Voltammetric Determination of Total Content of Parabens at a Carbon Fiber Microelectrode in Pharmaceutical Preparations

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Rapid, simple, and safe for the environment voltammetric method for direct determination of total content of esters of \( p \)-hydroxybenzoic acid (PHB), named as parabens, in pharmaceutical preparations has been developed. Performed determinations were based on differential pulse voltammetry (DPV) at a carbon fiber disk microelectrode (CF) in glacial acetic acid containing 20% acetonitrile (v/v) and 0.1 mol L\(^{-1}\) sodium acetate as a supporting electrolyte. Linear calibration plots for PHB, methylparaben and propylparaben were obtained over the corresponding concentration range of 0.75 - 47.05, 0.89 - 40.73 and 1.20 - 36.62 mg L\(^{-1}\) with limit of detection of 0.07, 0.08 and 0.10 mg L\(^{-1}\), respectively. This developed procedure allows the determination of parabens without the need for their separation from the matrices, and thereby aids in achieving desired accuracy, precision, and reproducibility of the results. This method has been successfully applied to their determination in commercially available pharmaceuticals.

Keywords: \( p \)-Hydroxybenzoic acid, Parabens, Acetic acid, Determination, Microelectrodes, Voltammetry

1. INTRODUCTION

An important issue in modern society is the feasibility in sustaining product quality for an acceptable duration, which has led to the addition of various types of preservatives. A group of these additives, named as parabens, are commonly used to preserve food, cosmetics and pharmaceutical samples [1-7]. Their molecular structures are shown in Scheme 1. This is a family of esters of \( p \)-hydroxybenzoic acid (PHB). Among them, methyl- (MP), ethyl- (EP), propyl- (PP) and butylparabens (BP) are best known.
Scheme 1. Chemical structure of \( p \)-hydroxybenzoic acid (PHB) and their esters: methyl- (MP), ethyl- (EP), propyl- (PP) and butylparaben (BP).

These esters have proved to be active antibacterial and especially antifungal agents. Their activity increases with the chain length of the alkyl group, while their solubility in water decreases [8,9]. In order to increase the preservative efficiency, they are often used in combination of two or more with different lipophilicity properties [1,10]. MP and PP, singly or in combination, are the most widely used preservatives. Parabens are recognized as the ideal preservatives because of their inexpensive production, good stability, biodegradability, neutral pH, lack of color, non-volatility, broad antimicrobial activity, as well as their relatively non-irritating and non-sensitizing properties [1,10]. For this reason, they are widely used, and humans are continuously exposed to their action. For many years, parabens have been regarded as preservatives of low toxicity. Recent studies indicate that their influence on the human health is not totally clear. For example, parabens were shown to induce allergic contact dermatitis [1,11], disrupt the human reproductive system, and produce inhibitory effect on mitochondrial respiratory capacities [10]. Some of these compounds were identified in human breast tumors [12,13]. Nevertheless, other studies indicate that parabens are safe for human health [1,2]. Due to unclear influence of parabens on human health, their content in different products must be closely controlled. Their use is permitted under the European Directives 1333/2008 and 1223/2009 in food and in cosmetic products. They can be used in cosmetics up to the maximum concentration of 0.4 % for one ester and up to 0.8 % (w/w) for their mixtures. The limited use of these compounds in foodstuffs and drugs should not exceed 0.1 and 1 % (w/w), respectively [1,2]. The total content of parabens is usually expressed as equivalent of PHB [1,2].

As many products, including pharmaceutical preparations, are preserved with parabens, their determination is very important for both the consumer safety and quality control. Various analytical methods have been used for determination of parabens in different matrices. They are based mainly on separation techniques, such as liquid chromatography, especially high performance liquid chromatography (HPLC) [3,5,14-16], gas chromatography [6,7,13], capillary electrophoresis [17,18], and spectrophotometry [19,20]. Only a few electroanalytical methods have been applied to this purpose [21-26]. The popularity of the chromatographic techniques is linked to their high sensitivity and selectivity as well as to their low detection limit. However, a lot of them need a sample clean-up, extraction, derivatization [27,28], and expensive instrumentation. Electrochemical methods are effective techniques for the determination of parabens. In comparison with the other methods, they offer several advantages such as simplicity, ease of sample preparation, short analysis time, and thus the lowering of their cost, comparable sensitivity, selectivity and detection limits. Therefore, electrochemical sensors are often used in conjunction with HPLC as well [5,14,15]. These methods are
based on the electrochemical activity of parabens. Their electrochemical properties were investigated mainly in water solutions [22-25] or in the mixtures with acetonitrile [5,26], methanol [14] or ethanol [21]. Unfortunately, all parabens were observed to yield a single irreversible oxidation peak at approximately 1.0 V vs. Ag/AgCl [14,21,22,25] indicating the inability of electroanalytical techniques in differentiating the parabens and therefore only their total content can be quantitatively estimated. The limited application of electroanalytical methods to determination of parabens is caused by the fouling of the electrode surface by the oxidation products of the phenolic compounds, which reduces the effective surface area, and thus diminishes the sensitivity and reproducibility [21,24,29,30]. The problem with the blocking of the working electrodes can be minimized by the modification of their surface or by appropriate choice of the solvents and composition of the solutions.

Recently, we reported [31] on the anodic oxidation of parabens at a carbon fiber (CF) disk microelectrode in glacial acetic acid containing acetonitrile (20%, v/v) and 0.1 mol L^{-1} sodium acetate as a supporting electrolyte. In our work, CV and DPV curves of these preservatives and parent PHB showed a single, well-defined and reproducible oxidation peak at approximately 1.05 V vs. Ag/AgCl. The differences between peak potentials characteristic of all parabens do not exceed 0.015 V and thus they cannot be electrochemically distinguished in this medium. The electrode process was characterized as being quasireversible, diffusion controlled, and proceeded with exchange of one electron and one proton (Scheme 2).

Scheme 2. Mechanism of the anodic oxidation of parabens.

The absence of a reduction peak on CV curves indicate that the phenoxy radicals as primary products are chemically unstable and participate in the successive irreversible homogenous reactions giving neutral dimers and oligomers. Thus, the anodic oxidation of parabens in the examined medium proceeds according to the $E_qC_1$ mechanism. The phenoxy radical formed in the electrode process for PHB is more stable in comparison with that characteristic of parabens and then can undergo a second irreversible exchange of electron (the second broad peak at about of 1.4 V) giving a quinone derivative as a final product of their oxidation. Very good reproducibility of the DPV curves indicates that the blocking of the electrode surface by the oxidation products is minimized in this medium.

Based on these results, we have developed a simple, rapid, and accurate voltammetric method for direct determination of total content of parabens in pharmaceutical preparations in the presence of their matrices. Glacial acetic acid has to-date not been applied as a medium to determination of these compounds. This solvent has several interesting properties, such as relatively wide potential window, and the ability to dissolve both hydrophobic organic compounds and their matrix as well as the necessary supporting electrolyte. Thus, it seems to be probable that acetic acid can also be a good medium for voltammetric determination of parabens in the presence of their matrix. A low dielectric
constant of this solvent ($\varepsilon = 6.2$ at 25 °C [32]) causes the appearance of significant ohmic potential drops, \textit{IR}. This problem can be overcome by the use of microelectrodes [33-36].

2. EXPERIMENTAL

2.1. Reagents

Chemicals including $p$-hydroxybenzoic acid, methyl $p$-hydroxybenzoate (methylparaben), propyl $p$-hydroxybenzoate (propylparaben), anhydrous sodium acetate (AcNa), were all of 99.0 % purity and were purchased from Fluka. Glacial acetic acid (HAc), p.a. ACS, acetonitrile (AN), p.a., all acquired from Merck were used as solvents in all electrochemical experiments. Potassium dihydrogen phosphate, sodium hydroxide of analytical reagent grade, and HPLC grade methanol, all purchased from Merck, were used in chromatographic analysis. All reagents and solvents were of high purity and used as received.

Pharmaceutical samples including Quinax (Alcon-Couvrer, Belgium), Zyrtec (UCB Pharma, Poland), Allertec (Polfa, Poland), Amertil (Biofarm, Poland), Levopront (Dompe Farmaceutici, Italy), Pulneo, (Aflofarm, Poland), Ibufen (Medana Pharma, Poland), and Ibum (Hasco-Lek, Poland) were purchased from local pharmacies. They were mainly in the form of solutions with exception of the Ibufen and Ibum which occurred as a suspensions. All of these preparations contained additionally a lot of ingredients, such as ibuprofen, phenoxyethanol, benzoic acid, sodium benzoate, citric acid, sodium citrate, boric acid, saccharose, glycerol, propylene glycole, sodium chloride, sunset yellow.

2.2. Preparation of the test solutions

In order to choose the most suitable medium for voltammetric determination of parabens, various compositions of the solutions were tested. It was found that of all solutions tested, HAc containing 20% AN (v/v) and 0.1 mol L$^{-1}$ AcNa gave well-formed voltammetric curves with relatively high sensitivity. The presence of acetate ions in the solutions improves the electrode process by binding the protons, and thus moving the equilibrium of this reaction in the direction of products (Scheme 2). In comparison with the solutions containing NaClO$_4$ as a supporting electrolyte, a decrease in the oxidation potentials of parabens was observed. This composition of the solutions has proved to be good medium for determination of parabens because of its ability to dissolve both analytes with different lipophilicity and their matrix. In opposite to water solutions, the experimentally chosen composition of this medium can be applied to determination of parabens even in the presence of hydrophobic matrix. This medium also avoids the passivation of the surface of the working electrode and ensures very good reproducibility of the voltammetric curves. The details of the selection of the solution composition were described previously [31].

In this work, 15 mg of PHB, MP, and PP were accurately weighed, transferred to separate 100 mL volumetric flasks and dissolved in the medium described above. In order to prepare the standards,
appropriate volumes of these stock solutions were diluted, yielding a final concentration in the 1 to 80 mg L$^{-1}$ range.

In order to test the voltammetric method, a solution with known amount of parabens was prepared. This solution contained pharmaceuticals Ibum (free from parabens, taken as a matrix) and MP in concentration of 6.08 mg L$^{-1}$ (5.52 mg L$^{-1}$ as PHB). The composition of the test solution was similar to this, which was obtained by dissolving of the real-life pharmaceuticals.

Solutions of real-life samples were prepared by dissolving accurately weighed 0.1 – 0.2 g pharmaceutical preparations in 25 mL of the same mixed solvent, which was described above. The content of the flask were sonicated for 15 min. In order to remove undissolved residues from the solutions, they were next filtered through an ordinary filtration paper. The obtained concentration of the drugs guarantees appropriate magnitude of the analytic signal for their quantitative analysis.

The solutions were directly analyzed without any extraction steps. All stock solutions were stored in a dark and cool place.

The mobile phase for HPLC analysis was an aqueous solution of 52.5% (v/v) methanol containing 0.2 M potassium dihydrogen phosphate, adjusted to pH 7.05 with 1 M sodium hydroxide. This composition of the solution for analysis of parabens in pharmaceuticals was proposed by Shabir [3]. The standards of MP and PP were obtained by appropriate dilution of more concentrated solutions of these preservatives in mobile phase.

The samples for HPLC analysis were prepared by dissolving accurately weighed pharmaceuticals (about 0.1 - 0.2 g) in 25 mL of the mobile phase.

The mobile phase and all solutions investigated were filtered through a 0.45 µm membrane filter and degased before use.

2.3. Apparatus and instruments

Voltammetric experiments were carried out with a three-electrode cell. A carbon fiber (CF) disk microelectrode of 35.4 µm diameter and platinum wire auxiliary electrode (both purchased from BASi, USA) were used as the working and counter electrode, respectively. All potentials were measured and reported against a Ag/AgCl reference electrode (Mineral, Poland) containing 1 mol L$^{-1}$ NaCl (aq). The reference electrode was isolated from the test solution by a salt bridge with a frit of Vicor Glass to avoid water and chloride ions leakage. Before each series of experiments, the surface of the working electrode was polished briefly using 0.05 µm alumina on a polishing cloth, rinsed with deionized water and dried. In order to exclude environmental noise, the electrochemical cell was enclosed in a grounded Faraday cage. A 5 mL glass electrochemical cell was used in the experiments.

All voltammetric measurements were performed using a Model M161E electrochemical analyzer connected with a Model M162 preamplifier and controlled via a PC computer using an mEALab Version 2.1 software (mtm-anko, Poland). The software used was equipped with a program for analytical determination by the standard addition method.
HPLC analysis was performed using a Model 210 Varian ProStar instrument (USA) consisting of a C-18 column (250 mm × 4.6 mm i.d.), a pump, injector equipped with a 20 µL sample loop, and UV-Vis detector. The chromatographic data were acquired by a version 6.30 LC Workstation.

2.4. Electrochemical measurements

Electrochemical measurements were performed with the use of differential pulse voltammetry (DPV). This technique is considered a convenient method because of the great speed of analysis, good sensitivity and selectivity, limited influence of adsorption phenomena on recorded curves, and thus good reproducibility, a wide range of linearity and the attainment of low quantification limit [37]. In order to establish the optimum conditions for the determination of parabens by means of DPV technique, various instrumental parameters were studied and the optimum conditions were: pulse amplitude of 20 mV, pulse width of 80 ms, and a scan rate of 20 mV s⁻¹. DPV curves were recorded in the range of potentials from 0 to 1.5 V vs. Ag/AgCl. It is to be noted that excellent reproducibility of the successive recorded curves was observed and thus the surface of the working electrode do not require to be polished.

Calibration was based on respective DPV curves of PHB, MP, and PP between 1 and 80 mg L⁻¹ (25 different concentrations). Since pharmaceutical preparations contain a mixture of MP and PP, only these preservatives were taken into account as well as PHB, which was applied as a standard solution in quantitative determinations.

Quantitative determination of parabens in the test solutions and in pharmaceutical preparations was conducted with the use of multiple standard addition method. PHB in concentration of about 50 mg L⁻¹ was used as a standard. Next, 2.0 mL of these solutions were transferred to electrochemical cell. DPV curves were recorded before and after addition of 100 µL of the standard of PHB. The addition of PHB was repeated four times for each sample. Three measurements were performed in the absence and in the presence of the added standard. Only a mean of measured currents was considered. The concentration of parabens in solutions was determined by linear regression. The amount of parabens was expressed as PHB concentration.

Electrochemical measurements were carried out at room temperature (25 ± 1 °C).

2.5. HPLC measurements

For comparison, the determination of parabens in pharmaceutical preparations was also performed by the HPLC method according to a procedure proposed by Shabir [3]. The chromatographic separation of parabens was carried out using an isocratic mode. A mixture of methanol and phosphate buffer pH 7.05 (52.5 : 47.5, v/v) was used as the mobile phase at a flow rate of 0.8 mL min⁻¹. The injection volume of the standard and sample solutions was 20 µL. Parabens were monitored with the use of UV detector at 254 nm. Chromatograms of the solutions containing pharmaceuticals gave good separation and resolution at retention time 6.60 and 19.50 min for MP and PP, respectively. Calibration plots based on the MP and PP peak area versus concentration were
constructed. These curves were used for quantitative determination of MP and PP in pharmaceutical preparations. The overall quantity of parabens was then expressed as concentration of PHB in these samples.

3. RESULTS AND DISCUSSION

3.1. Preliminary studies

Preliminary investigations on the determination of parabens in pharmaceutical samples consisted of identification of their voltammetric signals in solutions containing the standards and real-life samples. Figure 1(A) presents typical differential pulse voltammograms of the anodic oxidation of PHB, MP, PP and their mixture obtained at a CF disk microelectrode in HAc containing 20% AN (v/v) and 0.1 mol L\(^{-1}\) AcNa. As can be seen, the oxidation of the analytes proceeds in a single stage giving well-defined peaks between 1.05 to 1.065 V (these values are also tabulated in Table 1). Phenoxyl radicals are the primary products of this quasireversible process (Scheme 2). The broad peak observed at 1.39 V in the PHB trace is related to an irreversible second stage of the electrode reaction [31]. A quinone derivative is a final product of electrochemical oxidation. Owing to a difference of only 0.015 V between the peak potentials, as shown by the curve \(d\) obtained in a mixture of MP and PP in Fig. 1(A), it is difficult to distinguish the voltammetric peaks between parabens and PHB in acetic acid solutions. These results confirm the earlier observations taken from water solutions [21,22].

![Figure 1](image-url)

(Figure 1. (A) DPV curves of \(a\)) PHB, \(b\) MP, \(c\) PP (each 10.5 mg L\(^{-1}\)) and \(d\) the mixture of MP and PP (each 5.2 mg L\(^{-1}\)) recorded on CF in solutions of HAc containing 20% AN (v/v) and 0.1 mol L\(^{-1}\) AcNa. (B) DPV curves of the drugs (each 30.0 mg L\(^{-1}\)): \(a\) Levopront, \(b\) Amertil, \(c\) Ibufen, \(d\) Zyrtec, \(e\) Pulneo, \(f\) Allertec and \(g\) Quinax. Dashed line is residual current. DPV parameters: pulse amplitude 20 mV, pulse width 80 ms and scan rate 20 mV s\(^{-1}\).
These preliminary results indicate that only total content of parabens can be determined using HAc containing 20% AN (v/v) and 0.1 mol L\(^{-1}\) AcNa as a proposed medium for analysis. As a result of the close proximity of the peak potential for parabens and PHB (Table 1), this last one can be applied as a standard in determinations.

In the next stage of the experiments, we have attempted to identify the voltammetric signals characteristics for parabens in real-life samples. DPV curves of parabens at a CF disk microelectrode in pharmaceutical preparations of Levopront, Amertil, Ibufen, Zyrtec, Pulneo, Allertec, and Quinax are shown in Fig. 1(B). As can be seen, the peaks corresponding to oxidation of parabens can be easily identified. In all voltammograms, an oxidation peak at approximately of 1.05 V vs. Ag/AgCl was observed, and this is similar to the standards of parabens (Fig. 1(A) and Table 1). Additional oxidation peaks between 0.7 V and 1.0 V are likely to be attributed to the oxidation of other components in the pharmaceutical preparation matrix. Owing to these well-resolved peaks, they are unlikely to interfere with the oxidation signal of parabens. Thus, the determination without the need for their separation from the excipients should be possible.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PHB</th>
<th>MP</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak potential / V vs. Ag/AgCl</td>
<td>1.065 (1)</td>
<td>1.060</td>
<td>1.050</td>
</tr>
<tr>
<td></td>
<td>1.390 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linearity range / mg L(^{-1})</td>
<td>0.75 – 47.05</td>
<td>0.89 – 40.73</td>
<td>1.20 – 36.62</td>
</tr>
<tr>
<td>Correlation coefficient, (r) (n = 20)</td>
<td>0.9999</td>
<td>0.9998</td>
<td>0.9997</td>
</tr>
<tr>
<td>Slope / nA L mg(^{-1})</td>
<td>0.0260</td>
<td>0.0220</td>
<td>0.0180</td>
</tr>
<tr>
<td>Standard error of slope</td>
<td>0.0002</td>
<td>0.0003</td>
<td>0.0003</td>
</tr>
<tr>
<td>Intercept / nA</td>
<td>0.0032</td>
<td>0.0060</td>
<td>0.0040</td>
</tr>
<tr>
<td>Standard error of intercept</td>
<td>0.0022</td>
<td>0.0045</td>
<td>0.0048</td>
</tr>
<tr>
<td>(LOD) / mg L(^{-1})</td>
<td>0.07</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>(LOQ) / mg L(^{-1})</td>
<td>0.35</td>
<td>0.40</td>
<td>0.50</td>
</tr>
<tr>
<td>Repeatability of peak current (%RSD, n = 10)</td>
<td>1.50</td>
<td>1.40</td>
<td>1.50</td>
</tr>
<tr>
<td>Reproducibility of peak current (%RSD, n = 5)</td>
<td>1.70</td>
<td>1.70</td>
<td>1.80</td>
</tr>
</tbody>
</table>

3.2. Validation of the method

The applicability of the proposed voltammetric method to the determination of parabens was examined by measuring the peak current as a function of concentration of the analyte. From the voltammograms of the standards (Fig 2(A) – for MP as an example), linear calibration plots were obtained (Fig 2(B)). The characteristic of these relationships are summarized in Table 1. The linearity
range was found to be up to about 40 mg L\textsuperscript{-1} with the correlation coefficient, $r$ greater than 0.999. This correlation coefficient and the low standard error of the slope and the ordinate intercept were used to support the precision of the proposed method. As can be seen, the slopes of calibration graphs decrease with an increase in the chain length of the ester group and thus with an decrease of the diffusion coefficient. The limits of detection (LOD) and quantification (LOQ) were evaluated taking into account standard deviation of the blank ($\sigma_b$, $n = 10$) and the slope ($b$) of the calibration plots ($LOD = 3.29\sigma/b$, $LOQ = 5LOD$) [38]. These values ranged between 0.07 and 0.10 mg L\textsuperscript{-1} and 0.35 and 0.50 mg L\textsuperscript{-1}, respectively indicating the satisfactory sensitivity of the method developed.

![Graph](image)

**Figure 2.** (A) DPV curves recorded in solutions containing different concentrations of MP: a) 3.04, b) 6.22, c) 8.69, d) 12.16, e) 15.20, f) 20.27, g) 23.38, h) 27.64, i) 30.40, j) 38.00, k) 46.77, l) 55.27, m) 60.80, n) 67.56, o) 76.00 and p) 81.07 mg L\textsuperscript{-1}. Other parameters as in Fig. 1. (B) Calibration plots for PHB, MP and PP.

They are well below the legal levels allowed in pharmaceuticals [1,2]. The LOD values are comparable to these obtained using HPLC method with electrochemical detector [14] and they are lower than these obtained using HPLC with spectrophotometric detector [7] and with the use of other
voltammetric methods [21,26]. The comparison of linearity ranges and LOD values of the proposed method with the other reported methods are presented in Table 2.

It is to be noted that very good repeatability and reproducibility of the peak current and peak potentials were observed. The peak potentials were stable and repeatability for ten measurements of the peak currents for solutions of 12 mg L\(^{-1}\) was very good and RSD not exceeded 1.5%. The reproducibility of the peak currents was tested over 5 days with the use of the same solutions. The RSD values were not greater than 1.8% for all analytes investigated (Table 1).

**Table 2.** Comparison of the linearity range (LR) and limit of detection (LOD) for MP and PP obtained in this work with other methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>LR / mg L(^{-1})</th>
<th>LOD / mg L(^{-1})</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MP</td>
<td>PP</td>
<td></td>
</tr>
<tr>
<td>HPLC-UV(^{a})</td>
<td>3.0 – 100.0</td>
<td>1.0 – 75.0</td>
<td>0.30</td>
</tr>
<tr>
<td>HPLC-ECD(^{b})</td>
<td>lack</td>
<td>lack</td>
<td>0.05</td>
</tr>
<tr>
<td>CV (BDDE)(^{c})</td>
<td>0.30 – 15.82</td>
<td>3.60 – 25.23</td>
<td>0.23</td>
</tr>
<tr>
<td>CA (BDDE)(^{d})</td>
<td>1.52 – 12.27</td>
<td>1.80 – 14.42</td>
<td>0.11</td>
</tr>
<tr>
<td>SWV (Au)(^{e})</td>
<td>6.09 – 152.16</td>
<td>lack</td>
<td>0.26</td>
</tr>
<tr>
<td>DPV (GCE/PPy)(^{f})</td>
<td>1.52 – 152.16</td>
<td>lack</td>
<td>1.22</td>
</tr>
<tr>
<td>DPV (CF)</td>
<td>0.89 – 40.73</td>
<td>1.20 – 36.62</td>
<td>0.08</td>
</tr>
</tbody>
</table>

\(^{a}\)HPLC with UV detection, \(^{b}\)HPLC with electrochemical detection, \(^{c}\)Cyclic Voltammetry and, \(^{d}\)Chronoamperometry at a boron doped diamond electrode, \(^{e}\)Square wave voltammetry at a gold electrode, \(^{f}\)Differential pulse voltammetry at a glassy carbon electrode modified with polypyrrole.

### 3.3. Interference studies

The effects of some possible interferents were investigated by their addition to a solution containing MP in concentration of 10 mg L\(^{-1}\). The investigations were carried out for phenoxyethanol, benzoic acid, sodium benzoate, citric acid, sodium citrate, boric acid, saccharose, glycerol, propylene glycole, and sodium chloride. These compounds are commonly used together with the parabens in pharmaceuticals. The results were compared with those obtained in the absence of interferents. It was concluded that these compounds are not oxidized in the range of potentials characteristic of parabens and thus do not interfere in their quantification by the use of the proposed method. The changes in measured currents do not exceed ±2% for 10-fold excess of excipients and no changes of the peak potentials in their presence was observed. The presented voltammetric method can be considered specific.

### 3.4. Recovery studies

To develop the reliability and accuracy of the voltammetric method, a test solution with known amount of MP was prepared, as described above. In order to avoid the influence of excipients, the
determinations were performed by multiple standard addition method. DPV curves were recorded before addition of the standard solution of PHB and after each spike. Figure 3 presents responses obtained and the calibration plots for five independent determinations. As can be seen, the peak current of the anodic oxidation of MP increased with the concentration of PHB added and no changes in their potential was observed. In addition, these curves were excellently reproducible.

![Figure 3](image)

**Figure 3.** (A) DPV curves of a) pharmaceuticals Ibum (6.3 mg L\(^{-1}\), taken as a matrix) and b) after addition of MP (5.52 mg L\(^{-1}\) expressed as PHB – solution for control determination), c) - f): voltammograms as for b) but in the presence of PHB in concentration of 2.41, 4.59, 6.59 and 8.42 mg L\(^{-1}\), respectively. Other parameters as in Fig. 1. (B) Calibration plots for five quantification of parabens in control solution.

The calibration plots yielded good linearity, with a regression coefficient of 0.999. The content of parabens obtained from these curves is directly expressed as PHB concentration. All determinations were performed in five replications. The results achieved were statistically examined and are listed in Table 3. They are given with a confidence limit calculated for Student’s \(t\)-test coefficient equal to 95%. All quantitative data are expressed as \(x \pm \Delta x\) where \(x\) and \(\Delta x\) denote a mean value and confidence interval, respectively. The accuracy of the analysis was determined by calculating the relative error between the measured mean and the concentration involved, named as recovery trial (% \(R\)). The precision of the analysis was expressed as a relative standard deviation (% \(RSD\)). As can be seen from
Table 3, the $R$ and $RSD$ values are equal 100.0% and 1.0%, respectively. This indicates that the expected quantity of the analyte in solutions tested is in good agreement with the experimental results, thus the developed method is accurate and precise and is not affected by the interferences from excipients.

### Table 3. Results of the parabens determination in control and in pharmaceutical preparations by differential pulse voltammetry (DPV) compared with a reference HPLC method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount labeled$^b$ / mg mL$^{-1}$</th>
<th>Amount found / mg mL$^{-1}$</th>
<th>$t$-test$^c$ (2.31)$^d$</th>
<th>$F$-test$^c$ (6.39)$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPV method</td>
<td>HPLC method</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RSD / %</td>
<td>RSD / %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.02 ± 0.01 (100.0%)</td>
<td>1.08 ± 0.03 (105.9%)</td>
<td>1.9</td>
<td>5.79</td>
</tr>
<tr>
<td>A</td>
<td>1.98 ± 0.03 (100.5%)</td>
<td>1.93 ± 0.04 (98.0%)</td>
<td>1.7</td>
<td>2.79</td>
</tr>
<tr>
<td>B</td>
<td>1.34 ± 0.02 (100.0%)</td>
<td>1.39 ± 0.02 (103.7%)</td>
<td>1.3</td>
<td>3.95</td>
</tr>
<tr>
<td>C</td>
<td>1.34 ± 0.03 (100.8%)</td>
<td>2.96 ± 0.05 (222.6%)</td>
<td>1.4</td>
<td>75.34</td>
</tr>
<tr>
<td>D</td>
<td>0.42 ± 0.01 (97.7%)</td>
<td>0.46 ± 0.01 (107.0%)</td>
<td>2.2</td>
<td>6.02</td>
</tr>
<tr>
<td>E</td>
<td>lack</td>
<td>1.68 ± 0.03</td>
<td>1.8</td>
<td>7.63</td>
</tr>
<tr>
<td>F</td>
<td>lack</td>
<td>1.03 ± 0.01</td>
<td>1.11 ± 0.02</td>
<td>1.4</td>
</tr>
<tr>
<td>G</td>
<td>lack</td>
<td>0.059 ± 0.001</td>
<td>0.065 ± 0.002</td>
<td>2.5</td>
</tr>
</tbody>
</table>

The quantities of the drugs expressed as percentage of the label claim ($\%R$) are given in parentheses; $^a$A – Amer-til, B – Zyrtec, C – Levopront, D – Allertec, E – Ibufen, F – Pulneo, G – Quinax; $^b$Expressed as PHB equivalent in preparation; $^c$Calculated values; $^d$Values in parenthesis are tabulated $t$ and $F$ at $P = 0.05, n = 5$.

#### 3.5. Determination of parabens in pharmaceutical products

To evaluate the applicability of the proposed method, the parabens content was determined in seven commercially available samples. Preparation of the solutions tested was previously described. The analytical procedure was the same as for control determination. The representative DPV curves of pharmaceuticals are shown in Fig. 1(B). The appropriate peaks increased linearly with addition of the standard solution of PHB. Experimental results presented in Table 3 confirm very good precision ($RSD$ values were not greater than 2.7%) and accuracy ($R$ values for four preparations with known amount of
parabens were between 97.7% and 100.8%) and indicate that there is no interferences from the excipients. Additional experiments indicate that the solutions containing pharmaceutical preparations remained stable for at least ten weeks of storage at 4 °C. In this period no changes in peak potentials and peak currents were observed. The peak potentials were stable and RSD of the oxidation currents did not exceed 3% for n = 10.

For comparison, quantitative determination of parabens in pharmaceuticals was also conducted by HPLC. In contrast to voltammetric method, this analytical technique is able to differentiate these preservatives in their mixture. It has been demonstrated that all preparations tested contained a mixture of MP and PP. The content of these preservatives was separately determined and their sum was next expressed as concentration of PHB. This stage of HPLC analysis is more laborious and time-consuming in comparison with the proposed voltammetric procedure. The results were statistically examined in the same way as the voltammetric determinations and are listed in Table 3. As can be seen, the precision of the results obtained by HPLC is comparable with these of voltammetric method (the RSD values were not greater than 2.5% and 2.7%, respectively). This conclusion is confirmed by F-test. A comparison between the calculated and tabulated F-values indicates that there are no significant differences in precision between the methods. However, the accuracy of the HPLC analysis is worse. The experimental results for three preparations with known amount of parabens differ from these declared by manufacturers (the R values in the range from 98.0% to 107.0%). In addition, the concentration of these preservatives in formulation Levopront considerably differs from the amount labeled and from obtained with the use of voltammetric method. This can be probably a result of not separated signals characteristic for MP and for one of the excipients with similar polarity. The existence of the component of the matrix of Levopront with similar voltammetric properties to parabens can be seen on Fig. 1(B). It seems to be probable that this component can disturb the chromatographic analysis. The significant differences between the results obtained by proposed voltammetric and reference HPLC method was confirmed by Student’s t-test. As can be seen from Table 3, the experimental t-values exceed the theoretical ones. Because the differences in the amounts labeled and measured means (%R) are higher for the reference method, the voltammetric procedure can be recognized as accurate and more credible.

4. CONCLUSIONS

DPV of parabens at a carbon fiber disk microelectrode in glacial acetic acid containing acetonitrile (20%, v/v) and 0.1 mol L⁻¹ sodium acetate as supporting electrolyte was studied. This was then applied to the determination of overall paraben concentration in pharmaceutical preparations without the need for their separation from the matrices. The proposed method proved to be rapid, simple, sensitive, and precise, with a wide linearity range, a low detection limit and with satisfactory recovery. Preparation of the sample involves no other operation than dissolving and filtrating pharmaceutical samples. This procedure warrants very good reproductivity of the results and no interferences with the components of the matrix were observed. The results obtained with the use of
standard addition method are directly expressed as concentration of PHB, which is recognized as generally accepted mode to determine the amount of these preservatives [1,2].

The use of safe for environment solvents and the limitation of their amount by the application of microelectrode mean that this voltammetric method of determination of parabens is in accordance with the principles of green chemistry [39,40]. Consequently, the above-presented method can be a useful tool for quality control analysis of the drugs containing the parabens as preservatives.

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References
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