Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 1311–1318

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

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Direct determination of praziquantel in pharmaceutical formulations and human plasma by cathodic adsorptive stripping differential-pulse voltammetry

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Received 22 March 2002; received in revised form 19 July 2002; accepted 30 July 2002

Abstract

The polarographic and cyclic voltammetric behaviour of praziquantel was studied in B.R. buffers of different pH values. Contradictory to that mentioned in a previously published work, praziquantel is an electro-active compound. Its polarogram exhibited a single 2-electron irreversible reduction wave in B.R. buffer of pH 5, the wave height decreased on the increase of pH till it disappeared in solution of pH >7. This wave was attributed to the reduction of the C=O double bond. The quantitative trace determination of bulk praziquantel was studied at a hanging mercury drop electrode by cathodic adsorptive stripping differential-pulse voltammetry. A fully validated sensitive procedure based on controlled adsorptive accumulation of the drug onto a HMDE was developed for its direct determination without derivatization. Accumulation of praziquantel was found to be optimized in 0.1 M Na_2SO_4 solution as supporting electrolyte under the following conditions: accumulation potential, -1.2 V (vs. Ag/AgCl/KCl_s); accumulation time, 30 s; scan rate, 10 mV/s and pulse height 100 mV. The proposed procedure was applied successfully for determination of praziquantel in its pharmaceutical formulations and human plasma. The mean recoveries of the drug were 98.85–99.42% and 99.12–100.47% with RSD of 0.49–0.95% and 0.45–0.52% in pharmaceutical formulations and human plasma, respectively. Limits of detection and quantitation of 1.14×10^{-9} and 3.80×10^{-9} M praziquantel, respectively, were achieved.
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Keywords: Praziquantel; Polarography; Adsorptive stripping differential-pulse voltammetry; Determination in pharmaceutical forms and human plasma

1. Introduction

Praziquantel (I), a pyrazinoisoquinoline derivative: (2-(cyclohexyl-carbonyl)-1, 2, 3, 6, 7, 11b-hexahydro-4H-pyrazino [2,1-a] isoquinoline-4-one) is a synthetic heterocyclic anthelmintic agent. The drug has activity against many cestodes [1].

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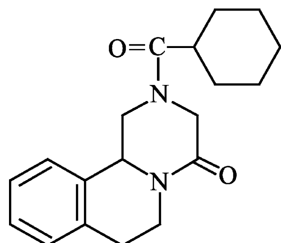
PII: S0731-7085(02)00457-0

Praziquantel is used for the treatment of schistosomiasis (bilharziasis) caused by all Schistosoma species pathogenic to humans. Praziquantel is effectively against all stages of Schistosoma infection including the acute phase and the chronic phase, which may be associated with hepatosplenic involvement. It is also used for the treatment of clonorchiasis caused by Clonorchis sinensis (Chinese liver fluke) and opisthorchiasis caused by Opisthorchis viverrini (liver fluke) [1].



I

Praziquantel is well absorbed following oral administration. Approximately 80% of an oral dose of the drug is adsorbed from the GI tract; however, because of extensive first-pass metabolism, only a small portion reaches systemic circulation as unchanged praziquantel. Peak serum concentrations of praziquantel occur approximately 1–3 h after oral administration of usual doses of the drug. In studies in rats, concentrations of free praziquantel in CFS were similar to those in serum. The concentration of the drug in CSF is reported to be 14–20% of the concurrent total plasma concentration. The drug is rapidly and extensively metabolized, principally in the liver via hydroxylation to monohydroxylated and polyhydroxylated metabolites. The drug and its metabolites are excreted mainly in urine. Following a single oral dose the drug, approximately 70–80% of the dose is excreted in urine within 24 h, principally as metabolites, less than 0.1% of an oral dose is excreted in urine unchanged [1].

Several procedures have been developed for the quantitative determination of praziquantel in tablets and biological fluids. These procedures include HPLC [2–10], GLC [11], TLC [12], spectrophotometry [13], PMR spectrometry [14], colorimetry [15,16], capillary electrophoresis [17] and ELISA [18]. It has been stated in a previously published work by Radi and Hassanein [19] that praziquantel is electro-inactive at the mercury electrode as evidenced by the absence of cathodic or anodic peak in the available potential range, that in our opinion is doubtful, whereas the nitrated-praziquantel exhibited a differential-pulse voltammetric peak due to the reduction of nitro group. The peak current was proportional to the nitrated drug concentration. Accordingly, the drug was indirectly determined in pharmaceutical formulations by cathodic adsorptive stripping differential-pulse voltammetry [19].

The present work aimed to reconsider the electrochemical behaviour of the drug in B.R. buffers at the dropping mercury electrode (DME). It is also aimed here to optimize an electrochemical procedure for the direct determination of the drug (without derivatisation) in pharmaceutical formulations and human plasma using cathodic adsorptive stripping differential-pulse voltammetry technique.

2. Experimental

2.1. Materials

Bulk praziquantel was obtained from Squibb Company Cairo, Egypt. Biltricide tablets (Bayer Co.) and Bilharzide tablets (Pharco Co.) were purchased from the local market of Egypt. Human plasma samples were obtained from healthy volunteers and stored frozen until assay.

2.2. Reagents and solutions

Stock solutions of concentrations 10^{-6}–10^{-4} M praziquantel were prepared in ethanol (Merck) and stored in dark bottles at 4 °C. The praziquantel solution is stable and its concentration does not change with time. A series of B.R. buffers of pH 2–11 was prepared according to Britton [20]. 0.2 M solutions of sodium sulphate, sodium nitrate, sodium perchlorate and potassium chloride as supporting electrolytes were also prepared by dissolving an accurate weight of the solid material (Analytical grade) in a specific volume of deionized water.

2.3. Spiked human plasma samples

Human plasma spiked with praziquantel was obtained by diluting aliquots of a stock standard praziquantel solution with human plasma. A 100 μl aliquot of this spiked solution was diluted to 1.0 ml with ethanol in a 10-ml centrifuge tube. The precipitated protein were separated by centrifugation for 5 min at 5000 r.p.m. The clear supernatant layer was filtrated through 0.45-μm milli-pore filter to produce protein free—spiked human plasma.

2.4. Tablets solution

Five tablets of each pharmaceutical formulation (Biltricide and Bilharzide), were weighed and finely grounded in Agate Mortar. A quantity of the finely grounded tablets equivalent to 100 mg of praziquantel was extracted with 20-ml successive portions of ethanol. The alcoholic extract was filtrated through 0.45-μm milli-pore filter (Gelman, Germany) into 100-ml volumetric flask and completed with ethanol. Aliquots of this solution were diluted with ethanol to produce 1×10^{-4}–1×10^{-6} M praziquantel, which were used as assay solutions.

2.5. Instrumentation

Polarograph Model 4001 (Sargent-Welch) was used for study the polarographic behaviour of praziquantel. The polarographic cell contains a DME as a working electrode ($m = 1.03$ mg/s, $t = 3.3$ s at mercury height = 60 cm) and a saturated calomel electrode (SCE) as a reference electrode.

The differential-pulse voltammograms were recorded using a Potentiostat/Galvanostat Model 263A—PAR or 394-PAR interfaced with PC-computer loaded with the software package 270/250 (PAR). The electrode assembly 303A (PAR) with a dark micro-electrolysis cell of a stationary mercury drop electrode as working electrode, an Ag/AgCl/KCl_s as a reference electrode and a platinum wire as a counter electrode was used.

The potentiostat/Galvanostat Model 173 incorporated with digital coulometer Model 179 (PAR) was used for the controlled-potential coulometric measurements. A dark coulometric cell incorporated with a Pt wire sealed through the cell bottom for contact with the mercury pool as a working electrode, a SCE as a reference electrode and a platinum gause immersed in a bridge tube as a counter electrode were used. The potential of the working electrode was maintained constant with respect to that of the reference electrode (potentiostatic control). The potential selected was adjusted to be equal the $E_{1/2}$ of the reactant polarographic wave plus 0.1 V or at the beginning of the limiting current of the wave (i.e. at the plateau). The total charge passed during the exhaustive electrolysis was obtained by integrating the current electronically. The charge due to the residual current was subtracted from the total measured charge in order to obtain the faradic charge for the electrode reaction of interest. Faraday's law relates the measured net charge Q (Coulombs) transferred to the amount of material electrolyzed as: $N = Q/nF$, where N is the number of moles of substance being electrolyzed, n is the number of electrons transferred per reactant molecule, and F is Faraday's constant (96 485 C/g eq.). In the present study, n was found to equal 2 per drug molecule in B.R. buffer of pH 5, that may be attributed to reduction of the C=O double bond.

Mettler balance (Toledo-AB104) was used for weighing the solid materials. Eppendorf centrifuge 5417C was used for separation of the precipitated proteins from the human plasma samples before the drug assay. Digital micropippetter (Volac) was used for the solutions transfer during the present electrochemical measurements. Deionized water was obtained from AquaMatic bidistillation system connected to a Purite Still Plus HP deionizer system.

2.6. Procedures

(i) For polarographic measurements, a known volume of praziquantel solution was pipetted into a 10 ml volume calibrating flask and then completed with Britton–Robinson buffer. The solution was transferred into the electrolysis cell and deoxygenated with pure nitrogen for 10 min, then the polarograms were recorded.

(ii) For stripping voltammetry measurements, a known volume of the analyte (reactant) solution was pipetted into 5 ml of 0.2 M of the supporting electrolyte solution and made up to 10 ml with deionized water, i.e. 0.1 M supporting electrolyte was achieved. The solution was introduced into the dark electrolysis cell, then deoxygenated with pure nitrogen for 10 min. The accumulation potential was applied to the working electrode for a selected time while the solution was stirred. At the end of the accumulation period the stirrer was stopped and 15 s was allowed for the solution to become quiescent. Then voltammograms were recorded by scanning the potential towards the negative direction using the differential-pulse waveform, the peak height being measured at −1.57 V. All data were obtained at room temperature.

3. Results and discussion

3.1. DC-polarography

The polarographic behaviour of praziquantel was studied in Britton–Robinson buffers of pH 2–10. Contradictory to the work published before by Radi and Hassanein [19], praziquantel was found to be electro-active at the mercury electrode, since its dc-polarogram exhibited a single 2-electron irreversible reduction wave in B.R. buffers of pH 5–7 (Fig. 1). The limiting current decreased on the increase of pH of the medium till it disappeared at pH > 7.0, that indicated a surface reaction [21]. The half-wave potential of the recorded wave was pH-independent. So, according to Zuman [22], the form predominated in the bulk of solution was transported to the electrode surface and was electroactive. A polarographic maximum was observed at the rising part of the reduction wave and covered most of the limiting current region. Its suppression with a lowest amount of Triton X-100 lead to a considerable decrease of the limiting current. The praziquantel reduction wave corresponds to the consumption of two electrons as confirmed by controlled potential coulometry experiment. This wave may be attributed to the reduction of the C=O double bond present in reactant molecule.

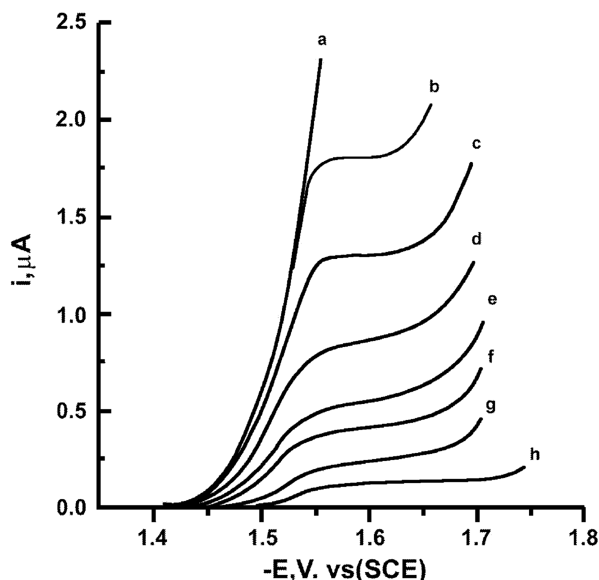


Fig. 1. DC-polarograms for 2×10^{-4} M praziquantel in B.R. buffers of different pH values: (a) 4.1; (b) 5.0; (c) 5.2; (d) 5.6; (e) 6; (f) 6.2; (g) 6.5; and (h) 7.

Logarithmic analysis [23] of the polarographic wave of praziquantel was performed at different pH values (5–6) by plotting $E_{d.e}$ vs. log $(i/i_d - i)$. The plots were straight lines with slope values of 50–55 mV, (slope = $0.0591/\alpha n_a$) from which αn_a values (1.08–1.18) were evaluated at different pH values. The αn_a values indicated that the number of electrons (n_a) involved in the rate-determining step should equal 2, and consequently values of the symmetry coefficient α at different pH values varied between 0.53 and 0.59, those confirmed the irreversible nature of praziquantel reduction process.

The limiting current (i_l) in B.R. buffer of pH 5 was proportional to the drug concentration. The plot of i_l (μA) versus concentration C(M) of the drug gave a straight-line that followed the equation: i_l (μA) = 4.906×10^3C + 0.26, $r = 0.996$ ($n = 10$). A limit of detection (LOD) of 3.1×10^{-6} M praziquantel was achieved using dc-polarography.

According to Zuman [22] for the electrode reactions of pH-independent-half-wave potential, no proton-transfer precedes the activation-energy-governed step, so the general sequence involved in such electrode reactions must be electron, electron,

proton, proton (e^-, e^-, H^+, H^+), which is the case for the present praziquantel electrode reaction.

3.2. Adsorptive stripping voltammetry

The cathodic adsorptive stripping differential-pulse (CAdSDP) voltammetric peak of praziquantel at mercury electrode was studied in different supporting electrolytes (such as 0.1 M sodium sulphate, 0.1 M potassium chloride, 0.1 M sodium nitrate, 0.1 M sodium perchlorate, and B.R. buffer of pH 2–10). A well-developed peak due to the reduction of C=O double bond was observed in 0.1 M sodium sulphate solution as supporting electrolyte (Fig. 2).

The effect of accumulation time (t_{acc}) on the peak current of the drug was studied. At $t_{acc} = 30$ s, an equilibrium surface concentration was occurred and the peak height reached the maximum response. Accordingly, a pre-concentration time of 30 s was adopted in the present study for the stripping analysis of praziquantel (Fig. 3).

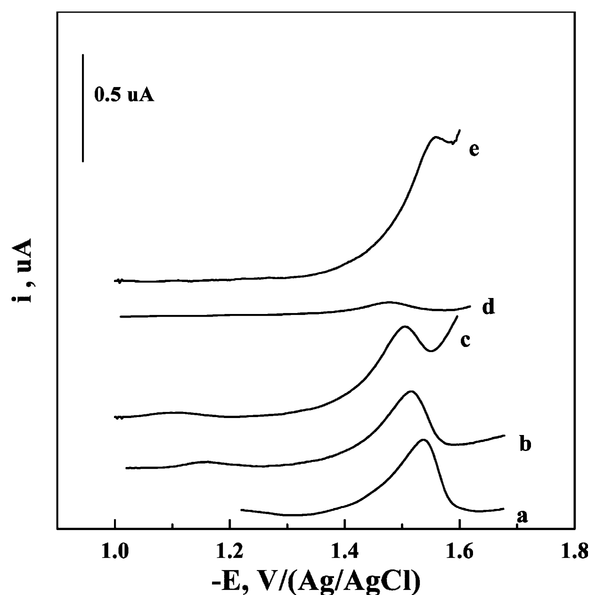


Fig. 2. CAdSDP voltammograms for 7×10^{-6} M praziquantel in different supporting electrolytes. (a) 0.1 M Na_2SO_4; (b) $NaClO_4$; (c) 0.1 M $NaNO_3$; (d) 0.1 M KCl; and (e) B.R. buffer of pH 5; $E_{acc} = -1.2$ V, $t_{acc} = 40$ s, scan rate = 10 mV/s, and pulse height = 100 mV.

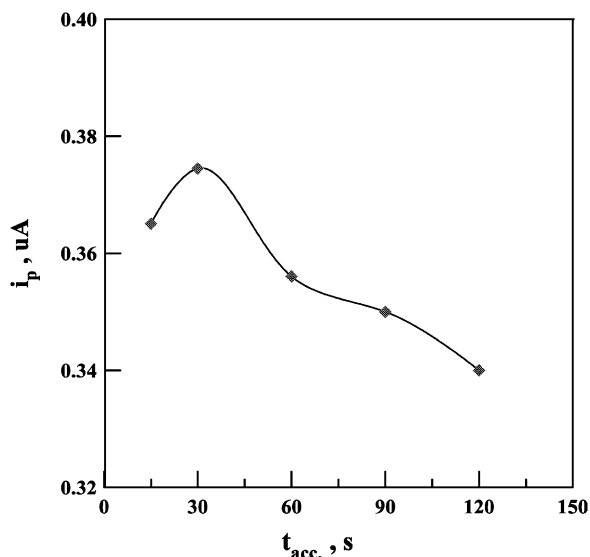


Fig. 3. Effect of accumulation time (t_{acc}) on the CAdSDP peak current for 7×10^{-6} M praziquantel in 0.1 M Na_2SO_4 solution; $E_{acc} = -1.2$ V, scan rate = 10 mV/s, and pulse height = 100 mV.

The effect of the accumulation potential ($E_{acc.}$) on the peak current of praziquantel was followed in 0.1 M Na_2SO_4 solution between −1.05 and −1.30 V (Fig. 4). The peak height almost decreased

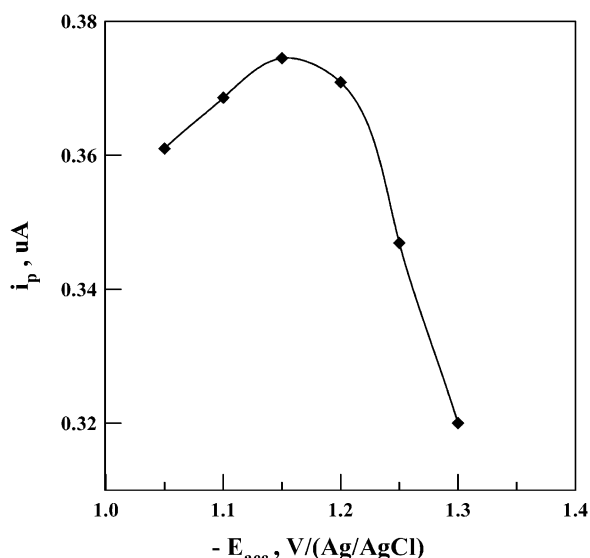


Fig. 4. Effect of accumulation potential on the CAdSDP peak current for 7×10^{-6} M praziquantel in 0.1 M Na_2SO_4 solution; $t_{acc} = 30$ s, scan rate = 10 mV/s, and pulse height = 100 mV.

with the shift of potential to more negative or to less negative value than −1.15 V. At lower or higher scan rate than 10 mV/s the peak current decreased (Fig. 5). The peak current for the adsorbed praziquantel was directly proportional to the pulse height.

Accordingly, the optimum conditions chosen for the present CAdSDP voltammetry determination of praziquantel were: accumulation potential (E_{acc}); −1.15 V (vs. Ag/AgCl/KCl_s), accumulation time (t_{acc}); 30 s, scan rate; 10 mV/s and pulse height; 100 mV in 0.1 M sodium sulphate solution as supporting electrolyte.

The applicability of the optimized cathodic adsorptive stripping differential-pulse voltammetry as an analytical procedure for the determination of praziquantel was tested as a function of i_p versus potential E at different concentration of the reactant. A calibration curve was constructed over the concentration range 2×10^{-7}–2×10^{-5} M praziquantel. The variation of peak current i_p (μA) with praziquantel concentration C (M) was represented by a straight-line equation: i_p (μA) $= 4.77485\times10^5$ C $+ 0.1064$, $r = 0.998$ ($n = 15$), where r is the correlation coefficient and n is the number of points.

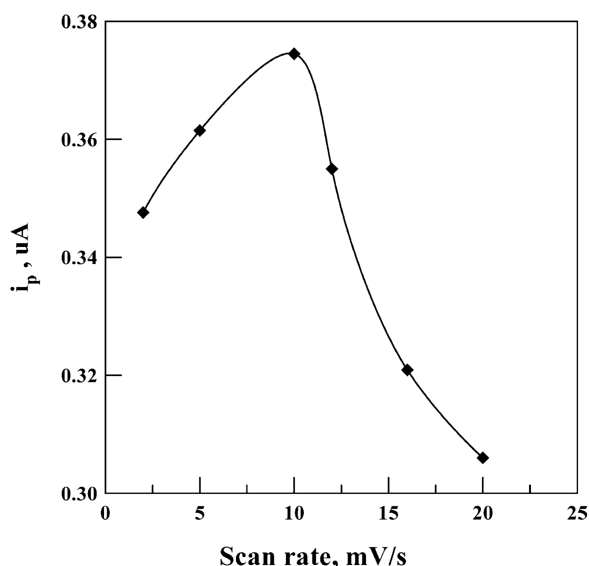


Fig. 5. Effect of scan rate on the CAdSDP peak current for 7×10^{-6} M praziquantel in 0.1 M Na_2SO_4 solution; E_{acc} = −1.15 V, t_{acc} = 30 s, and pulse height = 100.

3.2.1. Validation of the procedure

Validation of the procedure for the quantitative assay of the drug was examined via evaluation of the limit of detection (LOD), limit of quantitation (LOQ), repeatability, recovery, specificity, robustness and ruggedness. The LOD and LOQ were calculated from the calibration curves as $k\,SD_a/b$ [24] where $k = 3$ for LOD and 10 for LOQ, $S.D_a$ is the standard deviation of the intercept and b is the slope of the calibration curve. The values of LOD and LOQ were 1.14×10^{-9} and 3.80×10^{-9} M, respectively.

Repeatability and recovery were examined by performing five replicate measurements for the concentration of 2×10^{-7} and 8×10^{-7} M praziquantel after 30 s pre-concentration. The mean recoveries of $99.36\pm1.22\%$ and $99.38\pm0.57\%$ were achieved, respectively, that indicated high precision of the proposed procedure.

Specificity of the optimized procedure for assay of praziquantel was examined in presence of some common excipients in the same ratios usually used in pharmaceutical preparations (starch, gelatin, lactose, talc and magnesium stearate). The mean percentage recovery of 3×10^{-7} M praziquantel (98.55 ± 0.64) showed no significant excipients interference, thus the procedure was able to assay praziquantel in the presence of excipients and hence it can be considered specific.

Robustness [25] was examined by evaluating the influence of small variation of some of the most important procedure variables including pH, pre-concentration potential (E_{acc}) and pre-concentration time (t_{acc}). The results showed that none of these variables significantly affect the recovery of praziquantel. This provided an indication of the reliability of the proposed procedure for assay of the drug and it could be considered robust [25].

On the other hand, ruggedness [25] was examined by applying the developed procedure to assay the drug using two potentiostats (PAR-263A and 394) under the same optimized experimental conditions at different elapsed time. The results obtained due to lab-to-lab and even day-to-day variations were found reproducible since there was no significant difference between the recovery results.

Table 1
Assay of praziquantel in its pharmaceutical formulations using the proposed CAdSDPV and reported [3] methods

Brand name (producer)	Labeled concentration (drug)	% Recovery ± % RSD	
		Proposed CAdSDPV method	Reported HPLC method
Biltricide (Bayer)	600 mg/tablet	99.42 ± 0.491	100.46 ± 0.673
Bilharzide (Pharco)	600 mg/tablet	98.85 ± 0.954	101.32 ± 0.853

3.3. Applications

3.3.1. Assay of praziquantel in tablets:

The proposed procedure was successfully applied for the assay of praziquantel in its tablets (Biltricide and Bilharzide). The mean recoveries based on five replicated measurements were 98.85–99.42% with RSD of 0.49–0.95%. The results were favorably compared with those obtained for assay of the same tablet solution with HPLC method [3], Table 1.

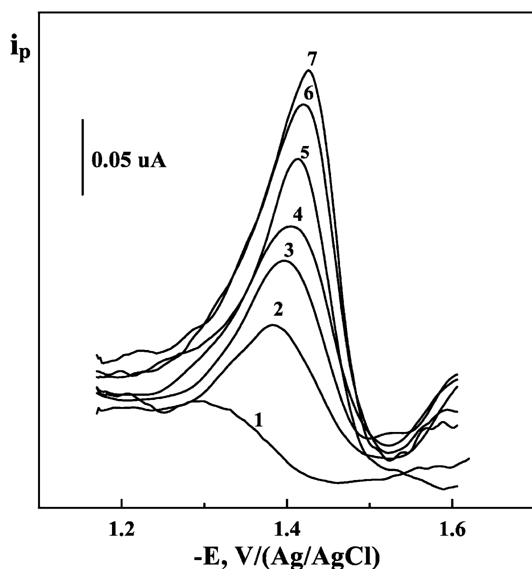


Fig. 6. CAdSDP voltammograms for different concentration of praziquantel spiked in human plasma: (1) Background; (2) 2×10^{-7}; (3) 4×10^{-7}; (4) 1×10^{-6}; (5) 2×10^{-6}; (6) 4×10^{-6}; (7) 5×10^{-6} M; in 0.1 M Na_2SO_4 solution, at $t_{acc} = 30$ s, $E_{acc} = -1.15$ V, scan rate = 10 mV/s and pulse height = 100 mV.

3.3.2. Assay of praziquantel in spiked human plasma

The optimized differential-pulse cathodic adsorptive stripping voltammetry procedure was also successfully used for the determination of praziquantel in protein-free spiked human plasma (Fig. 6). The variation of i_p(μA) vs. the praziquantel concentration C (M) was represented by a straight-line equation i_p (μA) = $5.66103 \times 10^5 C + 0.045$, $r = 0.997$ ($n = 15$). The calibration graph was rectilinear within the concentration range 1×10^{-7}–2×10^{-5} M. The reliability of the proposed procedure for the determination of praziquantel in human plasma was checked using different spiked human plasma samples. The mean recoveries of praziquantel based on four replicate measurements were found to be 99.12–100.47% with RSD of 0.45–0.52%. The LOD and LOQ for praziquantel spiked in human plasma were found 1.93×10^{-9} and 6.43×10^{-9} M, respectively.

4. Conclusion

Contradictory to the previously published work by Radi and Hassanein [19], praziquantel was found to be an electro-active compound, since it is reduced at the mercury electrode via a single 2-electron irreversible wave. The drug could be determined directly (without derivatization) in pharmaceutical formulations and human plasma with a good success using cathodic adsorptive stripping differential-pulse voltammetry. The optimized procedure for the direct determination of praziquantel offers a fast response, sensitive, low cost and easy analysis tool for clinical laboratories.

Acknowledgements

The authors express their gratitude to Alexander von Humboldt Foundation for the donation of the complete set of Potentiostat/Galvanostat Model 263A-PAR and the Eppendorf centrifuge.

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