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Differential pulse voltammetric method for the individual and simultaneous determination of antihypertensive drug metoprolol and its association with hydrochlorothiazide in pharmaceutical dosage forms

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ABSTRACT

The present study describes a convenient method for the individual determination of antihypertensive drug metoprolol (MTP) and its association with hydrochlorothiazide (HCTZ) using a cathodically pretreated boron-doped diamond (BDD) electrode and differential pulse voltammetry (DPV). Two very well-resolved and reproducible oxidative processes of HCTZ and MTP were found at 1.11 V and 1.32 V (*vs* Ag/AgCl (3.0 mol L^{-1} KCl)), respectively, in lactate buffer solution (pH 4.0). Under the optimum analytical experimental conditions, the voltammetric method exhibited linear response for individual determination of MTP in the concentration range 0.38–22 μmol L^{-1}, with detection limit of 0.034 μmol L^{-1}, while for a simultaneous determination of HCTZ and MTP, the concentrations range were 0.51–18.7 and 1.23–22.8 μmol L^{-1}, with detection limit of 0.376 and 0.077 μmol L^{-1} for HCTZ and MTP, respectively. The individual and simultaneous methods were successfully applied in the determination of HCTZ and MTP content in several pharmaceutical formulations, whose accuracy was attested by good agreement of the results (paired *t*-test at a 95% confidence level) with those obtained using high performance liquid chromatography. In order to indicate that the method is of potential application in biological fluids adequate recovery results were obtained for the determination of HCTZ and MTP in urine samples.

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1. Introduction

Hydrochlorothiazide (HCTZ; 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1 dioxide) is a thiazide diuretic (Fig. 1A). Metoprolol (MTP; 1-isopropylamino-3-[4-(2-methoxyethyl) phenoxy]propan-2-ol) is one drug classified as β-adrenergic receptor blocker (Fig. 1B). Combinations of these drugs are used for the treatment of arterial hypertension. MTP can also improve the heart's ability to relax and exhibiting calming neurological effects decreasing anxiety and stabilizing motor performance. HCTZ acts on the kidneys, increases the excretion of sodium, chloride and consequently of water [1]. Due to improved psychomotor performance these drugs are on the list of substances prohibited in athletic competitions by the World Anti-Doping Agency [2]. An overdose of MTP can lead to bronchospasm, cardiac failure, hypoglycemia, hypotension, and fatigue, while in overdose of HCTZ the patient loss fluid and electrolytes and the symptoms observed are dizziness, sedation/impairment of consciousness, hypotension and muscle cramps [1]. Therefore, it is very interesting the development of sensitive and selective analytical method for the individual determination of MTP and also for the simultaneous determination of MTP and HCTZ in pharmaceutical formulations and biological samples.

In recent years, increasing attention has been paid with the eco-friendly analytical methods to reduce the environmental pollution. Until now, the most analytical methods reported in the literature for the individual determination of MTP have been carried out by liquid chromatography tandem–mass spectroscopy [3,4], high performance liquid chromatography (HPLC) with different detection systems [5–8], gas chromatography with mass spectroscopic [9,10] and electron-capture detection [11], ultraviolet spectrophotometry (UV) [12–15] and capillary electrophoresis (CE) [16,17].

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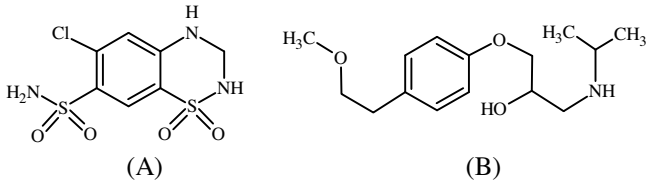

Fig. 1. Chemical structures of HCTZ and MTP.

Table 1
Analytical parameters for the voltammetric determination of MTP by DPV and SWV in BR buffer solution (pH 7.0) using a CP-BDDE.

	DPV	SWV
Peak potential (*V*)	1.22	1.24
Linear range (μmol L^{-1})	0.38–22	0.38–22
Determination coefficient, *r*	0.998	0.999
Slope (μA mol^{-1} L)	1.2×10^6	3.9×10^5
Intercept (μA)	0.94	0.059
Detection limit (μmol L^{-1})	0.034	0.076
Repeatability intra-day (RSD %)	3.0	2.9
Repeatability inter-day (RSD %)	3.2	3.5

The official method recommended by the British Pharmacopoeia for the determination of MTP involves the use of HPLC [18]. The use of this technique is preferred over the methods because of the possibility of simultaneous determination of MTP with other antihypertensive drugs, such as HCTZ [19–24]. Literature revealed that the simultaneous determination of MTP and HCTZ is not yet official in any pharmacopoeia. HPLC technique requires the use of toxic organic solvents, extensive preliminary sample pretreatment, and high implementation costs. Unfortunately, all these reliable methods are very expensive, and a different analytical method, which does not require expensive instrumentation, eco-friendly and which therefore could be used even in less highly developed areas, is required. In this sense, the development of eco-friendly, low cost and simpler analytical method is very important for quality control of pharmaceutical formulations and clinical diagnosis.

Voltammetric methods constitute a highly convenient alternative approach for the determination of several pharmaceutical drugs [25–31]. These methods are simple, rapid, highly sensitivity, selective, economical, and eco-friendly, which reduce the environmental pollution, important to drug analysis. To our knowledge there are no published reports regarding an electroanalytical method for simultaneous determination of MTP and HCTZ in pharmaceutical forms or biological fluids.

There are few reports available on the voltammetric procedures to individual determination of MTP. A stripping voltammetric method employing a nafion-carbon nanotube-nano-composite film modified glassy carbon (NAF-CNT-GC) electrode was used for the determination of MTP in pharmaceutical, serum and urine samples [32]. Voltammetric methods with and without adsorptive stripping modes were investigated for determination of MTP in tablets and spiked human serum samples [33]. In these studies a hanging mercury drop (HMD) electrode and a glassy carbon (GC) electrode were employed.

Several modified or unmodified electrodes have already been used as working electrodes for the individual determination of HCTZ in pharmaceutical formulations and biological samples. A GC electrode was used for the voltammetric determination of HCTZ in pharmaceutical formulations and biological fluids [34]. Electrochemical oxidation behavior of HCTZ was studied using a ferrocenedicarboxylic acid modified carbon paste [35] and a benzoylferrocene-modified carbon nanotubes paste electrodes [36]. Adsorptive stripping voltammetric methods employing a multiwalled carbon nanotubes modified electrode [37] and square-wave voltammetry (SWV) method employing an anodically pretreated boron-doped diamond (BDD) electrode [38] were used for individual determination of HCTZ in pharmaceutical samples.

Very few materials show as much versatility for electrochemical purposes as the BDD electrode. It can be used for electroanalysis with increased stability, accuracy, and can provide lower detection limits, and allow for individual or simultaneous analysis of a wide variety of organic compounds [38–41] which are electroactive without the interference of the water decomposition reactions due its wide potential window [41–47]. This electrode provide an electrochemical stability in conditions such as both strong alkaline and acidic media, a very low and stable background current, good response to certain analytes in aqueous solutions and non-aqueous with the conventional pre-treatment, and the possibility of measurement at high anodic potentials [43–47].

In the present study, the voltammetric individual determination of MTP and the simultaneous determination of HCTZ and MTP using differential-pulse voltammetry (DPV) and a cathodically pretreated BDDE without the need for prior separation step have been proposed. They may represent a simple, sensitive, rapid, economical, and eco-friendly alternative to chromatography methods. These methods were applied in the individual determination of MTP and its association with hydrochlorothiazide (HCTZ) in several pharmaceuticals (tablets) and recovery studies were performed in human urine sample, showing potential application of these methods in biological fluids. The obtained results in the case of tablets were compared with those from chromatography method [19].

2. Experimental

2.1. Chemicals and solutions

All chemicals were analytical grade, and the solutions were prepared using ultra-purified water (resistivity >18.2 MΩ cm) supplied by a Milli-Q system (Millipore®). Hydrochlorothiazide and metoprolol tartrate were obtained from Sigma–Aldrich. Acetic acid, boric acid, citric acid, lactic acid, ortophosphoric acid, and sodium hydroxide were obtained from Synth. Commercial pharmaceutical samples used in this studies were: MTP tablets (labeled 25 mg, 50 mg, and 100 mg MTP per tablet) and HCTZ:MTP tablets (labeled 12.5:100 mg HCTZ:MTP per tablet). These samples were purchased from local drugstore in city of Londrina in Brazil.

Britton–Robinson (BR) buffer solution was chosen as supporting electrolyte for pH study and individual determination of MTP. This solution was prepared by 0.04 mol L^{-1} in acetic, orthophosphoric, and boric acids, with pH adjusted to 7.0 with a 2.0 mol L^{-1} NaOH solution. MTP working solutions for individual determination were prepared by appropriated dilution of a 10 mmol L^{-1} MTP stock solution (prepared in methanol) with this supporting electrolyte.

A 0.1 mol L^{-1} lactate buffer was chosen as supporting electrolyte for the simultaneous determination of HCTZ and MTP. This solution was prepared by mixing 0.1 mol L^{-1} lactic acid solution and 2.0 mol L^{-1} NaOH solution with pH adjusted to 4.0. A 10 mmol L^{-1} stock solutions of HCTZ and MTP for simultaneous determination were prepared in methanol. Both HCTZ and MTP working solutions were prepared by appropriated dilution of these stock solutions with the 0.1 mol L^{-1} lactate buffer solution (pH 4.0) just before use.

2.2. Apparatus

All the electrochemical experiments were conducted in a three-electrode single-compartment glass cell, including a BDD electrode as working electrode, a platinum plate as auxiliary electrode, and an Ag/AgCl (3.0 mol L^{-1} KCl) as reference electrode to which all electrode potentials hereinafter are referred. The voltammetric

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