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Article

Novel Method for Determination of Trace Amounts of Citalopram in Tablets by Fast Fourier Continuous Cyclic Voltammetry at Au Microelectrode in Flowing Solutions

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Neste trabalho, é apresentada a técnica voltamétrica cíclica contínua com transformada de Fourier rápida, para o monitoramento de quantidades ultra-traço de citaloprama em um sistema de injeção em fluxo. A forma de onda de potencial consiste em pulsos de potencial para limpeza e uma rampa de potencial foi continuamente aplicada a um microeletrodo (disco de ouro, com raio de 12,5 μm). O método de detecção proposto tem algumas vantagens, sendo as maiores delas apresentadas a seguir: primeiro, não é mais necessário remover o oxigênio da solução do analito e segundo, esta é uma técnica muito rápida e apropriada para determinação de fármacos, em uma grande variedade de métodos cromatográficos de análise. O método foi linear no intervalo de concentração entre 7 e 116 pg mL^{-1} (r=0,9960), com um limite de detecção e quantização de 2,3 e 7 pg mL^{-1}, respectivamente. O método é preciso, sensível, e seletivo para determinação de citaloprama em pastilhas. As influências do pH do eluente, do potencial de acumulação, velocidade de varredura, e tempo de acumulação na determinação do citaloprama foram considerados. O método proposto foi aplicado para a determinação de citaloprama em preparações farmacêuticas.

In this work, fast Fourier transform continuous cyclic voltammetric technique for the monitoring of ultra trace amounts of citalopram in a flow-injection system has been presented. The potential waveform, consisting of the potential steps for cleaning, stripping and potential ramp, was continuously applied on an Au disk microelectrode (with a 12.5 μm in radius). The proposed detection method has some advantages, the greatest of which are as follows: first, it is no more necessary to remove oxygen from the analyte solution and second, this is a very fast and appropriate technique for determination of the drug compound in a wide variety of chromatographic analysis methods. The method was linear over the concentration range of 7 - 116 pg mL^{-1} (r = 0.9960) with a limit of detection and quantitation 2.3 and 7 pg mL^{-1}, respectively. The method has the requisite accuracy, sensitivity, precision and selectivity to assay citalopram in tablets. The influences of pH of eluent, accumulation potential, sweep rate, and accumulation time on the determination of the citalopram were considered. The proposed method was applied to the determination of citalopram in a pharmaceutical preparation.

Keywords: citalopram, fast Fourier transformation, flow injection analysis, Ultra microelectrode, cyclic voltammetry

Introduction

Citalopram (CIT) is one of the widely used anti-depressants of the selective serotonin reuptake inhibitors (SSRI) for the treatment of various affective disorders. It is active not only against major depression, but also anxiety, panic, obsessive compulsive disorder pathological laughing and crying. Its pharmacological effect is mainly due to the *S*-(+)-CIT enantiomer (escitalopram) while *R*-(“)-CIT considered to be inactive.[1] The antidepressants citalopram (CIT) belong to the class of selective serotonin (5-hydroxy-tryptamine; 5-HT) reuptake inhibitors (SSRIs). Therefore, SSRIs are currently widely prescribed medications. Although the toxicity of the SSRIs comparatively low, there have been case reports of capillary gas chromatography, Liquid-phase micro-extraction capillary electrophoresis (LPME-CE), micellar electrokinetic capillary chromatography (MEKC), High speed HPLC methods.[1-10] Voltammetric techniques are very rapid and economical in the determination of some organic

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and inorganic compounds in aqueous systems with a sensitivity range of parts-per-billion. Indeed, because of the movement of the analyte zone in an electrochemical flow cell in flowing solutions, the application of techniques like this require fast accumulation of the analyte and fast potential sweeping (which is not appropriate for large electrodes).[11,12] 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 electro-analysis in solutions with a very high resistance.[13] UMEs have, for instance, been applied as sensors in various techniques such as flow injection analysis.[14-16]

Now, our work describes a new electrochemical method based on FIA and FFT Cyclic voltammetry for determination of citalopram.

Experimental

Reagents

All solutions were prepared in double-distilled deionized water using analytical grade reagents. The reagents used to prepare the eluent solution for flow-injection analysis were obtained from Merck Chemicals. In all of the experiments, solutions were made up in the background electrolyte solution, and were used without removal of dissolved oxygen. Citalopram hydrobromide standard powder was a gift from the Center of Quality Control of Food and Drug (Tehran, Iran). Citalopram hydrobromide tablets containing a label claim of 10 mg citalopram that was purchased from a local pharmacy. In all experiments, solutions were made up in the background electrolyte solution, and were used without removal of dissolved oxygen.

Background electrolyte (BGE)

The running buffer or BGE was made by addition of 8.7 mL of phosphoric acid (85% m/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 and 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 citalopram (1 mg mL^{-1}) was prepared in distilled water. This solution was protected from light using aluminum foil and stored at 4 °C for 5 days and was found to be stable during this period.

Standard solutions for FIA

Aliquots of standard stock solution of citalopram were dispensed into 10 mL volumetric flasks and the flasks made up to volume with the running buffer to give final concentrations range of 7-116 pg mL^{-1}.

Assay sample preparation

Twenty tablets were weighed, finely powdered and portions equivalent to 10 mg citalopram were transferred into 1000 mL volumetric flask; 500 mL distilled water was added, shaken thoroughly to dissolve, made up to volume and mixed well. Suitable aliquots of solution were filtered through a Millipore filter (0.45 μm). 100 μL of the filtered solution was diluted with distilled water in a 100 mL volumetric flask. Then 100 μL of the resulting solution was added to a 10 μL volumetric flask and made up to volume with 0.05 mol L^{-1} phosphoric acid to yield starting concentration of 100 pg mL^{-1}.

Electrode preparation

Gold UMEs (with a 12.5 μm, in radius) were prepared by sealing metal micro-wires (Good fellow Metals Ltd., UK) into a soft glass capillary. The capillary was then cut perpendicular to its length to expose the wire. Electrical contacts were made using silver epoxy (Johnson Matthey Ltd., UK). 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 L^{-1}) 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 electrochemical cell used in flow-injection analysis is shown in Figure 1. The volume of the cell was 100 μL. In all experiments described in this paper, the flow rate of eluent solution was 3 mL min^{-1}.

Data acquisition and processing

All of the electrochemical experiments were done using a setup comprised of a PC PIV Pentium 900 MHz

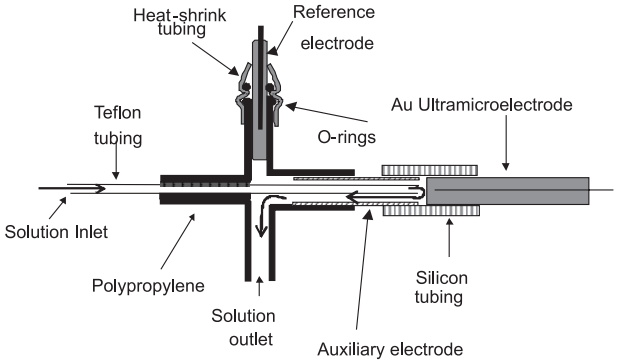

Figure 1. Diagram of the electrochemical cell.

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.

In Figure 2, the diagram of applied waveform potential during cyclic voltammetric measurements is shown. The potential waveform consists of three parts; (*i*) 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, (*ii*) E_c, where accumulation of analyte takes place, (*iii*) the final, part potential ramp, in which current measurements take place.

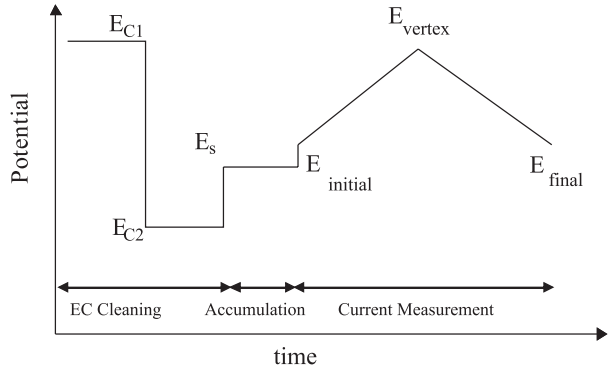

Figure 2. Diagram of the applied potential waveform.

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; (*i*) oxidation and reduction of adsorbed analyte, and (*ii*) 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. >20 V s^{-1}).

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. 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.[17-21] 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 potential waveform,[14] 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 obtain 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 formation of gold oxide and undergoes structural rearrangement,[22] The surface oxidation can be initiated by adsorption of water molecules and then at more positive potential AuOH forms leading to the formation of a two-dimensional phase of gold oxide:

$$Au(H_2O) \longrightarrow AuO + 2e^- + 2H^+ \qquad (1)$$

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 of 50 μL of 1.0×10^{-6} mol L^{-1} citalopram (in 0.05 mol L^{-1} H_3PO_4) into the eluent solution containing 0.05 mol L^{-1} H_3PO_4. The time axis of the graph represents the time of the flow injection experiment. This axis represents the total time of one run of experiment with a continuous applying of the potential waveform, which obtained graph containing a number of CVs of oxidation and reduction of the working electrode in the presence and absence of the analyte.

As shown clearly in three dimensional graphs in Figure 3a each injection of citalopram solution, only cause a decline in the oxidation/reduction peak of gold in acidic media, however, the shape of CVs in the absence of citalopram is typical for a polycrystalline gold electrode in acidic media.[23] Figure 3b shows the absolute current changes in the CVs curves after subtracting the average background 4 CVs (in absence of the analyte). This operation results one maximum and minimum for each injection (e.g. at the injection times 20, 80 and 140). As can be seen, this way of presentation of the electrode response gives more details about the effect of adsorbed 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 citalopram, 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 is present in sample.

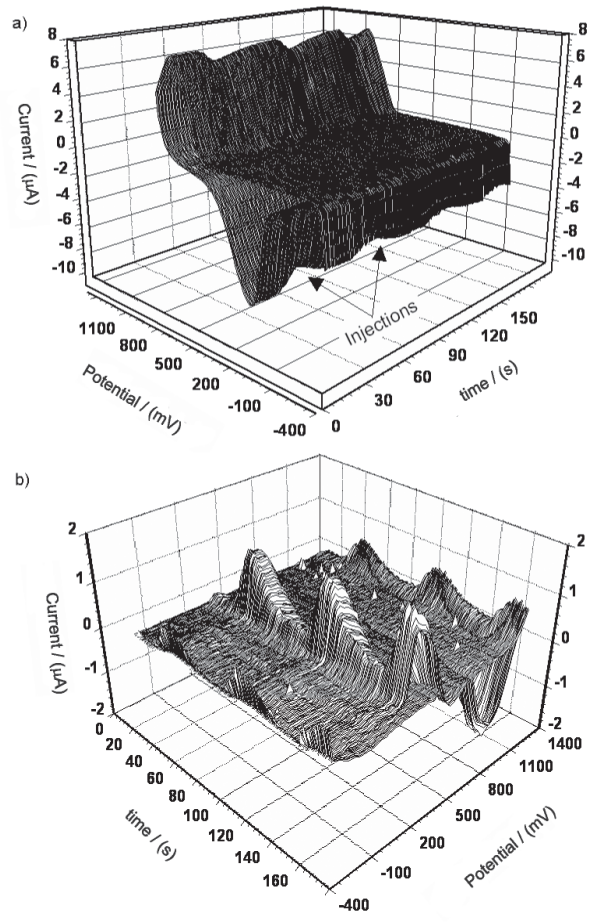

Figure 3. (a) Cyclic voltammograms at Au ultra-microelectrode recorded during the flow injection of 50 μL of 1.0×10^{-6} mol L^{-1} of citalopram at optimum conditions. The eluent was 0.05 mol L^{-1} H_3PO_4 and the flow rate was 3 mL min^{-1}. (b) Curves result from subtracting an average CV (in the absence of analyte) from test of the CVs in (a).

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 electrochemical behavior of the adsorbed species. 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 optimized.

Result and Discussion

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 S/N ratio was obtained between pH 2-3. In addition, the results shows that at pH values higher than 9 noises level in the baseline (ΔQ *vs.* time), 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 concentration of 9.0×10^{-9} mol L^{-1} citalopram were injected. At different scan rates (from 10 to 200 V s^{-1}) 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 maximum sensitivity at 70 V s^{-1} of scan

Table 1. Effect of pH on the response of microelectrode

pH	2.1	4	6	8	10	12
S/N	50	40	32	30	28	45

rates and 3.0 mL min^{-1} 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 citalopram 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 citalopram during the potential scanning, (because under this condition, the inhibition outcome of the adsorbed citalopram on the oxidation process can take place).

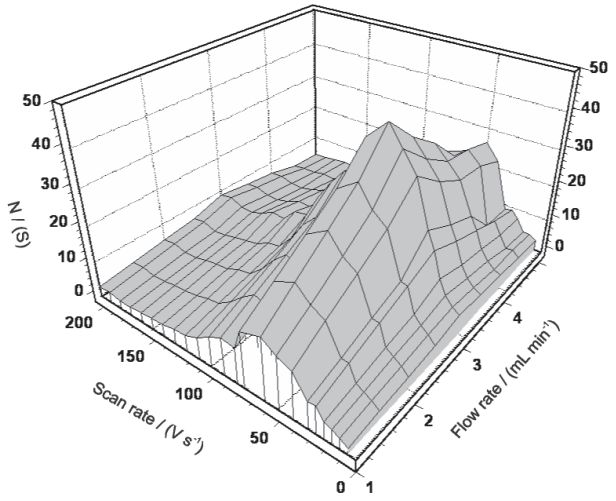

Figure 4. Effect of the sweep rate and effect of accumulation potential on the response of the Au microelectrode to injections of 9.0×10^{-9} mol L^{-1} citalopram in 0.05 mol L^{-1} H_3PO_4.

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 citalopram occurs at a potential that is very larger or smaller than E_i, this is very significant in this detection method. 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 solution of 9.0×10^{-9} mol L^{-1} citalopram, in 0.05 mol L^{-1} H_3PO_4 was studied. Figure 5 shows the detector response over the accumulation potential ranges -200 to 400 mV and accumulation time range from 0.05 s to 1.0 s. Based the figure accumulation potential 200 mV at 700 ms was chosen as the optimum condition. Because the surface of the electrode becomes saturated with the citalopram within 700 ms time window. In the case of more concentrated analyte (more than 9.0×10^{-9} mol L^{-1}) due to the very small surface area of the used gold microelectrode the saturation phenomena happen in a very short time (less than 700 s) and the correlation between concentration and sensitivity will be decreased drastically. In the other hand, in the case of using concentrated analyte the sensitivity of the detection system is independent of concentration of the analyte. Thus we have to use only very diluted analyte.

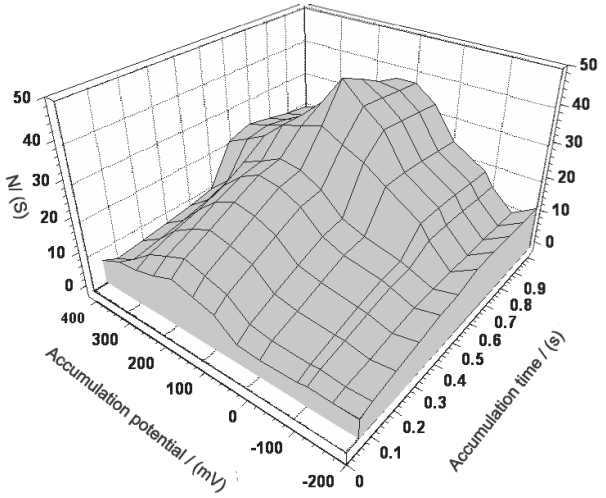

Figure 5. Effect of accumulation time on the electrode response to injections of 9.0×10^{-9} mol L^{-1} citalopram in 0.05 mol L^{-1} H_3PO_4.

On the electrode surface, the accumulation of citalopram 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 ultramicroelectrode is very small, and in a very short time the surface of the electrode can be saturated.

Validation

The method was validated with respect of linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, ruggedness/robustness, recovery and selectivity.[24-26]

Linearity

The Linearity was evaluated by linear regression analysis, which calculated by the least square regression

method.[27,28] The calibration curves constructed for citalopram were linear over the concentration range of 7-116 pg mL^{-1}. Peak areas of citalopram were plotted versus its concentration and linear regression analysis performed on the resultant curve. A correlation coefficient of R=0.9959 with % R.S.D. values ranging from 0.23-3.87% across the concentration range studied were obtained following linear regression analysis. Typically, the regression equation for the calibration curve was found to be Y = 151X + 6.9899**.** Figure 6 shows the calibration graph that obtained for the monitoring of citalopram in a 0.05 mol L^{-1} H_3PO_4.

LOQ and LOD

The LOD was measured as the lowest amount of the analyte that may be detected to produce a response which is significantly different from that of a blank. Limit of detection was approved by calculations based on the 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).[29] The LOD for citalopram was 2.3 pg mL^{-1}. The LOQ was measured as the lowest amount of analyte that can be reproducibly quantified above the baseline noise, for which triplicate injections resulted in a RSD ≤ 1.59%. A practical LOQ giving a good precision and acceptable accuracy was found to be 7 pg mL^{-1}.

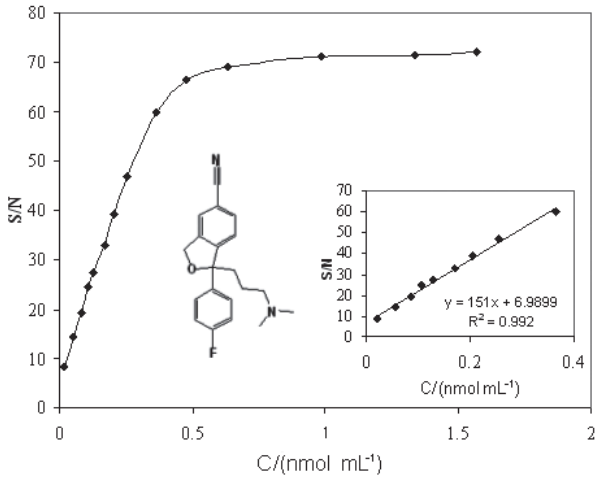

Figure 6. Calibration curves obtained for citalopram on the Au microelectrode in 0.05 mol L^{-1} H_3PO_4.

Precision

Precision of the assay was investigated with respect to both repeatability and reproducibility. Repeatability was investigated by injecting nine replicate samples of each of the 7, 50 and 116 pg mL^{-1} standards where the mean concentrations were found to be 7.25, 50.45 and 116.29 with associated % R.S.D.'s of 3.82, 1.21 and 0.31, respectively. Inter-day precision was assessed by injecting the same three concentrations over 3 consecutive days, resulting in mean concentrations of citalopram of 7.28, 50.65 and 116.43 pg mL^{-1} and associated % R.S.D. of 3.69, 2.21 and 1.11%, respectively.

Accuracy

Accuracy of the assay was determined by interpolation of replicate (n = 6) peak areas of three accuracy standards (7, 50 and 116 pg mL^{-1}) from a calibration curve prepared as previously described. In each case, the percent relevant error and accuracy was calculated. The resultant concentrations were 7.27 ± 0.26 pg mL^{-1}, 50.6 ± 0.66 pg mL^{-1}, and 116.4 ± 0.38 pg mL^{-1} with percent relevant errors of 3.81, 1.24 and 0.31%**,** respectively.

Ruggedness

The ruggedness of the method was assessed by comparison of the intra- and inter-day assay results for citalopram undertaken by two analysts. The % R.S.D. values for intra- and inter- day assays of citalopram in the cited formulations performed in the same laboratory by the two analysts did not exceed 3.9%, thus indicating the ruggedness of the method. Also the robustness of the method was investigated under a variety of conditions such as small changes in the pH of eluent, in the flow rate, in the buffer composition and in the laboratory temperature.[30] As can be seen in Table 2, the percent recoveries of citalopram were good under most conditions and did not show a significant change when the critical parameters were modified.

Recovery

A known amount of Citalopram potassium standard powder was added to samples of tablets, which was then

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

Parameter	modification	Citalopram / (% recovery)
pH	1.8	101.2
	2	101.4
	2.3	99.9
	3.0	100.9
flow rate / (mL min^{-1})	2.8	100.6
	3.0	101.3
	3.2	99.9
buffer composition / (mol L^{-1})	0.04	98.9
	0.05	101.2
	0.06	100.5
Lab. Temperature / (ºC)	20	99.9
	25	100.1
	30	101.2

Table 3. Comparison between the limit of detection of the proposed method with the other reported methods

Reference	Method	LOD / (μg L^{-1})
1	LC method with UV and polarimetric detectors connected in series	500
2	HPLC–MS/ESI	0.3
3	Capillary electrophoresis	60
4	HPLC method with UV detection	16
5	Capillary gas chromatographic method with flame ionization detection (FID)	27.5
6	Liquid-phase micro extraction capillary electrophoresis(LPME-CE)	1600
7	Micellar electro kinetic capillary chromatography (MEKC) with diode array detection (DAD)	10
8	High speed HPLC	0.96
9	HPLC	25
10	HPLC	6.25
This method	FFTCV	0.0023

extracted, diluted and analyzed as previously described. The final nominal concentration of citalopram was found to be 119.8 pg mL^{-1}. The assay was repeated (n=9) over 3 consecutive days to obtain intermediate precision data. The resultant % R.S.D. for this study was found to be 1.93% with a corresponding percentage recovery value of 99.83%.

Selectivity

The selectivity of the method was checked by monitoring standard solutions of citalopram in the presence of formulation components. The responses were not different from that obtained in the calibration. Hence, the determination of citalopram in this formulation is considered to be free from due to formulation components.

Assay of tablets

The method developed in the present study was applied for the determination of citalopram in tablets from the Iranian market. The results showed a percent recovery of 99.91% and a R.S.D. of 1.87%.

Comparison of the sensitivity of the method and other previously reported methods

Table 3 compares the limit of detection of the proposed method with the other reported methods. As it is obvious, the sensitivity of the method is superior to all previously reported methods. The data in Table 3 reveals that the detection limit of the method is about 400 times lower than the most sensitive reported method.

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