Scientific Paper

Sub-second FFT Continuous Stripping Cyclic Voltammetric Technique as a Novel Method for Pico-level Monitoring of Imipramine at Au Microelectrode in Flowing Solutions

Parviz Norouzi*, Mohammad Reza Ganjali, Behrouz Akbari-Adergani

Center of excellence in electrochemistry, Faculty of chemistry, University of Tehran, Tehran, Iran

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Abstract

A novel method for the determination of imipramine in flow-injection systems has been developed. The method was used for the fast determination of imipramine in its pharmaceutical formulations and biological samples. The developed technique is very simple, precise, accurate, time saving and economic as compared to all previously reported methods. The effects of various parameters on the sensitivity of the method were investigated. The best performance was obtained with the conditions, pH value of 2.0, and sweep rate value of 60 V s^{-1}, accumulation potential of 100 mV and accumulation time of 0.5s. In this work, we introduce a special computer based numerical method, for calculation of the analyte signal and noise reduction. The electrode response was calculated based on partial and total charge exchanges at the electrode surface after subtracting the background current from noise. The waveform potential consisting of potential steps for cleaning and accumulation of analyte, and potential ramp was applied on an Au disk microelectrode (with a radius of 12.5 μm). The method was linear over the concentration range of 14 – 22400 pg mL^{-1} (r = 0.999) with a limit of detection and quantitation 4.55 and 14 pg mL^{-1}, respectively. The method has the requisite accuracy, sensitivity, precision and selectivity to assay imipramine in tablets

Keywords: imipramine, antidepressant, continuous stripping, cyclic voltammetry.

1. Introduction

The tricyclic antidepressants are by far the most commonly prescribed drugs for the treatment of psychiatric patients suffering depressions. The monitoring of the level of the antidepressant drugs in plasma is important for various reasons, because it provides more insight into the relationship between the levels of the drug and its clinical effect. It is also useful for discriminating poor biological availability from fast metabolic degradation or unreliable drug administration to patients and finally it provides valuable information for pharmacokinetic studies.[1-4]

Tricyclic antidepressants (TCAs) have been widely used in clinical practice for the treatment of depression. Patients often show variability in clinical response to these drugs, so each dose must be individually determined for maximum effectiveness and safety. The measurement of serum levels is an effective way of determining if a patient is receiving an adequate therapeutic dose of a specific TCA.[5-7]

5H-Dibenz [b, f] azepine-5-propanamine, 10, 11-dihydro-N, N-Dimethyl-monohydro Chloride(Figure 4), is the most important antidepressant molecular shape. Imipramine is a widely prescribed tricyclic antidepressant that a few suitable methods for precisely quantitating plasma levels of it are available. Many of these methods are either time consuming or lack the sensitivity required to measure the low plasma concentration following single oral doses.[8-18]

In this work we wish to introduce a novel method for the fast and sensitive monitoring of imipramine in tablet dosage forms. To the best our knowledgements, this is the first report on the detection of Imipramine by the fast Fourier Transform cyclic voltammetry.

2. Experimental

2.1. 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. Imipramine hydrochloride working standard powder, (Figure 4) was a gift from the Center of Quality Control of Food and Drug (Tehran, Iran). Imipramine hydrochlorid tablet (Sobhan Daru Co., IRAN), containing a label claim of 10mg

imipramine hydrochloride, that was purchased from a local pharmacy. 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).

2.2. Background electrolyte (BGE)

The running buffer or BGE was made by addition of 8.7 mL of phosphoric acid (85% w/v) into a 1000 mL volumetric flask and 900ml of distilled water was added to the flask. The pH of the resulting solution was adjusted to 2.3 with sodium hydroxide (0.5M) and the resulted buffer diluted with distilled water to the mark. All solutions were freshly prepared and filtered using a Millipore filter (0.45μm) each day.

2.3. Standards and Sample Solutions

2.3.1. Standard stock solutions

A standard stock solution of imipramine hydrochloride (1mg mL^{-1}) was prepared in distilled water. This solution was freshly prepared each day.

2.3.2. Standard solutions for FIA

Aliquots of standard stock solution of imipramine were dispensed into 10 mL volumetric flasks and the flasks made up to volume with the running buffer to give final concentrations range of 14 - 22400 pg mL^{-1}.

2.3.3. Assay sample preparation

Twenty tablets were weighed, finely powdered and portions equivalent to 10 mg imipramine were transferred into 100 mL volumetric flask;50 ml distilled deionized water was added, shaken thoroughly to dissolve, made up to volume and mixed well. Suitable aliquots of solution were filtered through a Millipore filter (0.45μm). 10 µL of the filtered solution was added to a 100 mL volumetric flask and made up to volume with 0.05 mol L^{-1} phosphoric acid to yield starting concentration of 10000 pg mL^{-1}.

2.3.4. Determination of imiperamine in human urine and plasma

1 ml of untreated urine containing 40 ng/ml imiperamine was placed into a 25ml volumetric flask and diluted with water to the mark. A 1ml of this solution was diluted with pH 2 buffer solution to 25 ml into a volumetric flask. Then 20 µl aliquot was injected into the FIA system.

For the determination of imiperamine in plasma, 200 μl aqueous imiperamine solution (8 ng/ml) were added to 100 μl of untreated plasma. The mixture was vortexed for 30 s. In order to precipitate the plasma proteins, the plasma samples were treated with 20 μl perchloric acid $HClO_4$ 15%. After that, the mixture was vortexed 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.

The voltammograms were recorded according to the above recommended procedure. The voltammograms of samples without imiperamine do not show any signal that can interfere with the direct determination, so external calibration can be used.

2.3.5. Electrode preparation

Gold UMEs (with a 12.5 μm in diameter) 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, 1cm length and 0.5 mm in diameter.

2.3.6. 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 our previous papers[19-23]. The volume of the cell was 50 μL. In all experiments described in this paper, the flow rate of eluent solution was 6mL min^{-1}.

2.3.7. 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

The diagram of applied waveform potential and its describtion is explained in our previous papers[19-23].

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. >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. 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.[19-24] 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,[25] 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. 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.[26] 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;

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

An example of recorded CVs is shown in Figure 1 (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 60000 pgml^{-1} imipramine (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. In the absence of imipramine, the shape of the CV curves is typical for a polycrystalline gold electrode in acidic media.[27] Figures 1b 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 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 imipramine, 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.

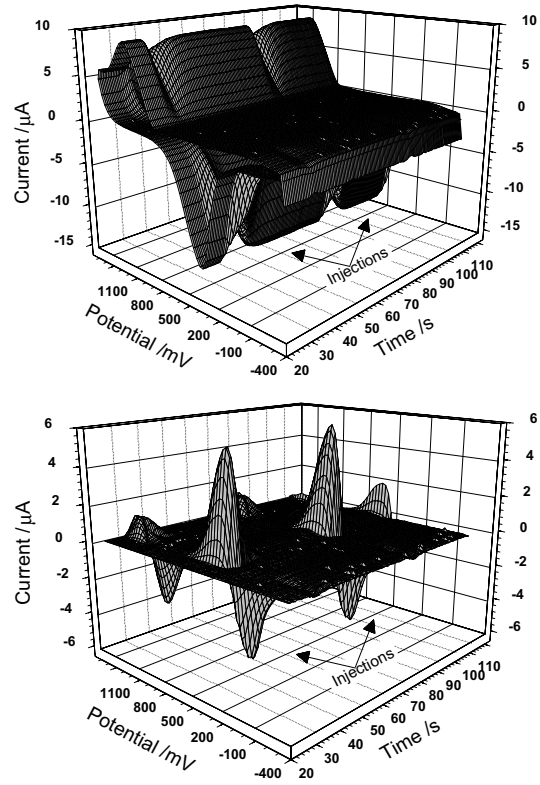

Figure 1. (a). Cyclic voltammogram at a 12.5μm Au ultramicroelectrode recorded during a Flow-Injection experiment. The eluent was 0.05 mol L^{-1} H_3PO_4, the flow rate was 6 mL min^{-1}, and the sweep rate was 60 V s^{-1}. Each scan was preceded by 100 ms (at 1600mV) and 100 ms (at 300 mV) conditioning, respectively. The accumulation time was 500 ms at 100 mV. The injected solution (50μl) contained 3.0 ×10^{-6} mol L^{-1} imipramine in 0.05 mol L^{-1} H_3PO_4. b) Curves result of subtraction of average CVs (in absence of the analyte) from test of the CVs in (a).

It must be noted that, theoretically, in this method, the thermodynamic and kinetic parameters of adsorption, the rate of mass transport and electrochemical behavior of the adsorbed species can affect the analyte response. 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.[28-32]

3. Results and Discussion

3.1. 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.

Table1. Effect of pH on the response of microelectrode

pH	2.0	4.0	6.0	8.0	10.0	12.0
S/N	600	520	410	350	270	310

Also, in order to investigate the influence of scan rate and the eluent flow rate on the sensitivity of the detector response, solutions having a concentration of 5660 pgml^{-1} imipramine were injected. At different scan rates (from 10 to 120 Vs^{-1}) and the eluent flow, the responses of the detector to the injected sample were recorded. The results are presented in Figure 2. As it is clear from the Figure 2, the detector exhibits the maximum sensitivity at 60 V s^{-1} of scan rate and 6 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 imipramine, 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 imipramine during the potential scanning, (because under this condition, the inhibition outcome of the adsorbed imipramine 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 imipramine 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

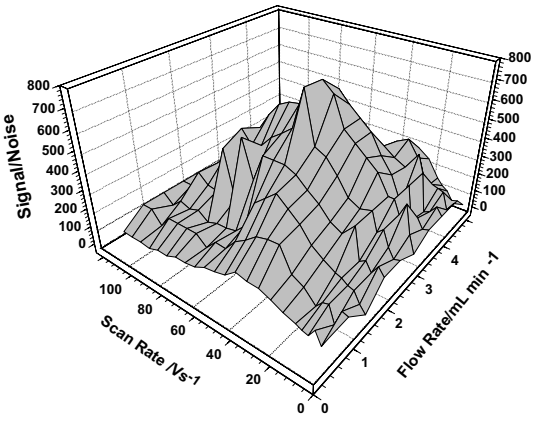

Figure 2. The effect of the sweep rate on the response of Au microelectrode (with a radius of 12.5μm) to injections 2.0 ×10^{-8} mol L^{-1} imipramine in 0.05 mol L^{-1} H_3PO_4 and the effect of flow rate.

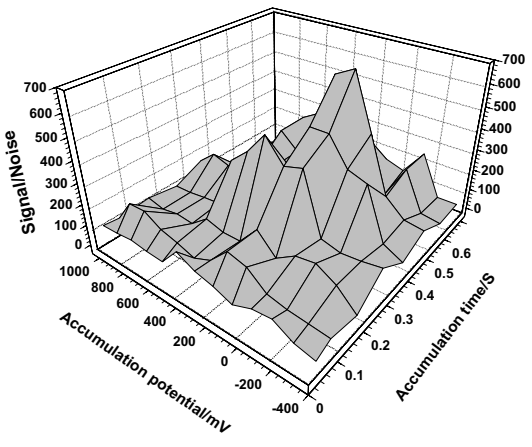

Figure 3. The effect of the accumulation potential and the effect of the accumulation time on the electrode response to injections 2.0 ×10^{-8} mol L^{-1} imipramine in 0.05 mol L^{-1} H_3PO_4.

accumulation potential and time on the response of the method for the injection of a solution of 5660 pgml^{-1} imipramine, in 0.05 mol L^{-} H_3PO_4, was studied. Figure 3 shows the detector response over the accumulation potential ranges -400 to 1000 mV and accumulation time range from 0.05s to 0.7s. Based the figure accumulation potential 100 mV at time 500 ms was chosen as the optimum condition. Because, the surface of the electrode becomes saturated with the imipramine within 500 ms time window.

On the electrode, the accumulation of imipramine 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.

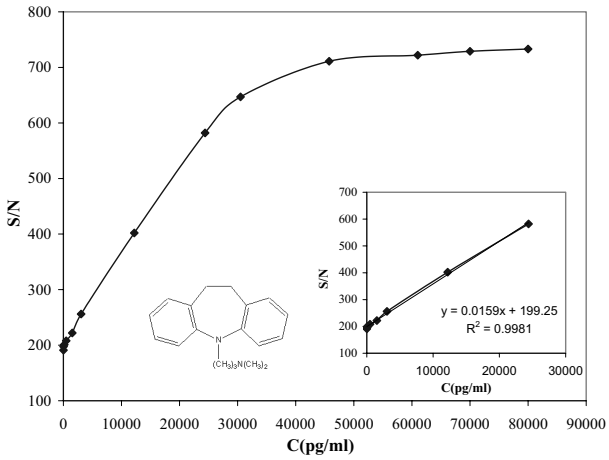

Figure 4. Calibration curves obtained for imipramine on the Au microelectrode in 0.05 mol L^{-1} H_3PO_4.

3.2. Validation

The method was validated with respect to parameters including linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, ruggedness/robustness, recovery and selectivity.[33–35]

3.3. Linearity

The Linearity was evaluated by linear regression analysis, which calculated by the least square regression method.[36,37] The calibration curves constructed for imipramine were linear over the concentration range of 14 – 22400 pg mL^{-1}. Peak areas of imipramine were plotted versus its concentration and linear regression analysis performed on the resultant curve. A correlation coefficient of 0.999 with %R.S.D. values ranging from 0.15 – 3.23 % across the concentration range studied were obtained following linear regression analysis. Typically, the regression equation for the calibration curve was found to be y = 0.01559 ± 0.002X + 199.25 ± 1.1 Figure 4 shows the calibration graph that obtained for the monitoring of imipramine in a 0.05 mol L^{-1} H_3PO_4.

3.4. LOQ and LOD

The LOQ and LOD were determined based on a signal-to-noise ratios and were based on analytical responses of 10 and 3 times the background noise, respectively.[38] The LOQ was found to be 14 pg mL^{-1} with a resultant %R.S.D. of 3.35 (n=6). The LOD was found to be 4.55pg mL^{-1}.

3.5. 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 14, 5000 and 22400 pg mL^{-1} standards where the mean concentrations were found to be 14.2, 5075 and 22301 with associated %R.S.D.'s of 3.65, 1.1 and 0.24, respectively. Inter-day precision was assessed by injecting the same three concentrations over 3 consecutive days, resulting in mean concentrations of imipramine of 14.25, 5070 and 22285 pg mL^{-1} and associated %R.S.D. of 3.45, 2.25 and 0.45 respectively.

3.6. Accuracy

Accuracy of the assay was determined by interpolation of replicate (n = 6) peak areas of three accuracy standards (14, 5000 and 22400 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 14.26 ± 0.49 pg mL^{-1}, 5140 ± 64.25 pg mL^{-1} and 22332 ± 78.16 pg mL^{-1} with percent relevant errors of 1.60, 2.85 and 0.30%, respectively.

3.7. Ruggedness

The ruggedness of the method was assessed by comparison of the intra- and inter-day assay results for imipramine undertaken by two analysts. The %R.S.D. values for intra - and inter – day assays of imipramine 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.[39] As can be seen in table 2, the percent recoveries of imipramine were good under most conditions and did not show a significant change when the critical parameters were modified.

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

Parameter	modification	Imipramine (% recovery)
pH	2.0	100.2
	2.3	99.9
	2.5	101.3
flow rate mL/min	5.8	102.0
	6.0	101.1
	5.2	100.5
buffer composition (M)	0.04	98.6
	0.05	99.0
	0.06	100.6
Lab. Temperature (°C)	20	100.0
	25	99.7
	30	100.8

3.8. Recovery

A known amount of imipramine standard powder was added to samples of tablets, which was then extracted, diluted and analyzed. The final nominal concentration of imipramine was found to be 11996pg 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 0.85% with a corresponding percentage recovery value of 99.96%**.**

3.9. Selectivity

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

3.10. Assay of tablets

The method developed in the present study was applied for the determination of imipramine in tablets from the Iranian market. The results showed a percent recovery of 100.05% and a R.S.D. of 1.15%.

3.11. Analytical applications

The proposed method was also applied to the determination of imiperamine in spiked urine and plasma samples. The results of analysis of spiked human plasma (n=6) and urine (n=6) is shown in Table 3.

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

Added (ng ml^{-1})	Interpolated concentration	R.S.D (%)	R.E. (%)
1 (plasma)	15.60 ± 0.45	3.55	3.85
10 (urine)	41.2 ± 0.7	3.7	4.1

The results are satisfactory, accurate and precise. No interference was noticed from the urine content after just dilution with the supporting electrolyte.

The major advantage of the method as applied to plasma and urine is that no prior extraction step is required.

3.12. Comparison of the sensitivity of the proposed method and other previously reported electrochemical detection methods

Table 3 compares the sensitivity (detection limit) of the proposed method with the other reported methods. As can be seen, the sensitivity of the method is superior to all previously reported methods. Table 4 shows that the detection limit of the method is about 200 times lower than the sensitive previously reported electrochemical method.

Table 4. Comparison of the detection limit of the proposed method and the best reported electrochemical detector

Ref.	D. L	Method
16	1×10^3 pg mL^{-1}	HPLC-Electrochemical detector
This Work	4.55 pg mL^{-1}	FFT-CV

4. Conclusion

In this method (FFTCV) the S/N ratio is enhanced by using of fast Fourier transform of the analyte and signal integration. FFTCV can be considered as a new sensitive, accurate and fast method for determination of similar drugs, with ability of adsorption gold surface, in chromatographic systems, such as HPLC and capillary electrophoresis. However, in order to obtain better sensitivity for a specific drug, experimental parameters should be optimized. Finally, such detection limit (in nanomolar level), make the method suitable for bio-analysis. For instance, this method was applied for determination of imiperamine in its tablets form and had good agreement with the reported values.

5. Acknowledgement

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6. References

1. F. L. Van der Mark, R. F. Adams, *Clin. Chem.* **1978,** *24*, 87–91.
2. A. S. Tamara, J. J. William, *Pharmaceutical Sciences* **1979,** *68*, 702–705.

3. L. F. Gram, B. Kvinesdal, N. Reisby, *Clin. Pharmacol. Ther.* **1983**, *33*, 335–342.
4. M. Chiba, S. Fujita, T. Suzuki, *J.Pharm. Sci.* **1990**, *79*, 281–287.
5. D. A. Ciraulo, J. G. Barnhill, J. H. Jaffe, *Clin. Pharmacol. Ther*. **1988**, *43*, 509–517.
6. M. Chiba, S. Fujita, T. Suzuki, *J Pharm. Sci.* **1988**, *77*, 944–947.
7. J. Anna, S. Barbara, *J. Pharm. Biomed. Anal.* **2003**, *31*, 795–799.
8. P. A. Reece, R. Zacest, *J. Chromatogr.* **1979,** *163***,** 310–314.
9. J. Fekete, P. Del Casttilho, J. C. Kraak, *J. Chromatogr.* **1981**, *204*, 319–327.
10. S. Sugita, A. Kobayashi, S. Suzuki, T. Yoshida, K. Nakazawa, *J. Chromatogr.* **1987**, *421*, 412–417.
11. T. B. Zeugin, K. Brosen, U. A. Meyer, *Anal. Biochem.* **1990**, *189*, 99–102.
12. J. S. Torano, A. Verbon, H. J. Guchelaar*, J. Chromatogr.* **1999**, *734*, 203–210.
13. L. Kristoffersen, A. Bugge, E. Lundanes, L. Slordal, *J. of Chromatogr. B* **1999**, 734, 229–246.
14. Y. Hideyuki, H. Keiko, I. Junichi, Y. Kenichiro, N. Hitoshi, Y. Masatoshi, *Anal. Chim. Acta* **2000**, *413*, 137–145.
15. F. Adriana, K. Elena, M. Robert, L. Jan, *Electrochem. Chem.* **2000**, *492*, 74–77.
16. T. A. Ivandini, B. V. Sarada, C. Terashima, T. N. Rao, D. A. Tryk, H. Ishiguro, Y. Kubota, A. Fujishima, *Electrochem. Chem.* **2002**, *521*, 117–126.
17. J. Rodriguez Flores, J. J. Berzas Nevado, A. M. Contento Salcedo, M. P. Cabello Diaz, *Talanta* **2005**, *65*, 155–162.
18. A. Chmielewska, L. Konieczna, A. Plenis, H. Lamparczyk, *J. Chromatogr. B* **2006**, *839*, 102–111.
19. P. Norouzi, M. R. Ganjali, P. Matloobi, *Electrochem. Communications* **2005**, *7*, 333–338.
20. M. R. Ganjali, P. Norouzi, M. Ghorbani, A. Sepehri, *Talanta* **2005**, *66*, 1225–1233.
21. P. Norouzi, M. R. Ganjali, A. Sepehri, M. Ghorbani, *Sens. Actuators B* **2005**, *110*, 239–245.
22. P. Norouzi, M. Namazian, A. Badiei, *Anal. Sci.* **2004**, *20*, 519–526.
23. P. Norouzi, G. R. Nabi Bidhendi, M. R. Ganjali, A. Sepehri, M. Ghorbani, *Microchim. Acta* **2005**, *152*, 123–129.
24. P. Norouzi, M. R. Ganjali, T. Alizadeh, P. Daneshgar, *Electroanalysis* **2006**, *18*, 947–954.
25. R. M. Wightman, D. O. Wipf, (Eds: A. J. Bard), Marcel Dekker, New York, **1989**, *15*.
26. J. Lipkowski, L. Stolberg, *Adsorption of Molecules at Metal Electrodes*, VCH, New York, **1992**.
27. J. O. M. Bockris, B. E. Conway, E. Yeager, *Comprehensive Treatise of Electrochemistry*, Plenum, New York and London, **1980**.
28. P. Norouzi, M. R. Ganjali, S. Shirvani-Arani, A. Mohammadi, *J. Pharm. Sci.* **2006**, *95*, 1.
29. P. Norouzi, S. Shirvani-Arani, P. Daneshgar, M. R. Ganjali, *Biosens. Bioelectron*. **2006** in press.
30. P. Norouzi, M. R. Ganjali, A. A. Moosavi–Movahedi, *J. Brazil. Chem. Soc*. **2006** in press.
31. P. Norouzi, M. R. Ganjali, P. Daneshgar, *Anal. Lett*. **2006** in press.
32. P. Norouzi, M. R. Ganjali, L. Hajiaghababaei, *Anal. Lett*. **2006**, *39*, 1941–1953.
33. J. Ermer, *J. Pharm. Biomed. Anal.* **2001**, *24*, 755–767.
34. G. A. Shabir, Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia
35. United States Pharmacopeia 28-NF 23. *United States Pharmacopeial Convention*, INC., Rockville, MD, **2005**, 2748.
36. J. C. Miller, J. N. Miller, *Statistics for Analytical Chemistry*, Ellis Horwood, Chichester, **1984**, *22*, 82.
37. Z. Al-Kurdi, T. Al–Jallad, A. Badwanamd, A. M. Y. Jaber, *Talanta* **1999,** *50***,** 1089–1097.
38. Internatioal Conference on Harmonisation (ICH) Topic Q2 B: Validation of Analytical Procedures: *Methodology, the European Agency for the Evaluation of Medicinal Products*, Geneva, **1996**.
39. Y. V. Heyden, A. Nijhuis, J. Smeyers-Verbeke, B. G. M. Vandeginste, D. L. Massaret, *J. Pharm. Biomed. Anal.* **2001**, *24*, 723–753.

Povzetek

Razvili smo novo metodo za določanje imipramina v farmacevtskih pripravkih in bioloških vzorcih s pretočno analizno tehniko. Tehnika je enostavna, točna, natančna in ni časovno zahtevna. Preverili smo vpliv nekaterih parametrov na občutljivost metode. Najboljši pogoji analize so pH 2,0, hitrost spreminjanja potenciala 60 V s^{-1}, akumulacijski potencial 100 mV in čas akumulacije 0,5 s. Uvedli smo tudi numerično metodo za izračun signala in za zmanjšanje šuma. Z uporabo mikroelektrode (polmera 12,5 μm) smo določili interval linearnosti 14 - 22400 pg mL^{-1} z mejo detekcije 4.55 in določljivosti 14 pg mL^{-1}.