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A validated stripping voltammetric procedure for quantification of the anti-hypertensive and benign prostatic hyperplasia drug terazosin in tablets and human serum

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Abstract

The electrochemical behavior of terazosin at the hanging mercury drop electrode was studied in Britton–Robinson buffer (pH 2–11), acetate buffer (4.5–5.5), and in 0.1 M solution of each of sodium sulfate, sodium nitrate, sodium perchlorate and potassium chloride as supporting electrolytes. The square-wave adsorptive cathodic stripping voltammogram of terazosin exhibited a single well-defined two-electron irreversible cathodic peak which may be attributed to the reduction of C=O double bond of the drug molecule. A fully validated, simple, high sensitive, precise and inexpensive square-wave adsorptive cathodic stripping voltammetric procedure was described for determination of terazosin in bulk form, tablets and human serum. A mean recovery for 1×10^{-8} M terazosin in bulk form, following preconcentration onto the hanging mercury drop electrode for 60 s at a −1.0 V (versus Ag/AgCl/KCl_s), of 99 ± 0.7% ($n = 5$) was obtained. Limits of detection (LOD) and quantitation (LOQ) of 1.5×10^{-11} and 5×10^{-11} M bulk terazosin were achieved, respectively. The proposed procedure was successfully applied to determination of the drug in its Itrin® tablets and human serum samples. The achieved LOD and LOQ of the drug in human serum samples were 5.3×10^{-11} and 1.8×10^{-10} M THD, respectively. The pharmacokinetic parameters of the drug in human plasma were estimated as: $C_{max} = 77.5\,ng\,ml^{-1}$, $t_{max} = 1.75\,h$, $AUC_{0-t} = 602.3\,ng\,h\,ml^{-1}$, $K_e = 0.088\,h^{-1}$ and $t_{1/2} = 11.32\,h$) which are favorably compared with those reported in literature.
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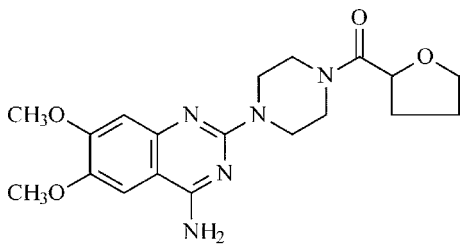
Keywords: Terazosin; Itrin® tablets; Human serum; Quantification; Square-wave; Adsorptive cathodic stripping voltammetry; Pharmacokinetic parameters

1. Introduction

Terazosin hydrochloride dihydrate (THD): 2-[4-(2-tetrahydrofuranyl carbonyl]-1-piperazinyl-6,7-dimethoxy-4-quinazolinamine monohydro-chloride dihydrate (I) is a highly selective potent α_1 adreno- receptor antagonist. It is an effective drug for hypertension [1–3] and



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benign prostatic hyperplasia [4–6]. Terazosin is rapidly and almost completely adsorbed from the gastro-intestinal tract after oral administration; the bioavailability is reported to be about 90%. It is metabolized in the liver and excreted in feces via the bile, and in the urine as unchanged drug and metabolites [7]. The pharmacokinetics of terasosin were shown to be linear in the range 0.1–7.5 mg orally and 1–5 mg intravenously [8,9]. The plasma level of the drug reach the peak concentration within about 1.5 h after oral administration, and then decline with a half-life of approximately 11 h [10], thus maintaining levels of therapeutic effect allowing the drug to be administrated only once daily.

The high potency of terazosin necessitated the development of a very sensitive assay in order to quantify the low plasma concentrations following a therapeutic dose of 1–5 mg THD. Different analytical methods have been reported for determination of terazosin in bulk form, pharmaceuticals and biological fluids following intravenous and oral dosage. These include spectrophotometry [11–14], spectrofluorimetry [14–16], high-performance liquid chromatography (HPLC) with UV detection [17], HPLC with fluorescence detection [9,18–28], normal phase HPLC—electrospray mass spectroscopy [29] and HPLC with photo-diode array detection [30]. To date no electrochemical procedure is reported for assay of terazosin. Prior to assay of the drug, the reported methods necessitate sample pretreatment and lengthy extraction procedures which either incorporate back or direct extraction of small volume into an organic layer which leads to column deterioration. Therefore, the clinical investigations of terazosin in biological samples still required the development of simple, sensitive, precise, selective and inexpensive analytical methods without the necessity for sample pretreatment or time-consuming extraction steps prior to the analysis.

Adsorptive stripping voltammetric analysis especially with the square-wave waveform is an extremely simple and sensitive technique that can be used for analysis of drugs without the necessity for extraction steps prior to the assay. Moreover, the square-wave voltammetry is a large-amplitude differential technique in which a waveform composed of a symmetrical square wave is applied to the working electrode [31]. The current is sampled twice during each square-wave cycle, once at the end of the forward pulse and once at the end of the reverse pulse. The resulting peak current is proportional to the concentration of the analyte. Excellent sensitivity accrues from the fact that the net current is larger than either the forward and reverse components. Coupled with the effective discrimination against the charging current, very low detection limits can be attained. Comparison of square-wave and differential-pulse voltammetry for reversible and irreversible cases indicated that the square-wave current are 4 and 3.3 times higher, respectively, than the analogous differential-pulse response [32,33]. The major advantage of square-wave voltammetry is its great speed. The effective scan rate is given by square-wave frequency f (in Hz) and the step height ΔE_s as $f\Delta E_s$.

Here a square wave adsorptive cathodic stripping voltammetric procedure was optimized for determination of terazosin in bulk form, pharmaceutical formulation and human serum. The utility of the developed procedure in a preliminary clinical study for determination of the pharmacokinetic parameters of terazosin in blood of healthy volunteers following an oral administration of a single dose of 5 mg THD was also demonstrated.

2. Experimental

2.1. Instrumentation

The Electrochemical Analyzers Models 394 and 263 A (PAR) were used for the voltammetric measurements. The electrode assembly (Model 303A—PAR) incorporated with a dark micro-electrolysis cell of three electrode system comprising 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 counter electrode, was used. Stirring of the solution in the micro-electrolysis cell was performed using a magnetic stirrer (305—PAR) with a star-shaped magnet to provide the convective transport during the preconcentration step. The whole measurements were automated and controlled through the programming capacity of the apparatus. The data were treated through a personal computer connected to the potentiostat and loaded with the 394 Analytical voltammetry software version 2.01—copyright© 1994 (PAR).

A Shimadzu UV-Vis Recording Spectrophotometer Model 160A was used for a comparative assay of the drug in tablets by means of a reported spectrophotometric method [11] and for the spectral study of terazosin.

A Mettler balance (Toledo-AB104) was used for weighing the solid materials. An Orion SA-720 pH-meter with combined glass and calomel (saturated KCl) electrodes was used for the pH measurements of the supporting electrolytes. A centrifuge (Eppendorf—5417 C) was used for separation of the precipitated proteins from the human serum samples before assay of the drug. A micopipetter (Eppendorf—Multipette® plus) was used for transfer of the reactant solutions throughout the present experimental work.

The de-ionized water used throughout the present study was supplied from a Purite-Still Plus de-ionizer connected to a Hamilton-AquaMatic bi-distillation water system.

2.2. Materials and reagents

Bulk terazosin hydrochloride dihydrate (THD) and Itrin® tablets (labeled as containing 2 and 5 mg THD per tablet) were supplied from Kahira Pharm. & Chem. Ind. Co., Egypt. A stock standard solutions (1×10^{-3} M THD) was prepared in methanol (Merck) and stored in a dark bottle at 4 °C. More dilute solutions (1×10^{-6} to 1×10^{-4} M THD) were prepared daily in methanol just before use. Terazosin solutions were

stable at the room temperature and their concentrations did not change with time.

Britton–Robinson (B–R) buffers of pH 2–11, acetate buffer, (pH 4.5–5.5) sodium sulphate (0.1 M), sodium nitrate (0.1 M), sodium perchlorate (0.1 M) and potassium chloride (0.1 M) solutions were prepared by dissolving the materials (analytical grade) in specific volumes of de-ionized water [34] and were used as supporting electrolytes.

2.3. Itrin® tablet solutions

Five Itrin® tablets (2 or 5 mg) were weighed and the average mass per tablet was determined. A portion of the finely ground terazosin was accurately weighed and transferred into a 100-ml volume calibrated flask contains 70 ml methanol (Merck). The content of the flask was sonicated for about 15 min and then made up to the volume with methanol. The solution was then filtered through a 0.45 μm milli-pore filter (Gelman, Germany), that to separate out the insoluble excipients, rejecting the first portion of the filtrate. The desired concentrations of the drug were obtained by accurate dilution with methanol and used as standard solutions. An aliquot volume of the solution was transferred into a 10-ml volume calibrated flask then made up to the volume with the supporting electrolyte. The solution was directly analyzed, according to the general analytical procedure without the necessity for sample pretreatment or any extraction steps.

2.4. Spiked human serum samples

Serum samples, obtained from healthy volunteers, were collected then mixed and stored frozen until assay. Aliquots of human serum sample (each of 100 μl) were fortified with THD in small tubes to achieve serum samples spiked with different concentrations of the drug (1×10^{-8} to 1×10^{-5} M). Then each of these samples was completed to a 1.0 ml volume with methanol (as a protein precipitating agent). After vortexing each of the serum samples for two min, the precipitated proteins were separated by centrifugation for 3 min at 14,000 rpm. The clear supernatant layer was filtered through a 0.45 μm milli-pore filter to obtain protein—free human serum samples which were used as standard spiked serum solutions. Then the analysis was followed up as indicated in the general analytical procedure. Then each of these samples was directly analyzed, according to the general analytical procedure without the necessity for sample pretreatment or any extraction steps.

2.5. Pharmacokinetic study

The study performed on two healthy volunteers at Ramadan Specialized Hospital, Tanta City, Egypt. The two volunteers gave their written informed consent prior to participating in the study (in this country, except the volunteer's written informed consent no ethical committee permission is required prior to participating in this study). Both volunteers fasted overnight for 8 h before dosing. Following an oral administration of a single dose of 5 mg THD (Itrin® tablet), 5 ml venous blood samples were aseptically aspirated from each volunteer at different time periods over 24 h and collected in appropriately labeled lithium-heparin tubes. The blood samples were centrifuged immediately at 2000 rpm for 10 min and the plasma fractions were rapidly separated and stored in coded polypropylene tubes at −20 °C until assayed. Each plasma sample was assayed in duplicate by using the proposed square-wave adsorptive cathodic stripping voltammetric procedure, and the mean of the two values provides the plasma concentration at the time period of collection of the blood sample.

2.6. General analytical procedure

A 10-ml volume of B–R buffer was introduced into a dark micro-electrolysis cell, then de-aerated with pure nitrogen for 10 min. A selected accumulation potential was then applied to the hanging mercury drop electrode for a selected preconcentration time period, while the solution was stirred at 400 rpm. At the end of the accumulation period, the stirring was stopped and a 5-s rest period was allowed for the solution to become quiescent. Then, the background voltammogram was recorded by scanning the potential toward the negative direction using the selected waveform. After recording the background voltammogram, an aliquot of the reactant solution was introduced into the micro-electrolysis cell and the voltammogram was then recorded at a new mercury drop. All the data were obtained at room temperature.

To study the reproducibility, accuracy and precision of the proposed procedure to determination of the drug in bulk form, tablets and human serum samples, recovery experiments were carried out, by means of both the calibration curve and standard addition methods.

2.7. Spectral study

A preliminary study for the sepectra (Absorbance—wave length λ) of 1×10^{-5} M terazosin in B–R buffers (pH 2–11) was carried out. The recorded spectra exhibited two bands at 245 and 330 nm within the entire pH range. The maximum absorbance of the first peak increased slightly while that of the second peak decreased practically with the same rate upon the increase of pH. From the S and Z shaped absorbance–pH curves for the first ($\lambda = 245$ nm) and second ($\lambda = 330$ nm) bands, respectively, the pK_a of terazosin was estimated and found to equal 6.2 and 6.5, respectively, with a mean pK_a value of 6.35.

3. Results and discussion

3.1. Effect of type and pH of the supporting electrolyte

The influence of pH on the square-wave voltammetric response for 5×10^{-7} M THD was examined in B–R buffers

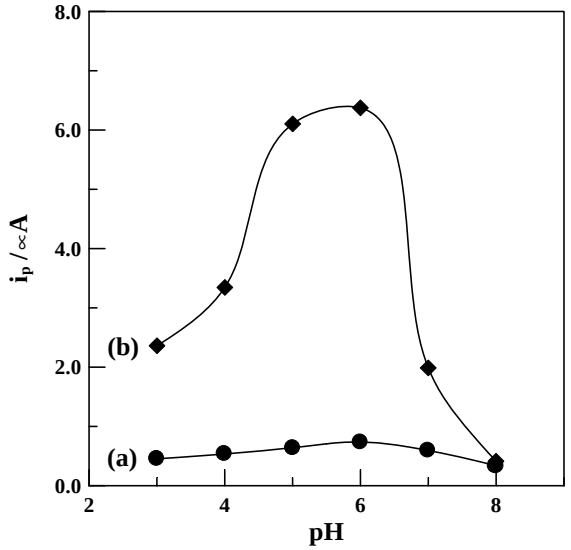

Fig. 1. Influence of pH (B–R buffers) on the SWAdCS voltammetric peak current (i_p) of 5×10^{-7} M THD; frequency $f = 120$ Hz, scan increment $\Delta E_s = 10$ mV and pulse-amplitude $E_{sw} = 25$ mV, without preconcentration (a) and following preconcentration for 30 s at −1.0 V (b).

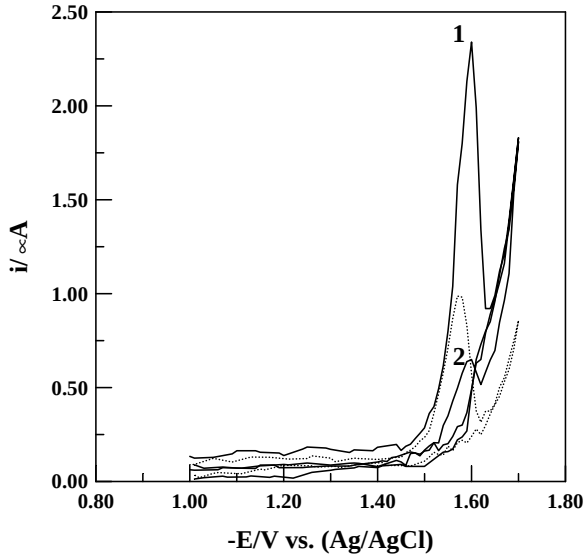

Fig. 2. Cyclic voltammograms of 5×10^{-6} M THD in B–R buffer of pH 5.5; without preconcentration ($t_{acc} = 0$ s) (dashed curve), following preconcentration for 30 s (curve 1) and its repetitive cycle at the same mercury drop (curve 2). Scan rate $\nu = 100$ mV s^{-1} and $E_{acc} = -1.0$ V.

of different pH values without pre-concentration ($t_{acc} = 0$ s) and following pre-concentration for 30 s. The voltammograms exhibited a single well-defined two-electron irreversible cathodic peak over the pH range 3–8. This peak may be attributed to the hydrogenation of C=O double bond via the consumption of two electrons per drug molecule, as confirmed by means of controlled-potential complete electrolysis experiment at a mercury pool electrode. The peak potential shifted to more negative values on the increase of pH of the medium denoting that protons are involved in the electrode reaction process and that the proton-transfer reaction precedes the electrode process proper [35]. As shown in Fig. 1, the peak current intensities (i_p) recorded at different pH values following pre-concentration for 30 s (curve b) are much more higher than those obtained without pre concentration (curve a). A much higher peak current intensity was achieved in B–R buffers of pH 5–6. Other supporting electrolytes such as acetate buffer (pH 4.5–5.5), sodium sulfate, sodium nitrate, sodium perchlorate and potassium chloride were also tested but the peak current intensity was less developed compared to that obtained in B–R buffer of pH 5–6. Therefore, B–R buffer of pH 5.5 was used as a supporting electrolyte in the rest of the present work.

3.2. Adsorptive character of the drug

Fig. 2 shows the cyclic voltammograms of 5×10^{-6} M THD in B–R buffer of pH 5.5 recorded without pre-concentration, $t_{acc} = 0$ s (dashed curve), following pre-concentration for 30 s (curve 1) and then its repetitive cycle at the same mercury drop (curve 2). A more developed peak current was achieved after pre-concentration of the drug onto the electrode surface, whereas the second cycle at the same mercury drop exhibited lower peak current intensity which may be due to the desorption of drug species out of the mercury electrode surface. This behavior indicated the interfacial adsorptive character of the drug onto the mercury electrode surface. The influence of the scan rate on the peak current (i_p) was studied within the range 25–500 mV s^{-1}. The cycles carried out for the increased values of scan rate (ν) under the above conditions gave rise to a cathodic peak with intensities that showed a linear increase with the scan rate, followed the relationship: $\log i_p = 0.94 \log \nu - 1.666$ ($r = 0.995$ and $n = 10$). Its slope value of 0.94 is very close to the theoretical value of 1.0 expected for an ideal electrode reaction of surface species [36].

Using a 5×10^{-6} M THD in R–R buffer of pH 5.5, the adsorptive saturation of the drug onto the mercury electrode surface was achieved after pre-concentration for 30 s. The response of surface-adsorbed drug at saturation was used to determine the amount of adsorbed drug species (surface coverage). The surface coverage (Γ_0) can be evaluated as $\Gamma_0 = Q/nFA$ [37], where Q is the charge (in Coulombs C) consumed by the surface process as calculated by the integration of the area under the peak, n is the total number of electrons transferred in the reactant electrode reaction ($n = 2$ per drug molecule as indicated from the controlled-potential complete electrolysis experiment), A is the mercury electrode surface area (0.026 cm^2) and F is the Faraday charge (96485C). The electrode surface coverage was obtained as 1.527×10^{-9} mol cm^{-2}. Each adsorbed THD molecule therefore occupied an area of 0.105 nm^2.

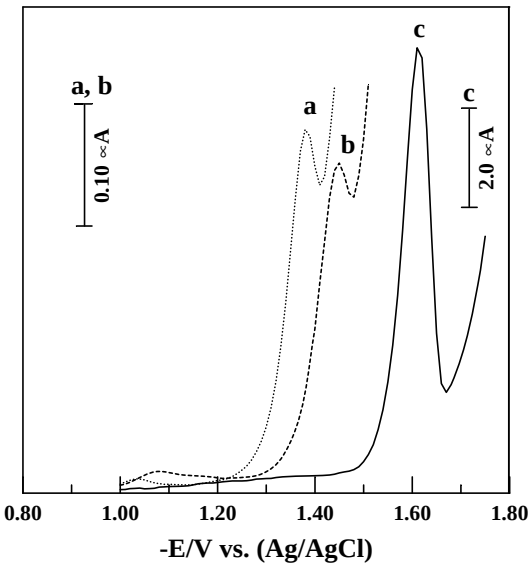

Fig. 3. Adsorptive cathodic stripping voltammograms of 5×10^{-7} M THD in B–R buffer of pH 5.5 recorded using: (a) differential- pulse ($v = 10\,\mathrm{mV\,s^{-1}}$, $E_{dp} = 25\,\mathrm{mV}$), (b) linear-sweep ($v = 100\,\mathrm{mV\,s^{-1}}$, $E_{ls} = 25\,\mathrm{mV}$) and (c) square-wave ($f = 120\,\mathrm{Hz}$, $\Delta E_s = 10\,\mathrm{mV}$, $E_{sw} = 25\,\mathrm{mV}$) waveforms, following preconcentration for 30 s at $E_{acc} = -1.0\,\mathrm{V}$.

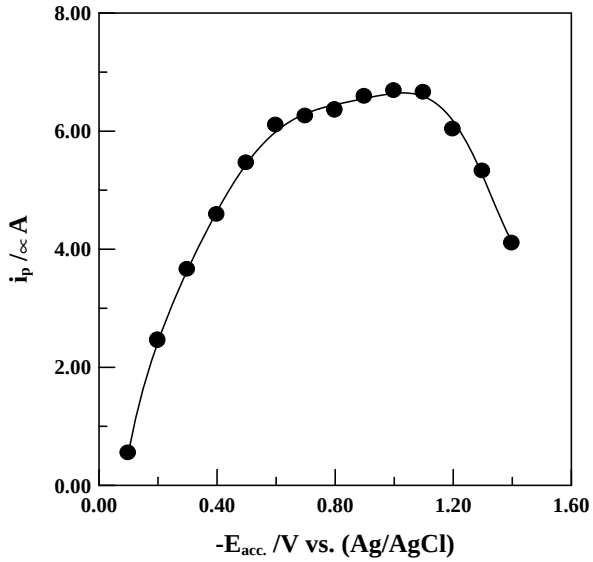

Fig. 4. Effect of accumulation potential (E_{acc}) on the SWAdCS voltammetric peak current (i_p) of 5×10^{-7} M THD in B–R buffer of pH 5.5; frequency $f = 120\,\mathrm{Hz}$, scan increment $\Delta E_s = 10\,\mathrm{mV}$ and pulse-amplitude $E_{sw} = 25\,\mathrm{mV}$, following preconcentration for 30 s.

3.3. Optimization of the proposed analytical procedure

The ability to readily assay nano-molar concentrations was attributed not only to the effective accumulation step (accumulation potential and accumulation time), but also to the improved sensitivity of the applied waveform for monitoring of the accumulated drug. Fig. 3 shows the recorded adsorptive cathodic stripping (AdCS) voltammograms of 5×10^{-7} M THD in B–R buffer of pH 5.5 following preconcentration for 30 s at -1.0 V (versus Ag/AgCl/KCl_s) using different waveforms: (a) differential-pulse (DP), (b) linear-sweep (LS) and (c) square-wave (SW). The signal intensity of SWAdCS voltammetry was found approximately 60 and 65 times higher than those of the DPAdCS and LSAdCS voltammetry, respectively. Therefore, the square-wave waveform was applied in the present assay in order to improve the sensitivity and the rapidity of assay of THD in pharmaceutical formulation and human serum. The advantage of application of square-wave waveform has been documented in connection with the trace analysis of several drugs [38–41].

The square-wave adsorptive stripping voltammetry response markedly depends on the parameters of the excitement signal. In order to reach a maximum developed SWAdCS voltammetric peak current, the optimum instrumental conditions (frequency f, scan increment ΔE_s and pulse amplitude E_{sw}) were studied for 5×10^{-7} M THD in B–R buffer of pH 5.5 following preconcentration for 30 s. At a scan increment of 10 mV and a pulse-amplitude of 25 mV, the peak current intensity increased linearly over the frequency range 20–120 Hz following the relationship: $i_p\ (\mu A) = 0.046f\ (\mathrm{Hz}) + 0.50$ ($r = 0.994$ and $n = 6$). At a frequency of 120 Hz and a pulse-amplitude of 25 mV, the peak current intensity increased linearly with the scan increment up to 10 mV, following the relationship: $i_p\ (\mu A) = 0.621\,\Delta E_s\ (\mathrm{mV}) + 0.291$ ($r = 0.996$ and $n = 5$). Also, at $f = 120\,\mathrm{Hz}$ and $\Delta E_s = 10\,\mathrm{mV}$, the peak current increased linearly with the increase of the pulse-amplitude from 25 to 100 mV, however the best peak morphology and sharper one was obtained at 25 mV. Therefore, the optimal instrumental operational conditions of the proposed square-wave procedure can be concluded as: frequency $f = 120\,\mathrm{Hz}$, scan increment $\Delta E_s = 10\,\mathrm{mV}$ and pulse amplitude $E_{sw} = 25\,\mathrm{mV}$.

On the other side, the effect of varying accumulation potential (E_{acc}) from -0.1 to -1.4 V on the peak current intensity of the SWAdCS voltammogram of 5×10^{-7} M THD in B–R buffer of pH 5.5 following preconcentration for 30 s was also evaluated (Fig. 4). A maximum developed peak current was achieved over the potential range of -0.8 to -1.1 V. The observed gradual decrease in peak current intensity may be attributed to the consequence of desorption of the drug at higher or lower potential values than the zero charge potential, where a maximum adsorption of uncharged organic molecules can be expected. Hence, a pre-concentration potential of -1.0 V (versus Ag/AgCl/KCl_s) was chosen throughout the present study.

The SWAdCS voltammograms of 1×10^{-8}, 5×10^{-8} and 1×10^{-7} M THD, were recorded under the optimal operational conditions at increased accumulation time. The peak current intensity showed a linear relationship with the accumulation time up to 500, 300 and 120 s for the concentration levels of 1×10^{-8}, 5×10^{-8} and 1×10^{-7} M terazosin, respectively. This means that the optimal accu-

Table 1
Characteristics of the calibration curves of the square-wave voltammetric determination of terazosin in B–R buffer of pH 5.5

t_{acc} (s)	Linearity range (M)	Least square equation[a]		Corr. coefficient (r)	LOD (M)	LOQ (M)
		Intercept (nA)	Slope (nA/nM)			
0	2×10^{-8} to 5×10^{-7}	28.87 ± 3.23	3.46 ± 0.05	0.996	2.8×10^{-9}	9.3×10^{-9}
60	8×10^{-9} to 1×10^{-7}	44.40 ± 5.55	83.22 ± 0.44	0.997	2.0×10^{-10}	6.7×10^{-10}
150	4×10^{-9} to 5×10^{-8}	52.60 ± 4.07	203.54 ± 1.11	0.999	6.0×10^{-11}	2.0×10^{-10}
300	1×10^{-9} to 3×10^{-8}	65.00 ± 1.82	363.68 ± 1.89	0.998	1.5×10^{-11}	5.0×10^{-11}

[a] Average of three determinations.

mulation time period should be chosen according to the concentration level of THD in the investigated sample (i.e. the higher the concentration, the shorter the accumulation time is).

3.4. Analytical applications

3.4.1. Validation of the analytical procedure

Validation of the proposed procedure for the quantitative assay of THD was examined via evaluation of the limit of detection (LOD), limit of quantitation (LOQ), repeatability, recovery, selectivity, robustness and ruggedness. Calibration curves within the concentration levels of 10^{-9} and 10^{-7} M THD were attempted following different accumulation time periods (0–300 s). The regression equations associated with the calibration plots exhibited good linearity (Table 1) that supported the validation of the proposed procedure for quantitation of terazosin drug. The limits of detection (LOD) and quantitation (LOQ) were calculated using the relation $k(\text{S.D.}_a)/b$ [42], 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. Both LOD and LOQ values, shown in Table 1, confirmed the high sensitivity of the proposed procedure compared to all the reported methods for analysis of terazosin.

The repeatability of the results obtained by means of the proposed SWAdCS voltammetric procedure was examined by performing five replicate measurements for 1×10^{-8} M THD following pre-concentration for 30 s. A mean recovery of 99 ± 0.8% ($n = 5$) was achieved, that indicated high precision of the proposed procedure for assay of the drug.

The selectivity of the optimized procedure for assay of THD was examined in the presence of some common excipients (usually present in formulations, e.g. starch, gelatin, lactose, talc and magnesium stearate). The mean recovery for 1×10^{-8} M THD was found to equal 98 ± 0.8% ($n = 5$), that showed no significant interference from excipients. Accordingly, the proposed procedure can be considered selective.

The robustness [43] of results of a procedure is the ability to remain unaffected by small changes in its operational parameters such as pH and accumulation potential. In the present work, this was examined by studying the effect of small variation of pH (pH 5–6) and accumulation potential ($E_{acc} = -0.9$ to -1.1 V). As shown in Table 2, the recovery values were not significantly affected by these variations and consequently the optimized procedure was reliable for assay of THD and it could be considered robust [43].

The ruggedness [43] of the measurements is defined as the degree of reproducibility of results obtained by analysis of the same sample under variety of normal test conditions such as different laboratories, different analysts, different instruments and different lots of reagents. This was examined by applying the proposed procedure to assay of THD under the same experimental conditions using two potentiostats, PAR—394 (lab 1) and 263A (lab 2), at different elapsed time by two different analysts (Table 2). The results obtained due to lab. (1) to lab. (2) and even day to day were found reproducible, since there is no significant difference between the recovery and S.D. values.

Table 2
Influence of small variations of some of the operational conditions of the proposed procedure on the mean recovery of 5×10^{-8} M terazosin; frequency = 120 Hz, scan increment = 10 mV and pulse-amplitude = 25 mV

Variables	Conditions	R ± R.S.D.% ($n = 3$)
Robustness results		
pH[a]		
5.0	$E_{acc} = -1.0$ V	100.5 ± 0.6
5.5	$t_{acc} = 60$ s	99.0 ± 0.7
6.0		99.0 ± 1
Accumulation potential (E_{acc})[a]		
−0.90 V	pH = 5.5	99.0 ± 1
−1.00 V	$t_{acc} = 60$ s	99.0 ± 0.7
−1.10 V		100.0 ± 0.6
Ruggedness results		
Lab (1): Potentiostat Model 394 (PAR)	pH = 5.5	99.0 ± 0.7
	$E_{acc} = -1.0$ V	
Lab (2): Potentiostat Model 263 A (PAR)	$t_{acc} = 60$ s	102.0 ± 1
Elapsed time[a] (day)		
1	pH = 5.5	99.0 ± 0.7
2	$E_{acc} = -1.0$ V	100.6 ± 1.1
4	$t_{acc} = 60$ s	99.4 ± 0.9

[a] Using Potentiostat Model 394 (PAR).

Table 3
Assay of terazosin in Itrin® tablets by the proposed procedure (t_{acc} = 60 s) and a reported visible spectrophotometric method [11]

Claimed (mg/tablet)	Recovery by the proposed method ($R \pm$ R.S.D.%)[a] (i)	Recovery by a reported method ($R \pm$ R.S.D.%)[a] (i)	Recovery by the proposed method ($R \pm$ R.S.D.%)[a] (ii)
2	98 ± 1 $F = 1.66$ $t = 0.64$	98 ± 1	98 ± 1
5	99 ± 1 $F = 4.27$ $t = 1.22$	100 ± 2	101 ± 1

The theoretical values of F and t-test at 95% confidence limit (for $n_1 = 5$, and $n_2 = 5$) are 6.39 and 2.30, respectively.

[a] Average of five determinations: (i) recovery using the calibration curve method. (ii) Recovery using the standard addition method; via addition of 1.0 mg of bulk terazosin to different concentrations of the drug in solution of Itrin tablets.

3.4.2. Analysis of Itrin® tablets

The developed square-wave adsorptive cathodic stripping voltammetric procedure was applied (t_{acc} = 60 s) for determination of THD in its pharmaceutical formulation (Itrin®—2 and 5 mg tablets) without the necessity for any extraction steps. The recoveries of THD in both dosage forms, based on the average of five replicate measurements are illustrated in Table 3. The results were statistically compared with those obtained by a reported visible spectrophotometric method [11]. Values of F-calculated, F-theoretical, t-calculated and t-theoretical are also included in Table 3. Since the calculated F value did not exceed the theoretical one, there was no significant difference between the proposed and reported methods with respect to reproducibility [44]. Also, no significant difference was noticed between the two methods regarding accuracy and precision as revealed by t value [44]. The accuracy of the proposed procedure was also judged by applying the standard addition method [45].

3.4.3. Analysis of human serum

Fig. 5 illustrates the square-wave adsorptive cathodic stripping voltammograms of successive additions of THD spiked in human serum. The variation of the peak current versus the concentration of the drug was represented by a straight line following the equation: i_p (nA) = 157.4C (nM) + 33 (r = 0.999 and n = 7). The mean recovery of THD in human serum, was found to equal 98 ± 1% (n = 5). The obtained LOD and LOQ values were found to equal 5.3×10^{-11} M and 1.8×10^{-10} M terazosin drug, respectively.

In a trial to prove the utility of the proposed SWAdCS voltammetric procedure in clinical analysis, the procedure was applied to determination of the pharmacokinetic parameters of terazosin drug in blood samples of two healthy male volunteers following an oral administration of a single dose of Itrin®—5 mg. Fig. 6 showed the plasma

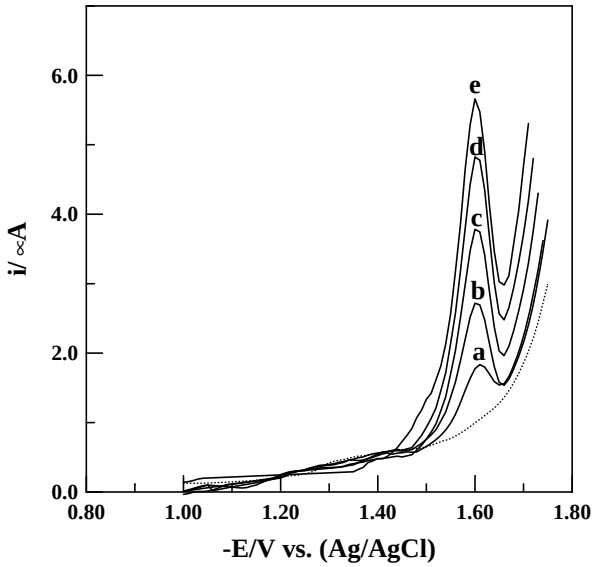

Fig. 5. SWAdCS voltammograms of different concentrations of THD spiked in human serum samples: the dotted line represents the background voltammogram; (a) 1×10^{-8}, (b) 2×10^{-8}, (c) 3×10^{-8}, (d) 4×10^{-8}, and (e) 5×10^{-8} M THD drug; t_{acc} = 300 s at $E_{acc} = -1.0$ V and B–R buffer of pH 5.5. Other square-wave operational parameters are as those given in Fig. 4.

concentration—time profile obtained by means of the proposed procedure at specified intervals. The obtained pharmacokinetic parameters are presented in Table 4, These parameters are: (C_{max}) the maximum measured plasma concentration of drug, (t_{max}) time to reach maximum measured plasma concentration of drug, (AUC_{0-t}) area under the plasma concentration–time profile calculated from time 0 until the last time point t, (K_e) the elimination rate constant and ($t_{1/2}$) the elimination half-life time. The obtained results were favorably compared with those obtained by a reported HPLC method [10], which can be considered as

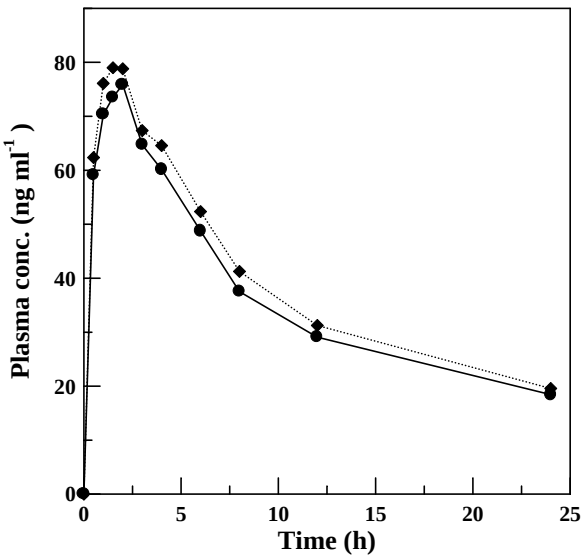

Fig. 6. Mean plasma concentration—time profile for two volunteers following administration of an oral single dose of Itrin®—5 mg THD.

Table 4
Estimated pharmacokinetic parameters of terazosin in plasma of two healthy volunteers following an oral administration of a single dose of 5 mg (Itrin-5 mg tablet)

Parameter (unit)	Volunteer (1)	Volunteer (2)	Mean value	Reported value [10]
C_{max} (ng ml^{-1})	75.88	79.11	77.50	69.0
t_{max} (h)	2.0	1.5	1.75	1.48
AUC (ng h ml^{-1})	588.74	615.80	602.27	511.85
K_e (h^{-1})	0.090	0.086	0.088	0.092
$t_{1/2}$ (h)	11.07	11.57	11.32	10.84

an indication for the reliability of the proposed procedure for assay of terazosin drug in real human plasma samples without a risk of interference from its metabolites.

4. Conclusion

A new highly sensitive, rapid, selective and reproducible square-wave adsorptive cathodic stripping voltammetric procedure for determination of terazosin drug in bulk form, pharmaceutical formulations and human serum was described. The proposed procedure showed clear advantages such as short period of real time of drug analysis and no pre-treatment or time-consuming extraction steps were required prior to the analysis. Moreover, because of its very low limits of detection and quantitation, the proposed procedure could be applied in clinical laboratories and pharmacokinetic studies.

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