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Electroanalytical determination of paroxetine in pharmaceuticals

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Abstract

Electroanalytical methods based on square-wave adsorptive-stripping voltammetry (SWAdSV) and flow-injection analysis with SWAdSV detection (FIA-SWAdSV) were developed for the determination of paroxetine (PRX). The methods were based on the reduction of PRX at a mercury drop electrode at −1.55 V versus Ag/AgCl, in a borate buffer of pH 8.8, and the possibility of accumulating the compound at the electrode surface. Because the presence of dissolved oxygen did not interfere significantly with the analysis, it was also possible to determine PRX using FIA-SWAdSV. This method enables analysis of up to 120 samples per hour at reduced costs. Both methods developed were validated and successfully applied to the quantification of PRX in pharmaceutical products.

Keywords: Paroxetine; Square-wave adsorptive-stripping voltammetry; Flow-injection analysis; Electrochemical detection; SSRI

1. Introduction

Paroxetine (PRX, Fig. 1), a selective serotonin reuptake inhibitor (SSRI), has been approved by the US Food and Drug Administration as safe and effective in the treatment of depression, generalized anxiety disorder, social anxiety disorder, panic disorder, obsessive compulsive disorder and posttraumatic stress disorder. Since its introduction to the market in 1991 a large number of analytical methodologies for PRX determination have been published. Because of the ever-increasing need for analytical methods with high sample-throughput, low limits of detection, and low maintenance costs, new methodologies are constantly being developed. During the past 6 years the majority of these methods were based on chromatography; high-performance liquid chromatography coupled with ultraviolet/diode array [1–11], mass [12–18], fluorimetric [19,20], and coulometric [21] detectors; gas chromatography with mass [22–24], flame-ionisation [25], nitrogen–phosphorus [26] and electron-capture [27] detection; micellar electrokinetic capillary chromatography [28]; and thin layer chromatography [9,29]. Capillary electrophoresis [30–32], flow-injection analysis with ultraviolet detection [33], and visible spectrophotometry [34,35] have also been applied in the analysis of PRX.

Although the electrochemical determination of the other SSRIs, fluoxetine [36], fluvoxamine [37,38], sertraline [39,40], and citalopram [41] by square-wave adsorptive-stripping (SWAdSV) has been described, only one electrochemical study of PRX, based on oxidation, has been published to date [6].

Adsorptive stripping voltammetry is a highly sensitive and selective technique for the analysis of organic compounds which can be accumulated at the hanging mercury drop electrode (HMDE) surface and afterwards stripped off by applying a potential scan [42]. This allows their selective pre-concentration at the electrode; a high sensitivity is also obtained, due to the use of a high frequency square-wave potential scan in the reduction step [43,44]. Furthermore, this type of potential scan decreases the interference of dissolved oxygen, enabling the inclusion of the HMDE in a flow-injection system without prior or on-line oxygen removal [45]. The previous concepts were used in the development of a SWAdSV method for PRX analysis under batch conditions. Furthermore, a flow-injection analysis method with SWAdSV detection (FIA-SWAdSV) was developed for the same purpose. The methods of analysis were optimised, validated, and successfully applied to quantification of PRX in pharmaceutical products.

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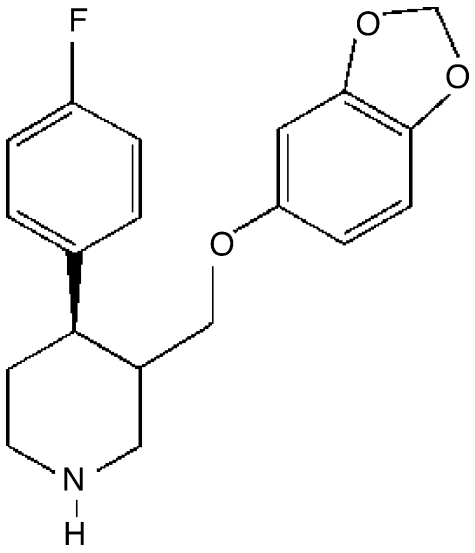

Fig. 1. Chemical structure of PRX.

2. Experimental

2.1. Apparatus

Voltammetric measurements using SWV and SWAdSV were performed with a Autolab PGSTAT12 potentiostat (Metrohm-EcoChemie), controlled by a PC by use of GPES 4.9 software from Metrohm-EcoChemie, and a Metrohm 663 VA stand containing a three-electrode cell (all Metrohm). This voltammetric cell consisted of a multimode mercury working electrode, used in the static mercury-drop position (SMDE), an Ag/AgCl/KCl 3 mol L^{-1} reference electrode, and a glassy-carbon auxiliary electrode. This system was used both under batch conditions and as the detector in the developed single-manifold FIA system.

In the FIA system, the solutions were transported to the voltammetric detection cell by a Gilson Minipuls 3 peristaltic pump, using PTFE tubing (i.d. 0.8 mm). Samples and standards (V_{inj} = 500 μL) were introduced into the carrier stream through a six-port Rheodyne type 5041 injection valve.

In the voltammetric flow cell used [45] the glass capillary of the SMDE is inserted into a PTFE adapter head and the flow is directed towards the mercury drop. This adapter head [46] enables reproducible repositioning of the glass capillary whenever the flow cell is dismounted and then remounted. The adapter head, the reference electrode, and the counter electrode are contained in a glass cell, which is filled with the solution used as the carrier stream. A drain ensures a constant level of solution to be maintained during the work.

2.2. Reagents

A PRX hydrochloride hemihydrate standard was provided by GlaxoSmithKline (Stevenage, Herts, UK) and used without further purification. Stock solutions of PRX were prepared by dissolution of precisely weighed amounts of the standard in water. These stock solutions were diluted with electrolyte solution to furnish the desired concentration for analysis. All other reagents, of p.a. quality, were purchased from Merck and Sigma–Aldrich. All solutions were prepared using deionised water (conductivity <0.1 μS cm^{-1}).

For optimisation of electrolyte pH several universal buffers (I = 0.3 mol L^{-1}) [47] between pH 1.9 and 11.6 were used. After optimisation a pH 8.8 borate buffer (0.2 mol L^{-1}) was used in both the SWAdSV method and the FIA-SWAdSV method.

2.3. Procedures

2.3.1. General procedure

To obtain the background voltammogram by use of SWAdSV a 10.00-mL volume of the supporting electrolyte was introduced in a voltammetric cell and purged with oxygen-free nitrogen for 300 s. The required accumulation potential (E_{acc}) was then applied to the electrode for a selected accumulation time (t_{acc}) while the solution was stirred. Finally, stirring was stopped and a square-wave voltammogram was recorded by applying a potential scan in the negative direction. After recording the background voltammogram, an aliquot of the analyte (standard or sample) solution was added and the procedure was repeated using a purge time of 10 s.

Background voltammograms using FIA-SWAdSV were obtained by simply applying, after the selected accumulation period, a square-wave potential scan in the negative direction while the electrolyte flowed through the adapter head.

2.3.2. Validation procedure

The linear range, limit of detection (LOD), limit of quantification (LOQ), repeatability, intermediate precision, recovery and selectivity were evaluated [48].

The linear range was determined by analysing PRX solutions in the ranges 1.00×10^{-7} to 3.90×10^{-6} mol L^{-1} and 3.00×10^{-6} to 2.10×10^{-5} mol L^{-1} for the SWAdSV and FIA-SWAdSV methods, respectively. The LOD and LOQ were calculated from the linear calibration curve [49].

Repeatability and intermediate precision were assessed at three concentrations. For the SWAdSV method concentration levels of 6.00×10^{-7}, 1.50×10^{-6}, and 2.40×10^{-6} mol L^{-1} (t_{acc} 10 s) and 2.00×10^{-7}, 5.00×10^{-7}, and 8.00×10^{-7} mol L^{-1} (t_{acc} 30 s) were used. The precision of the flow-injection method was evaluated by use of 4.50×10^{-6}, 9.00×10^{-6}, and 1.35×10^{-5} mol L^{-1} PRX solutions. To assess the repeatability five replicate measurements of each solution were made in a short period of time. To determine intermediate precision the solutions were each analysed five times per day for three consecutive days.

The accuracy of the procedure was verified by performing recovery assays at three concentrations, in triplicate. In the evaluation of the SWAdSV method solutions of the pharmaceuticals containing 8.0×10^{-7} mol L^{-1} PRX were spiked with PRX standard to furnish final concentrations of 1.1×10^{-6}, 1.4×10^{-6}, and 1.7×10^{-6} mol L^{-1} (i.e. additions of 3.0×10^{-7}, 6.0×10^{-7}, and 9.0×10^{-7} mol L^{-1}). For the flow-injection method spikes of 2.0×10^{-6}, 4.0×10^{-6}, and 6.0×10^{-6} mol L^{-1} were added to solutions of the pharma-

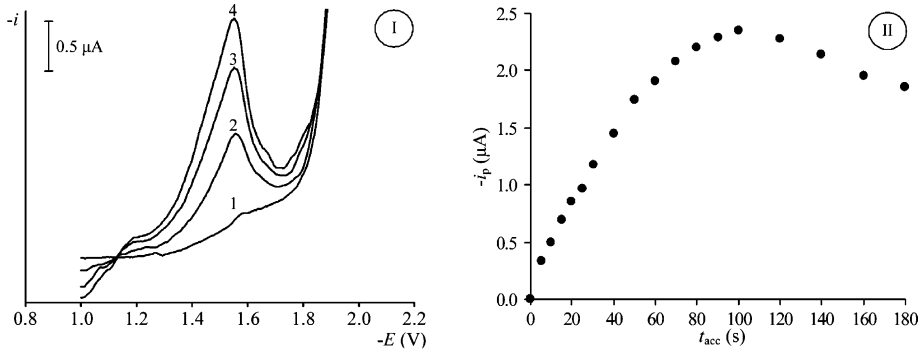

Fig. 2. Square-wave adsorptive-stripping voltammetry of a 5×10^{-7} mol L^{-1} PRX solution. $E_{acc} = -1.0$ V; $f = 500$ Hz; $\Delta E_s = 5$ mV; $\Delta E_p = 25$ mV. (I) Voltammograms for t_{acc} = (1) 0, (2) 20, (3) 40, and (4) 60 s. (II) Effect of t_{acc} on i_p.

ceuticals with a PRX concentration of 5.0×10^{-6} mol L^{-1}, to obtain final concentrations of 7.0×10^{-6}, 9.0×10^{-6}, and 1.1×10^{-5} mol L^{-1} PRX.

2.3.3. Pharmaceutical analysis

The adequacy of the developed methods was evaluated by quantifying PRX in several commercial pharmaceutical formulations available on the Portuguese market (labelled values of 20 mg PRX tablet^{-1}). For the analysis five tablets of each product were weighed, finely powdered, and a mass equivalent to about 10 mg of PRX was dissolved in 100.0 mL deionised water by sonication for 15 min.

For batch analysis the resulting solution was diluted 55-fold with water and an adequate volume of this diluted solution was added to 10.00 mL of electrolyte contained in the voltammetric cell. Standard additions between 0 and 1.10×10^{-6} mol L^{-1} were made by adding increasing quantities of PRX standard to the sample solution contained in the voltammetric cell. This procedure was repeated in triplicate for each pharmaceutical. There was no need for prior filtration of the sample because a large dilution was made and the standard addition method was used.

Prior to the flow-injection technique the sample solution was filtered to prevent obstruction of the manifold tubing. Seven standards were prepared by placing 165-μL sample aliquots in 10.00-mL volumetric flasks and standard additions between 0 and 6.00×10^{-6} mol L^{-1} were made. The volume was completed with electrolyte and the solutions were injected into the FIA system.

3. Results

3.1. Analysis of paroxetine using SWAdSV

The electrochemical activity of PRX, using SWV and SWAdSV, was only verified in the pH range from 5 to 9, in which the peak current intensity (i_p) and peak potential (E_p) were independent of pH. Suitable sensitivity, repeatability, and peak definition were obtained using a pH 8.8 borate buffer. Because the highest i_p was obtained by SWAdSV, this method was optimised for PRX analysis. For this purpose the accumulation potential (E_{acc}) was varied between −0.2 and −1.3 V using an accumulation time (t_{acc}) of 30 s. The highest signal was obtained at an E_{acc} of −1.0 V, which was used in the subsequent studies. At this E_{acc} the preconcentration of 5×10^{-7} mol L^{-1} PRX at the electrode followed by a SWV scan resulted in a single stripping peak at approximately −1.55 V (Fig. 2I) which increased linearly with t_{acc} for up to 60 s (Fig. 2II). For longer deposition times deviation of linearity was observed, indicating that saturation coverage of the drop is gradually achieved and that competitive adsorption may occur. Further optimisation was carried out using a 5×10^{-7} mol L^{-1} PRX solution and a t_{acc} of 30 s. To obtain maximum i_p and repeatability the influence of the square-wave frequency (f, 100–2000 Hz), pulse step (ΔE_s, 1–10 mV) and pulse amplitude (ΔE_p, 5–100 mV) on the stripping response were studied and optimised and led to the following optimum conditions: $f = 600$ Hz, $\Delta E_s = 6$ mV, and $\Delta E_p = 40$ mV.

Table 1 lists the achieved linear range, the LOD and LOQ values obtained in the voltammetric determination of PRX, under the optimised conditions, for accumulation times of 10 and 30 s. Although longer accumulation times increase sensitivity they also lead to smaller linear ranges and lower sample-throughputs, so they were not included further in this study.

Precision assays were carried out at three concentration levels for two accumulation times (10 and 30 s), the results obtained, expressed as the relative standard deviation (R.S.D.), ranged from 0.5% to 1.4% for repeatability and from 0.6% to 4.6% for intermediate precision (Table 2). These results indicate that the method is precise and also confirm the stability of the drug solutions during the evaluation period.

Table 1
Characteristics of the calibration curves of PRX obtained with SWAdSV

t_{acc} (s)	Regression equation ($-i_p$ (A); C_{PRX} (mol L^{-1}))	n	Linear range (mol L^{-1})	Correlation coefficient	LOD (mol L^{-1})	LOQ (mol L^{-1})
10	$-i_p = (1.13 \pm 0.01)C_{PRX} + (5.76 \pm 1.88) \times 10^{-8}$	14	3.00×10^{-7} to 2.90×10^{-6}	0.9995	8.7×10^{-8}	2.9×10^{-7}
30	$-i_p = (3.01 \pm 0.05)C_{PRX} + (3.57 \pm 2.80) \times 10^{-8}$	5	1.00×10^{-7} to 9.00×10^{-7}	0.9984	6.2×10^{-8}	2.1×10^{-7}

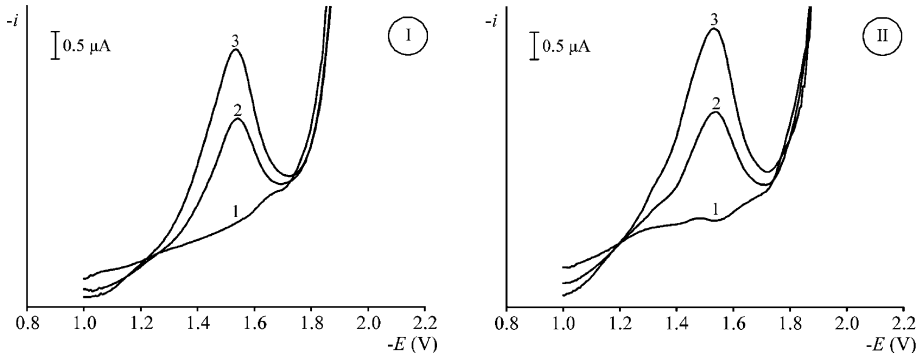

Fig. 3. Effect of dissolved oxygen on the voltammetric determination of PRX. PRX concentration: (1) 0; (2) 5×10^{-7}; (3) 1×10^{-6} mol L^{-1}. $E_{acc} = -1.0$ V; $t_{acc} = 30$ s; $f = 600$ Hz; $\Delta E_s = 6$ mV; $\Delta E_p = 40$ mV. Deoxygenation time: (I) 300 s and (II) 0 s.

Recovery assays were performed at three concentration levels (3.0×10^{-7}, 6.0×10^{-7}, and 9.0×10^{-7} mol L^{-1}) and the results obtained, 98–102%, indicate that the method is accurate and selective for PRX analysis in the used pharmaceutical products.

Because of the high square-wave frequency (600 Hz) used the time-consuming deoxygenation of the analysed solution could be avoided. In Fig. 3 voltammograms with and without oxygen removal of different PRX solutions are shown. It can be concluded that dissolved oxygen did not interfere significantly with the determination of PRX, enabling the inclusion of the HMDE in an FIA system, without prior or on-line oxygen removal, which leads to an increase in sample-throughput.

3.2. Determination of paroxetine in pharmaceutical products using SWAdSV

The method developed was applied in the determination of PRX in pharmaceutical products, using the procedure described previously. The results are reported in Table 3 and are in good agreement with the labelled values of the products (20 mg tablet^{-1}).

3.3. Determination of paroxetine using FIA-SWAdSV

On the basis of the results obtained in the batch study, an SWAdSV flow-detection system for the analysis of PRX was used in a single-manifold FIA system. The adsorption and measurement steps were performed while the sample solution slug was flowing through the adapter head containing the mercury electrode. No significant perturbation of the voltammetric signal because of the flow of electrolyte (borate buffer, pH 8.8) was observed. Dispersion of the injected sample was minimised by keeping the length of the tube between the injection valve and the HMDE as short as possible.

Table 2
Results from evaluation of the precision of SWAdSV

Property	t_{acc} (10 s)	t_{acc} (30 s)
Repeatability (R.S.D.%) ($n = 5$)	1.4 (0.60)	1.3 (0.20)
	1.0 (1.5)	0.5 (0.50)
	0.5 (2.4)	0.6 (0.80)
Intermediate precision (R.S.D.%) ($n = 3$)	2.1 (0.60)	4.6 (0.20)
	0.6 (1.5)	0.9 (0.50)
	2.0 (2.4)	0.7 (0.80)

Concentrations, in μmol L^{-1}, are given in parentheses.

Table 3
Determination of paroxetine in several drugs using SWAdSV

Product	C_{PRX} (mg tablet^{-1})[a]
Seroxat (GlaxoSmithKline)	20.1 ± 0.1
Paxetil (Bial)	20.4 ± 0.6
Paroxetina Sandoz	20.3 ± 0.3
Paroxetina Alpharma	20.0 ± 0.4
PaxPar (Merck)	20.6 ± 0.2
Paroxetina Merck genéricos	20.3 ± 0.1
Paroxetina Generis	20.0 ± 0.3

[a] Mean ± S.D. ($n = 3$).

A constant i_p value was obtained for flow rates between 1.1 and 1.7 mL min^{-1}, which decreased for higher flow rates. A flow rate of 1.5 mL min^{-1} provided suitable sample-throughput and sensitivity. Using this flow rate, an injection volume of 500 μL, and a tube length of 50 cm between the injector and the detector, a constant concentration profile between ±18 and 35 s after injection was obtained (Fig. 4). The length of this constant concentration zone can easily be controlled by changing the injection volume and the flow rate. The adsorption step and the voltammetric scan must be performed within this time interval.

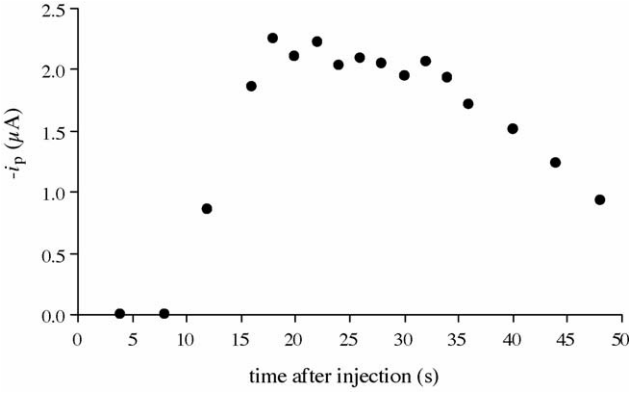

Fig. 4. Variation of i_p with time after sample injection for a 1×10^{-5} mol L^{-1} PRX solution, $t_{acc} = 3$ s.

Table 4
Precision of the FIA-SWAdSV method

Property	
Repeatability (R.S.D.%) ($n=5$)	2.4 (4.50) 1.7 (9.00) 2.3 (13.5)
Intermediate precision (R.S.D.%) ($n=3$)	2.0 (4.50) 0.4 (9.00) 4.7 (13.5)

Concentrations, in μmol L^{-1}, are given in parentheses.

The influence of the same parameters studied under batch conditions on i_p, repeatability and sample-throughput were optimised using a 1×10^{-5} mol L^{-1} PRX solution. The optimum E_{acc} was found to be −1.0 V, according to the result obtained in batch, and a linear variation of i_p with t_{acc} from 0 to 5 s was observed. For longer t_{acc} (up to 10 s) no further increase of i_p was verified, probably because of saturation coverage of the drop, competitive adsorption, or removal of adsorbed species by the flow. For the optimisation of f (50–1000 Hz), ΔE_s (1–10 mV), and ΔE_p (5–100 mV) a t_{acc} of 3 s was applied and the optimum conditions for determination of PRX were established as $f = 600$ Hz, $\Delta E_s = 6$ mV, and $\Delta E_p = 40$ mV. Up to 120 samples per hour could be analysed using the proposed flow system.

Using the optimised conditions the variation of i_p with C_{PRX} was represented by a straight-line equation: $-i_p = (0.196 \pm 0.003)C_{PRX} - (1.05 \pm 0.31) \times 10^{-7}$ ($r = 0.9995$; $n = 7$), valid within the linear range 3.00×10^{-6} to 1.70×10^{-5} mol L^{-1} PRX. The LOD and LOQ were found to be 4.8×10^{-7} and 1.6×10^{-6} mol L^{-1}, respectively. Precision assays were carried out at three concentration levels and the results obtained, expressed as the relative standard deviation (R.S.D.), ranged from 1.7% to 2.4% for repeatability and from 0.4% to 4.7% for intermediate precision (Table 4). These results indicate that the method is precise and also confirm the stability of the drug solutions during the evaluation period.

Recovery assays were performed at three concentration levels (2.0×10^{-6}, 4.0×10^{-6}, and 6.0×10^{-6} mol L^{-1}) and the results obtained, 97–105%, indicate that the method is accurate and selective for PRX analysis in the used pharmaceutical products.

Table 5
Determination of paroxetine in several drugs using FIA-SWAdSV

Product	C_{PRX} (mg tablet^{-1})[a]
Seroxat (GlaxoSmithKline)	20.5 ± 0.5
Paxetil (Bial)	19.8 ± 0.6
Paroxetina Sandoz	20.2 ± 0.8
Paroxetina Alpharma	20.2 ± 0.2
PaxPar (Merck)	19.8 ± 0.6
Paroxetina Merck genéricos	20.3 ± 0.4
Paroxetina Generis	19.4 ± 0.2

[a] Mean ± S.D. ($n = 3$).

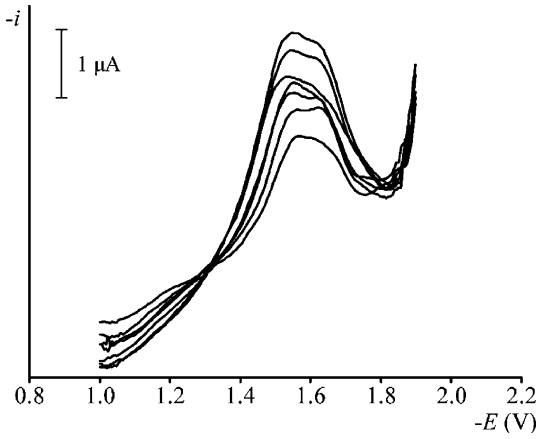

Fig. 5. Flow-injection voltammograms obtained in the determination of PRX in Paroxetine Sandoz using the standard addition method. Standard additions of PRX (10^{-6} mol L^{-1}): 0; 1.00; 2.00; 3.00; 4.01; 5.00; 6.00. $E_{acc} = -1.0$ V; $t_{acc} = 3$ s; $f = 600$ Hz; $\Delta E_s = 6$ mV; $\Delta E_p = 40$ mV; flow rate = 1.5 mL min^{-1}.

3.4. Determination of paroxetine in pharmaceutical products using FIA-SWAdSV

The results obtained in the determination of PRX in pharmaceutical products using FIA-SWAdSV can be seen in Table 5 and are in good agreement with the labelled values (20 mg $tablet^{-1}$). Flow-injection voltammograms obtained in the quantification of PRX in Paroxetina Sandoz are shown in Fig. 5.

4. Conclusions

SWAdSV and FIA-SWAdSV analytical methods have been developed for the determination of paroxetine. This drug was quantified in pharmaceutical products and the results are in good agreement with the labelled values. The FIA method has the advantage of high sample throughput, significantly reducing the analysis time and costs.

Further investigation is undertaken in order to determine PRX in biological samples using on-line sample extraction and using the HMDE flow cell in drug dissolution testing and as an HPLC detector.

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