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Electrochemical oxidation of CBS-113 A, a new anti-inflammatory drug: applicability to liquid chromatography-electrochemical detection ☆

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Abstract

The electrochemical properties of a new non-steroidal anti-inflammatory drug (2-hydroxy-4-methylphenyl-2-aminothiazole hydrochloride; CBS-113 A) have been studied using voltammetry in direct current and cyclic modes at glassy carbon disk electrodes. The results show an oxidative process with a diffusion-controlled and a reversible mechanism; these data agree with those obtained in a reversed-phase high-performance liquid chromatography (HPLC) system coupled either with amperometric (single glassy carbon electrode) or coulometric detection (two porous graphite electrodes in series) set at potentials of +0.65 V (vs. Ag/AgCl) and +0.4 V (vs. Pd/H_2), respectively. Similar electrochemical properties were found for 2-hydroxyphenyl-2-amino-5'-methylthiazole hydrochloride (RD-1546) which is a potential internal standard. An HPLC system coupled with a UV detector ($\lambda = 272$ nm) and an amperometric detector (+0.65 V) showed a gain in sensitivity of about 10 using electrochemical detection (ED) for the determination of CBS-113 A in human plasma. Linearity range, precision and accuracy were calculated and showed the potential application of HPLC-ED to pharmacokinetic studies of CBS-113 A in plasma.

Keywords: Amperometric detection; Anti-inflammatory drugs; Coulometric detection; Glassy carbon electrodes; Plamsa; Reversed-phase liquid chromatography; Voltammetry

1. Introduction

2-Hydroxy-4-methylphenyl-2-aminothiazole hydrochloride (CBS-113 A) is a new drug which presents a broad anti-inflammatory spectrum without the side-effects of steroids [1,2]. Many high-performance liquid chromatography (HPLC) methods have been reported to determine the level of non-steroidal anti-inflammatory drugs (NSAIDs) in biological matrices. UV spectrophotometry is mainly used as the detection mode. However, for increased sensitivity, other detection modes have been reported, e.g. direct fluorimetric detection for diclofenac [3], fluorimetric detection for indomethacin after precolumn alkaline hydrolysis [4] or postcolumn photolysis [5], chemiluminescence detection after precolumn fluorogenic labelling [6] and direct electrochemical detection (ED) for various NSAIDs [7–9]. These recent reports on the electrochemistry of NSAIDs mentioned different electro-oxidizable groups: a phenolic group for diflunisal [7], a hydroxyl

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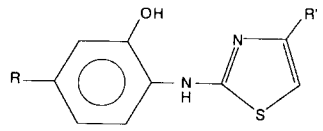

Fig. 1. Structures of CBS-113 A ($R = CH_3$ and $R' = H$) and RD-1546 ($R = H$ and $R' = CH_3$).

group substituted on an unsaturated heterocyclic structure for piroxicam and lornoxicam [7,8], an aromatic methoxy group for indomethacin and naproxen, an amino group for mefenamic acid [9] and a sulphone group for sulindac [7].

The chemical structure of CBS-113 A (Fig. 1) presents a phenolic group which is likely to be electrochemically oxidized. Since ED is a sensitive and selective detection mode for the determination of drugs in biological matrices [10], the electrochemical properties of CBS-113 A and that of a potential internal standard (2-hydroxyphenyl-2-amino-5'-methylthiazole hydrochloride; RD-1546) have been investigated by HPLC. First the electrochemical properties of CBS-113 A were examined by voltammetry at disk electrodes. Operating conditions for amperometric or coulometric detection in the HPLC of CBS-113 A and RD-1546 were then determined using standard solutions. Finally, the applicability of HPLC-amperometric detection to the measurement of CBS-113 A in plasma was investigated and compared to HPLC-UV detection.

2. Experimental

2.1. Chemicals and reagents

All chemicals and solvents were of analytical or HPLC reagent grade. CBS-113 A and RD-1546 were gifts from Chauvin Laboratory (Montpellier, France); 6-amino-*meta*-cresol and 2-aminothiazole were purchased from Aldrich (St. Quentin-Fallavier, France). Aqueous stock solutions of CBS-113 A and RD-1546 (500 μg ml^{-1} each) were prepared and kept at +4 °C for up to 30 days.

2.2. Voltammetric studies

Two voltamperographic analysers were used (model 174 A for direct (*dc*) and differential pulse (*dp*) modes and model 362 for cyclic voltammograms (*cv*) (EG & G, Princeton Applied Research, Princeton, NJ, USA). They were connected with a classical three-electrode stationary cell and an *X–Y* recorder (model RE 0074; Omnigraphic, Houston Instruments, TX, USA) to plot the current–potential curves. The working electrode was a rotating glassy carbon or platinum unit (model EDI & Controvit; Tacussel, Villeurbanne, France) with 3-mm and 7-mm diameter disks, respectively. The saturated calomel reference electrode (SCE) was placed into a compartment separated from the measuring cell by a porous glass bridge. The cell and compartment were filled with the same solution. A platinum wire was used as the auxiliary electrode. Current–potential curves were recorded under the following conditions.

(i) dc *Mode*

The rotating speed of the working electrode was varied within a 250–1500 rpm range; the scan rate was 2 mV s^{-1}.

(ii) dp *Mode*

The same conditions as above with a pulse height of 10 mV and a pulse repetition of 0.5 s.

(iii) cv *Mode*

The working electrode was not rotated and the scan rate was set at 200 mV s^{-1}.

For all experiments, a 5×10^{-4} M solution of each compound was prepared in methanol–phosphate buffer (50 mM) (50:50, v/v). The pH of the buffer ranged from 2.0 to 7.0. Experiments at pH 1.0 were performed in a solution of 0.10 M hydrochloric acid.

Coulometric experiments were performed using a potentiostat (model GCU, Tacussel), an integrator (model IGGN, Tacussel), a large surface annular platinum electrode (≈ 40 cm^2) as working electrode, a platinum wire as auxiliary electrode and an Ag/AgCl reference electrode. Anodic and cathodic compartments were separated by a porous glass filter. Electrolysis of CBS-113 A was realized in the mobile phase indicated below for HPLC, with addition of 0.2 M KCl. The applied potential was +0.65 V (vs. Ag/AgCl) and the current was integrated as a function of time. Electrolysis was assumed to be complete when the final current reached 1% of its initial value.

2.3. Electrochemical detection in the HPLC system

Preliminary experiments on aqueous standard solutions were conducted using an HPLC

pump (model 302, Gilson, Villiers-le-Bel, France) coupled with an autosampler (model PU 4247, Unicam, Cambridge, UK) fitted with a 20-μl loop. CBS-113 A and RD-1546 (internal standard) were separated on a column (250 × 4 mm i.d.) packed with 5-μm LiChrospher RP18 (Merck, Darmstadt, Germany). The mobile phase was methanol–phosphate buffer (50 mM, pH 3.0) (50:50, v/v) pumped at a flow-rate of 1.0 ml min^{-1}. The compounds were detected with a variable UV detector (model 1050, Hewlett Packard) set at 272 nm, placed in series prior to an amperometric detector equipped with a glassy carbon electrode as working electrode and an Ag/AgCl reference electrode (model Shimadzu L-ECD-6A, Touzart-et-Matignon, Vitry-sur-Seine, France). The hydrodynamic voltammogram was obtained from successive injections of the same solution of each compound (5 μg ml^{-1}) at different applied potential values ranging from +0.3 to +0.9 V (vs. Ag/AgCl). A coulometric detector (model ESA Coulochem 5100 A, Touzart-et-Matignon) equipped with a guard cell set at +0.5 V and an analytical cell with two porous graphite electrodes in series was used. The anodic wave was obtained from successive injections of a mixed standard solution of the two compounds (10 μg ml^{-1}) at variable potential values E_1 applied at the upstream electrode (from 0.0 to +0.5 V vs. Pd/H_2), the potential E_2 of the downstream electrode being set at 0.0 V (vs. Pd/H_2). The cathodic wave was obtained with variable applied potential values of E_2 (from 0.0 to −0.5 V vs. Pd/H_2), the potential E_1 being set at +0.4 V (vs. Pd/H_2). The effective conversion yields were calculated for each electrode by injecting a 1 μg ml^{-1} hydroquinone solution in order to correct the response of the electrodes.

For application to plasma samples, the same pumping system was used with a UV detector placed in series prior to the amperometric detector. The separation was carried out on a column (125 × 4 mm i.d.) packed with 5-μm LiChrospher RP18 (Merck).

Plasma samples were treated as follows. 20 μl of human plasma spiked with 40 μl of aqueous stock solution of CBS-113 A, suitably diluted, was transferred by pipette into 5-ml glass tubes; the plasma concentration of CBS-113 A ranged from 20 to 1000 ng ml^{-1}. After addition of 3 ml of ethyl acetate the tubes were vortexed for 10 min at 2000 rpm and frozen at −18 °C for 45 min. The organic layer was transferred into another tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The wall of the tube was rinsed with 0.5 ml of ethyl acetate. The resulting solution was evaporated to dryness and the residue was dissolved in 0.5 ml of the mobile phase. The reconstituted solution had to be used within 45 min. Blank plasma was treated according to the same procedure, replacing the addition of CBS-113 A solution by addition of water.

3. Results and discussion

3.1. Voltammetric studies

The electrochemical properties of CBS-113 A were investigated by recording current–potential curves at solid disk electrodes in order to facilitate the optimization of its electrochemical detection in the HPLC system. For this purpose, experiments were conducted under the following conditions: the analyte was dissolved in methanol–phosphate buffer mixtures, keeping the same proportions and ionic strength as the mobile phase used for the chromatographic separation; only positive potentials were scanned because of the lack of oxygen interference.

Well-defined anodic waves were obtained at positive potentials with a similar half-wave potential ($E_{1/2}$) in the *dc* mode when using either a platinum or a glassy carbon electrode; this suggests that the same electrochemical process occurred in both cases. Moreover, maximum potential peaks obtained in the *dp* mode were close to $E_{1/2}$ values ($\approx$ +0.4 V (vs. SCE) in methanol–phosphate buffer (pH 3.0) (50:50, v/v). Glassy carbon was selected as the material for the working electrode in further experiments because it is the material most used in HPLC-ED.

Cyclic voltammograms (*cv*) indicated a reversible oxidation reaction since the intensity values of the anodic and cathodic peaks are similar in magnitude (Fig. 2). Moreover, multiple *cv* experiment runs with the same solution did not show any significant variation in the curve indicating that the electrochemical reaction products formed at the surface of the electrode were not further involved in another electrochemical process.

A linear relationship was obtained by plotting (Fig. 3(I)) limiting current (i_{lim}) vs. the

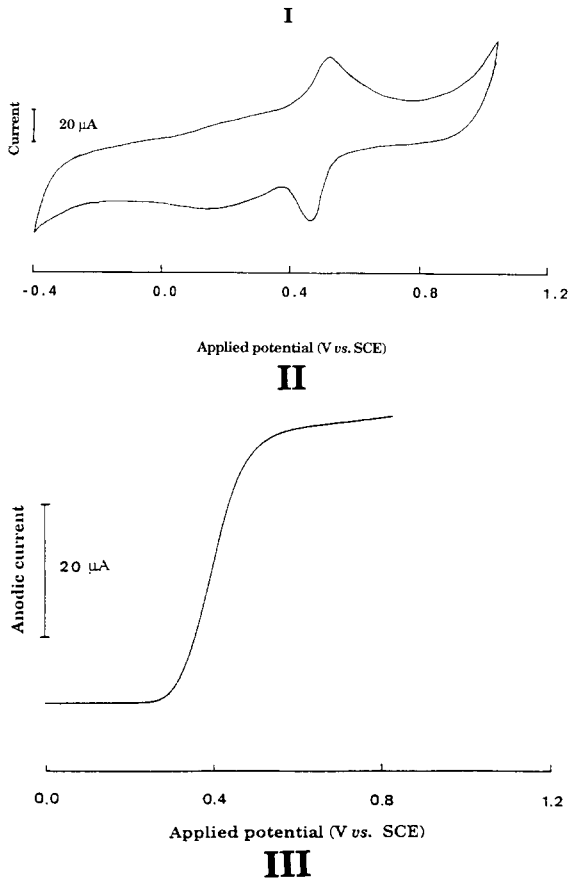

Fig. 2. Electrochemical study of CBS-113 A at a glassy carbon disk electrode: current–potential curves obtained in the cyclic voltammetry (I), direct current (II) and differential pulse (III) modes in methanol–phosphate buffer (50 mM, pH = 3.0) (50:50, v/v). (For other operating conditions see Experimental section).

square root of the rotating speed (ω) of the disk electrode, which demonstrated a diffusion-controlled electrochemical process with no kinetic complication, according to the Levich equation:

$$i_{lim} = k\omega^{1/2}$$

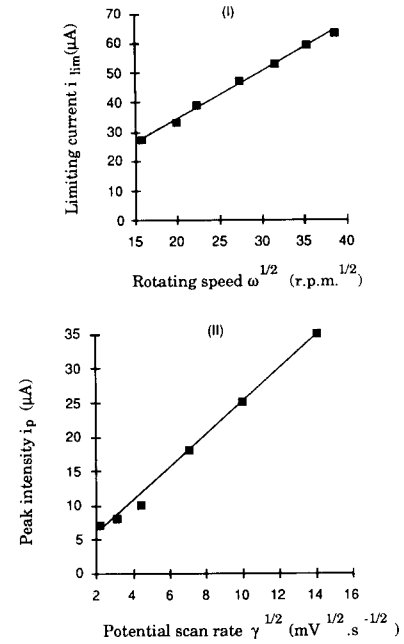

Fig. 3. Linear relationships during electro-oxidation of CBS-113 A at a glassy carbon disk electrode. (I) Limiting current value vs. the square root of the electrode rotating speed ($\omega^{1/2}$). (II) Peak current vs. the square root of the potential scan rate ($\gamma^{1/2}$). The measurements in the *dc* mode were carried out in methanol–phosphate buffer (50 mM, pH = 3.0) (50:50, v/v).

where k is a constant under well-defined experimental conditions.

A linear relationship was also obtained by plotting (Fig. 3(II)) peak current (i_p) vs. the square root of the potential scan-rate (γ) at a stationary disk electrode. For a reversible system, the equation of Randles Sevcik is

$$i_p = k'\gamma^{1/2}$$

with a peak potential value invariant with scan-rate. In the present case, unlike the peak current, the peak potential depends on the scan-rate which demonstrated some deviation from a real reversible redox reaction.

The influence of pH on the electrochemical reaction was tested by varying the pH value of the aqueous solution in the solvent mixture between 1.0 and 7.0. Two other compounds which represent the two main parts of the CBS-113 A structure, i.e. 6-amino-*meta*-cresol and 2-aminothiazole, were also tested for comparison. The results (Fig. 4) illustrate the dependence of the potential of the anodic wave on pH. For CBS-113 A, two linear regions, one between 1.0 and 3.0 and one between 5.0 and 7.0, are observed. The first trend is probably due to the progressive deprotonation of the

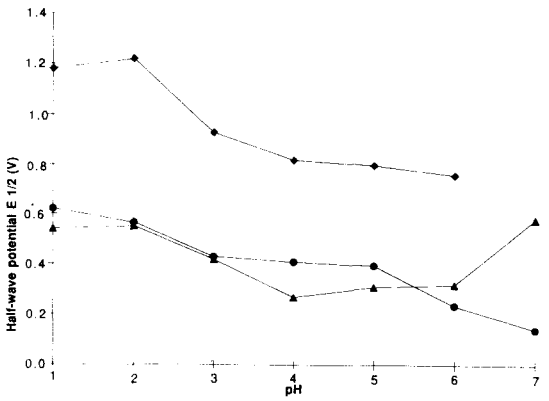

Fig. 4. pH dependence and half-wave potentials ($E_{1/2}$) of anodic waves obtained for CBS-113 A (●), 6-amino-*meta*-cresol (▲) and 2-aminothiazole (◆). Measurements were in the direct mode at a rotating glassy carbon disk electrode (750 rpm) in methanol–phosphate buffer (50 mM, pH = 2.0–7.0) or 0.1 M hydrochloric acid (50:50, v/v).

secondary amino group, resulting in an increasing ease of oxidation: the second trend at higher pH values may correspond to the loss of hydrogen ions with radical formation of either the amino or the phenolic group; similar results have been noted for amino compounds [11]. A comparison of the $E_{1/2}$ values found for these three compounds indicated similar electro-oxidative properties between CBS-113 A and 6-amino-*meta*-cresol in a pH range of 1–3; 2-aminothiazole exhibited higher $E_{1/2}$ values (>0.8 V vs. SCE) as previously reported for such a structure [12]. The "electrophore" of CBS-113 A can be assigned to the part of the structure containing 6-amino-*meta*-cresol. Among the different NSAIDs whose electrochemical properties have been studied, only piroxicam and lornoxicam are oxidized at similar low potential values [7,8].

Calculation of the number of electrons transferred during the electrochemical reaction (n) by measuring the difference between the anodic and cathodic potential peaks obtained in *cv* ($\Delta E = 57\ \text{mV}/n$) was not successful with a ΔE value higher than 57 mV. Hydroquinone and dopamine, which are known to exhibit a reversible oxidation process with a two-electron transfer, were tested under similar conditions. A voltage separation between the anodic and cathodic peaks larger than the value expected for an electrochemical reversible redox couple was obtained. However, this behaviour is typical of many quinone–hydroquinone systems on a carbon surface at intermediate values of pH and in mixed solvents, and is in agreement

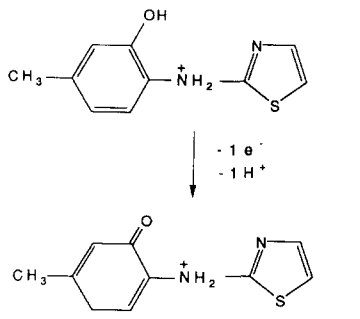

Fig. 5. Proposed electro-oxidation mechanism of CBS-113 A in acidic medium.

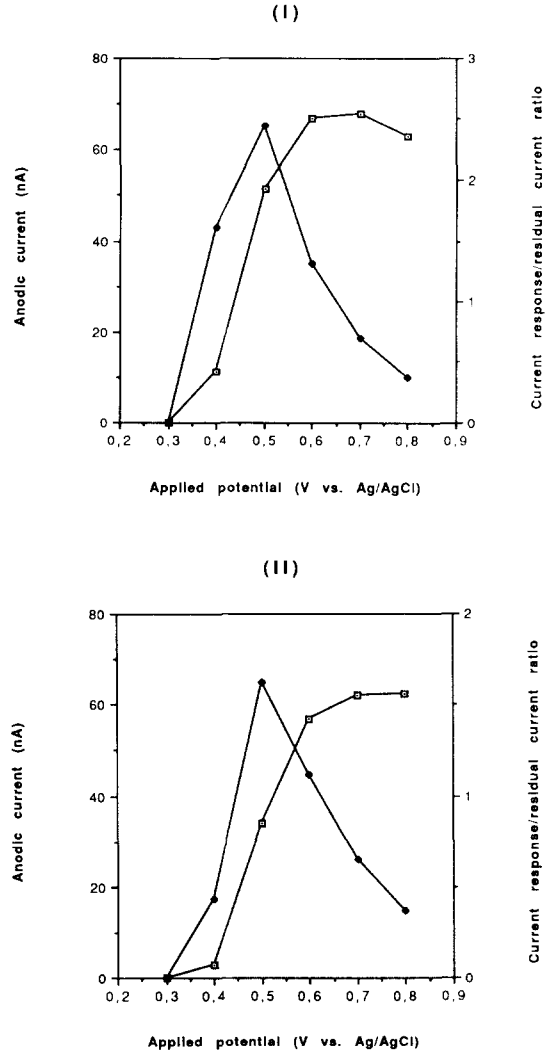

Fig. 6. Hydrodynamic voltammograms of CBS-113 A (I) and RD-1546 (II) using methanol–phosphate buffer (50 mM, pH = 3.0) (50:50, v/v) as mobile phase and an amperometric detector. (□) Current responses for the analyte. (◆) Ratio of analyte response to residual current values.

with the deviation observed in the Sevcik relationship and previous results [13].

Therefore, n was determined by coulometric electrolysis of CBS-113 A at a controlled potential, i.e. +0.65 V (vs. Ag/AgCl), in the mobile phase. A value of one electron was found. The number of protons involved in the oxidation process was determined from the slope of the $E_{1/2}$–pH curve. The electro-oxidation of CBS-113 A seems to be a $1e^- - 1H^+$ reversible process. A mechanism involving the phenolic group is proposed in Fig. 5.

This voltammetric study demonstrated the feasibility of using ED in an HPLC system. The diffusion-controlled process without any kinetic mechanism such as adsorption allows a reproducible signal. The influence of pH on the oxidation process should also be considered for optimized detection in chromatography, because it can affect the ruggedness of the method. Selectivity in biological matrices can be predicted from the low oxidation potential of the drug in a similar way to catecholamines.

3.2. Electrochemical detection in an HPLC system

First, the electrochemical oxidation of CBS 113-A and RD-1546 was assessed in the chromatographic system with an amperometric detector. The respective retention times were 13 min for RD-1546 and 15 min for CBS-113 A. Fig. 6 shows the hydrodynamic voltammograms. Similar shapes and diffusion plateau values are obtained for each compound; the position of the methyl group (structure shown in Fig. 1) does not affect their electro-oxidative behaviour. Because the background noise response is assumed to be proportional to the background response, the lower detection limit should be obtained at +0.5 V (vs. Ag/AgCl) corresponding to a maximum signal/residual current ratio, as shown in Fig. 6. However, for routine use and quantification, a potential of +0.65 V (vs. Ag/AgCl) on the diffusion plateau is preferable for ruggedness. In such conditions, the detection limit was about 10 pg of CBS-113 A injected (signal-to-noise ratio = 3) and was 40 times lower than UV absorption detection at 272 nm. Moreover, the repeatability of the peak areas ($n = 6$ injections of a 1.25 μg ml^{-1} standard solution) showed that no major adsorption problems occur at the electrode.

The reversibility of the oxidation process was then assessed by plotting the voltammograms corresponding to the oxidation and reduction reactions, using a coulometric detector with two electrodes placed in series (Fig. 7). The diffusion plateau for anodic oxidation was obtained at a potential of +0.4 V (vs. Pd/H_2). This low potential value, compared to that of amperometric detection, was expected because of the different nature of the reference electrode, which has a lower potential than Ag/AgCl. The peak area was corrected from the difference in conversion yield of the two electrodes calculated from the injection of a hydroquinone standard solution. For CBS-113 A and RD-1546, the amount of compound oxi-

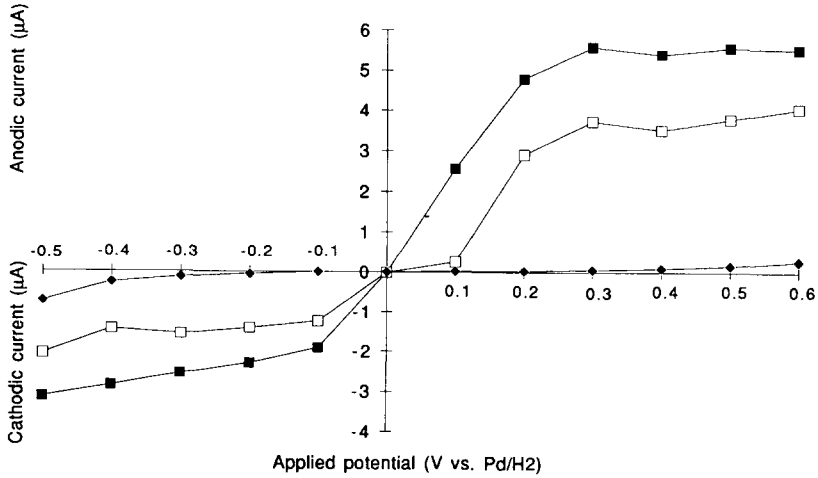

Fig. 7. Hydrodynamic voltammograms of CBS-113 A and RD-1546 using methanol–phosphate buffer (50 mM, pH = 3.0) (50:50, v/v) as mobile phase and the coulometric detector. (■, □) Current responses for CBS-113 A and RD-1546 respectively. (◆) Residual current level.

dized at the upstream electrode at +0.4 V (vs. Pd/H_2) was completely reduced at the downstream electrode at a potential of −0.5 V (vs. Pd/H_2). This reversibility may be of interest to increase the selectivity of detection in biological matrices.

3.3. Determination of CBS-113 A in plasma

The applicability of HPLC-amperometric detection to the determination of CBS-113 A in plasma samples was tested. Some aspects of validation were investigated.

Selectivity and sensitivity

Applying the extraction procedure described in the Experimental section, no interfering peaks were observed at the retention time of CBS-113 A in a chromatogram from a blank plasma, using either UV or amperometric detection. The comparison of UV and ED responses for a plasma sample spiked at different concentration levels shows the higher sensitivity of ED (Fig. 8).

Linearity

The linearity of ED response vs. concentration was assessed with plasma samples spiked with CBS-113 A at concentrations of 20–1000 ng ml^{-1} ($n = 8$). A linear regression was obtained ($r^2 = 0.9924$). The calibration line passed through the origin ($p = 0.05$). The regression equation was area = 0.0399 (±0.0035) concentration − 0.0641 (±1.681).

Repeatability, inter-day precision and accuracy

The repeatability of the whole procedure (extraction and chromatography) was assessed on plasma samples spiked at three concentration levels (20, 200 and 800 ng ml^{-1}). Table 1 shows the comparative results obtained using UV and amperometric detection. The repeatability of the method at 20 ng ml^{-1} together with the accuracy found at this concentration (−4.1% bias) show that this concentration can be considered as the limit of quantification [14]. Comparative RSD values were obtained for inter-day precision ($n = 3$), showing that no major problems occur at the working electrode.

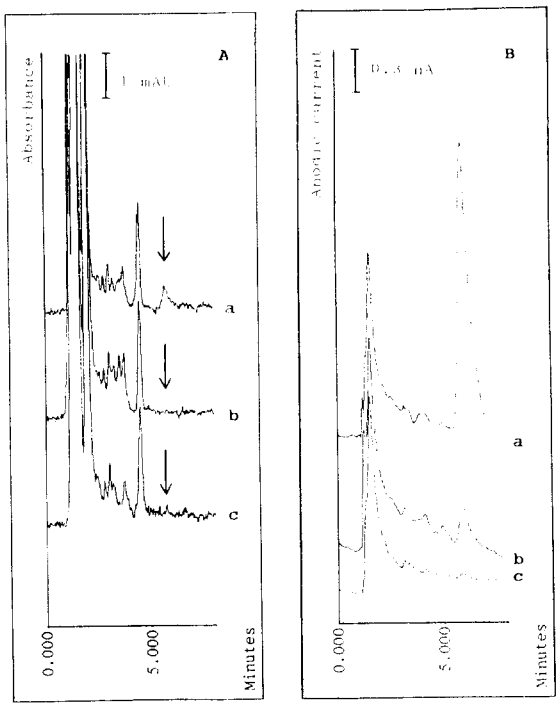

Fig. 8. Typical chromatograms from plasma samples recorded with (A) UV ($\lambda = 272$ nm) and (B) amperometric detection (+0.65 V). (a) Plasma spiked with 200 ng ml^{-1} of CBS-113 A. (b) Plasma spiked with 40 ng ml^{-1} of CBS-113 A. (c) Blank plasma.

Table 1
Repeatability of the overall assay of CBS-113 A in plasma

Concentration (ng ml^{-1})	n	RSD (%)	
		UV detection	Amperometric detection
20	6	[a]	16.5
200	5	[b]	8.9
800	6	6.7	2.2

[a] Not detected.
[b] Below the limit of quantification.

The sensitivity obtained compares favourably with UV absorption detection. The low oxidation potential and the reversibility of the electrochemical reaction allow highly selective detection of the analyte. Moreover, RD-1546 should be a good candidate as the internal standard in order to improve precision of the overall method.

4. Conclusion

ED can be used in an HPLC system for monitoring CBS-113 A in biological matrices.

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