

Analytical Methods

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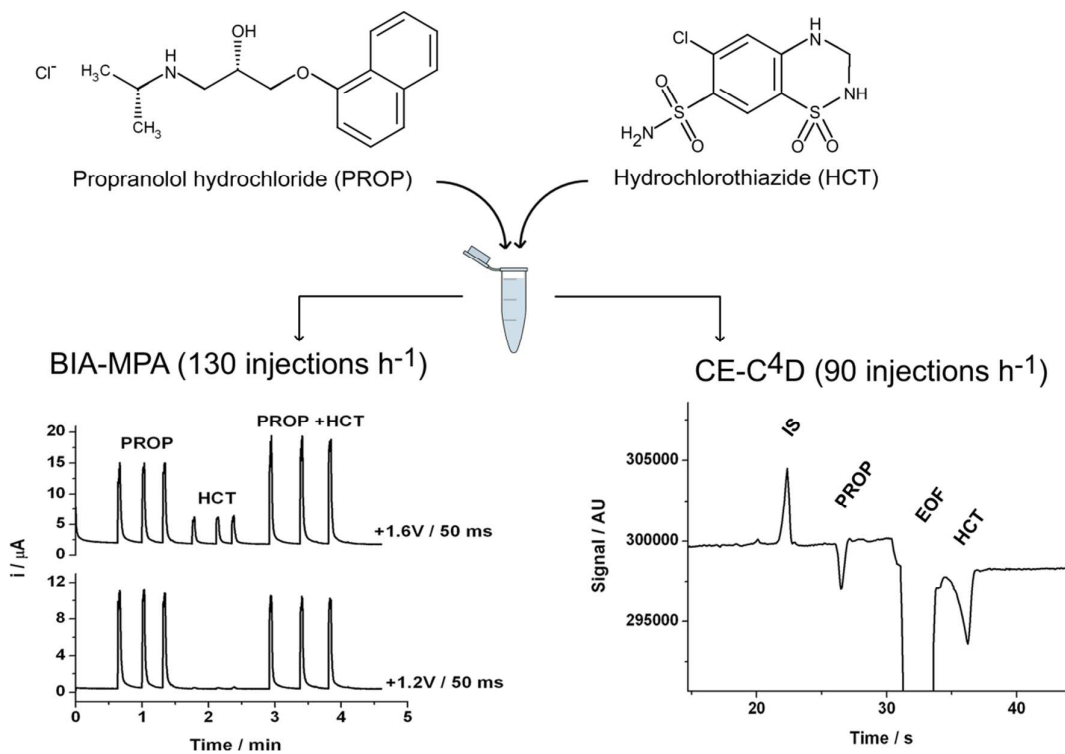
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Two new simple, accurate, and fast (> 90 injections h^{-1}) electrochemical methods for separation and selective determination propranolol and hydrochlorothiazide.



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3 **Determination of propranolol and hydrochlorothiazide by batch**
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5 **injection analysis with amperometric detection and capillary**
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7 **electrophoresis with capacitively coupled**
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9 **contactless conductivity detection**
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Abstract

We report two new electrochemical methods for the simultaneous determination of propranolol (PROP) and hydrochlorothiazide (HCT). One method is based on batch injection analysis with multiple pulse amperometric detection (BIA-MPA) and the other on capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C⁴D). The BIA-MPA procedure is highly-precise (RSD < 2.1%, n = 10, for both PROP and HCT), fast (130 injections h⁻¹) and has low detection limits (0.17 and 1.9 μmol L⁻¹ for PROP and HCT, respectively). The proposed CE-C⁴D method allows the determination of PROP and HCT in less than 1 minute with high precision (RSD < 2.8%, n = 10, for both PROP and HCT) and low detection limits (30 and 10 μmol L⁻¹ for PROP and HCT, respectively). Both of the proposed methods were applied to the determination of PROP and HCT in pharmaceutical samples.

Keywords: Propranolol, Hydrochlorothiazide, Multi-component analysis, Capillary electrophoresis, Batch injection analysis (BIA).

Introduction

Propranolol hydrochloride (PROP) is a beta-blocker drug that is widely prescribed for its antihypertensive properties, and for angina pectoris, cardiac arrhythmias and several other cardiovascular disorders.^{1,2} Hydrochlorothiazide (HCT) is a benzothiadiazine diuretic which inhibits the active reabsorption of sodium from distal tubulus.^{3,4} It is used for the treatment of both diuretic and hypertensive clinical indications. Due to the synergistic effect between HCT and PROP, both drugs are frequently combined in a single formulation and used in anti-hypertensive therapy.^{1,2} The combination of drugs may provide important therapeutic advantages; however, new challenges are raised for the pharmaceutical industry with respect to stability studies of combined drugs and their simultaneous determination.

Some analytical methods for the separation and simultaneous quantification of PROP and HCT were found in the literature.^{2,3,5-9} To date, only spectrophotometry combined with chemometric treatment,^{2,3} liquid chromatography (HPLC)⁵⁻⁷ and, thin-layer chromatography⁸ were explored for this purpose. However, most of these methods show high costs, requiring sample pretreatment and long periods of analysis. According to our knowledge, there are no reports on the simultaneous determination of these two compounds using electrochemical methods.

Capacitively coupled contactless conductivity detection (C⁴D) can be considered as a universal detection technique for CE that shows a good sensitivity for all ionic species.¹⁰⁻¹² C⁴D is based on the conductivity differences between the sample zone (analyte) and the background electrolyte (BGE)¹¹ and is constituted of two cylindrical electrodes positioned around the capillary column without any direct contact with the electrolytic solution. It can be easily positioned anywhere along the capillary.¹² The CE-C⁴D system offers excellent separation efficiency, short separation times, minimal

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3 sample volume requirements and simple instrumentation.¹³ CE-C⁴D has been widely
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5 applied in the analysis of inorganic cations, organic ions, pharmaceutical, amines,
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7 amino acids and other species.¹³
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10 The use of batch injection analysis (BIA) with amperometric detection has had
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12 success, mainly due to the combination of two factors: the hydrodynamic principle of
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14 the wall-jet configuration and the high and fast dilution of small volumes of samples or
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16 standard solutions (microliters) in a “blank” solution contained in a three-electrode
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18 electrochemical cell.^{14,15} Recently, an improvement in the BIA procedure was proposed.
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20 The system was coupled with multiple pulse amperometric (MPA) detection and
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22 simultaneous determinations of multi-analytes were possible using a single working
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24 electrode.¹⁶⁻¹⁸ This approach renders several desirable characteristics, such as the need
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26 for small sample volumes (typically 1–150 μ L), high sensitivity, low cost, and the
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28 possibility of multi-component analysis and can be performed in laboratories with
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30 minimal infrastructure or on-site analysis.^{14,15,19,20}
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34 In the present study, we report two new and fast electrochemical methods (BIA-
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36 MPA and CE-C⁴D) for the simultaneous determination of PROP and HCT. Results
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38 obtained with the two methods were evaluated in relation to linearity, repeatability
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40 (intra-day and inter-day studies), recovery studies, and detection and quantification
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42 limits.
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45 46 47 **Experimental**

48 49 **Reagents and samples**

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51 All reagents were of analytical grade and were used without further purification.
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53 Propranolol (PROP) and hydrochlorothiazide (HCT) were purchased from Attivos
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55 Magistrais (São Paulo, SP, Brazil), lithium hydroxide (internal standard),
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3 triethanolamine (TEA), 2-Amino-2-hydroxymethyl-propane-1,3-diol (Tris), and 3-[[2-
4 Hydroxy-1,1bis(hydroxymethyl)ethyl]amino]-1-propanesulfonic acid (TAPS) from
5 Sigma-Aldrich (Milwaukee, WI, USA) and sodium hydroxide and oxalic acid (OXA)
6 from Synth (Diadema, SP, Brazil). Deionized water (resistivity not less than 18 MΩ cm)
7 was obtained from a Direct-Q-System (Millipore, Bedford, MA, USA). Sulfuric acid
8 (0.1 mol L⁻¹) was used as the supporting electrolyte in the BIA-MPA experiments. A
9 buffer solution containing 11.3 mmol L⁻¹ TEA and 1.8 mmol L⁻¹ OXA (pH 8.7) was
10 used as a background electrolyte (BGE) in CE-C⁴D experiments. PROP and HCT
11 samples and standard stock solutions were prepared daily in acetone.
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24 Pharmaceutical formulations containing PROP (40 mg) and HCT (25mg) were
25 acquired at local drugstores. Ten tablets from each sample were accurately weighed
26 and powdered in a mortar. An adequate amount of the powder was dissolved in
27 acetone, after stirring and sonication for 10 min in an ultrasonic bath. The sample and
28 standard stock solutions were further diluted in a suitable electrolyte for subsequent
29 injection in the BIA-MPA system or in water if injected in the CE-C⁴D system.
30 Before injection in the CE-C⁴D system, all samples and standard stock solutions were
31 filtered through a membrane filter (pore size of 0.45 μm).
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44 **Instrumentation and apparatus**

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47 Electrochemical measurements were performed using μ-Autolab Type III
48 potentiostat/galvanostat (Metrohm Autolab, Utrecht, The Netherlands) connected to a
49 microcomputer and controlled by Autolab Software GPES version 4.9.007. The
50 reference and auxiliary electrodes were a miniaturized Ag/AgCl (saturated KCl)²¹ and
51 a platinum wire, respectively. A thin film (around 1.2 μm) of boron-doped diamond
52 (BDD) with a doping level of 8000 ppm on a polycrystalline silicon wafer (Adamant
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3 Technologies SA, La Chaux-de-Fonds, Switzerland) was used as the working
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5 electrode. Prior to first use, the BDD electrode was anodically pretreated by applying
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7 0.01 A for 1000 s in a 0.04 mol L⁻¹ Britton-Robinson buffer solution (pH = 2.0) and
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9 then cathodically pretreated by applying -0.01 A for 1000 s in a 0.1 mol L⁻¹ H₂SO₄
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11 solution. This electrochemical pretreatment is similar to that used in previously
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13 published works.^{22,23} After the first pretreatment, the BDD electrode was treated only
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15 cathodically once at the beginning of the workday. If the electrode was not used for a
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17 few days, both electrochemical pretreatments (anodic and cathodic) were repeated.
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21 The homemade BIA cell was previously described.²⁴ All experiments were
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23 carried out with the solution under stirring. A micro DC-motor was adapted to the
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25 BIA cell and used in the solution stirring.²⁰ The solutions (standards and samples)
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27 were injected into the BIA-MPA system with a motorized electronic pipette
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29 (Eppendorf[®] Multipipette stream) with a constant distance between the working
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31 electrode and the multipipette[®] combitip[®] (≈2 mm), as recommended in a previous
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33 work.¹⁴
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37 The electrophoretic analyses were performed using homemade CE equipment
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39 with two compact and high-resolution capacitively coupled contactless conductivity
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41 detectors (CE-C⁴D).^{12,25} A fused silica capillary with dimensions of 50 μm inner
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43 diameter, 375 μm outer diameter, 40 cm long, and effective length of 10 cm (Agilent,
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45 Folsom, CA, USA) was used. Before starting work, the capillary was preconditioned
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47 with deionized water for 10 min, 0.1 mol L⁻¹ NaOH for 15 min, with deionized water
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49 again for 10 min and finally with background electrolyte for 10 min. The samples
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51 were injected hydrodynamically for 0.5 s at 25 kPa. The separation potential adopted
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53 was 25 kV.
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Results and discussion

Batch injection analysis with multiple pulse amperometric detection (BIA-MPA)

Initially, the electrochemical behavior of PROP and HCT were investigated using cyclic voltammetry (not shown) at a BDD working electrode in different supporting electrolytes, such as sulfuric acid (pH 1.0), acetic acid/acetate (pH 4.7) and phosphate buffers (pH 2.1; 7.2 and 12.6) (all electrolytes at 0.1 mol L⁻¹). The best conditions with respect to the separation of oxidation peaks (about 200 mV), amperometric sensitivity and stability were obtained using sulfuric acid as the supporting electrolyte. Figure 1 presents the cyclic voltammograms obtained at a BDD electrode using sulfuric acid as the supporting electrolyte (0.1 mol L⁻¹) before (—) and after the addition of 1.0 mmol L⁻¹ of PROP (····) or HCT (----).

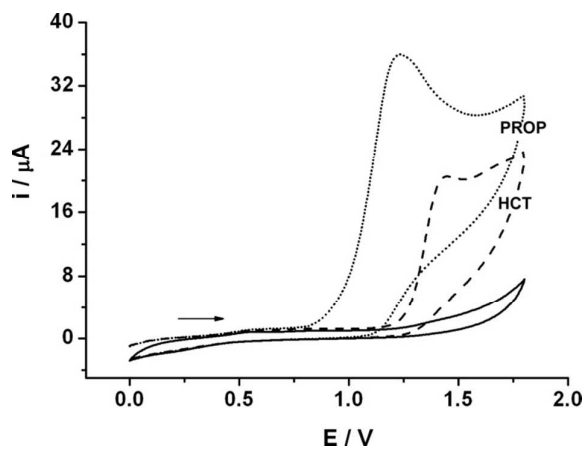


Fig. 1. Cyclic voltammograms of BDD working electrode in 0.1 mol L⁻¹ sulfuric acid before (—) and after addition of PROP 1.0 mmol L⁻¹ (····) or HCT 1.0 mmol L⁻¹ (----). Scan rate: 50 mV s⁻¹; step potential: 5 mV.

The obtained voltammogram for PROP presented one electrochemically irreversible anodic peak at +1.20V, which is in agreement with results previously reported.^{26,27} Two possible mechanisms for the electrochemical oxidation of propranolol

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3 were proposed in the literature. Bishop and Hussein²⁸ reported that the hydroxyl group
4 is oxidized, involving 2 protons and 2 electrons. In another work,²⁹ the authors
5 suggested that the secondary amine group is oxidized, involving the same number of
6 protons and electrons. HCT, in turn, was oxidized to chlorothiazide at about +1.4 V
7 involving the transfer of 2 electrons and 2 protons.³⁰ No peaks were observed in the
8 cathodic scan, pointing to the irreversibility of the oxidation process.

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16 The electrochemical behavior of PROP and HCT was also investigated using the
17 BIA-MPA system (hydrodynamic condition). For this, ten fast potential pulses (each for
18 70 ms) were applied continuously to the working electrode (BDD) positioned in the
19 BIA system; therefore, 10 amperograms were recorded in a single experiment (Figure
20 2A). The current at each potential pulse was monitored continuously during replicate
21 injections (n = 3) in the BIA system of solutions containing 100 $\mu\text{mol L}^{-1}$ of PROP (■)
22 or 53 $\mu\text{mol L}^{-1}$ of HCT (●). The average current peak (n=3) at each potential pulse was
23 measured and used to construct a hydrodynamic voltammogram for PROP and HCT
24 (Figure 2B).
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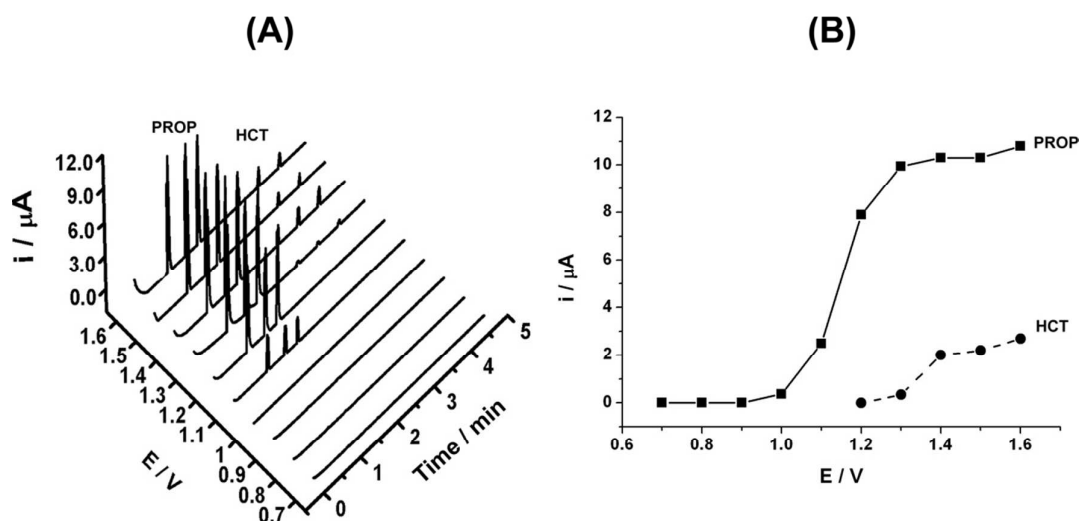


Fig. 2. (A) Amperograms obtained in BIA system after injections ($n = 3$) of a solution containing $100 \mu\text{mol L}^{-1}$ of PROP or $53 \mu\text{mol L}^{-1}$ of HCT. (B) Hydrodynamic voltammograms obtained for PROP (\blacksquare ; $100 \mu\text{mol L}^{-1}$) and HCT (\bullet ; $53 \mu\text{mol L}^{-1}$) by plotting peak current values as function of the corresponding applied potential pulse. Potential pulse times: 70 ms each; supporting electrolyte: $0.1 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$; dispensing rate: 4.5 mL min^{-1} ; injected volume: $200 \mu\text{L}$.

The hydrodynamic voltammograms revealed that PROP starts to oxidize at less positive potentials ($+1.1 \text{ V}$) than HCT ($+1.3 \text{ V}$). According to these hydrodynamic voltammograms, a potential of $+1.2 \text{ V}$ provides for a selective determination of PROP in the presence of HCT. If potential values higher than $+1.2 \text{ V}$ were employed, the electrochemical oxidation of both PROP and HCT would be verified. Then, a second potential pulse ($+1.6 \text{ V}$) was selected at which both compounds were electrochemically oxidized. The oxidation current from HCT was obtained by subtraction of the currents detected at the two potential pulses, similarly to previous studies.³¹⁻³³ A third potential pulse ($0.7 \text{ V} / 200 \text{ ms}$) was applied to avoid contamination/passivation of the working electrode surface. Figure 3 presents amperometric recordings obtained at $+1.2 \text{ V}$ (50 ms) and $+1.6 \text{ V}$ (50 ms) for the injection ($n = 3$) of solutions containing only $100 \mu\text{mol}$

L⁻¹ of PROP, only 53 μmol L⁻¹ of HCT and a mixture of 100 μmol L⁻¹ of PROP + 53 μmol L⁻¹ of HCT. In this study, the relationship between the PROP and HCT concentrations was similar to the relationship between these active ingredients in commercially available pharmaceutical samples.

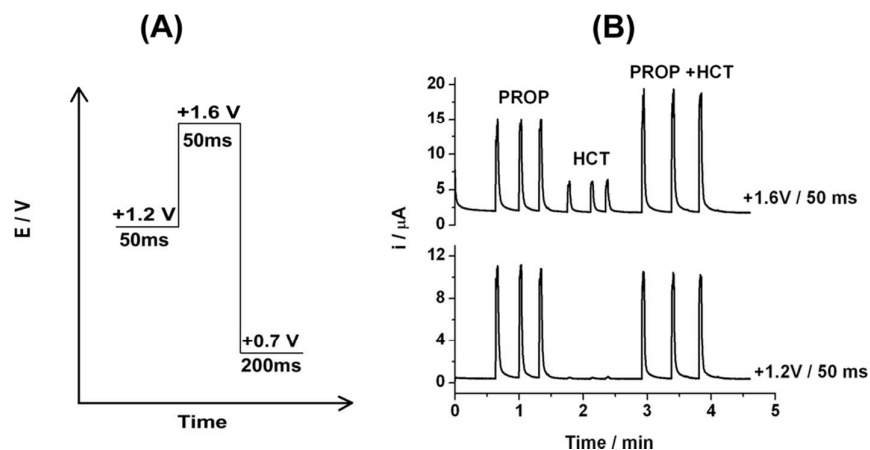


Fig. 3. (A) Potential pulse scheme; (B) Amperometric responses ($n = 3$) obtained after injections in the BIA-MPA system of solutions containing only PROP (100 μmol L⁻¹), only HCT (53 μmol L⁻¹) or PROP + HCT (100 + 53 μmol L⁻¹). Applied potential pulses: +1.2 V / 50 ms; +1.6 V / 50 ms; 0.7 V / 200 ms (cleaning potential pulse; signal not shown); dispensing rate: 4.5 mL min⁻¹; injected volume: 150 μL.

The amperometric recording registered at +1.2 V clearly shows that only PROP was oxidized at the BDD electrode, even in the presence of HCT, and that at +1.6 V, both molecules (PROP and HCT) were oxidized. Thus, PROP can be detected at +1.2 V without interference of HCT, while both PROP and HCT can be detected at +1.6 V. The oxidation current of HCT can then be obtained by subtraction of the currents detected at two potential pulses. However, as can be observed in Fig. 2B, the PROP oxidation current detected at +1.2 V (10.6 μA) is lower than the PROP current detected at +1.6 V (13.6 μA). Therefore, simple subtraction between the currents detected at the two potential pulses does not directly yield the HCT oxidation current. To bypass this

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3 problem, a correction factor (CF) was estimated based on the ratio of the oxidation
4 current for PROP registered at +1.2 and +1.6 V. This CF was obtained by the injection
5 of solutions containing only PROP into the BIA-MPA system and was determined using
6 the following equation:
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$$CF = i_{\text{PROP}+1.6\text{ V}} / i_{\text{PROP}+1.2\text{ V}} \quad (1)$$

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14 Afterwards, if a solution containing PROP + HCT is injected in the BIA-MPA
15 system, the current originating from HCT oxidation detected at +1.6V can be calculated
16 using the CF value and the following equation:
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$$i_{\text{HCT}} = i_{+1.6\text{V}} - (CF \times i_{+1.2\text{V}}) \quad (2)$$

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23 The CF value was obtained by injection in the BIA system of standard solutions
24 containing only PROP in the linear concentration range of the proposed method (10 to
25 $50 \mu\text{mol L}^{-1}$). Using the following optimized BIA parameters (dispensing rate = 4.5 mL
26 min^{-1} ; injection volume = $150 \mu\text{L}$), the CF value was calculated to be 1.30 ± 0.02 ($n =$
27 5) with a relative standard deviation of 1.5%. It is important to note here that the CF
28 value should still be determined for each calibration procedure (by injection of a
29 solution containing only PRO), because small variations may occur between analyses
30 conducted on different days.
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41 The stability of the proposed BIA-MPA system was evaluated (Figure 4) by
42 successive injections ($n=10$) of solutions containing $5.5 + 26.3 \mu\text{mol L}^{-1}$ (a) or $10 + 50$
43 $\mu\text{mol L}^{-1}$ (b) of PROP and HCT, respectively.
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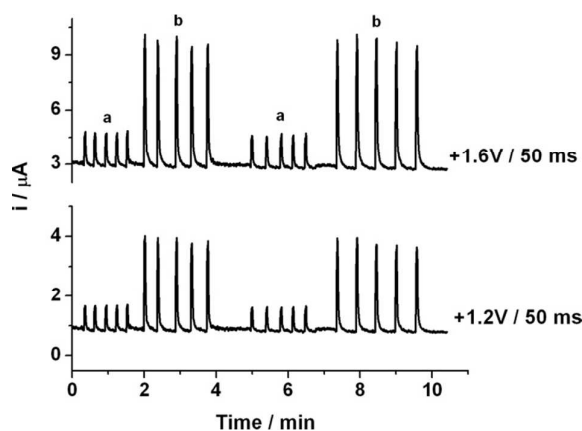


Fig. 4. Amperograms obtained from successive injections of standard solutions containing: $10 + 5.5 \mu\text{mol L}^{-1}$ (a) or $50 + 26.3 \mu\text{mol L}^{-1}$ (b) of PROP + HCT, respectively. Other conditions see Fig. 3.

The RSD were 0.93% (a) and 1.2% (b) for PROP and 1.9% (a) and 2.1% (b) for HCT (using the *CF*). These results indicate that the BIA-MPA system presented good repeatability, since no memory effect between successive injections was observed, even working with solutions with very different concentrations. These results were obtained by applying a third potential pulse (0.7 V for 200ms) alternately throughout the experiment. Probably, if the potential pulse is used, the adsorption of PROP or HCT or its oxidized products on the electrode surface are prevented (electrochemical cleaning step). In addition, if the cleaning potential pulse is used (0.7 V for 200ms), the contamination of the electrode does not occur during all time of the experiment because the contamination only occur during the application of the potential pulses used for the analytes oxidation (1.2 and 1.6 V; 50 ms each).

Figure 5A shows the amperograms obtained at 1.2 V and 1.6 V for triplicate injections of solutions containing only PROP which was used for calculation of the *CF*, five solutions containing increasing concentration of both PROP (a-e: $10.0 - 50.0 \mu\text{mol L}^{-1}$) and HCT (a - e: $5.3 - 26.3 \mu\text{mol L}^{-1}$), and two properly diluted samples (1 and 2).

The current responses of HCT were calculated using Eq. (2). The respective analytical curves are also presented for each analyte and were prepared by taking into consideration the concentration range for which the *correction factor* (*CF*) was relatively constant (1.30 ± 0.02 ; $n = 5$) and the concentration proportion found in commercial pharmaceutical samples (approximately 1.8-fold more of PROP than HCT).

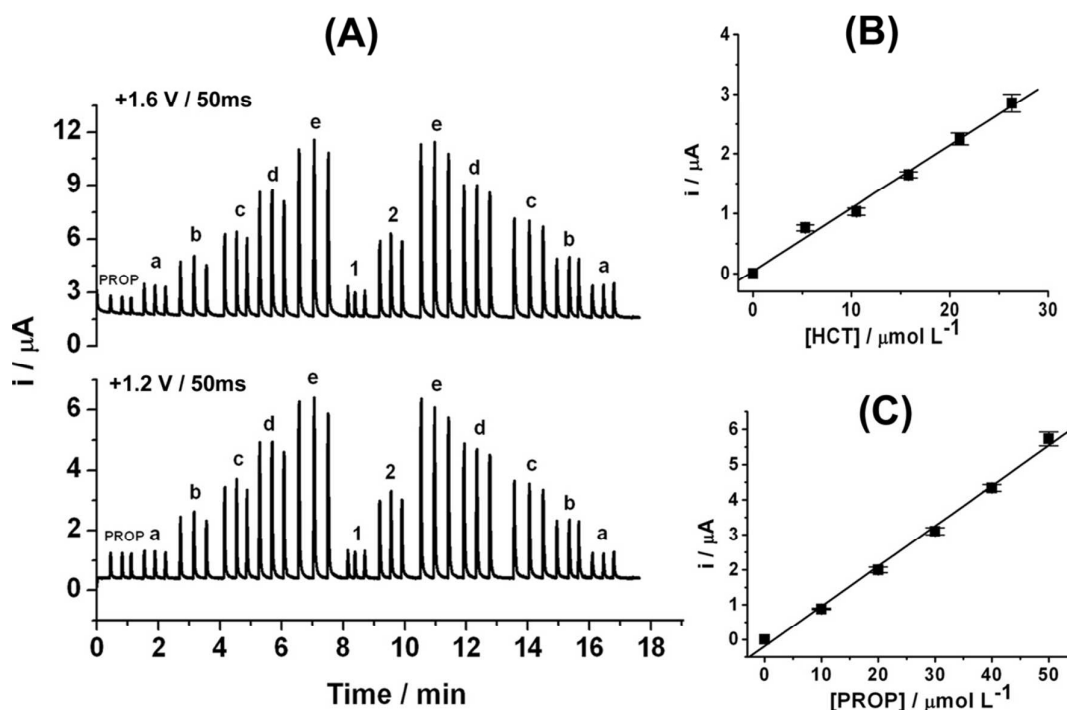


Fig. 5. (A) BIA-MPA amperograms obtained after injections of solutions containing only PROP ($10 \mu\text{mol L}^{-1}$); 5 standard solutions containing simultaneously increasing concentration of PROP (a – e: 10 to $50 \mu\text{mol L}^{-1}$) and HCT (a – e: 5.3 – $26.3 \mu\text{mol L}^{-1}$); and 2 appropriately diluted pharmaceuticals samples (1 and 2). Analytical curves for (B) HCT and (C) PROP. For other conditions see Fig. 3.

The standard solutions injected in ascending or descending order showed similar responses that confirm that the phenomenon of electrode contamination or memory effect was prevented. The analytical curves (Fig. 5B and C) showed good linearity in the investigated concentration range with the following calibration equations:

$$\text{PROP: } i (\mu\text{A}) = 0.04153 + 0.10514c (\text{mol L}^{-1}); r = 0.996$$

$$\text{HCT: } i (\mu\text{A}) = -0.19143 + 0.11469c (\text{mol L}^{-1}); r = 0.998$$

The detection limits were $0.17 \mu\text{mol L}^{-1}$ for PROP and $1.9 \mu\text{mol L}^{-1}$ for HCT, respectively.

Capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C⁴D)

Using a regular CE system, the simultaneous separation of cations (PROP; $\text{pK}_a = 9.5$)³⁴ and anions (HCT; $\text{pK}_{a1} = 7.9$ and $\text{pK}_{a2} = 9.2$)³⁵ can be performed in a condition where the electroosmotic flow (EOF) reached a high magnitude (BGE with $\text{pH} \geq 7.5$).³⁶ If normal EOF was used, cations were separated in co-EOF mode and anions with low mobility in counter-EOF mode (carried to the detector by the EOF). Simulations carried out using CurTiPot software³⁷ have indicated that in solutions with pH around 8.7, the mean charge of PROP species are positive (10% neutral and 90% with one positive charge) and HCT species are negative (13% neutral; 67% with one negative charge and 20% with two negative charges). Figure 6 shows typical electropherograms of a standard solution (A) containing PROP and HCT (400 and $211 \mu\text{mol L}^{-1}$, respectively) and of a pharmaceutical sample solution (B) adequately diluted in water. Lithium ($700 \mu\text{mol L}^{-1}$) was used as an internal standard (IS) in both solutions. These results were obtained using a BGE composed by TEA 11.3 mmol L^{-1} and OXA 1.8 mmol L^{-1} ($\text{pH} = 8.7$).

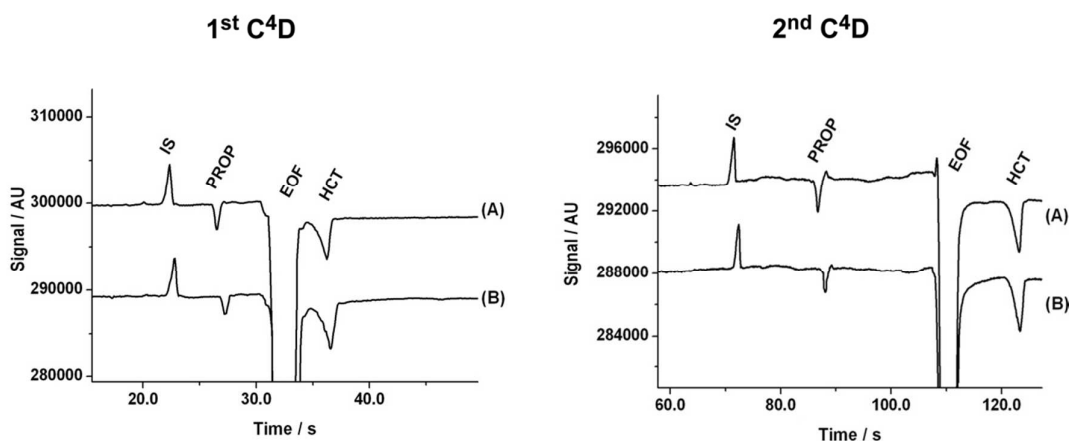


Fig. 6. Electropherograms obtained at the first and second detectors for injections of standard solution (A) containing PROP and HCT (400 and 211 $\mu\text{mol L}^{-1}$ respectively), 700 $\mu\text{mol L}^{-1}$ of lithium as an IS and a sample solution (B) with addition of the IS. CE conditions: 1.8 mmol L^{-1} oxalic acid and 11.3 mmol L^{-1} triethanolamine (pH=8.7) as BGE; separation voltage: 25 kV; hydrodynamic injection: 25 kPa for 0.5 s; effective capillary length: 10 and 30 cm for the first and second detectors, respectively.

As can be seen in Fig. 6, complete separation was possible in less than 40 s (0.6 min) at the first detector (10-cm effective length capillary). A better resolution was observed at the second detector (30-cm effective length capillary), but the analysis time increased slightly (0.6 to 2.0 min). BGE solutions with other pH values have also been tested (results not shown). If solutions with pH less than 8.7 were used as BGE, the HCT peak did not separate from the EOF mark at the first detector. On the other hand, if solutions with pH higher than 8.7 were used, an increase in analysis time was observed for both analytes. Furthermore, an electrolyte with lower mobility (Tris 20.0 mmol L^{-1} + TAPS 7.0 mmol L^{-1} ; pH = 8.7) was also evaluated as BGE for the simultaneous determination of PROP and HCT. Nonetheless, the Tris/TAPS buffer did not reach the deliver results since its conductivity was similar to both analytes and the sensitivity (conductimetric detector) of the method was compromised. For this reason, a buffer

with pH 8.7 and with higher mobility was selected for the following measurements (11.3 mmol L⁻¹ TEA and 1.8 mmol L⁻¹ OXA).

The stability of the CE-C⁴D system was evaluated by successive injections (n=10) of solutions containing 400 and 211 μmol L⁻¹ of PROP and HCT, respectively. Table 1 presents a comparison between the analytical characteristics of the two detectors of the CE-C⁴D system.

Table 1. Comparison between the analytical characteristics of the two detectors of the CE-C⁴D system.

Analyte	Migration time ^a (s)		Peak area ^a		R ^b	
	1 st C ⁴ D	2 nd C ⁴ D	1 st C ⁴ D	2 nd C ⁴ D	1 st C ⁴ D	2 nd C ⁴ D
PROP	25.8 ± 0.2	86.4 ± 0.3	44.7 ± 3.0	64.3 ± 2.8	2.2 ± 0.3	5.6 ± 0.5
HCT	37.2 ± 0.3	123.6 ± 0.4	125.0 ± 4.0	160 ± 3.0	1.0 ± 0.1	1.9 ± 0.2
EOF marker	33.7 ± 0.2	110.7 ± 0.3	-	-	2.6 ± 0.2	4.5 ± 0.5

^a(n = 10); ^bResolution between the corresponding peak and the previous one.

It can be seen that the proposed CE method presented appropriate reproducibility at both detectors. Thus, in the following measurements, the electropherograms acquired at the first detector were used (adequate resolution and faster analysis time). Figure 7 presents electropherograms and the respective analytical curves corresponding to injection of standard solutions containing increasing concentrations of PROP (a–e: 200 to 1000 μmol L⁻¹) and HCT (a–e: 105 to 527 μmol L⁻¹). Lithium was added in all solutions as an internal standard (IS).

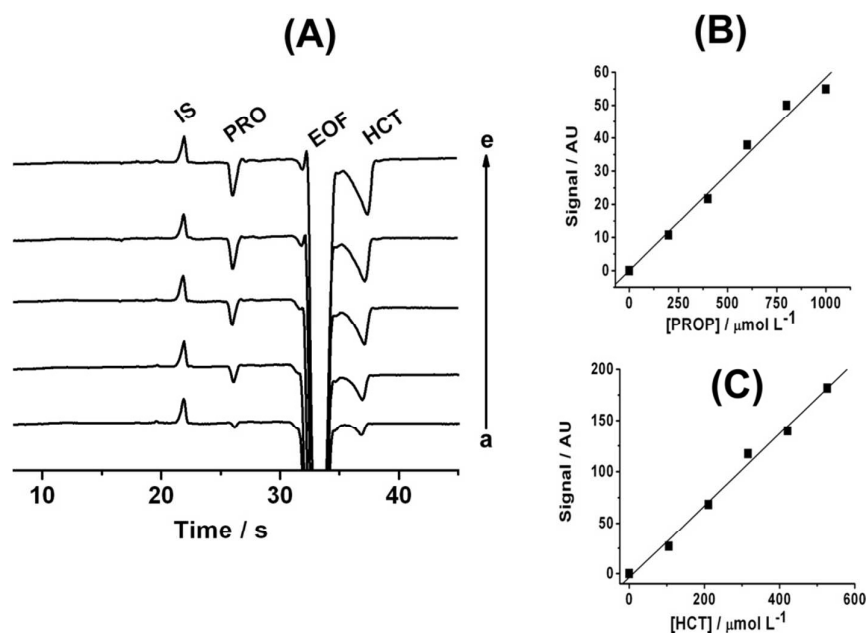


Fig. 7. (A) Electropherograms obtained from injection of standard solutions containing increasing concentrations of PROP (a – e: 200 to 1000 $\mu\text{mol L}^{-1}$) and HCT (a – e: 105 to 527 $\mu\text{mol L}^{-1}$). Lithium was used as an IS (700 $\mu\text{mol L}^{-1}$). (B and C) Analytical curves for PROP and HCT, respectively. For other conditions, see Fig. 6.

A linear relationship (Figure 7B and C) was observed between peak areas and concentrations of the PROP ($R = 0.996$) and HCT ($R = 0.993$). The calibration curves equations were:

$$\text{PROP: } S(\text{area}) = 0.1267 + 0.0584c \text{ (}\mu\text{mol L}^{-1}\text{);}$$

$$\text{HCT: } S(\text{area}) = -3.5239 + 0.3513c \text{ (}\mu\text{mol L}^{-1}\text{);}$$

The detection limits were 30 and 10 $\mu\text{mol L}^{-1}$ for PROP and HCT, respectively.

Comparison of the two proposed methods (BIA-MPA and CE-C⁴D)

Table 2 shows the results obtained for the analysis of four pharmaceutical samples by BIA-MPA and CE-C⁴D.

Table 2. Comparison of the results obtained for simultaneous determination of PROP and HCT by BIA-MPA and CE-C⁴D systems (n = 3).

Samples	Label value (mg / tablet)		Found value (mg / tablet)			
	PROP	HCT	BIA-MPA		CE-C ⁴ D	
			PROP	HCT	PROP	HCT
1	40	25	33.3 ± 0.1	25.9 ± 0.5	32.2 ± 1.6	26.7 ± 0.1
2	40	25	37.4 ± 0.2	29.1 ± 1.2	36.8 ± 3.6	27.9 ± 1.2
3	40	25	41.0 ± 0.1	29.1 ± 0.4	41.4 ± 2.2	30.3 ± 0.7
4	40	25	40.0 ± 0.2	25.2 ± 0.2	39.6 ± 0.2	25.9 ± 0.9

The results obtained using the BIA-MPA procedure were very close to those found by CE-C⁴D. The maximum difference was obtained for PROP in sample 1 (3.4%) and for HCT in sample 2 (4.3%). A significance test (null hypothesis) was applied to the results presented in Table 2. The calculated t-test values (triplicate determination) were between 0.00 and 2.45 for PROP and between 1.22 and 2.72 for HCT. At the 95% confidence level, the calculated *t-test* values were smaller than the critical value (2.78; n = 4). These results indicate that there were no significant differences between the results obtained with the two new proposed methods.

Studies were also performed to compare the results achieved with the proposed methods with HPLC. However, even using similar conditions to those described in a previous work⁶ which used a C₁₈ column (recently acquired), a mobile phase of

acetonitrile/phosphate buffer 0.05 mol L⁻¹ (17/83), pH 3.5, flow rate = 1.5 mL min⁻¹; λ = 270 nm, injection volume of 20 μ L, and same linear range, no adequate resolution was obtained between both compounds. This study suggests that this analysis is not easy by HPLC. On the other hand, here, we propose two new options for rapid and simultaneous determination of PROP and HCT.

Finally, in Table 3, a comparison between the analytical characteristics of the two new proposed methods is shown.

Table 3. Comparison of the analytical characteristics of the two new proposed methods.

Characteristics	BIA-MPA		CE-C ⁴ D	
	PROP	HCT	PROP	HCT
LR (μ mol L ⁻¹)	10 to 50	5.3 to 26.3	200 to 1000	105 to 527
r	0.996	0.998	0.996	0.993
LOD (μ mol L ⁻¹)	0.17	1.9	30	10
AN (h ⁻¹)	130	130	90	90
Intra-day RSD (n=10)	< 1.2%	< 2.1%	2.8%	1.3%
Inter-day RSD (n=3)	6.6%	11.0%	7.5%	5.0%
RT (%)	104 \pm 6	98 \pm 1	99 \pm 3	106 \pm 4

LR: linear range; r: correlation coefficient; LOD: Limit of detection; AN: analytical frequency; RSD: relative standard deviation; RT: recovery test (n = 4); Confidence interval = 95%.

Both proposed methods showed correlation coefficients of better than 0.99, high analytical frequency (130 h⁻¹ for BIA and 90 h⁻¹ for CE), similar intra-day precision (< 2.8 %), and recovery values between 98 and 106 %. The linear range and the limit of detection obtained with the CE-C⁴D system are higher than those obtained by BIA-MPA. However, in pharmaceutical analysis, low detection limits may not be as useful. Excessive dilution of samples can become a source of error.

Conclusions

In this study, we demonstrated that BIA-MPA and CE-C⁴D are suitable electrochemical techniques for the rapid and simultaneous quantification of PROP and HCT in pharmaceutical samples. Both methods have several desirable characteristics, such as the fact that they are very fast (90 h⁻¹ by CE and 130 h⁻¹ by BIA), require low reagent and sample consumptions, have good precision, simple sample preparation procedures (only dilution for BIA-MPA and dilution + filtration for CE-C⁴D), and exhibit good linearity ($r > 0.99$ in all cases). The limits of detection were 0.17 and 1.9 $\mu\text{mol L}^{-1}$ (BIA) and 30 and 10 $\mu\text{mol L}^{-1}$ (CE) for PROP and HCT, respectively.

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