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Simultaneous determination of antihypertensive drugs by flow injection analysis using multiple pulse amperometric detection with a cathodically pretreated boron-doped diamond electrode

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ABSTRACT

A flow injection analysis (FIA) and multiple pulse amperometric detection (MPA) using a cathodically pretreated boron-doped diamond electrode (CPT-BDD) were employed for simultaneous determination of the antihypertensive drugs hydrochlorothiazide (HTZ) and enalapril (ENP). A dual-potential waveform as a function of time, previously optimized, was used: $E_{det.1} = +1.50$ V / 150 ms (pulse potential at which HTZ was oxidized) and $E_{det.2} = +1.80$ V / 150 ms (pulse potential at which oxidation of both analytes – HTZ and ENP – occurs). The analytical curves presented good linearity from 0.40 to 8.00 μmol L^{-1} for HTZ and from 0.03 to 1.00 μmol L^{-1} for HTZ, and the detection limits were 0.20 μmol L^{-1} for HTZ and 0.01 μmol L^{-1} for ENP. The proposed flow method presented an analytical frequency of 89 determinations per hour, good precision and accuracy, was successfully applied to the simultaneous determination of HTZ and ENP in pharmaceutical samples and the results obtained using the FIA–MPA method were in agreement with those obtained using a comparative method (HPLC) at a 95% confidence level.

1. Introduction

Hypertension is an important chronic disease in which the blood pressure in the arteries is elevated. It is considered a worldwide public-health challenge because of its high frequency and high economic and social cost, due to concomitant risks of cardiovascular and kidney diseases [1]. Several studies have shown that in many cases of hypertension, monotherapy alone is not enough to reduce the arterial pressure. Thus, a current trend is the introduction of a combination of two or more antihypertensive drugs that may, by synergistic and/or additive effects, achieve the goal of treatment faster [2,3].

Enalapril (ENP) and hydrochlorothiazide (HTZ) are two different antihypertensive drugs that are sometimes administered together for more effective treatment of hypertension. ENP (Fig. 1A) is a monoethyl ester derivative of two amino acids, L-alanine and L-proline, which is hydrolysed in the liver to produce an active diacid form, enalaprilat, an angiotensin-converting enzyme (ACE) inhibitor. Therefore, it is considered a prodrug with good bioavailability and rapid absorption [2,3]. ENP's excretion is renal, with 40% of the dose excreted in the urine as enalaprilat and the remainder as enalapril [2–4].

HTZ (Fig. 1B) is a diuretic belonging to the class of thiazides. Diuretics are substances that increase urine flow and act to reduce the capacity for tubular reabsorption of sodium and water. HTZ has wide indications in the treatment of diseases such as renal tubular acidosis, hepatic and cardiac oedema as well as in hypertension, nephrogenic diabetes insipidus and hypercalcemia [5]. It is effective via oral administration, absorbed well from the gastrointestinal tract and excreted mainly via the renal route in the urine without being metabolized [6].

In this context, the widespread use of these combined compounds and the need for clinical and pharmacological studies require the development of simple, rapid and sensitive analytical methods for the simultaneous determination of these compounds in different samples. Most of the methods described in the literature for simultaneous determination of HTZ and ENP employ chromatographic techniques [7–12] that use toxic reagents, need relatively expensive instrumentation, and may require a long time for the analysis and processing of samples. Electroanalytical methods are less used for this kind of determination, but are a good alternative, since they could present some advantages compared to chromatographic methods; for example, lower reagent consumption, shorter analysis time and lower instrumentation cost. Furthermore, the use of organic solvents is unnecessary and the determination can be done in aqueous solutions. Very few electrochemical methods for individual determination of HTZ are found in the literature [13–15]. Furthermore, in the case of ENP, just two studies involving individual electrochemical determination have been described [16,17]. To the best of our knowledge, there are no articles reporting on the simultaneous electrochemical determination of HTZ and ENP.

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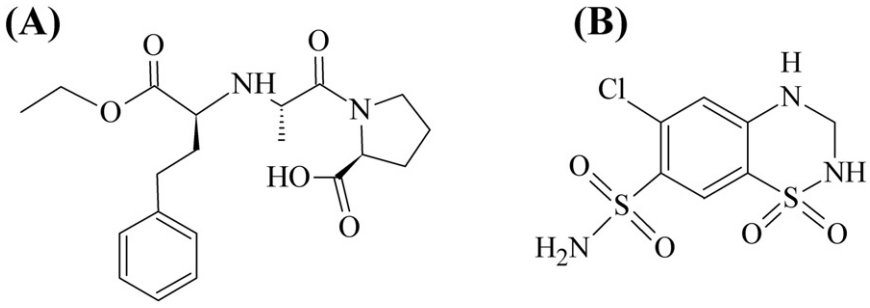

Fig. 1. (A) ENP and (B) HTZ molecular structures.

Pulsed amperometry is an electroanalytical technique in which different pulses of potential are applied sequentially and continuously to the working electrode as a function of time. In the potentiostat marketed by Metrohm, the technique is provided by GPES software and is known as multiple pulse amperometry (MPA). This software allows the application of up to 10 different potential pulses with the acquisition of current versus time for each potential pulse (which corresponds to the acquisition of 10 different amperograms simultaneously) [18].

A flow injection analysis (FIA) system can be easily associated with amperometric detection and presents some advantages when compared to conventional methods using voltammetric techniques, including greater sensitivity and reduced surface contamination of the electrode [18]. Furthermore, FIA present other advantages such as the low cost of system components, high analytical frequency and the possibility of automation.

On the other hand, flow-injection analysis coupled to multiple-pulse amperometric detection (FIA–MPA) has an advantage by making it possible to apply two or more pulses of potential in which one pulse is used to detect the analyte and the other is used to perform cleaning and/or activation of the working electrode surface during the experiment. Moreover, FIA–MPA systems have been used for the simultaneous determination of different analytes and, with the application of an appropriate waveform of potentials on a simple working electrode, allow the determination of two analytes without requiring sophisticated prior sample treatment, separation of the analytes on a chromatographic column or even chemometric treatments [19–23].

In addition to the advantages presented by FIA–MPA techniques, the use of a boron doped diamond electrode (BDD) as the working electrode eliminates the need to use a potential for cleaning of the electrode, since one of the characteristics of this electrode is low adsorption of inorganic and/or organic compounds. Moreover, BDD electrodes have several unique properties, such as stable and low background currents due to low adsorption of the test substances, wide potential window, long-term stability in acid and alkaline solutions and low sensitivity to dissolved oxygen [24,25]. However, the analytical performance of these electrodes for a given analyte greatly depends on their surface termination, i.e., whether they are hydrogen or oxygen terminated [26,27]. In our research group, lower electroanalytical detection limits for different organic analytes have been attained after a cathodic pretreatment [21,22,28,29].

Thus, in this study, we report a novel, simple, inexpensive and clean electroanalytical method for the simultaneous determination of HTZ and ENP in pharmaceutical products using a FIA–MPA with a cathodically pretreated boron-doped diamond electrode.

2. Experimental

2.1. Apparatus

The amperometric and voltammetric measurements were carried out using a potentiostat/galvanostat μAutolab type III (Ecochemie) controlled with the GPES software (version 4.9). Multiple pulse amperometric flow measurements were performed using a homemade three-electrode wall-jet electrochemical cell, which is described in detail elsewhere [28,29]. In the flow experiments, a stainless steel tube was used as counter electrode, along with a miniaturized Ag/AgCl (3.0 mol L^{-1} KCl) reference electrode.

The BDD film (8000 ppm) was obtained from Adamant Technologies, Switzerland. Prior to use, the BDD electrode surface was cleaned with isopropanol and rinsed with ultra-pure water. After that it was cathodically pretreated (CPT) in a 0.50 mol L^{-1} H_2SO_4 solution by applying 0.5 A cm^{-2} during 30 s and sequentially applying −0.5 A cm^{-2} during 180 s; thus, the BDD surface was made predominantly hydrogen terminated. The BDD pretreatment procedure was carried out once per day before performing the measurements.

The simultaneous determination of HTZ and ENP by a chromatographic comparative method was carried out using a LC-10 AT Shimadzu system with a UV–Vis detector (SPD-M10-AVP) set at 210 nm for both compounds. A Shim-Pack CLC-ODS (4.6 × 150 mm, 5 μm) chromatographic column (C_{18}) was used. The mobile phase, optimized empirically, was a 50:25:25 (v/v/v) mixture of acetonitrile, methanol and triethylammonium phosphate buffer (0.1% orthophosphoric acid was prepared and its pH was adjusted to 2.5 with a triethylamine solution), respectively, at a flow rate of 0.3 mL min^{-1}, while the injection volume was 20 μL [30].

2.2. Reagents and Standards

Stock solutions (1.0×10^{-2} mol L^{-1}) of HTZ and ENP (Sigma-Aldrich) were prepared in acetone and methanol, respectively, stored in a refrigerator at 4 °C and protected from light. For dilutions, the supporting electrolyte was used. A 0.5 mol L^{-1} H_2SO_4 stock solution was prepared and appropriate dilutions were made for a supporting electrolyte/carrier solution.

Other reagents used were of analytical grade, and all solutions were prepared using ultra-purified water of resistivity not less than 18 MΩ cm obtained with a Millipore® Milli-Q system.

2.3. Pharmaceutical Samples

Commercial samples of pharmaceutical formulations containing HTZ and ENP were purchased in a local market. Ten tablets of each analysed pharmaceutical formulation were accurately weighed and finely powdered in a mortar and pestle and transferred into a calibrated flask, which was completed to volume with methanol to prepare a stock solution. Afterward, appropriate aliquots were diluted with the supporting electrolyte/carrier solution. The concentration of each analyte was determined by interpolation on the analytical curve.

2.4. Measurement Procedure

First, cyclic and hydrodynamic voltammograms were obtained for HTZ and ENP.