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Voltammetric study of secnidazole and its determination in pharmaceutical tablet using 1, 4-benzoquinone modified carbon paste electrode

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ABSTRACT

In this study voltammetric behaviour of secnidazole (SCZ) at 1, 4-Benzoquinone Modified Carbon Paste Electrode (1,4-BQMCPE) was investigated in Britton Robinson buffer solution using cyclic voltammetric technique. A well-defined cathodic peak was observed for the SCZ in the entire pH range. The current increases steadily with scan rate and the results indicated that the process is irreversible reduction and adsorption controlled. The number of electrons transferred and different kinetic parameters like transfer coefficient and rate constant were calculated by using cyclic voltammetry technique. Differential pulse voltammetric method has been used for the determination of SCZ content in pharmaceutical tablet. This method enabled to determine SCZ in the concentration range 1.0×10^{-8} to 4.0×10^{-4} M. The limit of detection (LOD) and limit of quantification (LOO) were found to be 2.13×10^{-9} and 2.85×10^{-9} respectively. The method was applied to determine the content of SCZ in different sample solutions of SCZ tablet with excellent recovery and relative standard deviation results (99.892±1.53 respectively) for spiked standard SCZ in tablet sample solutions. The selectivity of the method for SCZ was further studied in the presence of selected potential interferents such as fluconazole, azithromycin etc and confirmed the potential applicability of the developed method for the determination of SCZ in real pharmaceutical tablets.

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Capsule Summary: SCZ undergoes adsorption controlled irreversible reduction reaction when it was investigated using cyclic voltammetric technique at 1,4 BQMCPE. Differential pulse voltammetric method showed excellent selectivity, precision and accuracy for the quantitative determination of SCZ in pharmaceutical formulations.

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INTRODUCTION

Secnidazole (SCZ), 1-(2-methyl-5-nitroimidazole-1-yl) propan-2-ol, is a nitroimidazole medicinal drug which is used in treatment of amoebiasis (Bohbot et al., 2010) and is

particularly effective in the treatment of giardiasis, trichomoniasis and bacterial vaginosis. It is an an active drug against trichomoniasis, amaebiasis and infectionwith anaerobic bacteria (Edwards, 1993; Jokipiiet al., 1985; Gillis and Wiseman, 1996; Tripathi, 2008; Bohbot et al., 2010).



Fig. 1: Cyclic voltammograms of (a) CPE in BRB solution containing no SCZ, (b) CPE in BRB solution containing 0.1mM SCZ and (c) 1,4-BQMCPE in BRB solution containing 0.1mM SCZ



Fig. 2: Cyclic voltammograms of 1,4-BQMCPE in BRB containing 0.1mM SCZ at various scan rates (a-k:10, 20, 40, 60, 80, 100, 120, 140, 160, 180 and; 200 mV/s, respectively).

Considering its huge importance, the SCZ has studied by different approaches like spectrophotometry (Khieret al., 2008; kumar et al., 2012; Sonpetkaret al., 2012; Youssef et al., 2015), HPLC (Baraka et al., 2014; Ali et al., 2016; Sharminet al., 2016) and electrochemical techniques (Radi andHassanein, 2000; El-Sayedet al., 2010; Elqudabyet al., 2013). But most of the conventional methods reported are sensitivity poor, require expensive instruments and a much longer detection time and not environmentally friendly.

Therefore, development of other better alternative method which is selective, sensitive, cheap and environmentally friendly is necessary. Recently, electrochemical techniques including cyclic voltammetry, differential pulse voltammetry etc are attracting attention of researchers throughout the world for the detection of pharmaceutical formulations. This is because they provide fast response time and high sensitivity for measuring analytes. Further; they require only compact instruments and straightforward operations (Harvey, 2000).

Electrodes modified by polymers have received special attention by researchers in recent years. This is because such electrodes have good stability, reproducibility, increase in active sites, homogeneity in electrochemical deposition and strong adherence to electrode surface (Radhi, 2016). Particularly those electrodes modified by benzoquinone had shown remarkable advantages from their low noise levels and higher sensitivity (Akliluet al., 2008).

In this work, SCZ was studied using 1,4-BQMCPE and yielded a well-defined and a very sensitive reduction peak. Compared with that at the bare CPE, the reduction peak increased remarkably. The electrode process was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) and influence of different experimental parameters was studied. Based on this, an electrochemical method which has advantages such as low detection limit, a rapid response, excellent reproducibility simplicity and low cost is proposed for direct determination of SCZ.

MATERIAL AND METHODS

Instruments and apparatus

CHI760D Electrochemical Workstation, (CHI Instruments, USA) was used for all electrochemical measurements. A bare CPE or 1,4-BQMCPE as a working electrode, saturated silversilver chloride (Ag/AgCl, KCl saturated) as a reference electrode and platinum wire as a counter electrode were employed for a conventional three-electrode system. The pH of the buffer solutions was measured with a Jenway model 3310 pH meter (Denver instrument). An electronic balance (Denver instrument) was used for measuring mass of different chemicals and samples. A magnetic stirrer with a hot plate form was used for stirring in pH adjustments. Other materials include; Spatula, filter paper, beaker, wash bottle, Mortar with pestle, volumetric flask of different sizes, micropipette (John Poulten Ltd) and Graduated cylinder.

Chemicals and reagents

Standard SCZ was purchased from Emmellen Biotech Pharmaceuticals Limited. Britton-Robinson buffer (BRB) solutions were prepared using Boric acid (BIO-lab laboratories LTD), phosphoric acid (Veeni chemicals) and glacial acetic acid(Blulux laboratories reagent) and adjusted to the desired pH with 1.0 M NaOH(Blulux laboratories reagent). 1, 4-Benzoquinone (Riedel-de Haën), graphite powder (BDH-Laboratory supplies Poole) and paraffin oil-



Fig. 3: Plot of variation of *I*^{*P*} versus (a) scan rate, (b) square root of scan rate



Fig. 4: Cyclic voltammograms of 0.1 mM SCZ in BRB solution of different pH values (a-g: 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 respectively). Scan rate: 100 mV s^{-1}



Fig. 5: Plot of reductive (a) peak current and (b) peak potential versus pH for 0.1 mM SCZ in 0.1 M BRB solution of different pH values at 1,4-BQMCPE . Scan rate: 100 mV/s

(Abron Chemicals) were used for the preparation of the working electrodes. All solutions were prepared from distilled water except the stoke solutions which were prepared from 50% of ethanol with distilled water.

Procedures

(a) Preparation of working electrodes

Unmodified carbon paste electrode (CPE) (100 mg) was prepared by mixing graphite powder with paraffin oil. The composition of the paste was 75 % (w/w) graphite powder and 25 % (w/w) paraffin oil. The mixture was homogenized with mortar and pestle for 30 minutes and allowed to rest for 24 hours. The homogenized paste was packed in to the tip of a plastic syringe (3 mm diameter, 7 mm deep). A copper wire was inserted from the backside of the syringe to provide electrical contact. Then the surface of the electrode was smoothed against a smooth white paper with a light manual pressure until a shiny surface is emerged.

Modified carbon paste (100 mg) was prepared by mixing graphite powder with 1,4- benzoquinone in paraffin oil. To 20 mg of 1,4-benzoquinone and 60 mg of carbon powder initially mixed with a mortar and pestle for 5 minutes, 23 μ L (20 mg) of paraffin oil were added and thoroughly mortared together for 30 minutes. The resulting paste was packed in to the tip of the syringe by extruding a small amount of paste from the tip of the previously prepared unmodified carbon paste electrode.

(b) Preparation of standard solutions

Supporting electrolyte of Britten Robinson buffer (BRB) solution) in the pH range 2-10 composed of equi-molar mixture of 0.1 M acetic acid, boric acid, and phosphoric acid were prepared. The pH of the solutions was adjusted using 1 M sodium hydroxide solution.

Stock solution of 0.01 molar standard SCZ was prepared by dissolving 0.185 gram of standard SCZ in 100 mL volumetric flask using 50% of ethanol with distilled water and stored under refrigeration. The required SCZ working solutions were prepared by diluting the stock solution with the BRB solution.

(c) Preparation of pharmaceutical tablet sample solutions

SCZ tablet (labeled as 500 mg per tablet) was collected from local pharmacy and stock solution of the tablet was prepared by accurately weighing 5 tablets and finely powdering in a porcelain mortar. 0.215 gram of this powder, corresponding to a stock solution of concentration 0.01 M, was weighed and transferred into a 100 mL flask and dissolved with 50% of ethanol solution.

Then, the tablet solution was filtrated using a Whatman® filter paper. Then, 25.0, 50.0, 75.0 and 100 μ M sample solutions were prepared from the stock solution using BRB solutions.



Scheme 1: The proposed reduction reaction mechanism of SCZ on 1,4-BQMCPE.

Furthermore, the recovery of the developed method was studied by comparing the experimental current response with the expected current for the sample solutions spiked with standard SCZ. For this purpose, the four solutions of SCZ tablet were spiked with 3 mg standard SCZ. All experiments were carried out at room temperature.

RESULTS AND DISCUSSION

Electrochemical investigation of SCZ at1, 4-BQMCPE

To study the electrochemical behavior of SCZ, cyclic voltammetric technique was used. As can be seen from figure 1, the voltammograms of SCZ in BRB solution using CPE and 1,4-BQMCPE showed the SCZ undergoes irreversible reduction reaction on both electrodes. But figure 1 showed there was an excellent improvement in the voltammograms when 1,4-BQMCPE was used. The enhancement in the peak current is due to the catalytic property of benzoquinone as indicated in different literatures (Abraham et al., 2011).

Effect of scan rate on peak current and peak potential

To study the effect of scan rate (ν) on peak current and peak potential, it was varied from 10 mV to 220 mV and the voltammograms in figure 2 were recorded. Therefore figure 2 showed shifting of peak potential to ward more negative potential value which is another confirmation for irreversibility of the reduction reaction of SCZ at 1,4-BQMCPE (Wang, 2006).

The dependence of peak current on scan rate and square root of scan rate was studied to investigate whether the reduction of SCZ at1,4-BQMCPE is diffusion controlled or surface confined process. Considering the value of the correlation coefficients of the plots in figure 2,the electrochemical reduction reaction of SCZ at1,4-BQMCPE is adsorption controlled process in the selected scan rate range (Wang, 2006).

Kinetics of the electrochemical reaction of SCZ at1,4-BQMCPE

The number of electrons transferred on the electrode process (n) during the reduction reaction of SCZ was calculated using the following equations (Wang, 2006; Bard and Faulkner, 2001).

$$I_{\rm p} = \frac{(\alpha n) n^2 F^2 A \Gamma v}{2.718 \text{RT}}$$
(1)

$$\left| \mathbf{E}_{\mathbf{p}} - \mathbf{E}_{\frac{\mathbf{p}}{2}} \right| = 1.85 \frac{\mathbf{RT}}{\alpha \mathbf{nF}} \mathbf{v} = \frac{0.048}{\alpha \mathbf{n}} \mathbf{v} \text{ at } 25 \ ^{\circ}\mathbf{C}$$
(2)
$$\Gamma = \frac{\mathbf{Q}}{\mathbf{nFA}}$$
(3)

Where, I_P is peak current, Γ is surface concentration of the electroactive species, α is the transfer coefficient, ν is scan rate, R is the universal gas constant, n is number of electrons transferred in the electrode reaction, A is the electrochemical active area, F is the Faraday constant, T is the Kelvin temperature, E_P is peak potential, $E_{P/2}$ is half peak potential and Q is charge consumed and was obtained from integral of peak area. (α n) was calculated from equation (2) to about 2.789 for a scan rate of 100mV. Using equation (3) and equation (1), n can be calculated as follow:

$$n = \frac{2.718I_{P}RT}{(\alpha n)FQv}$$
(4)

n was calculated to be 5.764 using equation (4) which indicated approximately six electrons were involved in the electrochemical reduction of SCZ on the 1,4-BQMCPE, which is comparable with previous reported work (El-Sayedet al., 2010). Therefore the value of α is calculated to be 0.484 for SCZ which is another confirmation for the irreversibility of the reduction of SCZ on 1,4-BQMCPE.

Ep was found to be linearly proportional to the logarithm of ν (ln ν) with a linear regression equation of $Ep(V) = -359.09 \text{mV} \cdot 20.37 \text{ ln}\nu$ (mVs⁻¹) and correlation coefficient of 0.926. Standard rate constant (K_o) for an irreversible reduction reaction is calculated by equation (5) (Laviron, 1979).

$$E_{P} = E^{o} + \frac{RT}{\alpha \ n \ F} \ln\left(\frac{RTK_{o}}{\alpha \ nF}\right) - \frac{RT}{\alpha nF} \ln \ \nu$$
(5)



Fig. 6: Plot of differential pulse voltammetric peak current response of 1,4-BQMCPE for 0.1 mM SCZ versus (a) accumulation potential, (b) accumulation time at E_{acc} = -250 mV.



Fig. 7: the effect of amount of 1,4-benzo quinone(modifier) on the reduction peak current 0.1 mM SCZ. Amplitude = 40 mV scane rate = 35 mV/s.



Fig. 8: DPV of 1,4-BQMCPE (corrected for background) in pH 7 BRB solution of different concentrations of SCZ (0.01, 0.1, 10, 25, 75, 125, 250, 300 and 400 μ M, respectively). Inset: Calibration curve of peak current (I_P) versus concentration (C) of SCZ in BRB solution on 1,4-BQMCPE.

Where, E^o is the formal potential and was found equal to -415.74 mV determined from the intercept of the plot of E_P versus v. The value of K_o calculated from the intercept of the plot of Ep versus ln v as the equation below was calculated to be 5.1356 × 10⁴ s⁻¹.

$$E^{\circ} + \frac{RT}{\alpha n F} \left[ln \left(\frac{RTK_{\circ}}{\alpha nF} \right) \right] = -359.09 mV$$

Effect of solution pH on peak current and peak potential

pH of the solution was varied from 2.0 to 10.0 to investigate its effect on peak current and peak potential. Cyclic voltammograms recorded for different values of pH in figure 4 showed no anodic peak in the entire pH range was observed. As expected, as can be seen from figure 5(a), peak current was pH dependent and a maximum peak current was recorded at pH solution of equal to 7.0. Therefore this pH value was selected as the optimum pH for the subsequent experiments which is in agreement with a previous report (Elqudabyet al., 2013).

Peak potential of SCZ is strongly pH dependent in the acidic and neutral media. As pH solution increase, peak potential shifted towards more negative values up to pH 7.0 and then remain unchanged from pH 8.0-10.0 (voltammograms of pH 9.0 and 10.0 not included in figure 4).

Shifting of peak potential to more negative value is an indication that protons take part at the electrode reaction in acidic and neutral media.

To estimate the number of protons take part in the electrochemical reduction reaction of SCZ on 1,4-BQMCPE, solution pH was plotted against its respective peak potential and figure 5(b) was obtained with a linear equation and correlation coefficient of Ep(V) = -0.2804 - 0.0622pH and R² = 0.9478, respectively. A slope of 0.0622 V/pH suggests that the number of protons taking part in the electrode reaction is similar to the number of electrons. Hence, the reduction of SCZ involves six electrons and six protons (Scheme 1) as suggested by (El-Sayedet al., 2010):

Optimization of differential pulse voltammetric (DPV) parameters

Since DPV has a much higher current sensitivity and better resolution than cyclic voltammetry, the application of 1,4-BQMCPE for the quantitative analysis of SCZ has been investigated using DPV. Therefore the effects of different DPV parameters on the current response of 1,4-BQMCPE in 0.1 mM SCZ were studied.

DPV pulse amplitude and scan rate

The influence of differential pulse voltammetry parameters was investigated. First, the amplitude was varied in the range of 10-60 mV, fixing the scan rate at 50 mV/s and peak current increased with increasing amplitude in this range. However, the slope changes to a lower value after amplitude of 40 mV.

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Table 1: Amount of SCZ detected in two brands of tablets using the developed method

Sample	Expected (µM)	Detected *		Labeled	value	Measured %
Solution		In µM	mg/tablet	mg/tablet (mg/tablet)		
а	25.0	24.403	488.060	500		97.612
b	50.0	47.976	479.760	500		95.952
С	75.0	72.689	484.593	500		96.919
d	100	97.158	485.790	500		97.158

* Mean of triplicate measurements

Table 2: Percentage recovery of SCZ from pharmaceutical tablets

Sample Solution	Present (mg)	SCZ Added (mg)	SCZ	Expected (mg)	SCZ	Found (mg) *	Recovery (%)±%RSD
а	0.113	3		3.113		3.105 ± 0.003	99.743±1.117
b	0.222	3		3.222		3.218±0.001	99.876±1.714
С	0.337	3		3.337		3.337±0.006	100±1.202
d	0.450	3		3.450		3.448±0.006	99.942±2.106

* mean of double measurements.

Table 3: Interference study of SCZ with different selected potential interferents

	Concentration (in µM)	Recorded signal (Ι _P /μΑ)	Signal Change (%)
SCZ	100	2.217	
Interferents added			
microcrystalline cellulose	50	2.203	0.631
wheat starch	50	2.199	0.182
Calcium hydrogen phosphate	50	2.181	1.624
Sodium starch glycolate	50	2.157	2.706
Fluconazole	50	2.191	1.173
Azithromycin	50	2.203	0.631
Tinidazole	50	2.513	13.351
Metronidazole	50	2.462	11.051

Table 4: Comparison between the developed method and other reported methods.

Electrode	Method	Linear range (M)	Detection limit	Recovery	References
			(M)	(%± R)	
HMDE	ASV	$1 \times 10^{-8} - 1 \times 10^{-7}$	5 × 10 ⁻⁹	98.5±2.5	Radi and Hassanein (2000)
GCE	ASV	4 ×10 ⁻⁶ - 1.2 ×10 ⁻⁴	1.2 ×10 ⁻⁶	100.91±1.82	El-Sayedet al. (2010)
GCE	DPV	2.7 ×10 ⁻⁴ - 4.1×10 ⁻³	2 ×10 ⁻¹⁰	98.5±4.7	Elqudabyet al. (2013)
1,4-BQMCPE	DPV	1.0×10 ⁻⁸ - 4.0×10 ⁻⁴	2.13 ×10 ⁻⁹	99.892±1.53	This work

This indicates that, the dependence of the peak current on amplitude is being distorted due to an accompanying peak broadening. Hence, 40mV was chosen as the square-wave amplitude. Upon increasing scan rate from 10-60 mV/s, a

linear increase in the peak current was observed accompanied by peak broadening in particular when the scan rate was greater than 35mV/s. Thus, 35 mV/s of scan rate was chosen as an optimum value.

The effect of adsorption character

Since the reduction of SCZ at 1,4-BQMCPE is governed predominantly by surface confined kinetics, the effects of accumulation time and accumulation potential were studied, by varying one of them and maintaining the other constant.

Accumulation potential (Eacc): *Eacc* was varied in the range of -50 to -400 mV to study its effect on the peak current of 0.1 mM SCZ. Initially the peak current increased when the accumulation potential was increased and reaches maximum at -250 mV as shown in figure 6(a). Therefore -250 mV was chosen as the optimum *Eacc* for further work.

Accumulation time (tacc): At a potential of -250 mV, the effect of *tacc* on the peak current of 0.1 mM SCZ was also investigated in the range of 5 and 50 s. the result showed that the peak current increased with the increase in accumulation time up to 35 s and then almost leveled off (figure 6(b)). Therefore 35s was selected as an optimum *tacc* for further experiments. Increasing the peak current with increase in accumulation time is another indication for the accumulation of SCZ at the surface of the 1,4-BQMCPE

Effect of modifier composition

The effect of 1,4-benzoquinone on the peak current was studied by varying its content on CPE from 0% to 30 % (w/w). As can be seen from figure 7, peak current of 0.1 mM SCZ increased with increasing 1,4-benzoquinone composition from 0% to to 20 % (w/w). Therefore, 20 % (w/w) was taken as the optimum modifier composition throughout this work.

Calibration Plot for SCZ

Using the optimal DPV parameters described, the calibration curve for the determination of SCZ concentration was established in pH 5.0 BRB at 1,4-BQMCPE. As can be seen from figure 8 and its inset, the reduction peak current was found to increase proportionally with increasing concentration of SCZ in the range from 1.0×10^{-8} to 4.0×10^{-4} M.

The calibration curve for nine average data points (n = 9) was found to be linear with R = 0.994 and a regression equation of Ip (μ A)= 0.227 + 0.019C (μ M). LOD and LOQ calculated using the equations below were found to be 2.13 ×10⁻⁹ and 2.85 ×10⁻⁹ respectively.

$$LOD = \frac{3S}{m}$$
(6)

$$LOQ = \frac{10S}{m}$$
(7)

Where, *S* is the standard deviation for the blank (n = 9) and m is the slope of the calibration curve.

Determination of SCZ in pharmaceutical tablets

The applicability of the developed method for the determination of SCZ in real sample was investigated. The

developed modified electrode was used for the determination of SCZ content in pharmaceutical tablet sample solutions.

Four different tablet sample solutions with concentration of 25, 50, 75 and 100 μ M were prepared from the stock solution as indicated in section 2.3(c). Finally differential pulse voltammograms sample solutions were recorded. The determination of SCZ in these samples was carried out according to the linear regression equation formulated for the calibration curve and the results are summarized in table 1. As can be seen from the table, the results for the tablet sample solutions are in good agreement with the marked content (500 mg per tablet).

Validation parameters for the quantitative analysis

Validation of the procedure for the quantitative determination of SCZ was examined by the DPV technique viaevaluation of the repeatability, reproducibility, stability, accuracy, precision and inference of the results obtained using 1,4-BQMCPE.

Reproducibility, Repeatability and Stability of 1,4-BQMCPE

To study the reproducibility of the electrode three 1, 4-BQMCPEs were prepared independently and triplicate measurements for 0.1 mM SCZ were taken using the electrodes. The relative standard deviation of the measurements was found to be 1.03% showing excellent reproducibility of the method.

Repeatability of 1,4-BQMCPE was also investigated by making eight repetitive determinations of 0.1 mM SCZ. The relative standard deviation (RSD) for the successive measurements was 2.54 which revealed an excellent repeatability. The 1,4-BQMCPE showed high stability. As it is shown during the experiment, there has been no significant difference in the peak current responses for the same electrode over a period of two months.

Recovery analyses

To confirm accuracy and precision of the developed method for the determination of SCZ additional recovery experiments were conducted. The recovery procedures were carried out by spiking the already analyzed samples of tablet solutions (section 3.4) with a known amount of standard SCZ. These results are shown in table 2. According to the obtained results, it can be concluded that the proposed method is sufficiently accurate and precise to be applied for SCZ analyses in real samples. High-percentage recovery data show that the proposed method is free from the interferences of the common excipients used in the tablet formulations. The average recovery and relative standard deviation (R.S.D) recorded for a duplicate measurement were 99.892±1.53 respectively showing the results obtained using the proposed method are almost comparable or even better than the results obtained by a method which used a very expensive electrodes (Radi and Hassanein, 2000; El-Sayedet al., 2010; Elqudabyet al., 2013).

Interference analyses of the method

To study selectivity of the method for SCZ different potential interferents were taken. The selected interferents were drugs which could be prepared mixed together with SCZ tablet (fluconazole and azithromycin), drugs which have structural similarities with SCZ (metronidazole and tinidazole) and excipients of SCZ tablet.

A 50 μ M solution of each interferent was added to 100 μ M concentration of secnidazole solution and a voltammogram was recorded. Table 3 indicated that most of the selected interferents did not significantly affect the peak current response for the SCZ which is comparable with a result reported somewhere (Elqudabyet al., 2013; Youssef et al., 2015). But metronidazole and tinidazole showed positive interference on the peak current for SCZ and this is because both metronidazole (Nikodimos and Amare, 2016) and tinidazole (Taye and Amare, 2016; Nikodimos and Hagos, 2017) contain the same reductive groups that can be reduced near the potentials of SCZ.

Comparison of the proposed method with previously reported methods

The detection performances of 1,4-BQMCPE was compared with other electrodes. Table 4 presents summary of performance of the developed method against reported works on electrochemical determination of SCZ. As can clearly be seen from the table, the present approach which uses relatively cheap and easily available electrode showed a comparable limit of detection, linear range and recovery with the methods used expensive electrodes like HMDE and GCE.

CONCLUSIONS

The developed electrochemical technique is simpler, faster, and requires less expensive equipment for the determination of secnidazole using an environmentally friendly electrodemodifier. The results obtained allow us to conclude that the proposed method can be used with some advantages for the selective and quantitative determination of secnidazole. As the proposed method has lower limits of detection and quantification, compared to those works which have used very expensive electrodes, it could be used to determine SCZ even in complex matrix systems with satisfactory results.

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