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Flow-injection biamperometric determination of epinephrine

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Abstract

A flow-injection manifold is proposed for the determination of epinephrine. The experimental procedure is based on the indirect biamperometric detection of the drug by using Fe(III)–Fe(II) as an indicating redox system and a flow-through detector with two polarized Pt wire electrodes. The calibration graph is linear over the range 0.3–20 μg ml^{-1} of epinephrine. The relative standard deviation for the determination of 10 μg ml^{-1} of epinephrine is 1.5% ($n = 25$) and the sample throughput is 153 h^{-1}. The method was applied to the determination of epinephrine in two commercially available pharmaceutical preparations. © 1997 Elsevier Science B.V.

Keywords: Biamperometry; Flow injection; Catecholamines; Epinephrine; Adrenaline

1. Introduction

Epinephrine [1-(3,4-dihydroxyphenyl)-2-methyloaminoethanol] is a catecholamine and is a drug extensively employed in medicine in the treatment of cardiac arrest, heart block with syncope, bronchical asthma and cardiac surgery. Several analytical methods have been proposed recently for the determination of epinephrine and other catecholamines in pharmaceutical preparations, mainly spectrophotometric, fluorimetric, kinetic, photokinetic and chromatographic ones [1–8].

Flow injection analysis (FIA) has advantages that make it suitable for use in various fields of routine analyses including the pharmaceutical one. Flow injection procedures have already been used for the determination of epinephrine. Epinephrine is readily oxidized to adrenochrome with various agents such as iodine, potassium hexacyanoferrate(III), potassium permanganate and manganese dioxide [9] and this characteristic has been the basis of a few FIA methods for determining epinephrine using mainly detection techniques such as spectrophotometry and spectrofluorimetry. The use of spectrofluorimetric detection was based on the oxidation of the drug to adrenochrome which in turn is converted by alkali into adrenolutine, a fluorescent substance. In flow injection spectrofluorimetric procedures oxidation was carried out by means of an oxidative solid-phase reactor of manganese dioxide incorporated in polyester resin beads [10] and solid-phase reactor of iodine prepared by the 'impregnation' of

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the flexible pump tubing with the reagent [11]. The solid-phase reactor of microcrystalline manganese dioxide has been used for spectrophotometric determination [12]. In the spectrophotometric procedure the oxidation of epinephrine by metaperiodate was also applied [13]. Fluorimetric determination based on the strong inhibition by epinephrine on the photochemical reaction between ploxin and ethylenediaminetetraacetic has also been proposed [14].

On the other hand, the application of amperometric detection with two platinum electrodes, also called biamperometry, provides a good and not too much exploited mode of detection in FIA for the determination of different analytes [15–19] including the determination of drugs in pharmaceutical preparations [20].

The aim of this study was to develop a simple, sensitive and rapid flow injection method for the determination of epinephrine with two polarized indicating electrodes.

2. Experimental

2.1. Apparatus

Biamperometric detection was carried out by means of a purpose built programmable potentiostat-galvanostat which was designed for the study of electric phenomena occurring in membranes [21]. The apparatus was controlled by software working in the Windows (Microsoft) environment.

The design of the flow-through cell has been described earlier [18]. Platinum electrodes were made of Pt wire 0.5 mm in diameter and 4 cm long. The exposed length of wire electrodes to the flowing solution was usually 1.5 cm. The surface of the platinum electrodes was cleaned electrochemically with the aid of the potentiostat-galvanostat mentioned above. The polarization was alternated between +4.5 and −4.5 V every 10 s over a period of 10 min and the electrodes were in contact with an alkaline phosphate solution (5 g Na_2HPO_4 and 2 g KOH in 100 ml of water) at 80°C. Such treatment of the working electrodes allowed us to obtain a maximum sensitivity of detector response and it was repeated every day before using the measuring system.

Fig. 1. shows the flow-injection manifold used. A model 5021 rotary injection valve (Rheodyne, Cotai, CA) and a Gilson Minipuls 2 peristaltic pump were used. The flow system was made of poly(tetrafluoroethylene) (PTFE) tubing 0.5 mm i.d.

2.2. Reagents

Epinephrine [1-(3,4-dihydroxyphenyl)-2-methylaminoethanol)] was obtained from SIGMA. The selected oxidant $Fe(NO_3)_3.9H_2O$ came from Loba Feinchemie (Austria). All other reagents were of analytical grade from POCh (Gliwice, Poland). All solutions were prepared with double distilled water.

2.3. Recommended procedure for calibration

The samples, 248 μl epinephrine in $1 \cdot 10^{-3}$ mol l^{-1} hydrochloric acid, was injected into the carrier stream of $1 \cdot 10^{-3}$ mol l^{-1} hydrochloric acid delivered at a flow rate of 3.8 ml min^{-1}. This stream was merged with the stream of $1 \cdot 10^{-1}$ mol l^{-1} $Fe(NO_3)_3$ in 1.0 mol l^{-1} hydrochloric acid pumped at a flow rate 1.1 ml min^{-1}. Flow-injection measurements were carried out at 60°C with a 110 cm reaction coil in the system and polarization of the working electrodes with a potential difference of 150 mV. A calibration graph was prepared by plotting the peak height (μA)

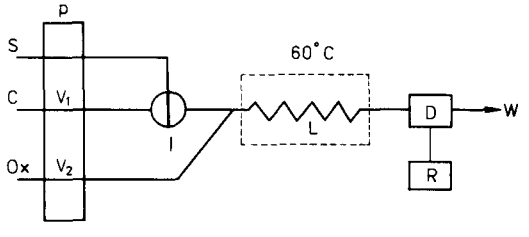

Fig. 1. The flow-injection manifold proposed for the determination of epinephrine: P, peristaltic pump; S, sample; C, carrier stream; Ox, oxidant stream; V_1, carrier flow-rate; V_2, oxidant flow-rate; I, sample injection valve; L, reaction coil in thermostat; D, biamperometric flow-through detector; R, recorder; W, waste.

versus epinephrine concentration over the range 0.3–20 μg ml^{-1}.

3. Results and discussion

3.1. Preliminary studies with different indicating redox systems

The indirect determination of epinephrine is based on the linear dependence of current on the concentration of reduced species formed in the reaction of analyte with the oxidized form of the indicating reversible redox couple being in large excess. The intensity of current passing through two identical inert electrodes is measured when a small potential difference is applied.

The preliminary studies with different indicating reversible redox systems were carried out in the FIA assembly shown in Fig. 1. A 185 μl volume of epinephrine solution in $1 \cdot 10^{-1}$ mol l^{-1} hydrochloric acid was injected into the carrier stream (distilled water) flowing at 3 ml min^{-1} which was then merged with the acidified oxidant solution pumped at the same flow rate. The mixed solutions were passed through a reaction coil (PTFE tubing 0.5 mm i.d. and 110 cm long) to the flow-through detector (two platinum wire electrodes 0.5 mm i.d. and a distance between them of 0.5 cm). The polarizing voltage was 100 mV. Three different oxidizing species were examined, Fe(III), hexacyanoferrate(III) and iodine. With each indicating system an optimization of chemical parameters (concentration of oxidant and concentration of acid in the oxidant solution) was carried out for three concentrations of epinephrine (10, 30 and 50 μg ml^{-1}) in order to evaluate the influence of the study parameters on the linear response of the detector.

The effect of Fe(III) concentration was tested within the range $1 \cdot 10^{-2}$–$4 \cdot 10^{-1}$ mol l^{-1}. The peak height increased with the Fe(III) concentration up to $1 \cdot 10^{-1}$ mol l^{-1}, above which it reached an almost constant value. A $1 \cdot 10^{-1}$ mol l^{-1} concentration of Fe(III) was chosen. The acidity of the Fe(III) solution appeared to be a very important parameter. The effect of the acid concentration on the signal magnitude was studied by using different concentrations of hydrochloric acid. The peak height increased with the hydrochloric acid concentration up to about $5 \cdot 10^{-1}$ mol l^{-1}, above which it levelled off. A 1.0 mol l^{-1} hydrochloric acid concentration was used in further measurements. Distilled water and different hydrochloric acid solutions were used as a carrier stream. For $1 \cdot 10^{-3}$, $1 \cdot 10^{-1}$ and 1 mol l^{-1} hydrochloric acid solutions similar signals were observed but higher than for the distilled water. A $1 \cdot 10^{-3}$ mol l^{-1} hydrochloric acid solution was chosen as a carrier stream—the same medium in which epinephrine was prepared because it showed good stability against oxidation during the storeage period.

Using hexacyanoferrate(III) as an oxidant it was found, that its optimum concentration in the reagent stream was $5 \cdot 10^{-2}$ M in the presence of 2 M HCl, which in this case was employed as a carrier stream instead of water because of high instability of the oxidant in the acidic solution. A blue precipitate appeared on the walls of the glass container during a few hours storage of the solution of hexacyanoferrate(III) in 2 mol l^{-1} hydrochloric acid. A neutral solution of the oxidant is very stable for long time. The greatest drawback of this system was intereference with the flow-injection signals by a negative pre-peak.

In the iodine–iodide system both the acid and oxidant concentrations were optimized in the range $1.5 \cdot 10^{-2}$–1.5 M and $3.2 \cdot 10^{-1}$–3.2 mM respectively. A $9.6 \cdot 10^{-1}$ mM iodine solution also containing $1 \cdot 10^{-1}$ M sulphuric acid were assumed as the best values. The carrier stream was $1 \cdot 10^{-3}$ M HCl. The results obtained for the indicating redox systems studied are summarized in Table 1.

The iodine–iodide system has drawbacks from the point of view of sensitivity, short linear range and lower sample throughput, whereas Fe(III) and hexacyanoferrate(III) present comparable behaviours. The excellent base line obtained in the system Fe(III)–Fe(II) and the best tolerance of this redox couple to ascorbic acid and bisulphite as potential interferences [20], which are present as antioxidants in almost all pharmaceutical preparations containing epinephrine, have decided its choice. Due to these advantages further opti-

Table 1
Comparison of different indicating redox systems for the biamperometric determination of epinephrine

Indicating system	Linear range (μg ml^{-1})	Equation	Correlation coefficient	R.S.D. (%)	Detection limit ($\mu g\ ml^{-1}$)	Sample throughput (h^{-1})	Base line
Fe(III)–Fe(II)	1–40	$\mu A = 0.2912 + 0.3528\ X$	0.9978	0.9[a] ($n = 20$)	0.4	180	very good
$Fe(CN)_6^{3-}$ – $Fe(CN)_6^{4-}$	1–40	$\mu A = 0.1016 + 0.4578\ X$	0.9999	0.5[b] ($n = 20$)	0.3	185	good
I_2–I^-	0.5–10	$\mu A = 0.1671 + 0.3064\ X$	0.9950	0.9[a] ($n = 15$)	0.1	138	good

[a] For 40 μg ml^{-1} of epinephrine; [b] for 60 μg ml^{-1} of epinephrine.

mization of the FIA parameters was carried out with Fe(III) as an oxidant.

3.2. Optimization of flow-injection parameters with the Fe(III)–Fe(II) indicating system

Flow-injection parameters were optimized by the univariate method in order to achieve a compromise between the peak height, sample throughput and reproducibility. The variables studied were: the injected sample volume, length of the reaction coil and flow rates of the oxidant and of the carrier stream. The optimization of all hydrodynamic parameters mentioned above was carried out using a 19.5 $\mu g\ ml^{-1}$ solution of epinephrine. The design of the FIA manifold was the same as that used in the preliminary studies with platinum wire electrodes 0.5 mm in diameter, 0.5 cm distance between electrodes and 100 mV polarizing voltage. The reagent concentrations used in these experiments were as follow: reagent line, $1 \cdot 10^{-1}$ mol l^{-1} Fe(III) in 1 M hydrochloric acid; and carrier line, $1 \cdot 10^{-3}$ mol l^{-1} of hydrochloric acid.

The volume of sample injected was varied from 107 to 499 μl by changing the length of the loop in the injection valve. No significant differences were observed above 155 μl except for an increase in the width of the peak and therefore a decrease

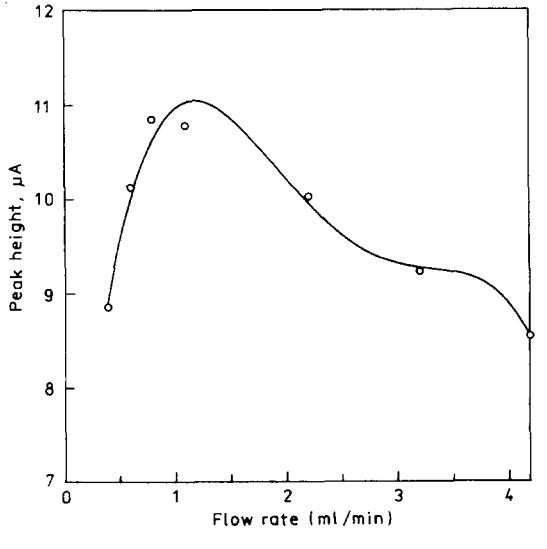

Fig. 2. Influence of the flow rate of the oxidant stream on peak height in flow-injection measurements.

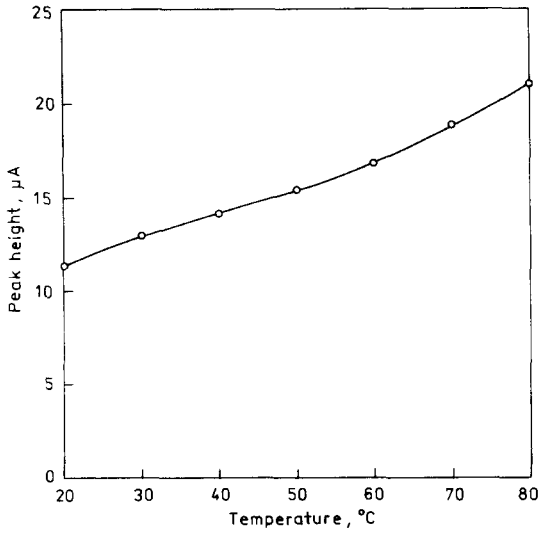

Fig. 3. Influence of the temperature of the reaction coil on peak height in flow-injection measurements.

in the sample throughput. A 248 μl sample volume was assumed as the optimum.

The effect of the length of the reaction coil on the signal magnitude was examined within the range of 35–210 cm. For 35 cm the peaks were slightly higher but they were deformed by turning on/off the injection valve due it's proximity to the detector as well as to insufficient mixing of the carrier and oxidant flows. A 110 cm coil was selected as the most appropriate.

Increasing the oxidant flow rate over the range $4 \cdot 10^{-1}$–4.2 ml min^{-1} produced an increased signal up to about 0.8 ml min^{-1}, above which it decreased (Fig. 2). Transient signals for different carrier flow rates were obtained over the range $2 \cdot 10^{-1}$–8.4 ml min^{-1}. The peak height increased to about 3.8 ml min^{-1}, above which it levelled off. The sample throughput was strongly dependent on these last parameters. A flow rate of 3.8 ml min^{-1} for the carrier and 1.1 ml min^{-1} for the oxidant were considered to be a sensible choice and provided a suitable sampling rate. A flow rate of 1.1 ml min^{-1} for the oxidant was chosen as a compromise between sensitivity and sample throughput (the highest peaks were similar to those obtained for a flow rate of 0.8 ml min^{-1}).

The temperature was the last parameter studied concerning the FIA-assembly. The influence of this parameter on the system was investigated by immersing the reaction coil in a water bath and by varying the temperature. A linear increase of the peak height was observed when the temperature was raised from 20 to 80°C (Fig. 3). Above 60°C a significant increase in the noise amplitude of the base line was observed and the detection limit became worse; hence 60°C was chosen as the optimum temperature.

3.3. Optimization of the electrochemical parameters

To a significant extent the magnitude of the flow-injection signal in determining epinephrine by biamperometric detection also depends on several electrochemical parameters such as the diameter and the material of the electrodes, applied polarizing voltage and distance between the two electrodes in the flow-cell. The influence of these parameters was studied for three different concentration of epinephrine (10, 20 and 50 μg ml^{-1}) in order to obtain information about the sensitivity and linear response of the system. Four different electrodes were tested, two platinum and two gold of 0.5 and 0.3 mm diameter for each material.

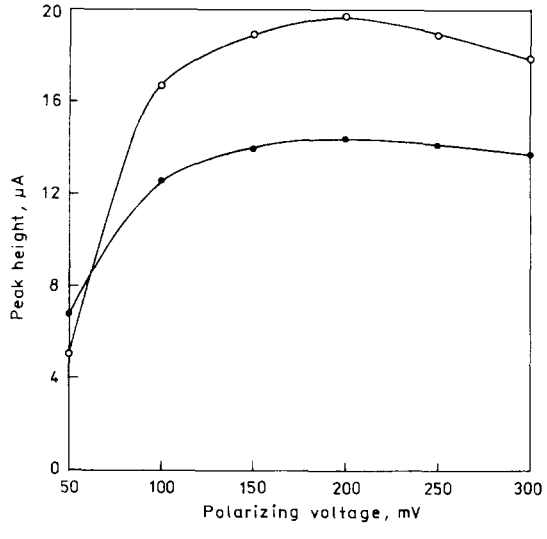

Fig. 4. Influence of the magnitude of polarizing voltage applied to Pt electrodes of 0.5 (○) and 0.3 mm (●) diameter on peak height in flow-injection measurements.

Table 2
Selected analytical parameters obtained from the optimization experiments

Optimized parameter	Optimum value
Fe(III) concentration (M)	10^{-1}
HCl concentration in Fe(III) stream (M)	1.0
HCl concentration in carrier stream (M)	10^{-3}
Flow rate V_1 (ml min^{-1})	3.8
Flow rate V_2 (ml min^{-1})	1.1
Sample injection volume (μl)	248
Length of reaction coil (cm)	110
Polarizing voltage (mV)	100
Distance between electrodes (mm)	2
Temperature (°C)	60

The influence of the potential difference used for the polarization of the indicating electrodes was investigated within the range 50–300 mV. The best results were obtained with the Pt electrodes. For the Au electrodes the recorded FIA-peaks were deformed and the reproducibility was poor, the base line was wider and not stable, and measurements above 150 mV were not possible due to an increase in of all these disadvantages. For Pt electrodes a large increase in the peak height with the potential difference applied was observed up to 100 mV. A further increase in the applied potential resulted in a smaller increase of the flow-injection signal and above 150 mV a plateau was observed; therefore 100 mV was selected as the optimum potential difference for further experiments. Fig. 4 shows the influence of the applied potential for two different electrode diameters. Pt wire electrodes of 0.5 mm diameter provided the best FIA signals as expected considering the bigger surface area. On the other hand, this size allowed us to work with lower polarizing voltage, which has to be considered from the point of view of selectivity.

Lastly an electrochemical factor, the distance between the electrodes, was studied under the conditions previously established as the optimum (Pt electrodes of 0.5 mm in diameter and 100 mV as potential difference). This parameter determined the exposed surface of the wire electrodes in the flowing solution. An increase in distance between electrodes resulted in a diminution of the exposed surface and therefore a decrease in peak height was observed, for the distances tested, in the following order: 2 mm, 5 mm, 1 cm and 1.5 cm. 2 mm was selected as the optimum.

The selected values for the analytical parameters of the flow-injection biamperometric determination of epinephrine are given in Table 2.

4. Analytical applications

4.1. Analytical characteristics

A study of the analytical application of the flow-injection procedure was carried out to establish the application range, reproducibility, detection limit and sample throughput. Due to the increase in the sensitivity and noise amplitude of the base line with temperature, two sets of experiments, at room temperature and at 60°C allowed us to compare the analytical characteristics of the method under both conditions. The calibration graphs were linear over the range 0.3–20 μg ml^{-1} of epinephrine and the detection limit for S/N = 3 was $1 \cdot 10^{-1}$ μg ml^{-1} in both cases. The calibration graph could be described at room temperature by the equation $\mu A = 0.0958 + 0.6498\ X$, with a correlation coefficient of 0.9986 (where μA is the current in micro amperes and X the concentration of epinephrine in $\mu g\ ml^{-1}$). In order to check the day-to-day reproducibility, 7 calibration graphs were obtained at 60°C on different days; the arithmetic medium of the slopes obtained was 0.8628 with R.S.D. = 4.3%.

The R.S.D. of the proposed procedure and the sample throughput were determined by repeatedly injecting a sample containing 10.3 $\mu g\ ml^{-1}$ of epinephrine. The results obtained were 1.1% and 147 h^{-1} at room temperature and 1.5% and 153 h^{-1} at 60°C.

4.2. Influence of interferences

The tolerance of the method to foreign compounds which can be found in typical pharmaceutical samples containing epinephrine was investigated by using solutions containing 10 μg ml^{-1} of the drug and adding various concentrations of the interfering compounds. Errors were

calculated by comparing the peak height with that obtained by injecting an aqueous solution of pure epinephrine. The tolerated level was taken as the measured signal variation ± 5%. The results obtained are listed in Table 3. The method shows a good tolerance to the interferents tested (e.g. weaker reductants such as sugars or complexing agents of the indicating system such as EDTA), excepting sodium bisulphite and an ascorbic acid. The addition of sodium carbonate and sodium metaborate to the epinephrine solution caused the oxidation over time of the drug due to the basic pH of the medium. However these two interferences were easily stopped by adding HCl so that the pH remained acidic. In the case of sodium carbonate it was necessary to shake the solution prior to the injection step. This procedure in turn allows removal of the CO_2 formed and prevents the CO_2 bubbles from reaching the detector.

Many attempts have been made to remove the main interferences which are present practically in all available formulations of epinephrine (e.g. by heating at different temperatures or by preparing the epinephrine solutions in different acid concentrations). The increase of HCl concentration and heating the solutions containing sodium bisulphite and ascorbic acid respectively, allowed a slight reduction, but not effectively, of these two remarkable interferences.

Table 3
Influence of foreign compounds on epinephrine determination

Interferent	Tolerated concentration μg ml^{-1}	Error (%)
Glucose	32 000	−1.4
Lactose	64 000	−0.7
NaCl	56 000	−4.4
Ascorbic acid	0.68	+4.3
$NaHSO_3$	2.02	+4.0
H_3BO_3	32 000	−3.7
EDTA	5000	+2.7
$NaHCO_3$	30 000	0.0
$Na_2B_4O_7 \cdot 10H_2O$	12 500	−1.5
$NaBO_2 \cdot 4H_2O$	40 000	−1.8
Sodium citrate	10 000	−3.0
Formaldehyde	790	+1.5
$ZnSO_4 \cdot 7H_2O$	20 000	−2.7
Picric acid	8000	−0.9

Epinephrine concentration 10 μg ml^{-1}.

4.3. Determination of epinephrine in pharmaceutical preparations

The epinephrine content of Adrenalinum Solution 0.1% (from CEFARM Gdansk) and Injec. Adrenalini 0.1% (from POLFA Warsaw) were determined. The first attempt to determine epinephrine in these pharmaceutical preparations by appropriate dilution of the original solutions yielded errors of 60.6 and 31.9% for Adrenalinum Solution and Injec. Adrenalini respectively. These unacceptable errors were caused by the antioxidants present in both preparations. Therefore in the next determinations we prepared solutions containing the same amount of 'antioxidant matrix' as the pharmaceuticals in the following way: aliquots of 5 ml (Adrenalinum Solution) or the content of 5 ampoules (Injec. Adrenaline) were transferred into a flask, 5 ml of HCl $1 \cdot 10^{-1}$ M were added and diluted with distilled water to 100 ml; 5 ml of this solution were introduced into the flasks with standards and mixed with 1 ml of KOH $1 \cdot 10^{-1}$ M. In this medium the epinephrine contained in the sample is oxidized and only the antioxidants (sodium bisulfite or ascorbic acid) accompanying the drug remain. After 45 min 5 ml of HCl $1 \cdot 10^{-1}$ M were transferred into each flask and appropriated aliquots from the concentrated solution of epinephrine were added and then diluted to 50 ml in order to obtain a standard solution in the linear range. The sample solutions were prepared by means of the same procedure except for the addition of KOH $1 \cdot 10^{-1}$ M.

Four determinations were carried out for each pharmaceutical preparation and the results obtained were consistent with those certified on the labels. The errors were + 5.5 and 0.2% for Injec. Adrenalini and Adrenalinum Solution respectively. Epinephrine was also determined in 'epinephrine inhalation' (a drug without antioxidant) prepared according to the Pharmacopoeia [22]. The results were compared with the weighed and dissolved drug. The relative error for four determination was 1.2%.

5. Conclusions

A FIA procedure is proposed for the determination of epinephrine with application to the control analysis of pharmaceuticals. The method is based on the indirect determination of the drug using Fe(III)–Fe(II) as an indicating redox system and two polarized Pt wire electrodes between which a small potential difference is applied. Several parameters in relation to the through detector can be selected which allows the determination of a wide family of drugs susceptible to oxidation and they increase the selectivity. The proposed continuous procedure presents some additional advantages for epinephrine determination, namely a low linear application range and a high injection throughput.

References

[1] W.I. Mohamed and F.B. Salem, Anal. Lett., 17 (1984) 191.
[2] F.B. Salem, Talanta, 34 (1987) 810.
[3] D. Amin, Analyst, 111 (1986) 255.
[4] M.J. Rodriguez-Dopazo, M. Silwa and D. Perez-Bendito, Microchem. J., 39 (1989) 235.
[5] H. Nohta, M.K. Lee and Y. Ohkura, Anal. Chim. Acta, 267 (1992) 137.
[6] M. Camona, M. Silva and D. Perez-Bendito, Analyst, 116 (1991) 1075.
[7] C. Martinez-Lozano, T. Perez-Ruiz, V. Tomas and O. Val, Analyst, 116 (1991) 857.
[8] M.V. Camanas, M.S.Mallos, R.T. Lapasio and G. Ramisramos, Analyst, 120 (1995) 1767.
[9] H.D. Szulczewski and Wen-hai Hong, in K. Florey (Ed.), Analytical Profiles of Drug Substances, Vol. VII, Academic Press, Orlando, 1982.
[10] A. Kojło and J. Martinez Calatayud, Anal. Lett., 28 (1995) 239.
[11] A. Kojło and J. Martinez Calatayud. Anal. Chim. Acta, 308 (1995) 334.
[12] A. Kojło and J. Martinez Calatayud. J. Pharm. Biomed. Anal., 8 (1990) 663.
[13] J.J. Nevado, J.M. Gallego and P. Laguna. Anal. Chim. Acta, 300 (1995) 293.
[14] T. Perez Ruiz, C. Martinez Lozano, V. Tomas and O. Val. Talanta, 40 (1993) 1625.
[15] M. Trojanowicz and J. Michałowski, J. Flow Injection Anal., Vol. 11, No. 1 (1994).
[16] A. Hulanicki, W. Matuszewski and M. Trojanowicz, Anal. Chim. Acta, 194 (1987) 119.
[17] W. Matuszewski and M. Trojanowicz, Anal. Chim. Acta, 207 (1988) 59.
[18] J. Michałowski, A. Kojlo, M. Trojanowicz, B. Szostek and E.A.G. Zagatto, Anal. Chim. Acta, 271 (1992) 239.
[19] J. Michałowski and M. Trojanowicz, Anal. Chim., Acta, 281 (1993) 299.
[20] J. Michałowski, A. Kojło, B. Magnuszewska and M. Trojanowicz, Anal. Chim. Acta, 289 (1994) 339.
[21] S. Kalinowski and Z. Figaszewski, Meas. Sci. Technol, 6 (1995) 1050.
[22] Martindale, The Extra Pharmacopoeia. Pharmaceutical Press, 30th edn., 1993.