CROATICA CHEMICA ACTA
CCACAA, ISSN 0011-1643, e-ISSN 1334-417X
Croat. Chem. Acta **83** (2) (2010) 135–142.
CCA-3403

Original Scientific Article

Trace Detection of Diphenhydramine by Adsorption on a Microelectrode at Flow Injection System by Fast Fourier Transform Continuous Cyclic Voltammetry

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RECEIVED JUNE 24, 2008; REVISED AUGUST 8, 2009; ACCEPTED SEPTEMBER 10, 2009

Abstract. A continuous cyclic voltammetric study of diphenhydramine at gold micro electrode was carried out. Some investigations were also done to find the effects of various parameters on the sensitivity of the proposed method. The experiments were performed under the following conditions: pH = 2, the scan rate = 40 V / s (v), accumulation potential = 500 mV (E), and accumulation time = 0.2 s (t). The drug in phosphate buffer (pH = 2.0) is adsorbed at optimized condition on the surface of electrode, giving rise to change in the current of well-defined oxidation peak of gold in the flow injection system. The proposed detection method is a very fast and appropriate technique for determination of the drug compound in a wide variety of chromatographic analysis methods. Signal-to-noise ratio has significantly increased by application of discrete Fast Fourier Transform (FFT) method, background subtraction and two-dimensional integration of the electrode response over a selected potential range and time window.
The linear concentration range was from 4.0×10^{-7} to 1.0×10^{-11} mol dm^{-3} ($r = 0.9987$) with a limit of detection and quantitation 5×10^{-12} and 4×10^{-11} mol dm^{-3}, respectively. The method has the requisite accuracy, sensitivity, precision and selectivity to assay diphenhydramine in tablets.

Keywords: diphenhydramine, countinuous cyclic voltammetry, flow injection, fourier transformation

INTRODUCTION

Diphenhydramine, 2-(diphenylmethoxy)-*N*,*N*-dimethylamine, (Figure 1) is a conventional antihistaminic specie in the H1 group (receptor antagonist) that can block most anti-allergic, anti-emetic and anti-tussive drugs found in many pharmaceutical preparations. Like other antihistaminic species, local anaesthetic activities have been observed. It is usually given orally in a preparation of tablet, capsule or syrup. It may be administered by intramuscular or intravenous injections in severe allergies and applied topically for local allergic reactions in preparations of lotion and cream.[1] Various anti-motion-sickness medications, which many of them are available over the counter, are commonly used to ameliorate motion sickness. Many antihistamines dimenhydrinate, meclizine and promethazine have been effective anti-motion-sickness drugs, however, these drugs are antihistamine-H1 receptor antagonists that cause sedation as the most common subjective side effect. Wood and Garybiel demonstrated that D-amphetamine improves tolerance to Coriolis stimulation of the vestibular system. They found that the antihistamines produced an increase in treatment effectiveness and reduced sedation when D-amphetamine was added. However, many anti-motion-sickness drugs, alone or in combination are only partially effective, and their adverse effect cannot be ruled out. Therefore, it is highly desirable to look for a drug that is effective for prevention of motion sickness and which is relatively free of side effect.[2]

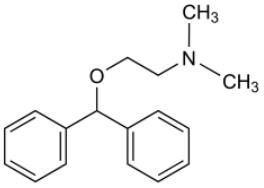

Figure 1. The structure of diphenhydramine.

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Several methods have been proposed for determining diphenhydramine in pharmaceutical preparations including titrimetry, fluorometry electrochemical analysis and spectrophotometry, which a batch-wise has been applied.[1–4] Chromatographic methods have been used such as gas chromatography[5] and HPLC,[6] and the necessity to ensure the quality of pharmaceutical polydrugs like diphenhydramine and consequently the safety and efficacy of the final marketed product, has led to the development and evaluation of new techniques that reduce the time and cost of analysis such as capillary electrophoresis.[7–9]

Some of techniques described above are not simple for direct application a large scale routine analysis and require expensive instruments. As far as voltammetric techniques are considered, they are generally rapid and economical in the determination of some organic and inorganic compounds in aqueous system with a sensitivity range of part-per-billion. Indeed, because of the selective detector, voltammetric techniques are useful for the samples. The use of voltammetric techniques have been further stimulated by the advent of UMEs, due to their steady state currents, higher sensitivity, increased mass transport and their ability to be used in electroanalysis in solution with high resistance.[10] UMEs, for instance, have been applied as sensors in various techniques such as flow injection analysis,[11,12] cardiovascular monitoring and organic compound analysis.[13,14] Now, our work describes a new electrochemical method based on FIA and FFT Cyclic voltammetry for determination of diphenhydramine.

EXPERIMENTAL

Reagents and Materials

Double-distilled deionized water was used for preparation of samples by using analytical grade reagents (Merck Chemicals). The reagents used for preparation of the running buffer or background electrolyte (BGE) solution for flow-injection analysis (0.05 M H_3PO_4 and 1 M NaOH used for adjusting pH of the eluent), were obtained from Merck Chemicals. Diphenhydramine standard powder was a gift from the Center of Quality Control (Tehran, Iran). In all experiments, solutions were made up in the background electrolyte solution, and were used without removal of dissolved oxygen.

Background Electrolyte

The background electrolyte was made by addition of 8.7 mL of phosphoric acid (85 % *w*/*v*) into a 1000 ml volumetric flask and dilution to a constant volume with distilled water. The pH was adjusted to 2 with sodium hydroxide. All solutions were freshly prepared and filtered using a Millipore filter (0.45 μm) each day.

Standards and Sample Solutions

Standard stock solutions

A standard stock solution of diphenhydramine (mass concentration, $\gamma = 1$ mg / ml) was prepared in the distilled water. This solution was protected from light using foil and stored at 4 °C and it is to be stable during this period.

Standard solutions for FIA

Aliquots of standard stock solution of diphenhydramine were dispensed into 10 mL volumetric flasks and the flasks made up to volume with the running buffer to give final concentrations range of 4.0×10^{-7} to 1.0×10^{-11} mol dm^{-3}.

Sample Preparation of Human Urine and Plasma

Plasma was obtained from Tehran University Hospital, Tehran, Iran and kept frozen until use after gentle thawing. Urine was also collected from healthy volunteers (males, around 30 years old).

One mL of untreated urine containing 10 μg / mL diphenhydramine was placed into a 50 mL volumetric flask and diluted with water to the mark. A 1 mL of this solution was diluted with pH = 2 buffer solution to 20 mL into a volumetric flask. Then 20 μL aliquot was injected into the FIA system.

For the determination of diphenhydramine in plasma, 100 μL aqueous diphenhydramine solutions (100 ng / mL) were added to 1 mL of untreated plasma. The mixture was vortexes for 30 s. In order to precipitate the plasma proteins, the plasma samples were treated with 20 μL perchloric acid $HClO_4$ 20 %. After that, the mixture was whirled for a further 30 s and then centrifuged at 6000 rpm for 5 min. Then 20 μL aliquot of the obtained supernatant was injected into the FIA system.

Electrode Preparation

Gold UMEs (with a 25μm in diameter) were prepared as described in previous papers[15–20] Before each experiment the electrode surface was polished for 1 minute using extra fine carborundum paper and then for 10 minutes with 0.3 μm alumina. Prior to being placed in the cell the electrode was washed with water. In all measurements, an Ag(s)|AgCl(s)|KCl(aq, 1 mol dm^{-3}) reference electrode was used. The auxiliary electrode was made of a Pt wire, 1 cm length and 0.5 mm in diameter.

Flow Injection Setup

The equipment for flow injection analysis included a 10 roller peristaltic pump (UltrateckLabs Co., Iran) and a four way injection valve (Supelco Rheodyne Model 5020) with a 50 μL sample injection loop. Solutions were introduced into the sample loop by means of a plastic syringe. The volume of the cell was 100 μL. In all experiments described in this paper, the flow rate (u) of eluent solution was 2 mL / min.

Data Acquisition and Processing

All of the electrochemical experiments were done using a setup comprised of a PC PIV Pentium 900 MHz microcomputer, equipped with a data acquisition board (PCL-818HG, Advantech. Co.), and a custom made potentiostat. All data acquisition and data processing programs were developed in Delphi 6® program environment.

RESULTS AND DISCUSSION

In Figure 2 the diagram of applied waveform potential during cyclic voltammetric measurements is shown. The potential waveform consists of three parts; a) Potential steps, E_{c1} and E_{c2} (which are used for oxidizing and reduction of the electrode surface, respectively), by which electrochemical cleaning of the electrode surface takes place, b) E_c, where accumulation of analyte takes place, c) the final, part potential ramp, in which current measurements take place.

Signal Calculation in this method is established based on the integration of net current changes over the scanned potential range. It must be noted that in this case, the current changes (result of injected analyte) at the voltammograms can be caused by various processes, which take place at the electrode surface. Those processes include; a) oxidation and reduction of adsorbed analyte, and b) inhibition of oxidation and reduction of the electrode surface by the adsorbed analyte. Indeed, in order to see the influence of the adsorbed analyte on the oxidation and reductions peaks of the gold surface, the scan rate must be set at very high rates (*e.g.* $v > 20$ V / s).

However, during the scan, some of the adsorbed analyte molecules are desorbed. Depending on the rate of those processes and scan rate, the amount of the desorption analyte molecule (during the scan) can be changed.[21–29] The important point here is that part of the adsorbed analyte molecule still remaining on the electrode surface that can inhibit the red/ox process of the electrode surface. In this method, ΔQ is calculated based on the all current changes at the CVs. However, the selectivity and sensitivity of the analyte response expressed in terms of ΔQ strongly depends on the selection of the integration limits. One of the important aspects of this method is application of a special digital filtration, which is applied during the measurement. In this method at the first, a CV of the electrode was recorded and then by applying FFT on the collected data, the existing high frequency noises were indicated. Finally, by using this information, the cutoff frequency of the analog filter was set at a certain value (where the noises were removed from the CV).

Since the crystal structure of a polycrystalline gold electrode, strongly depends on the condition of applied

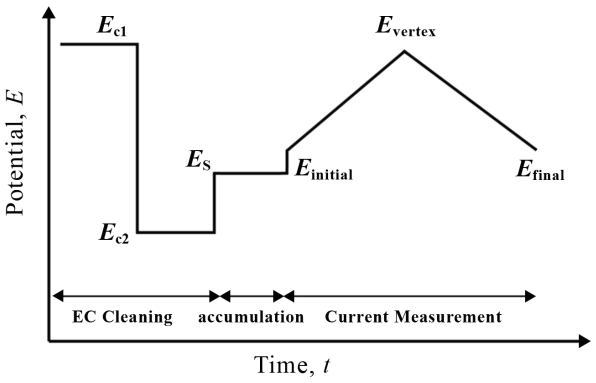

Figure 2. The diagram of the applied potential waveform.

potential waveform,[13] therefore various potential waveforms were examined in order to obtain a reproducible electrode surface (or a stable background signal). In fact, application of cyclic voltammetry for determination of electroactive compound mainly face to low stability of the background signal, due to changes occurring in the surface crystal structure during oxidation, and reduction of the electrode in each potential cycle. In this work, after examination of various potential wave forms, the best potential waveform for obtains a stable background during the measurement was the waveform shown in Figure 2. As mentioned above, in this work, the potential waveform was continuously applied during an experiment run where the collected data were filtered by FFT method before using them in the signal calculation.

The electrochemical oxidation process of gold surface started with electrosorption of hydroxyl ion, which at more positive potentials form gold oxide and undergoes structural rearrangement,[20] The surface oxidation can be initiated by adsorption of water molecule and then at more positive potential AuOH forms leading to the formation of a two-dimensional phase of gold oxide;

$$2Au + 3H_2O \longrightarrow Au_2O_3 + 6e^- + 6H^+ \qquad (1)$$

An example of recorded CVs is shown in Figure 3 (a, b). Figure 3a shows a sequence of CVs recorded during the flow analysis for determination of the drug. The volume of the injection was 50 μL of 5.0×10^{-5} M diphenhydramine (in 0.05 M H_3PO_4) into the eluent solution containing 0.05 M H_3PO_4. The time axis of the graph represents the time of the flow injection experiment. In the absence of diphenhydramine, the shape of the CV curves is typical for a polycrystalline gold electrode in acidic media.[21] Figure 3b shows the absolute current changes in the CVs curves after subtracting the average background 4 CVs (in absent of the analyte). As can be seen, this way of presentation of the electrode response gives more details about the effect of adsorbed

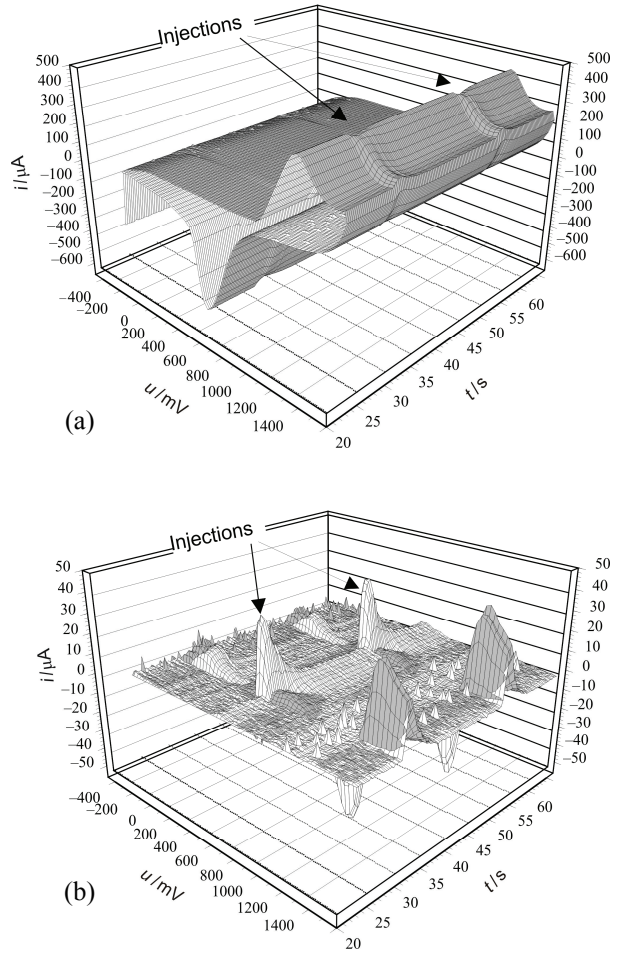

Figure 3. (a) Cyclic voltammograms at Au ultra-microelectrode recorded during the flow injection of 50 μL of 5.0×10^{-5} M of diphenhydramine at optimum conditions. The eluent was 0.05 M H_3PO_4 and the flow rate was 2 mL / min. (b) Curves result from subtracting an average CV (in the absence of analyte) from test of the CVs in (a).

ion on currents of the CV. The curves show that current changes mainly take place at the potential regions of the oxidation and reduction of gold. When the electrode-solution interface is exposed to diphenhydramine, which can adsorbed on the electrode, the oxide formation process becomes strongly inhibited. In fact, the inhibition of the surface process causes significant change in the currents at the potential region, and as a consequence the profound changes in the shape of CVs take place. Universality of the detector in this mode is very advantageous for chromatographic analysis, where a mixture of compounds presents in sample.

It must be noted that, theoretically, in this method, the analyte response can be affected by the thermodynamic and kinetic parameters of adsorption, the rate of mass transport and other means if the component contains hetroatoms such as S or N it thermodynamicly is more suitable for stronger adsorption because these atoms have better interaction with gold electrode orbitals. Furthermore this system the adsorption and desorption have a frequency which make the kinetic of phenomena. So if the component has a vary fast adsorption/desorption the system is not able to detect it. The free energy and the rate of adsorption depend on the electrode potential, the electrode material, and to some extent, on the choice of the concentration and type of supporting electrolyte. By taking points into consideration, in order to achieve maximum performance of the detector, the effect of experimental parameters (such as; pH of the supporting electrolyte, potential and time of the accumulation and potential scan rate) must be examined and optimize.[20–29]

Optimizing the experimental parameters

The effect of eluent pH on performance of the detector was examined the results are shown in Table 1. As shown, the best ΔQ was obtained with pH between 2 and 3. In addition, the results shows that at pH values higher than 9 noises level in the baseline (ΔQ *vs*. *t*), is higher up to 12 % compared to acidic solution.

Also, in order to investigate the influence of scan rates and the eluent flow rate on the sensitivity of the detector response, solutions having a diphenhydramine concentration of 2.0×10^{-7} mol dm^{-3} were injected. At different scan rates (from 10 to 60 V/s) and the eluent flow, the responses of the detector to the injected sample were recorded. The results are presented in Figure 4. As it is clear from the Figure 4, the detector exhibits the

Table 1. pH effect on the microelectrode response

pH	2.1	4	6	8	10	12
ΔQ / μC	350	300	200	195	190	180

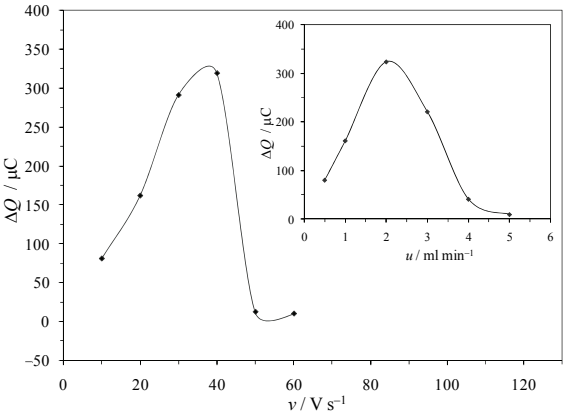

Figure 4. Effect of the sweep rate, *v*, on the response of the Au microelectrode to injections of 2.0×10^{-7} M diphenhydramine in 0.05 M H_3PO_4.

maximum sensitivity at 40 V / s of scan rate and 2 mL / min of the flow rate. The effects of the sweep rate on the detection performance can be taken into consideration from three different aspects: first, speed in data acquisition, second, kinetic factors of adsorption of the diphenhydramine, and finally the flow rate of the eluent which controls the time window of the solution zone in the detector. The main reason for application of high scan rates, is prevention from desorption of the adsorbed diphenhydramine during the potential scanning, (because under this condition, the inhibition outcome of the adsorbed diphenhydramine on the oxidation process can take place.

Indeed, the use of this detection method in conjunction with fast separation techniques such as capillary electrophoresis also requires the employment of high scan rates. From this point of view, checking how the sensitivity of the method is affected by the sweep rate is necessary. To detect the amount of the adsorbed analyte on the electrode surface, high sweep rates must be employed, so that the potential scanning step is short in comparison with the accumulation period. Notably, when the accumulation of diphenhydramine occurs at a potential very larger or smaller than E_i, more better sensivity (low detection limit) will be obtained. However, sensitivity of the detection system mainly depends on the potential sweep rate mainly due to kinetic factors in adsorption, and instrumental limitations.

Due to this fact that any changes in the parameters related to adsorption process shows a strong dependence upon the applied potential and the time and the potential of accumulation strongly affect the sensitivity of the measurement. Therefore, the influence of the accumulation potential and time on the response of the method for the injection of a 2.0×10^{-7} M solution of diphenhydramine, in 0.05 M H_3PO_4, was studied. Figure 5 shows the detector response over the accumulation potential ranges, E from −400 to 900 mV and accumulation time range, t, from 0.1 to 1.0 s. Based the figure accumulation potential 500 mV at time 200 ms was chosen as the optimum condition. Because, the surface of the electrode becomes saturated with the diphenhydramine within 200 s time window

On the electrode, the accumulation of diphenhydramine takes place during the accumulation step (assuming that an appropriate potential is selected). In fact, the difference in the time of saturation of the various compounds can be related to the existing differences in their kinetics of the electron transfer and mass transport. As mentioned above, the surface of the gold microelectrode is very small, and in a very short time the surface of the electrode can be saturated.

Validation

The investigation of validity was performed with respect of linearity, limit of detection (LOD), precision, accuracy, ruggedness/robustness, recovery and selectivity.[30–32]

Linearity

Linear regression analysis of least square method was used to evaluate the linearity.[33,34] The linear range of 0.4−0.00001 μmol dm^{-3} was conspicuous in constructed calibration curve. Peak areas of diphenhydramine were plotted versus its concentration and linear regression analysis performed on the resultant curve. A correlation coefficient of R = 0.9987 with RSD values ranging from 0.14 to 3.65 % across the concentration range stu-died were obtained following linear regression analysis. Typically, the regression equation for the calibration curve was found to be $y = 0.7875\,x + 188.19$. Figure 6 shows the calibration graph that obtained for the monitoring of diphenhydramine in a 0.05 M H_3PO_4.

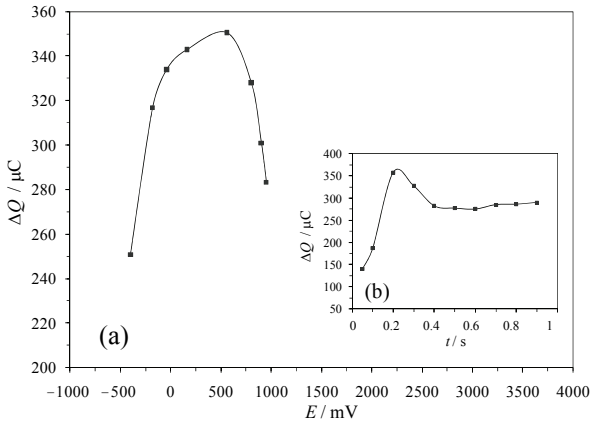

Figure 5. Effect of accumulation potential (a) and the effect of accumulation time (b) on the electrode response to injections of 2.0×10^{-7} M diphenhydramine in 0.05 M H_3PO_4.

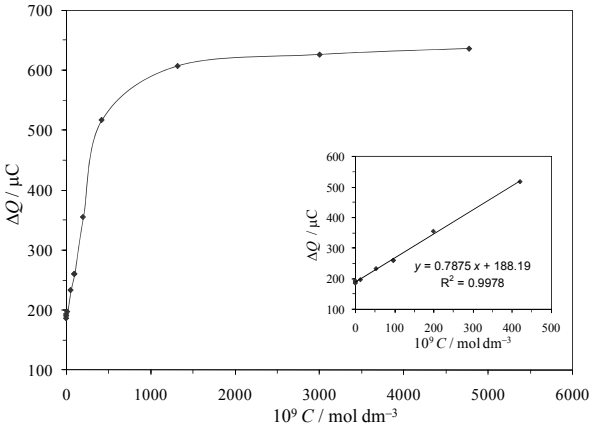

Figure 6. Calibration curves obtained for diphenhydramine on the Au microelectrode in 0.05 M H_3PO_4.

Table 2. Influence of the changes in the experimental conditions on the performance of the FIA system

Parameter	Modification	Diphenhydramine recovery (expressed in %)
pH	1.8	100.3
	2.0	101.1
	2.3	99.9
	3.0	99.8
flow rate, u / ml min^{-1}	1.8	100.6
	2.0	101.3
	2.2	99.9
buffer composition, $C_{b.c.}$ / mol dm^{-3}	0.04	97.9
	0.05	101.6
	0.06	100.4
$\theta_{lab.}$ / ºC	20	101.3
	25	99.9
	30	100.8

Table 3. Application of the proposed method to the determination of diphenhydramine in spiked human plasma and urine

$\gamma_{diph.}$/ ng mL^{-1} (added)	$\gamma_{diph.}$/ ng mL^{-1} (interpolated)	RSD (in %)	RE (in %)
10 (plasma)	9.89 ± 0.2	1.5	1.02
100 (urine)	101.1 ± 0.5	1.0	1.4

Data obtained from five replicates at each concentration. Interpolated concentration data expressed as mean ± SD.

Limit of Detection

The lowest amount of the analyte that may be detected to produce a response is defined as LOD. Based on the calculation of standard deviation of the response (δ) and the slope (S) of the calibration curve at the levels approaching the limits according to equation LOD = 3.3 (δ/S),[35] the limit of detection that found to be 0.005 nmol dm^{-3}, was approved.

Precision

Precision was investigated by injecting nine replicate samples of each of the 0.2, 0.005 and 0.0005 μmol dm^{-3} standards. The final mean concentrations were found to be 0.19, 0.006 and 0.0005 μmol dm^{-3} with associated RSDs of 0.05, 0.2 and 1.0 %, respectively. The inter-day precision was assessed by injecting the same three concentrations for 3 consecutive days, resulting in mean diphenhydramine concentrations of 0.2, 0.005 and 0.0006 μmol dm^{-3} with associated RSD. values of 0.6, 0.7 and 0.9 %, respectively.

Accuracy

Interpolating of replicate (n = 6) peak areas of three accuracy standards (0.2, 0.005 and 0.0005 μmol dm^{-3}), the accuracy of the method was assessed by a calibration curve prepared as previously described. In each case, the percent relevant error and accuracy was calculated. The resultant concentrations were 0.2 ± 0.01, 0.005 ± 0.0001 and 0.0006 ± 0.00003 μmol dm^{-3} with relevant error percentage of 0.6, 0.9 and 0.85 %, respectively.

Ruggedness

Comparing of the intra- and inter- day assay results for tow diphenhydramine analytes was used to check the ruggedness of the method. The RSD values for intra- and inter- day assays of diphenhydramine in the cited formulations performed in the same laboratory by the two analysts did not exceed 3.5 %, this way the ruggedness of the method is illustrated. The robustness was also examined while the parameters values (the pH of the eluent, the flow rate, the buffer composition and the laboratory temperature) were being slightly changed.[35] According to Table 2, the diphenhydramine recovery percentages were satisfactory in most cases, without presenting any important changes during the alteration of the critical parameters.

Recovery

In order to perform the recovery test, diphenhydramine standard powder at concentration of 1.0×10^{-8} mol dm^{-3} was added to samples of known amounts at 0.2, 0.005 and 0.0005 μmol dm^{-3} and then the voltammograms were recorded. The assay was repeated (n = 9) over 3 consecutive days to obtain intermediate precision data. The resultant RSD for this study was found to be 0.9 % with a corresponding percentage recovery value of 99.95 %.

Selectivity

Standard solutions of diphenhydramine, was exploited to determine the sensitivity of the method in the presence of formulation components. As expected, the responses were not different from that obtained in the calibration. We found that the formulation compounds have no interference to the determination of diphenhydramine due to the well fixed optimized parameters.

Determination of Diphenhydramine in Real Samples

The voltamograms were recorded according to the above recommended procedure. The voltamograms of samples

Table 4. Influence of the changes in the experimental conditions on the performance of the FIA system

Method	LOD	Ref. No.
Native fluorescence flow-through optosensor in Phamaceuticals	0.02 mg/ml	1
LC-MS/MS	1 ng/ml	2
Flow injection spectrophotometriy	75 mg/ml	3
spectrophotometriy	0.03 mg/ml	4
GC	0.4 mg/ml	5
Atomic emission spectrometric based on formation of ion-associates with ammonium reineckate	2.7 mg/ml	6
Capillary electrophoresis	2.5 mg/ml	7
Indirect Atomic Absorption based on formation of ion-associates with Potassium tetraiodometrcurate	5.6 mg/ml	36
Nonaqueous Capillary electrophoresis	0.6 mg/ml	37
FFTCV	1.0 pg/ml	This work

without diphenhydramine do not show any signal that can interfere with the direct determination, so external calibration can be used. The result has been shown in Table 3. The major advantage of the method as applied to plasma and urine is that no prior extraction step is required.

Comparison of the Method's Sensitivity and Other Previously Reported Methods

The detection limit of the proposed method is compared with the other reported methods. The results are shown in Table 4. In comparison to other reported methods, the sensitivity of this method is considerably more than previously reported methods. As can be seen in Table 4, the detection limit of the method is 1000 times lower than the most sensitive reported method.[1–7,36,37]

Acknowledgment. The authors express their appreciation to the Research Council of the University of Tehran for the financial support of this work.

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SAŽETAK

Određivanje tragova difenhidramina adsorbiranih na površini mikroelektrode kontinuiranom cikličkom voltametrijom s brzim Fourierovim transformacijama u protočnom sustavu s ubrizgavanjem analita

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Opisana je kontinuirana ciklička voltametrija difenhidramina na zlatnoj mikroelektrodi. Istraženi su utjecaji parametara pobude na osjetljivost metode. Optimalni uvjeti su fosfatni pufer (pH = 2), brzina promjene potencijala (v) 40 V / s, potencijal akumulacije 0,5 V i trajanje akumulacije 0,2 s. Adsorpcija analita na površinu zlatne elektrode mijenja odziv aksidacije zlata u protočnom sustavu s ubrizgavanjem uzorka. Opisana elektroanalitička metoda je pogodna za određivanje difenhidramina različitim kromatografskim tehnikama. Korištenjem brze Fourierove transformacije povećava se omjer signala i šuma odbijanjem osnovne struje i dvodimenzionalnom integracijom elektrodnog odziva u odabranim područjima potencijala i vremena. Ovisnost odziva o koncentraciji analita linearna je u rasponu od 1×10^{-11} do 4×10^{-7} mol dm^{-3}, a granice detekcije i kvantifikacije su 5×10^{-12} mol dm^{-3} i 4×10^{-11} mol dm^{-3}. Predloženom metodom određen je sadržaj difenhidramina u tabletama