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Voltammetric determination of Imatinib (Gleevec) and its main metabolite using square-wave and adsorptive stripping square-wave techniques in urine samples

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Abstract

The voltammetric behaviour of Imatinib (STI 571) and its main metabolite (*N*-demethylated piperazine derivative) were studied by square-wave techniques, resulting in to two methods for their determination in aqueous and urine samples at pH 2. The application of the square-wave (SW) without the adsorptive accumulation and voltammetric stripping (AdSV) exhibit a peak at a reduction potential of -0.70 V for an accumulation potential of -0.45 V. The sensitivity was higher for the stripping technique because a signal four times higher than that provided by the square-wave method without the previous accumulation was obtained. Due to the fact that Imatinib and its metabolite show the same voltammetric reduction process, some experiments were performed in order to compare the voltammetric response of Imatinib and its main metabolite in a similar ratio than that of the therapeutic concentration. The calibration curve for Imatinib in urine was linear in the range from 1.9×10^{-8} to 1.9×10^{-6} M in stripping mode with an accumulation time (t_{acc}) of 10 s. The relative standard deviations obtained for concentration levels of Imatinib as low as 2.0×10^{-7} M for square-wave was 2.17% ($n = 9$) and for stripping square-wave was 2.65% ($n = 9$) in the same day. The limits of detection for square-wave and stripping square-wave were 5.55×10^{-9} and 5.19×10^{-9} M, respectively. Thus, the presented method are straightforward, rapid and sensitive and has been applied to the determination of Imatinib and its main metabolite altogether in urine samples from real patients.

Keywords: Voltammetry; Stripping voltammetry; Square-wave; Gleevec; Imatinib; STI 571; Clinical urine analysis

1. Introduction

Gleevec (Imatinib mesylate) is a new chemotherapy drug indicated for the treatment of patients with chronic myeloid leukemia (CML) in blast crisis, accelerated phase, or in chronic phase after failure of interferon-alpha therapy. It is the first of its kind developed to fight cancer by turning off an enzyme that causes cells to become cancerous and multiply.

Recently, signal transduction inhibitor 571 (STI 571 or Gleevec) was approved by the food and drug administration (FDA) in record time [1] for the treatment of patients at any of the three stages of CML.

Gleevec or Imatinib mesylate, is chemically designated as 4-[(4-methyl-1-piperazinyl)methyl]-*N*-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate (Fig. 1). It is an inhibitor of the tyrosine kinase protein, which inhibits the Bcr-Abl tyrosine kinase at the in vitro, cellular, and in vivo levels. The compound selectively inhibits proliferation and induces apoptosis in Bcr-Abl positive cell lines as well as fresh leukemic cells from Philadelphia chromosome positive CML and acute lymphoid leukemia (ALL) patients [2].

The recommended dosage of Imatinib is 400 mg per day for patients in chronic phase CML and 600 mg per day for patients in accelerated phase or blast crisis. The prescribed dose should be administered orally. The treatment should be continued as long as the patient continues to benefit. The dose

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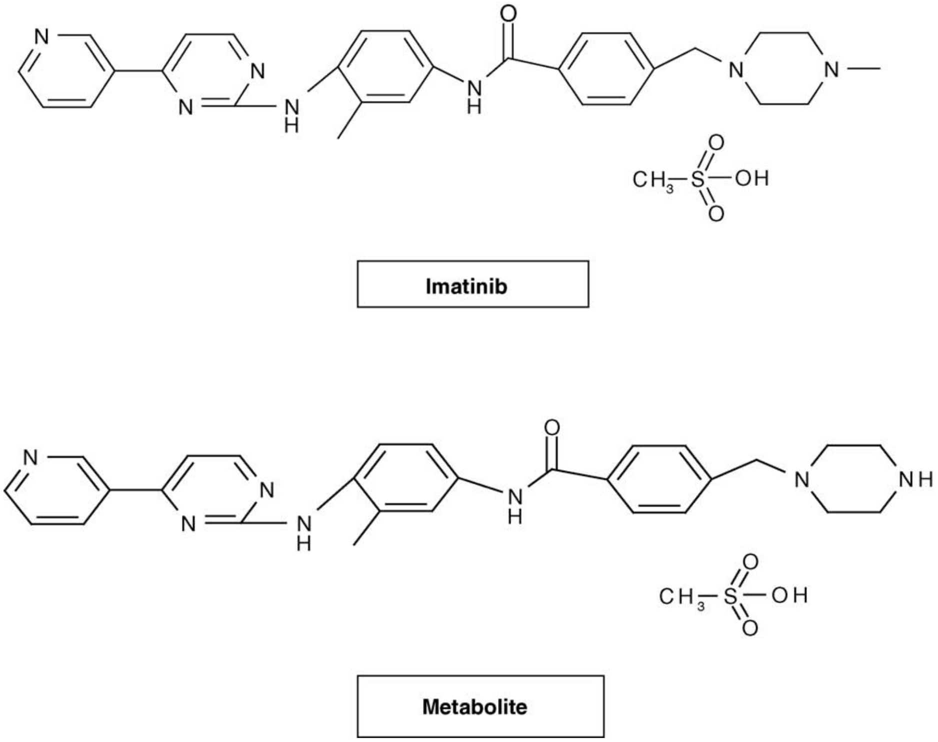

Fig. 1. Chemical structures of Imatinib and its main metabolite.

decreases from 400 to 100 mg in patients with severe adverse drug reaction.

In addition, Imatinib is an inhibitor of the receptor tyrosine kinases for platelet-derived growth factor (PDGF) and stem cell factor (SCF), c-kit, and inhibits PDGF- and SCF-mediated cellular events. In vitro, Imatinib inhibits proliferation and induces apoptosis in gastrointestinal stromal tumor (GIST) cells, which express an activating *kit* mutation.

The main circulating active metabolite in humans is the *N*-demethylated piperazine derivative (Fig. 1). It shows in vitro potency similar to the parent compound, Imatinib. The plasma concentration for this metabolite is about 15% that of Imatinib and the terminal half life is approximately 40 h at a steady state. There is no change in the kinetics of this metabolite on repeated dosing and it did not unexpectedly accumulate after long-term administration of Imatinib to patients [3].

Based on the recovery of compounds after an oral ^{14}C-labelled dose of Imatinib, approximately 81% of the dose was eliminated within 7 days in faeces (68% of dose) and urine (13% of dose). Unchanged Imatinib accounted for 25% of the dose (5% urine, 20% faeces), the remaining being metabolites [4].

Liquid chromatography-tandem mass spectrometry has shown to be a useful technique for the study and determination of the anti-leukemia drug (Imatinib) and its main metabolite in human plasma [5] and in monkey plasma using a semi-automated solid phase extraction procedure [6].

The determination of Imatinib and its main metabolite by capillary zone electrophoresis (CZE) in urine without any previous treatment of the biological sample was reported by Rodriguez et al. [7].

This paper shows that Imatinib and its main metabolite (*N*-demethylated piperazine derivative) experience a strong adsorption process onto an static mercury electrode. By using this phenomenon and by accumulation of these compounds at a hanging mercury dropping electrode (HMDE) prior to square-wave voltammetric measurement, higher sensitivities can be readily achieved in order to achieve determination of the total content. The influence of several experimental (pH, ionic strength) and instrumental (step increment, pulse amplitude, frequency, drop size) variables on the square-wave and on the stripping square-wave response of these drugs has been evaluated. Finally, the proposed method was applied to the determination of Imatinib and its metabolite in urine.

2. Experimental

2.1. Materials

All solvents and the reagents were analytical reagent grade unless indicated otherwise. Solutions for voltammetric study were prepared with deionized water (Milli-Q quality). Stock solutions of 0.012 M $HClO_4$ were prepared using suprapure grade reagent.

Imatinib and its main metabolite (*N*-demethylated piperazine derivative) were obtained from Novartis Pharma AG, Basel, Switzerland. Standard solutions were prepared with deionised water (Milli-Q quality) and stored at 4 °C.

2.2. Apparatus

Voltammograms were obtained with a Princeton applied research (PAR) (Princeton, NJ) model 384B polarographic analyser combined with a PAR Model 303^a hanging mercury drop electrode (HMDE) using an Ag/AgCl reference electrode, a PAR Model 305 magnetic stirrer, an HP computer, using 384 software, and an HP 510 printer.

Cyclic voltammograms were obtained with a PAR Model 264A polarographic analyser/stripping voltammeter combined with the PAR 303A stand and a Yokogawa 3022 A4 X-Y recorder. A crison micropH 2002 was used for the pH measurements.

2.3. Procedure

For the voltammetric experiments, 10 mL of perchloric acid (12 mM) was purged with oxygen-free nitrogen for 10 min (and for 2 min in subsequent runs). The required accumulation potential (E_{acc}) of −0.45 V was then applied to the electrode for a selected accumulation time ($t_{acc} = 10$ s) while the solution was stirred at 600 rpm and after a 10 s rest period a square-wave scan started in the negative direction (stripping square-wave method).

2.4. Results and discussion

The preconcentration of Imatinib at the HMDE and the application of a subsequent differential pulse voltammetric (DPV) scan in the negative direction gave rise to on stripping reduction peak ($E_p = -0.520$ V) at pH 2.0 ($HClO_4$) (Fig. 2). As can be seen in Fig. 2, a higher peak is obtained after only 10 s of accumulation time for a concentration of 4.0×10^{-7} M of the drug. On the contrary, the solution phase response (direct response, $t_{acc} = 0$ s) shows a very small reduction peak as expected for such a low concentration. These facts indicate that the Imatinib undergoes an adsorptive preconcentration at the mercury electrode before its reduction process.

In Fig. 2 it can be readily seen that a square-wave form applied to the stripping of the adsorbate provides high analytical signals (the most sensitive reduction peak was placed at $E_p = -0.700$ V, $I_p = 2466$ nA), compared with applying a differential pulse wave form ($E_p = -0.520$ V, $I_p = 134$ nA) after an accumulation step of 10 s.

At this point, the nature of the two electrochemical processes was also studied by cyclic voltammetry (CV). Fig. 3 shows two different cyclic voltammograms for a 1.0×10^{-6} M solution of Imatinib without accumulation and after a step of 10 s of accumulation time (scan rate 100 mV/s). The reduction process ($E_p = -0.700$ V) was not accompanied by anodic waves, which indicates that the redox reactions are totally irreversible. When the potential was scanned at increasing rates from 10 to 100 mV/s, under the same experimental conditions, a linear relationship was observed

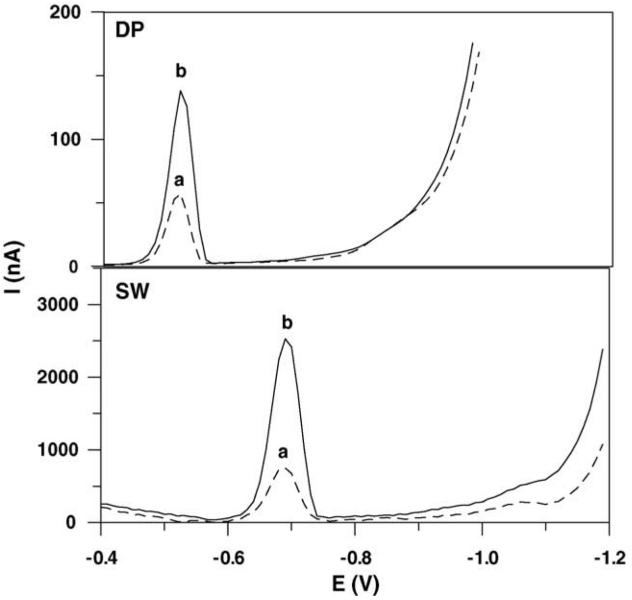

Fig. 2. Differential pulse (DP) and square-wave (SW) adsorptive stripping voltammograms of a solution containing 2.0×10^{-7} M of Imatinib at pH 2. Accumulation time: (a) 0 s and (b) 10 s. Experimental conditions: (DP) accumulation potential −0.35 V, equilibration time 10 s, scan rate 20 mV s^{-1}, pulse amplitude −40 mV, scan increment 10 mV and step/drop 0.5 s; (SW) accumulation potential −0.35 V, equilibration time 10 s, frequency 120 Hz, pulse amplitude −40 mV and scan increment 10 mV.

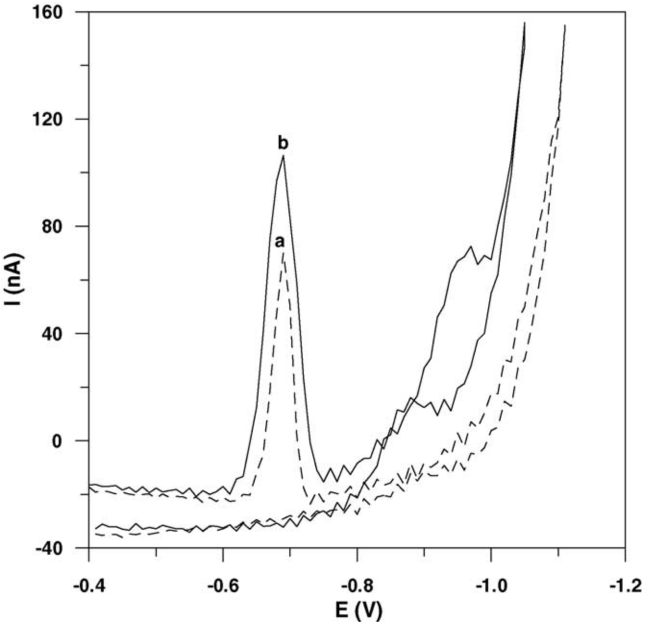

Fig. 3. Cyclic voltammogram obtained for a 1.0×10^{-6} M Imatinib solution at pH 2. Accumulation time: (a) 0 s and (b) 10 s. Experimental conditions: accumulation potential −0.45 V, equilibration time 10 s, scan rate 100 mV s^{-1}, scan increment 10 mV and step/drop 0.1 s.

between the peak intensity I_p (nA) and the scan rate V_b (mV/s), demonstrating that the phenomenon is adsorption-controlled

$$I_p = (14.0 \pm 3.7) + (0.70 \pm 0.07)V_b$$
$$(r^2 = 0.991, n = 8, t_{acc} = 0\,s)$$

Apart from this increase in the analytical response caused by the use of higher scan rates, the significant improvement in sensitivity, achieved by employing a square-wave scan can be described in terms of the characteristic parameters governing this technique.

The influence of pH on the voltammetric response of Imatinib was studied, in order to optimise this parameter. For this purpose, the voltammetric response was recorded at pH values ranging from 2.0 up to 5.5 by using perchloric acid or the adequate buffers (acetic and phosphate buffers). The highest peak intensity was obtained for 2.0 which was provided by $HClO_4$. That is why this pH was selected for further steps of this study. Moreover, it is remarkable that a peak shift towards more negative values is observed as the pH increases. This fact indicates that the protons were involved in the reduction process.

2.4.1. *Square-wave voltammetry SWV*

The checked parameters for SW were the ionic strength of the supporting electrolyte, the initial potential, the pulse height, the frequency and the pulse increment. These parameters are interrelated and have a combined effect on the response but only the general trends will be examined.

The ionic strength of the supporting electrolyte ($HClO_4$–$NaClO_4$) was studied in the range from 0.01 to 0.10 M for a 3.2×10^{-7} M of Imatinib solution. A low ionic strength (0.01 M) was seen to provide the best and the largest response as expected in adsorptive phenomena. The peak potential (E_p) remained constant all along this study. The lowest ionic strength was very effective for the adsorptive accumulation of Imatinib on the electrode, as described by Wang on the behaviour of riboflavine in a 1 mM NaOH medium [8].

In Table 1 the optimum conditions selected by the studied technique and the range tested for each parameter are summarized.

The voltammograms at different concentrations of Imatinib were recorded using the optimal conditions. From this experiment, the linearity range was checked from 2.0×10^{-8} up to 1.0×10^{-6} M and the resulting calibration curve, calculated by the least-squares method, was:

$$I = (45 \pm 34) + (185.0 \times 10^7 \pm 7.7 \times 10^7)C,$$
$$r^2 = 0.996, \quad n = 13 \qquad (1)$$

where C is the concentration (molar) of the Imatinib, I is the peak intensity (nA, corresponding reduction process) and r^2 denotes determination coefficient.

Table 1
Optimised conditions to determine Imatinib by SWV

Parameter	Variation range	Optimum value
Accumulation potential	−0.35 to −0.55 V	−0.45 V
Pulse amplitude	10–60 mV	40 mV
Scan increment	2–10 mV	10 mV
Frequency	10–120 Hz	120 Hz
Electrode area	1.0–2.5 mm^2	2.5 mm^2

The relative standard deviations obtained for concentration levels of Imatinib as low as 2.0×10^{-7} M with square-wave was 0.72% ($n = 9$) in the same day.

2.4.2. *Square-wave adsorptive stripping voltammetry (SWAdSV)*

Considering that the response obtained in the stripping of Imatinib by SWAdSV is better than the SWV response (Fig. 2), the SWAdSV was expected to be successfully applied to the determination of this drug. Consequently, the parameters governing this voltammetric mode were studied.

Several preconcentration-stripping voltammograms were recorded for accumulation potentials varying from −0.35 to −0.55 V at a t_{acc} of 10 s. The peaks showed a big intensity for an accumulation potential of −0.45 V, and therefore this value was chosen as optimal accumulation potential.

Further experiments were made by varying the pulse amplitude (10–60 mV), scan increment (2–10 mV), frequency (10–120 Hz) and drop size (1.0–2.5 mm^2), in order to find the best analytical signal. The values selected as optimal were 40 mV, 10 mV, 120 Hz and the largest size of the electrode, respectively.

Different voltammograms with increasing accumulation times were recorded for a solution containing 8.0×10^{-8} M of Imatinib using the selected conditions. The resulting peaks showed (Fig. 4) a linear relationship between peak intensity and accumulation time up to 90 s for the studied solution. Although the peak intensity still increases for accumulation times over 90 s, it does with a lower slope due to the saturation of the electrode area. Thus, when certain coverage of the electrode is reached, the interactions among the molecules in the adsorbed state become noticeable. Finally, the calibration curve was obtained using 10 s accumulation time.

Voltammograms at different concentrations of Imatinib were recorded using the optimal conditions. From this experiment, the linearity range was tested between 1.0×10^{-8} and 4.8×10^{-7} M and the calibration curve, calculated using the least-squares method, was:

$$I = (70 \pm 34) + (628 \times 10^7 \pm 15 \times 10^7)C,$$
$$r^2 = 0.999, \quad n = 11 \qquad (2)$$

where C is the concentration (molar) of Imatinib, I is the intensity peak (in nA corresponding reduction process) and r^2 denotes determination coefficient.

The relative standard deviations obtained for concentration levels of Imatinib as low as 2.0×10^{-7} M with square-wave was 1.39% ($n = 9$) in the same day.

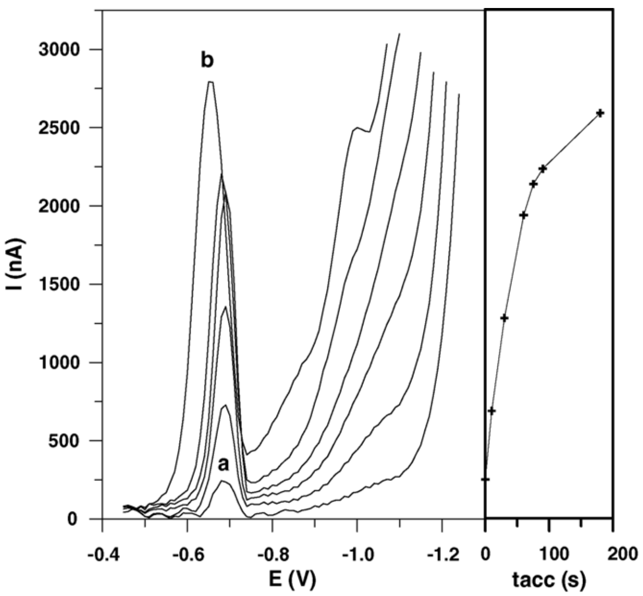

Fig. 4. Effect of the accumulation time on the SW adsorptive stripping signal for a solution containing 8.0×10^{-8} M of Imatinib (pH 2). Accumulation time: (a) 0 s and (b) 180 s. Experimental conditions: accumulation potential −0.45 V, equilibration time 10 s, frequency 120 Hz, pulse amplitude −40 mV and scan increment 10 mV.

2.4.3. Determination of Imatinib and its main metabolite in urine

As stated above, the *N*-demethylated piperazine derivative is the main metabolite of Imatinib, and that is why both compounds are always present in the urine of a patient who has been treated with Imatinib. Because Imatinib and its metabolite show the same voltammetric reduction process, some experiments were performed in order to compare the voltammetric response of Imatinib and its main metabolite in a similar ratio to the therapeutic concentration.

The linearity of the voltammetric response at two accumulation times (0 and 10 s) were checked in aqueous medium for the metabolite. The regression lines, calculated using the least-squares method, were:

- SW

$$I = (83 \pm 50) + (185.0 \times 10^7 \pm 7.6. \times 10^7)C,$$
$$r^2 = 0.996, \quad n = 14 \;\; (1.9 \times 10^{-8} \text{ to } 1.9 \times 10^{-6}) \qquad (3)$$

- SWAdSV

$$I = (82 \pm 83) + (666 \times 10^7 \pm 37 \times 10^7)C,$$
$$r^2 = 0.995, \quad n = 10 \;\; (1.9 \times 10^{-8} \text{ to } 4.6 \times 10^{-7}) \qquad (4)$$

where C is the concentration (molar) of metabolite, I is the peak intensity (nA, corresponding reduction process) and r^2 denotes determination coefficient.

An analysis of variance (ANOVA) test was performed to compare the different regression lines obtained, to determine whether the data could be combined to enable an estimation of appropriate quantities by using a comprehensive calibration curve [9,10]. The analysis of variance values are shown in Table 1. If the experimental value of F is lower than the theoretical value, there are no significant differences between variances, whereas, if F_{theor} is lower than the experimental value of F, there are significant differences between variances. The t-test was used to compare the different slopes of the calibration curves. If the experimental value of t is lower than the theoretical value, there are no significant differences between slopes.

Table 2
Calibration curves and analysis of variance (ANOVA)

Comparison regression line	F_{exp}	F_{theor}	t_{exp}	t_{theor}
(1) and (3)	3.000	3.430	0.061	2.190
(2) and (4)	5.710	3.438	2.230	2.306
(5) and (7)	1.980	2.950	3.218	2.090
(6) and (8)	5.252	3.179	3.131	2.095
(5) and (9)	1.070	3.478	0.002	2.090
(6) and (10)	1.119	3.179	0.277	2.095
(5) and (11)	1.040	3.478	24.740	2.090
(6) and (12)	0.195	3.179	3.329	2.095

As shown in Table 2, no significant differences were found between the calibration curves obtained for Imatinib and its main metabolite (comparison of t_{exp} (between (1) and (3)) and (between (2) and (4))). Therefore, the overall concentration of the two compounds by voltammetric techniques can be determined. Due to this, the calibration curves shown in successive steps of this paper, were obtained with the concentration of Imatinib exclusively, because both compounds show similar response and analytical behaviour.

The linearity of the voltammetric response using different amount of urine (25 or 50 μL), different urine type (female o male) and fresh or unfrozen urine in the voltammetric cell (10 mL) with respect to the overall Imatinib and metabolite concentration was checked. The calibration curves, calculated using the least-squares method, were:

- 25 μL female fresh urine (in voltammetric cell with 10 mL of $HClO_4$)
  - SW

$$I = (23 \pm 11) + (159.0 \times 10^7 \pm 2.5 \times 10^7)C,$$
$$r^2 = 0.999, \quad n = 13 \;\; (2.0 \times 10^{-8} \text{ to } 1.0 \times 10^{-6}) \qquad (5)$$

  - SWAdSV

$$I = (101 \pm 80) + (410 \times 10^7 \pm 28 \times 10^7)C,$$
$$r^2 = 0.992, \quad n = 11 \;\; (2.0 \times 10^{-8} \text{ to } 6.0 \times 10^{-7}) \qquad (6)$$

- 50 μL female fresh urine (in voltammetric cell with 10 mL of $HClO_4$)
  - SW

$$I = (1 \pm 20) + (149 \times 10^7 \pm 74 \times 10^7)C,$$
$$r^2 = 0.996, \quad n = 10 \;\; (6.0 \times 10^{-8} \text{ to } 6 \times 10^{-7}) \qquad (7)$$

- SWAdSV

$$I=(56\pm 55)+(362\times 10^{7}\pm 19\times 10^{7})C,$$
$$r^{2}=0.995,\quad n=11\ \ (4.0\times 10^{-8}\ \text{to}\ 6.0\times 10^{-7})\qquad (8)$$

- 25 μL male fresh urine (in voltammetric cell with 10 mL of $HClO_4$)
  - SW

$$I=(26\pm 12)+(160.0\times 10^{7}\pm 2.8\times 10^{7})C,$$
$$r^{2}=0.999,\quad n=13\ \ (2.0\times 10^{-8}\ \text{to}\ 1.0\times 10^{-6})\qquad (9)$$

  - SWAdSV

$$I=(125\pm 78)+(405\times 10^{7}\pm 27\times 10^{7})C,$$
$$r^{2}=0.992,\quad n=11\ \ (2.0\times 10^{-8}\ \text{to}\ 6.0\times 10^{-7})\qquad (10)$$

- 25 μL female unfrozen urine (in voltammetric cell with 10 mL of $HClO_4$)
  - SW

$$I=(42\pm 11)+(128.0\times 10^{7}\pm 2.6\times 10^{7})C,$$
$$r^{2}=0.999,\quad n=13\ \ (2.0\times 10^{-8}\ \text{to}\ 1.0\times 10^{-6})\qquad (11)$$

  - SWAdSV

$$I=(68\pm 53)+(360\times 10^{7}\pm 18\times 10^{7})C,$$
$$r^{2}=0.992,\quad n=11\ \ (2.0\times 10^{-8}\ \text{to}\ 6.0\times 10^{-7})\qquad (12)$$

where C is the concentration (molar) of Imatinib, I is the peak intensity (nA, corresponding reduction process) and r^2 denotes determination coefficient.

The ANOVA test was performed to compare the different calibration curves obtained. As presented in Table 2, the calibration curves obtained for 25 and 50 μL of urine in the cell (comparison between (5) and (7) and (6) and (8)) and between fresh and unfrozen urine (comparison between (5) and (11) and (6) and (12)) were significantly different. This fact could be due to changes in the matrix composition. But, in contrast, no significant differences were found between female and male urine. For this reason, in all cases, the standard addition method must be used.

Two different samples containing 8.0×10^{-8} M Imatinib were prepared and analysed in two different days using the optimised method. The repeatability was studied by performing a series of nine voltammograms of each sample. The reproducibility was studied by recording nine voltammograms of another sample, 24 h later, under the same conditions. The results showed that the repeatability for Imatinib within each day is satisfactory (Table 3). The comparison of averages with the Snedecor test did not provide any significant difference between the 2 days series for $\alpha = 0.05$ ($n = 9$) [11].

Table 3
Repeatability and reproducibility

	t_{acc} (0 s)		t_{acc} (10 s)	
	Day 1	Day 2	Day 1	Day 2
Intensity peak (nA)	130.0	134.0	323.0	328.0
S.D.	3.0	2.7	9.1	4.4
R.S.D. (%)	2.3	2.0	2.8	1.3
F_{exp}	1.26		4.23	
F_{theor}	4.43		4.43	

Table 4
Recoveries in urine samples

Sample	Imatinib (mol L^{-1})[a]	Recovery (%) SW		Recovery (%) SWAdSV	
		Fresh	Unfrozen	Fresh	Unfrozen
S1	2.0×10^{-5}	110 ± 5	108 ± 6	103 ± 4	94 ± 5
S2	2.80×10^{-5}	104 ± 2	107 ± 5	105 ± 6	96 ± 3
S3	3.64×10^{-5}	108 ± 3	103 ± 4	96 ± 4	100 ± 3

[a] Concentration of Imatinib in the urine. In all cases the standard addition method was used for the drug determination.

The relative standard deviation (R.S.D.) obtained for concentration levels of Imatinib as low as 2.0×10^{-7} M was 2.17% ($n = 9$) for square-wave and 2.65% ($n = 9$) for stripping square-wave in the same day. These R.S.D. values are higher than those obtained for spiked water, but they are still satisfactory, taking into account that they have been obtained by direct measurement in biological samples.

In order to test the accuracy of the proposed method, a pool of different urine samples ($n = 5$) was spiked with Imatinib and its metabolite at three different concentrations. Then five independent analysis were performed for each concentration in both fresh and frozen urine because there were significant differences between the calibration graphs. The voltammograms were recorded using the optimal conditions for their analytical determination. In both cases the standard addition method was used for the drug determination. The results obtained for the determination of Imatinib in urine samples are given in Table 4. As can be seen, the recoveries obtained by square-wave adsorptive stripping voltammetry were better than by square-wave voltammetry.

2.4.4. Limits of detection (LODs) and quantitation (LOQs)

The equations in order to calculate the LOD and LOQ are:

$$\text{LOD}=Y_{B}+3S_{B},\qquad \text{LOD}=Y_{B}+10S_{B}$$

where Y_B is the ordinate in the origin of calibration curves; S_B the standard deviation of blank.

LODs were about 5.55×10^{-9} and 5.19×10^{-9} M for square-wave and stripping square-wave, respectively. The LOQs were estimated to be about 1.85×10^{-8} and 1.73×10^{-8} M for square-wave and stripping square-wave, respectively.

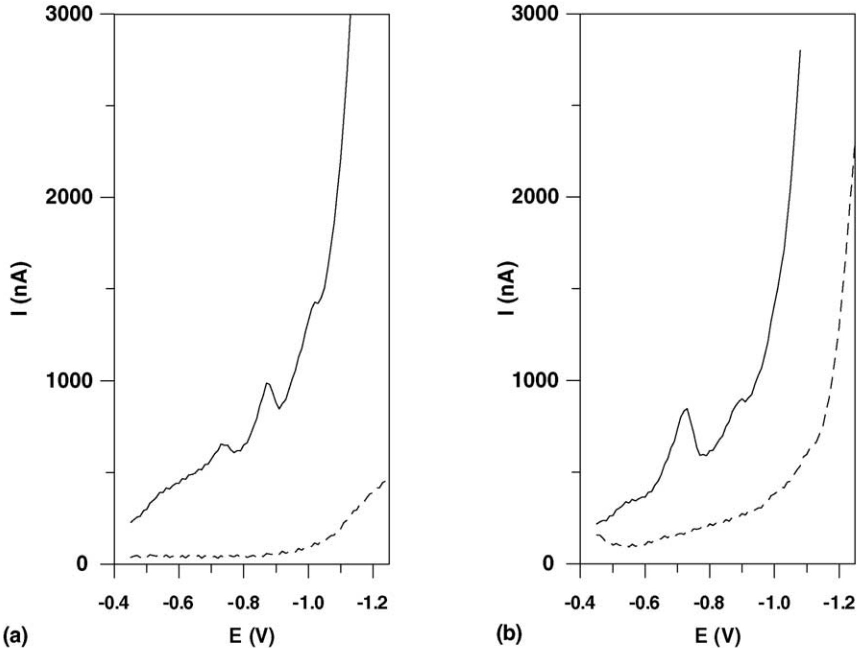

Fig. 5. Square-wave voltammograms corresponding to urine sample from patient A (dosages Imatinib 100 mg per day) at pH 2 and different accumulation times (a) 0 s and (b) 10 s. Experimental conditions: accumulation potential −0.45 V, equilibration time 10 s, frequency 120 Hz, pulse amplitude −40 mV and scan increment 10 mV.

2.4.5. Application to real samples

The methods were applied for the determination of Imatinib and its main metabolite in urine samples (kept frozen) from two patients undergoing medical treatment with Imatinib (real samples). In all cases the standard addition method was used for the drug determination. 25 μL urine sample of patient A undergoing medical treatment with Imatinib (100 mg/day during 1 year) was added to 10 mL of perchloric acid (12 mM) over the voltammetric cell and the total concentration found using this method was 1.99×10^{-5} and 2.04×10^{-5} M (dilution factor 1:400 (urine:water), corresponding to a cell concentration of 5.02×10^{-8} M), for SW (t_{acc}, 0 s) and SWAdS (t_{acc}, 10 s), respectively. Fig. 5 shows the voltammograms corresponding to the urine sample of patient A undergoing medical treatment with Imatinib (100 mg/day).

Our second patient (patient B) took a dosage of 600 mg/day during 6 months. In this case, 5 μL of urine sample of patient B was added to10 mL of perchloric acid (12 mM) in the voltammetric cell. In this case the total concentration found was 1.02×10^{-4} and 1.04×10^{-4} M (dilution factor 1:2000 (urine:water), corresponding to a cell concentration of 2.4×10^{-7} M), for t_{acc} 0 and 10 s, respectively. As can be seen, the concentration found in patient B's urine was five times higher than in patient A's urine, due to the higher dose taken by patient B.

The results provided by both voltammetric methods were similar to those obtained by using a capillary electrophoresis method [7], as shown in Table 5.

The main advantage of the voltammetric method is the lower LOD obtained (40 times lower than the obtained by CE technique [7]). Others advantages were the low level of interferences due to an accumulation step before to AdSV measure and the small time necessary to get a stripping voltammogram (less than 20 s).

These advantages permit to do a determination of Imatinib in urine after a long period time after its administration in the patients.

Accordingly, the voltammetric methods resulted to be useful for an immediate analysis for Imatinib and its main metabolite in urine.

Moreover, it is remarkable the little volume of urine needed (5–25 μL), as well as the low analysis time, which is never over 1 min.

Table 5
Recoveries in real urine samples and comparison with the results obtained by CE

Sample	CE[a]	SW		SWAdSV	
		[a]	Recovery (%)	[a]	Recovery (%)
Patient A	1.98×10^{-5}	1.99×10^{-5}	100 ± 2	2.04×10^{-5}	103 ± 4
Patient B	9.61×10^{-5}	1.02×10^{-4}	106 ± 4	1.03×10^{-4}	107 ± 5

[a] Concentration (molar) of Imatinib and its metabolite in the urine. In all cases the standard addition method was used for the drug determination.

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