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Adsorptive cathodic stripping voltammetric assay of the estrogen drug ethinylestradiol in pharmaceutical formulation and human plasma at a mercury electrode

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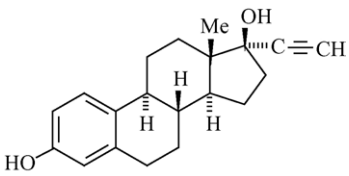
Abstract

The electroreduction of ethinylestradiol at the hanging mercury drop electrode in the Britton–Robinson universal buffer of pH 2–11 was studied and its interfacial adsorptive character onto the mercury electrode surface was identified. A validated simple, rapid, sensitive, specific, precise and inexpensive square-wave voltammetric procedure is described for the determination of ethinylestradiol following its accumulation onto a hanging mercury drop electrode in a Britton–Robinson universal buffer of pH 7. The optimal procedural conditions were: accumulation potential $E_{acc} = -0.7$ V versus Ag/AgCl/KCl$_s$, accumulation duration = 60 s, pulse-amplitude = 70 mV, scan increment = 10 mV and frequency = 120 Hz. Limits of detection (LOD) and quantification (LOQ) of 5.9×10^{-10} M and 1.9×10^{-9} M bulk ethinylestradiol, respectively, were achieved. The proposed procedure was successfully applied to the quantification of ethinylestradiol in pharmaceutical formulation (Ethinyl-oestradiol® tablets) and in human serum and plasma without the necessity for sample pretreatments and/or time-consuming extraction or evaporation steps prior to the analysis. LOD of 8.7×10^{-10} M and 3×10^{-9} M and LOQ of 2.9×10^{-9} M and 1×10^{-8} M of ethinylestradiol were achieved in human serum and plasma, respectively.

Keywords: Ethinylestradiol; Ethinyl-oestradiol® tablets; Adsorptive square-wave stripping voltammetry; Human serum and plasma

1. Introduction

Ethinylestradiol (ETE) is a semisynthetic estrogen female sex hormone which is used as a contraceptive. Oral contraceptives have had an enormous positive impact on public health for the past three decades and, overall, there has been a remarkably low incidence of troublesome side-effects [1].



(STRUCTURE OF ETE)

Several analytical methods have been reported for the determination of ETE in surface water, underground water, waste water, pharmaceutical formulations and biological fluids, including spectrophotometry [2–6], liquid chromatography [7–10], gas chromatography [11–20], high-performance liquid chromatography [20–26], thin-layer chromatography [27], micellar electrokinetic capillary chromatography [28–30], isotope dilution mass spectrometry [31], electrophoresis [32], radioimmunoassay [33,34], biosensor-immunoassay [35], argentometry [36] and amperometry [37]. Most of the above reported methods for the quantification of ETE require sample pretreatment and/or time-consuming extraction steps prior to analysis of the drug. However, no attempts were made to date to assay ETE using any of the electroanalytical techniques.

The formulations of such a semisynthetic estrogen, present at an extremely low concentration, presents a challenging analytical problem. Therefore, a rapid, sensitive, accurate

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and low cost analytical procedure is desired for determination of ETE and testing content uniformity of its dosage form. Since the ETE molecule has a characteristic 17-ethinyl group (–C≡CH) which is a promising electroactive center, which might be successfully used for its trace determination in formulations and human serum and plasma by means of the stripping voltammetric technique. Adsorptive stripping voltammetry is an extremely sensitive technique that utilizes a bulk electrolyte to accumulate the analyte from the sample solution onto the working electrode surface. The accumulation process can be viewed as an effective electrochemical extraction step in which the analyte is accumulated onto the working electrode surface to a much higher level than it exists in solution. The combination of an accumulation step with advanced voltammetric measurement generates the extremely favorable signal-to-background ratio that characterizes stripping analysis [38]. We describe here a square-wave adsorptive stripping voltammetric procedure for the determination of ETE in bulk form, pharmaceutical formulation, human serum and in real human plasma samples.

2. Experimental

2.1. Material and solutions

Ethinylestradiol sample was kindly supplied from Schering AG (Berlin, Germany) and was used without further purification. A standard stock solution of 1×10^{-3} M ETE was prepared by dissolution in methanol and stored at 4 °C. A Mettler balance (Toledo-AB 104, Switzerland) was used for weighing the solid material. Working solutions (10^{-6}–10^{-4} M ETE) were prepared daily by appropriate dilution.

2.2. Supporting electrolyte

A series of Britton–Robinson (B–R) universal buffers of pH 2–11 as supporting electrolyts was prepared [39]. A pH-meter (Crison, Barcelona, Spain) was used for the pH measurements. Deionized water was supplied from a Purite-Still Plus Deionizer connected to AquaMatic bidistillation water system (Hamilton, UK). All the chemicals used (Merck) were of analytical-reagent grade and were used without further purification.

2.3. Electrochemical apparatus and software

The votammetric measurements were carried out using computer-controlled Electrochemical Analyzers Models 394 and 263A-PAR (Princeton Applied Research, Princeton, NJ, USA), using 270/250 software. An electrode assembly (303A-PAR) of a micro-electrolysis cell incorporating a three electrode configuration system consisting of a hanging mercury drop electrode (HMDE) as a working electrode (area = 0.026 cm^2), an $Ag/AgCl/KCl_s$ reference electrode and a platinum wire auxiliary electrode, was used in the voltammetric measurements. Stirring of the solution in the electrochemical cell was performed using a magnetic stirrer (305-PAR) and a stirring bar to provide the convective transport during the accumulation step. The measurements were automated and controlled through the programming capacity of the apparatus.

A potentiostat/galvanostat model 173-PAR incorporating a digital coulometer model 179-PAR was used for the controlled-potential electrolysis of ETE. A coulometric cell of a mercury pool as a working electrode, a saturated calomel electrode as a reference electrode and a platinum gauze immersed in a bridge tube as a counter electrode was used. The potential selected was adjusted to a value equal to the $E_p \pm 0.1$ V of the cyclic voltammogram of ETE recorded at 100 mV/s scan rate. The charge Q (Coulombs) passed during the exhaustive electrolysis of ETE was obtained by integrating the current electronically. The number of electrons (n) transferred per reactant molecule was determined using Faraday's relation $N = Q/nF$ (where N is the number of moles of ETE substance being electrolyzed) and found to equal two electrons per ETE molecule, which attributed to reduction of the 17-ethinyl group (–C≡CH), probably yielding 17-vinyl-estradiol (–CH=CH_2).

2.4. Procedures for analysis

2.4.1. Analysis of bulk ETE

A known volume of standard ETE solution was pipetted into a 10 ml calibrating flask, and then made to the volume with the pH 7 B–R universal buffer. The percentage of methanol in the final solution was 1%. The solution was transferred into the electrolysis cell, and then deoxygenated with pure nitrogen for 10 min in the first cycle and 30 s for each successive cycle; the nitrogen was then kept over the solution during measurements. Then ETE was accumulated onto the HMDE for 60 s at −0.8 V while stirring the solution at 400 rpm. After an equilibrium duration of 5 s was allowed for the solution to become quiescent, the voltammograms were recorded by scanning the potential towards the negative direction using the square-wave waveform under the following instrumental parameters: pulse amplitude = 70 mV; frequency = 120 Hz and scan increment = 10 mV.

2.4.2. Analysis of ETE in tablets

Twenty-five tablets of Ethinyl-oestradiol® (Kahira Pharm. & Chem. Ind. Co., Egypt) labeled to contain 50 μg ETE per tablet were powdered. An adequate amount of the homogenous powder, corresponding to 1×10^{-4} M, was accurately weighed and transferred into a calibrated flask and then dissolved in 25 ml of methanol by sonication for 10 min, followed by mechanical shaking for 10 min and lastly centrifuged for 5 min at 14,000 rpm. A portion of the clear solution was diluted with the supporting electrolyte to achieve the desired concentration. Then ETE was quantified by means of the proposed stripping voltammetric procedure.

2.4.3. Analysis of ETE in spiked human serum and plasma

Serum and plasma samples obtained from a healthy volunteer were stored frozen until the assay. Into each of seven centrifugation tubes, 1 ml of spiked human serum or plasma, containing various concentrations of ETE, was transferred and then mixed well with 4 ml of methanol to precipitate the proteins. The precipitated proteins were separated by centrifugation for 5 min at 14,000 rpm. The clear supernatant layer was filtered through 0.45 μm Milli-pore filter. A 0.1 ml volume of the supernatant liquor was transferred into the voltammetric cell then completed to a 10 ml volume with a pH 7.0 B–R universal buffer. Then ETE was quantified by means of the proposed stripping voltammetric procedure.

2.4.4. Analysis of real plasma samples

Two volunteers (aged 30–33 years) of regular menstrual cycles and with negative tests for pregnancy were involved in the present study under the medical care of Ramadan Specialized Hospital, Tanta City, Egypt. Volunteers refrained from taking any medication including hormonal therapy, from 2 weeks before the study commenced until study completion. Both volunteers fasted overnight for 8 h before dosing. The two volunteers gave their written informed consent prior to participating in the study (no more permission is required from any ethical committee). Following an oral administration of a single dose of 50 μg ETE (Ethinyl-oestrodiol® tablet), venous blood samples (5 ml) were aseptically aspirated from each volunteer at different time periods over 5 h (1, 1.5, 2.5, 3.5 and 5 h) and collected in appropriately labeled lithium-heparin tubes. The blood samples were centrifuged immediately at 15,000 rpm for 10 min and the plasma fractions were rapidly separated and stored in coded polypropylene tubes at −20 °C until assayed. The plasma samples of the two volunteers were analyzed in parallel by means of the proposed stripping voltammetric procedure and a reported HPLC method [22].

Quantifications of ETE in the different analyte samples were performed by means of both the calibration curve and standard addition methods.

3. Results and discussion

3.1. Cyclic voltammetry

Cyclic voltammetric behavior of ETE showed a single 2-electron irreversible cathodic peak in B–R universal buffers of pH 4–11 which was attributed to reduction of 17-ethinyl group (–C≡CH), probably yielding 17-vinyl-estradiol (–CH=CH_2). The peak potential shifted linearly to more negative values with increasing scan rate, which indicated the irreversible nature of the reduction process of ETE. The interfacial adsorptive character of ETE onto the HMDE was identified by recording cyclic voltammograms for 1×10^{-6} M ETE in a B–R universal buffer of pH 7 at 200 mV/s at open circuit (Fig. 1, curve a), following accumulation onto the HMDE for 60 s at −0.8 V (Fig. 1, curve b), and the second cycle at the same mercury drop (Fig. 1, curve c). As shown in Fig. 1, accumulation of ETE onto the HMDE gave a substantial enhancement of the cathodic peak signal (first scan, curve b) compared with that of the second cycle at the same mercury drop (curve c), indicating a rapid desorption of ETE species from the mercury surface. Increased scan rate ν (25–500 mV s^{-1}) showed increased peak current intensity. A linear plot of $\log i_p$ versus $\log \nu$ following the equation: $\log i_p$ (μA) = 0.853 $\log \nu$ − 2.2 (r = 0.997) was obtained; its slope value (0.853) is very close to one which is the expected value for an ideal reaction of surface species [40]. The dependence of the peak potential (E_p) on the decimal logarithm of scan rate was linear according to the equation: E_p (mV) = −11.9–42.3 $\log \nu$ (mV/s), its slope value corresponds to a value of αn_a = 1.39. A symmetry coefficient α value of 0.69 was obtained when n_a = 2.

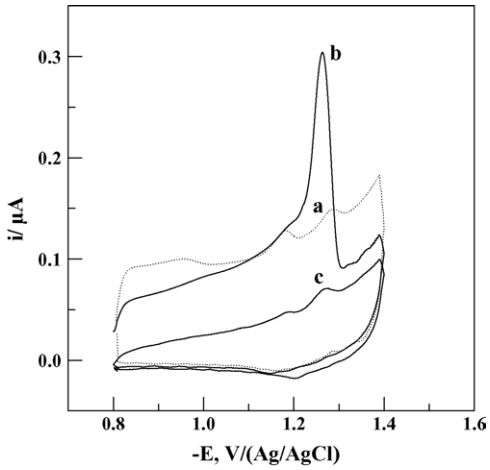

Fig. 1. Cyclic voltammograms for 5×10^{-6} M ETE in pH 7 B–R universal buffer (a) at open circuit, (b) following accumulation for 60 s at E_{acc} = −0.8 V and (c) the repetitive cycle at the same mercury drop; scan rate = 200 mV/s.

The electrode surface coverage (Γ^0 mol cm^{-2}) was calculated using the relation: $\Gamma^0 = Q/nFA$, where Q (Coulombs) is the amount of charge consumed by the surface process as calculated by the integration of the area under the peak of the cyclic voltammogram corrected to residual current [41], n the number of electrons consumed in the reduction process (n = 2), and A is the electrode surface area (0.026 cm^2). On dividing the number of coulombs transferred, 0.325 μC, by the conversion factor (nFA) yielded a monolayer surface coverage of 6.48×10^{-11} mol cm^{-2}. Each adsorbed ETE molecule therefore occupies an area of 0.256 nm^2.

3.2. Square-wave stripping voltammetry

3.2.1. pH of the medium

Square-wave voltammograms of 5×10^{-7} M ETE in the B–R universal buffer of pH 4–11 showed a single cathodic peak. The response preceding by accumulation increases extensively and a maximum peak current intensity was

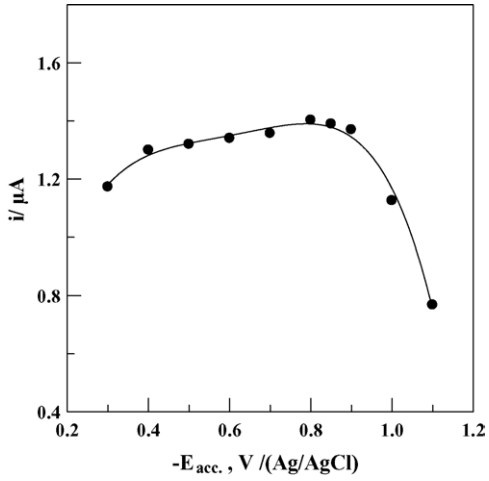

Fig. 2. Effect of accumulation potential (E_{acc}) on SWAdCS voltammetric peak current (i_p) of 5×10^{-7} M ETE in pH 7 B–R universal buffer, t_{acc} = 30 s, f = 120 Hz, ΔE = 10 mV, a = 25 mV, stirring rate = 400 rpm and rest period = 5 s.

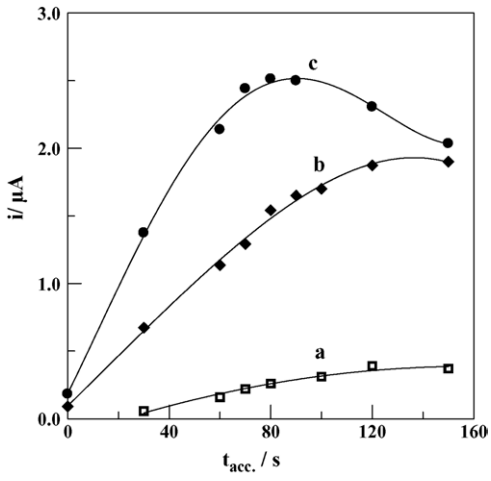

Fig. 3. Effect of accumulation times (t_{acc}) on SWAdCS voltammetric peak current of various concentrations of ETE: (a) 5×10^{-8} M, (b) 1×10^{-7} M and (c) 5×10^{-7} M in a B–R universal buffer of pH 7 at $E_{acc} = -0.8$ V, a = 70 mV. Other operational parameters were as those indicated in Fig. 2.

achieved in a B–R universal buffer of pH 7, which was chosen for determination of ETE.

3.2.2. Accumulation potential and duration

The dependence of the stripping voltammetric peak current (i_p) of 5×10^{-7} M ETE in pH 7 B–R universal buffer on the accumulation potential (E_p) was evaluated over the range −0.3 V to −1.1 V following accumulation onto the HMDE for 30 s. A potential of −0.8 V was chosen as the accumulation potential which gives a well-defined peak and a more developed peak current (Fig. 2). The effect of accumulation duration (t_{acc}) on the peak current following accumulation at −0.8 V was studied for various concentrations of ETE: 5×10^{-8} M, 1×10^{-7} M and 5×10^{-7} M. The peak current was found to increase linearly with the accumulation duration up to 60–70 s (Fig. 3). An accumulation duration of 60–70 s was found reasonable for the present analytical study.

3.2.3. Instrumental parameters

The peak current for 5×10^{-7} M ETE in pH 7 B–R universal buffer following accumulation for 60 s at −0.8 V was optimized by changing the pulse-amplitude a, scan increment ΔE_s and frequency f over the range 10–100 mV, 2–10 mV, and 10–120 Hz, respectively. The dependence of peak current (i_p) on the frequency (f) was linear according to the equation: i_p (µA) = 0.044f (Hz) − 0.32 (r = 0.996). A pulse-amplitude of 70 mV was chosen which gives a well-defined peak and a more developed peak current. Since the peak current intensity increased linearly with the increase of scan increment a scan increment of 10 mV was considered a reasonable condition for the present analytical study. As expected, an increase of the area of the HMDE yields an increase in the stripping voltammetric peak current, so a large HMDE area (0.026 cm^2) was considered suitable in the present work. The influence of the rest time on the peak current was also considered and a value of 5 s was chosen. Accordingly, the established conditions of the square-wave adsorptive cathodic stripping (SWAdCS) voltammetric procedure were: E_{acc} = −0.8 V, t_{acc} = 60–70 s, ΔE_s = 10 mV, a = 70 mV, f = 120 Hz (effective scan rate, $\Delta E_s f$ = 1.2 V s^{-1}), HMDE area = 0.026 cm^2, rest period = 5 s, and a B–R universal buffer of pH 7 as a supporting electrolyte.

3.2.4. Validation of the procedure

In the present work, quantification of ETE was based on the extent of the dependence of the peak current upon its concentration in the analyzed solution under the optimal procedural conditions. Validation of the present proposed SWAdCS voltammetric procedure for trace assay of ETE was examined via linearity, limit of detection, limit of quantification, reproducibility, repeatability, specificity, robustness and ruggedness.

The calibration curve for bulk ETE was established using the proposed SWAdCS voltammetric procedure after its accumulation onto the HMDE for 60 s at −0.8 V. Variation of the peak current (i_p) with the concentration of bulk ETE gave a straight line following the equation: i_p (µA) = 0.10197C (nM) + 0.24 (r = 0.997, n = 15). The linearity was shown over the concentration range 1.9×10^{-9}–6×10^{-7} M ETE. Limits of detection (LOD) and quantification (LOQ) of bulk ETE were estimated from the following equations [42]: LOD = 3S.D./m and LOQ = 10S.D./m (where S.D. is the standard deviation of the blank or intercept and m is the slope of calibration curve) and were found to equal 5.9×10^{-10} M and 1.9×10^{-9} M for bulk ETE, respectively.

Performing five successive measurements for three different concentrations of bulk ETE demonstrated the reproducibility of the results obtained by the proposed procedure. For intra-assay precision, recoveries were calculated from

Table 1
Analytical precision and accuracy of bulk ethinylestradiol determination by the proposed method; t_{acc} = 60 s; n = 5

[Taken] (M)	Intra-day			Inter-day		
	R%	Bias%	R.S.D.%	*R*%	Bias%	R.S.D.%
5×10^{-9}	99.0	−1.0	1.58	101.4	1.4	2.1
2×10^{-8}	100.4	4.0	2.35	98.5	−1.5	2.0
4×10^{-8}	102.5	2.5	1.97	100.7	0.70	1.5

Table 2
Assay of ethinylestradiol by the proposed stripping voltammetric procedure and a reported spectrophotometric method [4] using calibration curve (A) and standard addition (B) methods, t_{acc} = 60 s; n = 5

Claimed (μg/tablet)	50
Proposed method (*R*% ± R.S.D.) (A)	99.7 ± 0.87
Proposed method (*R*% ± R.S.D.) (B)	101.3 ± 1.4
Reported method (*R*% ± R.S.D.) (B)	100.4 ± 1.2

Calculated *F*-value = 5.15. and *t*-test = 0.48. The theoretical *F*-value and *t*-test at 95% confidence limit are 6.39 and 2.30, respectively.

repeated analysis during 1 day and for inter-assay precision, recoveries were calculated from repeated analysis for 5 days over a period of 1 week (Table 1). Accuracy of the results expressed as bias% (bias% = {(concentration found − concentration taken) × 100/(concentration taken)} within and between days were less than 5% at low and high concentrations (Table 1).

The specificity of the proposed procedure was tested by analysis of 5×10^{-7} M bulk ETE and a standard tablet solution containing 5×10^{-7} M ETE, following accumulation onto the HMDE for 60 s at −0.8 V. No significant differences in the main percentage recoveries (*R*%) or the relative standard deviation (R.S.D.%) of the two solutions were observed, indicating the absence of interference from excipients. Thus, the proposed procedure can be considered specific [43].

The robustness, is a measure of the capacity of the proposed analytical procedure to remain unaffected by small but deliberate variations in procedural parameters and provides an indication of its reliability during normal usage [43]. A study of the effect of small variation of some of the procedural operational conditions such as pH (7–8), accumulation potential, E_{acc} (−0.7 V to −0.8 V) and accumulation duration, t_{acc} (60–70 s) on the recovery and standard deviation of 5×10^{-7} M bulk ETE was carried out. The obtained mean percentage recoveries (*R*%) and relative standard deviations (R.S.D.%) were not significantly affected within the studied ranges of variations in the procedural operational conditions, and consequently the proposed procedure can be considered robust [43].

Ruggedness is the degree of reproducibility of results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different elapsed assay time and different instruments [43]. This was examined by the assaying concentrations of 5×10^{-7} M ETE using the PAR-Potentiostats models 263 A (Lab. 1) and 394 (Lab. 2) under the same operational conditions. The mean percentage recoveries obtained due to Lab. (1) and Lab. (2) were found to be reproducible, since there were no significant differences between the recoveries or the standard deviations obtained in both laboratories.

3.2.5. Application to tablets

The proposed SWAdCS voltammetric procedure was successfully applied for assay of ETE in its pharmaceutical formulation (Ethinyl-oestradiol®) without the necessity for samples pretreatment or any time-consuming extraction or evaporation steps prior to the analysis.

The results obtained using both calibration curve and standard addition methods (Table 2) are favorably compared to those obtained by means of a reported spectrophotometric method [4]. The calculated *F*-value and *t*-test did not exceed the theoretical values (95% confidence limits for five degrees of freedom), which verified the accuracy of the proposed procedure for assay of ETE in tablets.

3.2.6. Application to spiked human serum and plasma

The possibility of applying the proposed procedure for the determination of ETE in spiked human serum (Fig. 4) and plasma was tested. Linear calibration plots were obtained over the concentration ranges of 2.9×10^{-9}–5×10^{-7} M and 1×10^{-8}–9×10^{-8} M ETE following an accumulation duration of 60 s at −0.8 V. The intra-day and the inter-day precisions were evaluated by five replicate measurements for human serum and plasma samples spiked with various concentrations of bulk ETE. The accuracy of the proposed procedure was determined as bias% (Table 3). LOD of 8.7×10^{-10} M and 3×10^{-9} M and LOQ of 2.9×10^{-9} M and 1×10^{-8} M ETE were achieved in spiked human serum and plasma, respectively.

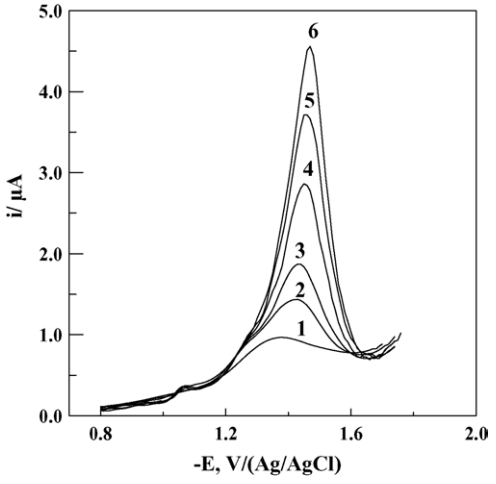

Fig. 4. Representative SWAdCS voltammograms for various concentrations of ETE spiked in human serum: (1) background; (2) 5×10^{-8} M; (3) 1×10^{-7} M; (4) 2×10^{-7} M; (5) 3×10^{-7} M and (6) 4×10^{-7} M ETE; t_{acc} = 60 s, pulse height = 70 mV. Other operational parameters were as those indicated in Fig. 2.

Table 3
Analytical precision and accuracy of Ethinylestradiol determination in spiked human: (A) serum and (B) plasma; $t_{acc} = 60$ s; $n = 5$

[ETE] (M)	Intra-day						Inter-day					
	R%		Bias%		R.S.D.%		*R*%		Bias%		R.S.D.%	
	A	B	A	B	A	B	A	B	A	B	A	B
2×10^{-8}	99.0	98.0	−1.0	−2.0	1.7	2.2	98.0	97.5	−2.0	−2.5	2.0	2.3
4×10^{-8}	101.3	98.7	1.3	−1.3	2.6	1.9	98.2	98.4	−1.8	−1.6	2.6	2.1
6×10^{-8}	98.2	101.6	−1.8	1.6	2.2	2.4	90.2	98.9	−1.0	−1.1	1.5	2.0

Table 4
Average results for the analysis of real plasma samples of the two volunteers (after administration of a 50 μg single dose of Ethinyl-oestradiol ®) obtained by the proposed SWAdCSV voltammetric procedure and a reported HPLC method [22]

Time (h)	Average plasma concentration (μg ETE/ml)	
	SWAdCSV	HPLC
1.0	1.31	1.25
1.5	1.50	1.43
2.5	1.71	1.63
3.5	1.61	1.54
5.0	1.05	0.97

3.2.7. Application to real plasma

Samples from the two volunteers which were collected and pretreated as described in Section 2.4.4 were assayed in duplicate using the proposed SWAdCS voltammetric procedure parallel to analysis of the same plasma sample by a reported HPLC method [22]. The average results obtained by both analytical methods (Table 4) agree well confirming the reliability of the SWAdCS voltammetric procedure for assay of ETE in real plasma samples.

4. Conclusion

A validated, simple, precise and specific SWAdCS voltammetric procedure is described for the trace determination of ethinylestradiol in bulk form, tablets and human serum and plasma samples without the necessity for sample pretreatments and/or time-consuming extraction or evaporation steps prior to the analysis. The procedure had adequate accuracy and consequently is recommended for ETE analysis in clinical and quality control laboratories.

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References

[1] T.A. Ternes, M. Stumpf, J. Mueller, K. Haberer, R.D. Wilken, M. Servos, Sci. Total Environ. 228 (1999) 87–98.
[2] J.J. Berzas, J. Rodrigues, G. Gastaneda, Anal. Sci. 13 (1997) 1029–1032.
[3] J.J.B. Nevado, J.R. Flores, G.C. Penalvo, F.J.G. Bernardo, Anal. Lett. 30 (1997) 2221–2233.
[4] J.J.B. Nevado, J.R. Flores, G.C. Penalvo, Anal. Chim. Acta 340 (1997) 257–265.
[5] J.J. Berzas, J. Rodriguez, G. Castaneda, Analyst 122 (1997) 41–44.
[6] M.A. Korany, F.A. Elyazbi, O. Abdelrazak, M.A. El-Sayed, Pharm. Wekblad-Scientfic Ed. 7 (1985) 163–166.
[7] Y. Fan, M. Shang, S.L. Da, Y.Q. Feng, Analyst 130 (2005) 065–069.
[8] S. Zuehlke, U. Duennbier, T. Heberer, B. Fritz, Ground Water Monit. Remediation 24 (2004) 78–85.
[9] N.C. Twaddle, M.I. Churchwell, R.R. Newbold, K.B. Delclos, D.R. Doerge, J. Chromatogr. B 793 (2003) 309–315.
[10] Y. Ishii, S. Okita, M. Torigai, S.J. Yun, Bunseki Kagaku 49 (2000) 753–758.
[11] J.B. Quintana, J. Carpinteiro, I. Rodriguez, R.A. Lorenzo, A.M. Carro, R. Cela, J. Chromatogr. A 1024 (2004) 177–185.
[12] A. Mouatassim-Souali, S.L. Tamisier-Karolak, D. Perdiz, M. Cargouet, Y. Levi, J. Sep. Sci. 26 (2003) 105–111.
[13] S. Muller, M. Moder, S. Schrader, P. Popp, J. Chromatogr. A 985 (2003) 99–106.
[14] T.A. Ternes, H. Anderson, D. Gilberg, M. Bonerz, Anal. Chem. 74 (2002) 3498–3504.
[15] A.A. Durant, C.A. Fente, C.M. Franco, B.I. Vazquez, A. Cepeda, J. Agric. Food Chem. 50 (2002) 436–440.
[16] P. Spengler, W. Korner, J.W. Metzger, Environ. Toxicol. Chem. 20 (2001) 2133–2141.
[17] E. Daeseleire, A. Deguesquiere, C. Vanpeteghem, J. Chromatogr. Biomed. Appl. 562 (1991) 673–679.
[18] A.A. Durant, C.A. Fente, C.M. Franco, B.I. Vazquez, S. Mayo, A. Cepeda, J. Chromatogr. B 766 (2002) 251–256.
[19] H.M. Kuch, K. Ballschmiter, Environ. Sci. Technol. 35 (2001) 3201–3206.
[20] M. Esperanza, M.T. Suidan, F. Nishimura, Z.M. Wang, G.A. Sorial, Environ. Sci. Technol. 38 (2004) 3028–3035.
[21] A. Laban, S. Markovic, M. Stankov, P. Djurdjevic, Anal. Lett. 37 (2004) 273–282.
[22] M.I.R.M. Santoro, N.M. Kassab, M. Hasegawa, E.R.M. Kedor-Hackmann, Drug Dev. Ind. Pharm. 28 (2002) 741–747.
[23] N. Fernandez, J.J. Garcia, M.T. Teran, M. Sierra, J. Chromatogr. Biomed. Appl. 619 (1993) 143–147.
[24] A.H.N. Ahmed, S.M. Elgizawy, N.M. Omer, Anal. Lett. 24 (1991) 2207–2216.
[25] W. Backe, Archiv der Pharm. 321 (1988) 431–432.

[26] L.S. Mao, C.J. Sun, Y.X. Li, D.S. Wu, J.H. Zhao, Chin. J. Anal. Chem. 33 (2005) 33–36.
[27] J. Molnar, M. Gazdag, G. Szepesi, Pharmazie 37 (1982) 836–838.
[28] J.J. Berzas, G. Castaneda, M.J. Pinilla, Anal. Lett. 32 (1999) 2453–2469.
[29] J.J. Berzas, B. Del Castillo, G. Castaneda, M.J. Pinilla, Talanta 50 (1999) 261–268.
[30] J.J. Berzas, J. Rodriguez, G. Castaneda, M.J. Pinilla, Chromatographia 49 (1999) 65–70.
[31] L. Siekmann, A. Siekmann, F. Bidlingmaier, K. Brill, M. Albring, Eur. J. Endocrinol. 139 (1998) 167–177.
[32] B. Allner, G. Wegener, T. Knacker, P. Stahlschmidt-Allner, Sci. Total Environ. 233 (1999) 21–31.
[33] W. Kuhnz, T. Louton, D.J. Back, K. Michaelis, Drug Res. 43 (1993) 16–21.
[34] E. Rattenberger, W. Gedek, P. Matzke, Arch. Lebensmittelhyg. 34 (1983) 48–51.
[35] M.A. Johansson, K.E. Hellenas, Analyst 126 (2001) 1721–1727.
[36] F.I. Khattab, F.M. Ashour, M.M. Amer, J. De Pharm. De Belgique 38 (1983) 147–155.
[37] L.H. Wang, S.F. Jseng, J. Liq. Chromatogr. Relat. Technol. 28 (2005) 1367–1381.
[38] J. Wang, Analytical Electrochemistry, second ed., Wiley–VCH, New York, 2000, pp. 75–84.
[39] H.T.S. Britton, Hydrogen Ions, fourth ed., Chapman and Hall, London, 1952, p. 113.
[40] E.J. Laviron, J. Electroanal. Chem. 112 (1980) 11–23.
[41] A. Webber, M. Shah, J. Osteryoung, Anal. Chim. Acta 157 (1984) 17–29.
[42] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, fourth ed., Eellis-Howood, New York, 1994, p. 115.
[43] The United States Pharmacopoeia, Convention Inc., 2003, p. 2446.