some electroanalytical studies[1,3,13,14,26–29] reported in the literature showed higher sensitivity than the developed method. However, it should be pointed out that all reported works chemically modified electrodes (based on glassy carbon and carbon paste electrodes) were used. Despite the higher sensitivity of chemically modified electrodes, they have some drawbacks, such as poor reproducibility, high costs and long-time preparation. In this work, using of the BDD electrode without any modification presented good performance including sufficient sensitivity, simplicity, rapidity and low cost for TRH quantification in pharmaceutical form and urine samples.

The precision of the developed method was assessed in terms of the intra- and inter-day repeatability under the optimum experimental conditions. The intra-day repeatability of the magnitude of oxidation peak current was deter-

(CC) 2020 SCS.

mined by ten times repeated measurements of 0.25 μg mL^{-1} for TRH solution. The results indicated a relative standard deviation (*RSD*) of 4.51 % demonstrating that the results are repeatable. Then, the inter-day repeatability was assessed by measuring the magnitude of oxidation peak current response of the BDD electrode for five consecutive working days for the same level of TRH concentration and the *RSD* was calculated to be 5.33 %. Bearing in the mind the obtained precision values, the BDD electrode was proven a suitable electrochemical sensor for repeatable quantification of TRH in pharmaceutical formulations.

TABLE I. Comparison of the efficiency of the BDD electrode with literature electrodes for TRH determination. Analyte: TRH, tramadol hydrochloride; ACP, acetaminophen; CAF, caffeine; electrode: CVE, coated wire electrode, La^{+3}CuO/MWCNT/GCE, lanthanum doped CuO multiwalled carbon nanotube glassy carbon electrode; D50wx2/GNP/GCE, dowex50wx2 gold nanoparticle glassy carbon electrode; $NiFe_2O_4$NPs/GR/CPE, $NiFe_2O_4$ nanoparticles graphene carbon paste electrode; PdNPs/GCE Pd nanoparticles glassy carbon electrode; CNPs/GCE carbon nanoparticles glassy carbon electrode; Nafion®/CTAB-Au/GCE, nafion cetyl trimethylammonium bromide Au nanoparticles glassy carbon electrode; PNB/GCE, poly(Nile Blue) glassy carbon electrode; MIP/MWCNT/CPE, molecularly imprinted polymer multiwalled carbon nanotube carbon paste electrode; P@SG/MIP/fMWCNT/GCE polypyrrole sol–gel molecularly imprinted polymer carboxylic acid functionalized multiwalled carbon nanotube glassy carbon electrode; MWCNT/GCE multiwalled carbon nanotube glassy carbon electrode; Sb_2O_3NPs/MWCNTs/GCE, antimony oxide nanoparticles/multiwalled carbon nanotubes/glassy carbon electrode

Analyte	Electrode	Linear range mol L^{-1}	*LOD* mol L^{-1}	Sample	Ref.
TRH	PdNPs/GCE	5.0×10^{-8}–2.0×10^{-4}	1.5×10^{-8}	Drug, plasma, urine	1
TRH + ACP	La^{+3}CuO/MWCNT/ /GCE	5.0×10^{-7}–9.0×10^{-4}	1.4×10^{-8}	Drug, urine	3
TRH	Modified CPE	9.2×10^{-6}–1.0×10^{-1}	6.2×10^{-6}	Drug, urine, milk	12
TRH	MIP/MWCNT/CPE	1.0×10^{-8}–2.0×10^{-5}	4.0×10^{-9}	Drug, urine	13
TRH+ACP+CAF	PNB/GCE	2.0×10^{-7}–1.6×10^{-5}	8.0×10^{-8}	Drug	14
TRH	CWE	1.0×10^{-5}–1.0×10^{-1}	1.2×10^{-6}	Drug	23
TRH+ACP	CNPs/GCE	1.0×10^{-5}–1.0×10^{-3}	1.0×10^{-6}	Drug, plasma	24
TRH +ACP	MWCNT/GCE	2.0×10^{-6}–3.0×10^{-4}	3.6×10^{-7}	Drug, urine, serum	25
TRH+ACP	D50wx2/GNP/GCE	3.3×10^{-8}–4.2×10^{-5}	1.1×10^{-8}	Drug, blood serum	26
TRH+ACP	$NiFe_2O_4$NPs/GR/ /CPE	1.0×10^{-8}–9.0×10^{-6}	3.6×10^{-9}	Drug, blood serum	27
TRH	P@SG/MIP/ /fMWCNT/GCE	2.0×10^{-10}–2.0×10^{-9}	3.0×10^{-11}	Drug, urine	28
TRH	Sb_2O_3NPs/MWCNTs/GCE	4.0×10^{-8}–3.0×10^{-5}	9.5×10^{-9}	Drug, blood serum	29
TRH	BDD	8.3×10^{-7}–1.7×10^{-4}	2.4×10^{-7}		This work

(CC) 2020 SCS.

Effect of interfering compounds

Prior to the analyses of the real samples, the influence of potentially interfering compounds, mostly present in the pharmaceutical formulation or biological samples were examined by SW-AdSV for 2.5 μg mL^{-1} TRH under the same experimental conditions. The tolerance limit was defined as the maximum concentration of the selected interfering compounds that caused an approximately ±10 % relative error for the oxidation peak current of TRH. It was found that inorganic ions, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, Ti^{4+}, NO_3^-, Cl^- and SO_4^{2-}, do not affect the oxidation signal even at 100 times the concentration of TRH. As for the carbohydrate compounds such as glucose, fructose, sucrose, and lactose, they did not have a significant effect on TRH oxidation peaks even at 100 times excess of TRH. The effect of the agents present in the pharmaceutical formulations, such as cornstarch, magnesium stearate, microcrystalline cellulose on the oxidation current responses of TRH were also found to be negligible. The effect of ascorbic acid (AA), dopamine (DOP), Fig. 6A, and uric acid (UA), Fig. 6B, which could be present in biological fluids, were evaluated in molar concentrations at the ratio (TRH solution:interfering agent) of 1:1, 1:10 and 1:50.

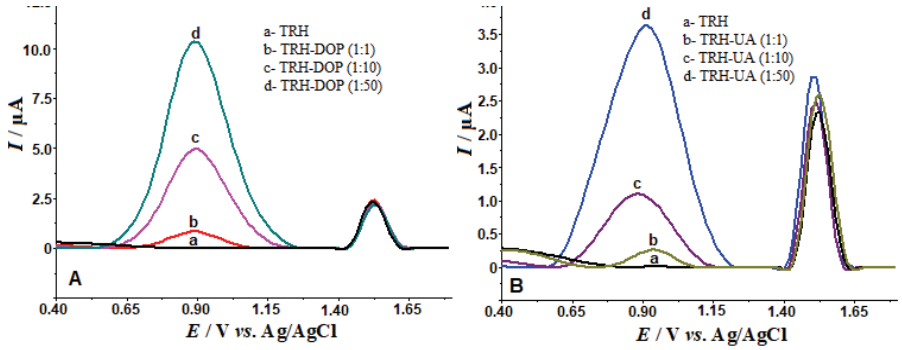

Fig. 6. The stripping voltammograms of TRH (2.5 μg mL^{-1}) mixture in the presence of (A) equimolar concentration, 10-fold and 50 fold excess DOP and (B) equimolar concentration, 10-fold and 50 fold excess UA. Other operating conditions were as indicated in Fig. 4.

Obviously, the oxidation peak current of TRH was not affected by the oxidation peak currents of the individual solutions of AA, DOP and UA in working concentrations. Furthermore, Paracetamol (100-fold excess), coexisting of TRH in some pharmaceutical formulations, did not affect the oxidation peak current of TRH. These results show that the developed approach could be successfully applied to real samples.

Analytical application

Based on the obtained results, in the final step, pharmaceutical and urine samples were used for the quantification of TRH as examples of the applicability

(CC) 2020 SCS.

of the developed method. First, the practical applicability of the BDD electrode for SW-AdSV quantification of TRH was verified by analysis in a pharmaceutical formulation (injectable solution). The injectable solution was diluted with water to obtain the required concentration for assay. The analysis of the sample was undertaken using the calibration curve method from the related regression equation. The TRH content of the injectable solution, which was declared by the producer to be 50 mg mL^{-1}, was found by the developed method to be 48.80 mg mL^{-1} (*RSD* of 3.86%). The validity of the developed approach was also assessed by applying recovery experiments. Recovery studies were performed by adding standard TRH solutions (0.25, 1.0 and 2.5 μg mL^{-1}) prepared in the supporting electrolyte to 10 mL of sample solution in voltammetric cell and the SW-AdSV responses were assessed. Acceptable recoveries were obtained in the range from 97.15 to 104.62 %, demonstrating that the BDD electrode response is not influenced by the sample matrix. Secondly, satisfactory sensitivity and good selectivity of the proposed approach was also used for the quantification of TRH in human urine samples with more complex matrices in comparison with pharmaceutical forms. The preparation of the samples is described in Experimental section in detail. TRH was spiked to the urine samples since TRH was collected from the donor who did not use the TRH medication. Quantification was performed by means of the standard addition method for urine sample spiked with TRH (2.5 μg mL^{-1}). The reached results have been summarized in Table II. Thus, it could be concluded that the developed approach is suitable for the quantification of TRH in urine samples from the obtained results and recovery values. The measurement values in urine samples by standard addition method are depicted in Fig. 7A.

TABLE II. Measurement results for addition and recovery of TRH from urine sample using proposed method; values reported are the average of three independent analysis of the same sample

TRH added, μg mL^{-1}	TRH found[a], μg mL^{-1}	Recovery±*RSD*, %
2.50	2.44	97.60 ±2.57

[a]Calculated by use of the standard addition method

It could be concluded that the oxidation peak that appeared at about +1.55 V was due to TRH oxidation since its peak current increased after each TRH standard addition. In the absence of TRH, there were no detectable oxidation peaks in the working potential range where the analytical peak is observed (Fig. 7B). On the other hand, an unknown oxidation peak at about 1.00 V was observed in blank urine samples, which could be due to the oxidation of uric acid (UA).[30,31] After several standard additions of UA, an increase was observed on this oxidation peak. Since its peak potential was well differentiated from that of TRH, it did not interfere with its quantification.

(CC) 2020 SCS.

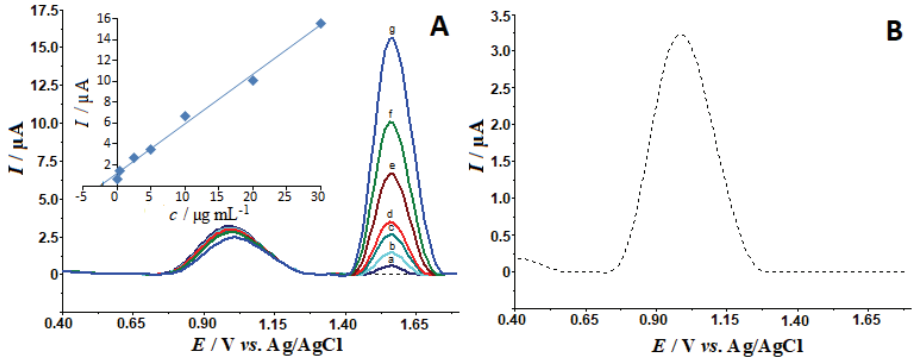

Fig. 7. The stripping voltammograms of the urine sample (diluted with supporting electrolyte in the ratio of 5:100, v/v): (- - -) in the absence of TRH (dashed line), (a) in the presence of 2.5 μg mL^{-1} TRH, (b–g) after standard additions of 0.5, 2.5, 5.0, 10, 20, 30 μg mL^{-1} TRH in 0.1 mol L^{-1} BR buffer solution (pH 3.0) in the presence of 8×10^{-4} mol L^{-1} SDS. Inset depicts the result of analysis by standard addition method (A). The stripping voltammograms of the diluted urine sample under the experimental condition (B). Other operating conditions were as indicated in Fig. 4.

CONCLUSIONS

Hitherto, antecedent published electroanalytical works dealing with the determination of TRH are generally based on modification of the carbon electrodes. In this study, the BDD electrode was used for the first time to develop a simple, alternative and novel voltammetric approach for electroanalytical quantification of TRH. The electrochemical behaviour of irreversibly oxidized TRH on the BDD electrode was examined by CV and SW-AdSV techniques. The obtained results showed that the oxidation peak currents of TRH could be affected by the anionic surfactant SDS. Using the optimized experimental conditions, the developed voltammetric method exhibited a limit of detection in the 2.40×10^{-7} mol L^{-1} concentration level in 0.1 mol L^{-1} BR buffer (pH 3.0) solution with 8×10^{-4} mol L^{-1} SDS. The methods were sufficiently selective with negligible effects of possible interference. The applicability of the proposed methodology for TRH determination has been demonstrated in pharmaceutical formulation and model human urine successfully.

ИЗВОД

ОДРЕЂИВАЊЕ ТРАМАДОЛА У ФАРМАЦЕУТСКИМ ОБЛИЦИМА И УЗОРЦИМА УРИНА ПРИМЕНОМ БОРОМ ДОПИРАНЕ ДИЈАМАНТСКЕ ЕЛЕКТРОДЕ

ERTUĞRUL KESKIN[1], SHABNAM ALLAHVERDIYEVA[2] и ESMA ŞEYHO[3] и YAVUZ YARDIM[3]

[1]*Department of Analytical Chemistry, Faculty of Pharmacy Adıyaman University, 02040 Adıyaman, Turkey,* [2]*Department of Biochemistry, Faculty of Science Van Yüzüncü Yıl University, 65080 Van, Turkey и* [3]*Department of Analytical Chemistry, Faculty of Pharmacy Van Yüzüncü Yıl University, 65080 Van, Turkey*

У овом раду је описано електроаналитичко испитивање и нова волтаметријска метода за економично, брзо и једноставно одређивање трамадола (TRH) применом

(CC) 2020 SCS.

бором допиране дијамантске електроде. Применом цикличне волтаметрије, TRH показује један добро дефинисан иреверзибилни и адсорпционо контролисан оксидациони пик на потенцијалу 1,58 V (према Ag/AgCl) у Британ–Робинсоновом пуферу (BR, 0,1 mol L^{-1}, pH 3,0). Изражен је утицај pH на оксидационе пикове у волтаграму TRH и примећено је значајно повећање осетљивости пикова у присуству сурфактаната (натријум-додецил-сулфат, SDS). У оптимизованим експерименталним условима, применом волтаметрије правоугаоних таласа, обогаћивањем и растварањем добијена је одлична корелација између струје оксидационог пика и концентрације TRH у опсегу од 0,25 до 50,0 μg mL^{-1} ($8{,}34\times10^{-7}$–$1{,}67\times10^{-4}$ mol L^{-1}), са детекционом границом од 0,072 μg mL^{-1} ($2{,}40\times10^{-7}$ mol L^{-1}) у 0,1 mol L^{-1} BR пуферу (pH 3,0), који садржи 8×10^{-4} mol L^{-1} SDS на 1,52 V (после 30 s акумулације у условима отвореног кола). Развијен приступ се може применити за квантификацију TPX у фармацеутским формулацијама и обогаћеним узорцима урина са прихватљивим „recovery“ вредностима.

(Примљено 6. септембра, ревидирано 8. новембра, прихваћено 16. децембра 2019)

REFERENCES

1. F. Fathirad, A. Mostafavi, D. Afzali, *Anal. Chim. Acta* **940** (2016) 65 (http://dx.doi.org/10.1016/j.aca.2016.08.051)
2. K. S. Lewis, N. H. Han, *Am. J. Heal. Pharm.* **54** (1997) 643 (http://dx.doi.org/https://doi.org/10.1093/ajhp/54.6.643)
3. M. M. Foroughi, S. Jahani, H. Hassani Nadiki, *Sensors Actuators, B* **285** (2019) 562 (http://dx.doi.org/10.1016/j.snb.2019.01.069)
4. M. Nobilis, J. Kopecký, J. Květina, J. Chládek, Z. Svoboda, V. Voříšek, F. Perlík, M. Pour, J. Kuneš, *J. Chromatogr., A* **949** (2002) 11 (http://dx.doi.org/10.1016/S0021-9673(01)01567-9)
5. T. Madrakian, A. Afkhami, H. Mahmood-Kashani, M. Ahmadi, *Talanta* **105** (2013) 255 (http://dx.doi.org/10.1016/j.talanta.2012.12.039)
6. N. A. Ghalwa, H. M. Abu-Shawish, F. R. Zaggout, S. M. Saadeh, A. R. Al-Dalou, A. A. Abou Assi, *Arab. J. Chem.* **7** (2014) 708 (http://dx.doi.org/10.1016/j.arabjc.2010.12.007)
7. G. Saccomanni, S. Del Carlo, M. Giorgi, C. Manera, A. Saba, M. Macchia, *J. Pharm. Biomed. Anal.* **53** (2010) 194 (http://dx.doi.org/10.1016/j.jpba.2010.03.016).
8. I. Y. Zaghloul, M. A. Radwan, *J. Liq. Chromatogr. Relat. Technol.* **20** (1997) 779 (http://dx.doi.org/10.1080/10826079708014142)
9. S. Glavanović, M. Glavanović, V. Tomišić, *Spectrochim. Acta, A* **157** (2016) 258 (http://dx.doi.org/10.1016/j.saa.2015.12.020)
10. S. N. Ding, J. J. Xu, W. J. Zhang, H. Y. Chen, *Talanta* **70** (2006) 572 (http://dx.doi.org/10.1016/j.talanta.2006.01.017)
11. V. Gambaro, C. Benvenuti, L. De Ferrari, L. Dell'Acqua, F. Farè, *Farmaco* **58** (2003) 947 (http://dx.doi.org/10.1016/S0014-827X(03)00153-8).
12. H. M. Abu-Shawish, N. A. Ghalwa, F. R. Zaggout, S. M. Saadeh, A. R. Al-Dalou, A. A. A. Assi, *Biochem. Eng. J.* **48** (2010) 237 (http://dx.doi.org/10.1016/j.bej.2009.10.019)
13. A. Afkhami, H. Ghaedi, T. Madrakian, M. Ahmadi, H. Mahmood-Kashani, *Biosens. Bioelectron.* **44** (2013) 34 (http://dx.doi.org/10.1016/j.bios.2012.11.030)
14. S. Chitravathi, N. Munichandraiah, *J. Electroanal. Chem.* **764** (2016) 93 (http://dx.doi.org/10.1016/j.jelechem.2016.01.021)
15. Y. Yardım, A. Levent, E. Keskin, Z. Şentürk, *Talanta* **85** (2011) 441 (http://dx.doi.org/10.1016/j.talanta.2011.04.005)

(CC) 2020 SCS.

16. S. Pysarevska, L. Dubenska, S. Plotycya, Ľ. Švorc, *Sensors Actuators, B* **270** (2018) 9 (http://dx.doi.org/10.1016/j.snb.2018.05.012)
17. Ľ. Švorc, K. Borovská, K. Cinková, D. M. Stanković, A. Planková, *Electrochim. Acta* **251** (2017) 621 (http://dx.doi.org/10.1016/j.electacta.2017.08.077)
18. Y. Li, J. Hao, G. Li, *J. Dispers. Sci. Technol.* **27** (2006) 781 (http://dx.doi.org/10.1080/01932690500461172)
19. A. M. Santos, F. C. Vicentini, L. C. S. Figueiredo-Filho, P. B. Deroco, O. Fatibello-Filho, *Diam. Relat. Mater.* **60** (2015) 1 (http://dx.doi.org/10.1016/j.diamond.2015.10.005)
20. O. Yunusoğlu, S. Allahverdiyeva, Y. Yardım, Z. Şentürk, *Electroanalysis* **32** (2020) 429 (http://dx.doi.org/10.1002/elan.201900452)
21. M. Soleimani, M. G. Afshar, A. Shafaat, G. A. Crespo, *Electroanalysis* **25** (2013) 1159 (http://dx.doi.org/10.1002/elan.201200601)
22. E. Mynttinen, N. Wester, T. Lilius, E. Kalso, J. Koskinen, T. Laurila, *Electrochim. Acta* **295** (2019) 347 (http://dx.doi.org/10.1016/j.electacta.2018.10.148).
23. S. Y. Al Samarrai, F. M. Abdoon, K. K. Hashim, *Microchem. J.* **146** (2019) 588 (http://dx.doi.org/10.1016/j.microc.2019.01.041)
24. F. Ghorbani-Bidkorbeh, S. Shahrokhian, A. Mohammadi, R. Dinarvand, *Electrochim. Acta* **55** (2010) 2752 (http://dx.doi.org/10.1016/j.electacta.2009.12.052)
25. A. Babaei, A. R. Taheri, M. Afrasiabi, *J. Braz. Chem. Soc.* **22** (2011) 1549 (http://dx.doi.org/10.1590/S0103-50532011000800020)
26. B. J. Sanghavi, A. K. Srivastava, *Anal. Chim. Acta* **706** (2011) 246 (http://dx.doi.org/10.1016/j.aca.2011.08.040)
27. A. Afkhami, H. Khoshsafar, H. Bagheri, T. Madrakian, *Anal. Chim. Acta* **831** (2014) 50 (http://dx.doi.org/10.1016/j.aca.2014.04.061)
28. B. Deiminiat, G. H. Rounaghi, M. H. Arbab-Zavar, *Sensors Actuators, B* **238** (2017) 651 (http://dx.doi.org/10.1016/j.snb.2016.07.110)
29. E. Çidem, T. Teker, M. Aslanoglu, *Microchem. J.* **147** (2019) 879 (http://dx.doi.org/10.1016/j.microc.2019.04.018)
30. R. Kiran, E. Scorsone, P. Mailley, P. Bergonzo, *Anal. Chem.* **84** (2012) 10207 (http://dx.doi.org/10.1021/ac301177z)
31. F. Dönmez, Y. Yardım, Z. Şentürk, *Diam. Relat. Mater.* **84** (2018) 95 (http://dx.doi.org/10.1016/j.diamond.2018.03.013).

(CC) 2020 SCS.