

Square-Wave Voltammetric Determination of Acetylsalicylic Acid in Pharmaceutical Formulations using a Boron-Doped Diamond Electrode without the Need of Previous Alkaline Hydrolysis Step

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Neste trabalho descreve-se a determinação do ácido acetilsalicílico (ASA) em formulações farmacêuticas empregando voltametria de onda quadrada (SWV) e um eletrodo de diamante dopado com boro (BDD). Neste método, ASA é determinado diretamente em solução de H_2SO_4 $0,01 \text{ mol L}^{-1}$, sem a necessidade da hidrólise alcalina. Foi obtido um único pico de oxidação no potencial de $1,97 \text{ V vs. Ag/AgCl}$ ($\text{KCl } 3,0 \text{ mol L}^{-1}$) com características de uma reação irreversível. A curva analítica obtida é linear na faixa de concentração ASA de $2,50 \times 10^{-6}$ - $1,05 \times 10^{-4} \text{ mol L}^{-1}$, com um limite de detecção de $2,0 \mu\text{mol L}^{-1}$. O desvio padrão relativo foi menor que 1,4% para uma solução de ASA $45 \mu\text{mol L}^{-1}$ ($n = 10$). O método proposto foi aplicado com sucesso para a determinação de ASA em várias formulações farmacêuticas e os resultados obtidos foram concordantes com um método oficial da Farmacopéia Britânica, a um nível de confiança de 95%.

In this paper the determination of acetylsalicylic acid (ASA) in pharmaceutical formulations using square-wave voltammetry (SWV) and a boron-doped diamond electrode (BDD) is described. By this proposed method, ASA is directly determined in a $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ solution without the need of a previous time-consuming alkaline hydrolysis step. A single oxidation peak at a potential of $1.97 \text{ V vs. Ag/AgCl}$ ($3.0 \text{ mol L}^{-1} \text{ KCl}$) with the characteristics of an irreversible reaction was obtained. The obtained analytical curve is linear in the ASA concentration range 2.50×10^{-6} - $1.05 \times 10^{-4} \text{ mol L}^{-1}$, with a detection limit of $2.0 \mu\text{mol L}^{-1}$. The obtained relative standard deviation was smaller than 1.4% for a $45 \mu\text{mol L}^{-1}$ ASA solution ($n = 10$). The proposed method was applied with success in the determination of ASA in several pharmaceutical formulations; the obtained results were in close agreement, at a 95% confidence level, with those obtained using an official method of the British Pharmacopoeia.

Keywords: acetylsalicylic acid determination, boron-doped diamond electrode, square-wave voltammetry, pharmaceutical formulations

Introduction

Acetylsalicylic acid (ASA), shown in Figure 1, more popularly known as aspirin, is one of the oldest medicines that still plays an important role in modern therapeutics. It is widely employed in pharmaceutical formulations for the relief of headaches, fever, muscular pain, and inflammation due to arthritis or injury. It was first synthesized in 1897, by Felix Hoffmann, in the Farbenfabrik Freidrich Bayer laboratories, in Elberfeld, Germany.^{1,2} The high consumption of this substance all over the world^{3,4} indicates the importance of the development of

new analytical methods to assess not only the quality but also the authenticity of the product.

The rate of decomposition of ASA to salicylic acid (SA) and acetic acid (AA) is dependent on solution pH and temperature. In the pH range 11-12 ASA is quickly hydrolyzed, in the pH range 4-8 its hydrolysis rate is slow, and maximum stability is attained at pH 2-3.⁵ Commonly, ASA is indirectly determined after its conversion to SA

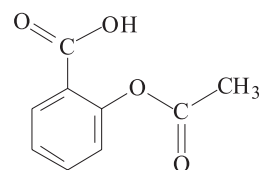


Figure 1. Chemical structure of acetylsalicylic acid (ASA).

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and AA by alkaline hydrolysis. According to a British Pharmacopoeia method,⁶ the ASA sample is dissolved in a sodium hydroxide solution and heated under reflux for 10 min; after cooling the solution to ambient temperature, the NaOH excess is titrated with a HCl standardized solution. Another reference method for the determination of SA is the spectrophotometric method based on the Trinder reaction,⁷ which involves the formation of a deep blue-violet complex between salicylate and Fe(III). However, this is not an indicated method to determine ASA in samples that have dyes in their formulation, because serious interferences might be expected.

Several other methods for ASA determination in pharmaceuticals formulations, some of them combined with a flow-injection system, were also proposed; for example, the quantitative reflectance spot test,⁸ spectrophotometric,⁹⁻¹¹ fluorimetric,¹² and chromatographic^{13,14} methods. The use of HPLC is preferred over other methods because of the possibility of simultaneous determination of ASA and SA. However, this technique involves expensive apparatus and a usually difficult and tedious preparation of the samples.

Electroanalytical methods such as potentiometric and amperometric ones were also developed for the analysis of acetylsalicylic acid in pharmaceuticals formulations. Ferreira *et al.*¹⁵ constructed an electrode of the second kind, Pt|Hg|Hg₂(salic)₂|graphite, sensitive to salicylate, for the determination of ASA, after its hydrolysis to salicylate, with a sensitivity of 58.66 mV decade⁻¹ in the ASA concentration range 6.0×10^{-4} mol L⁻¹ to 1.0×10^{-1} mol L⁻¹ at pH 6.0 and ionic strength of 0.50-3.0 mol L⁻¹, adjusted with NaClO₄. Kubota *et al.*¹⁶ developed a potentiometric flow-injection system for ASA determination after an on-line hydrolysis of an alcoholic ASA solution; the used tubular potentiometric electrode was based on Aliquat-salicylate as ion-exchanger. This electrode showed a linear response to ASA in the concentration range 4.0×10^{-3} mol L⁻¹ to 4.0×10^{-2} mol L⁻¹, with an analytical frequency of 28 samples h⁻¹. Fernandes *et al.*¹⁷ used a batch injection analysis (BIA) system for the potentiometric ASA determination in tablets, with a salicylate ion selective electrode. This system showed a linear response to ASA in the concentration range 7.5×10^{-3} mol L⁻¹ to 7.5×10^{-2} mol L⁻¹, with an analytical frequency of 90 samples h⁻¹. Quintino *et al.*¹⁸ determined ASA, after its hydrolysis to salicylate in a 0.10 mol L⁻¹ NaOH solution, by using amperometric detection with a copper electrode in a BIA system. This method presented a linear analytical curve in the range 1.0×10^{-6} mol L⁻¹ to 1.0×10^{-3} mol L⁻¹, using an injected volume of 100 μ L, with a detection limit of 0.48 μ mol L⁻¹. Majdi *et al.*¹⁹

developed a nickel hydroxide-modified nickel electrode for determination of ASA in alkaline medium. The analytical curve was linear in the ASA concentration range 4.0×10^{-4} to 6.0×10^{-3} mol L⁻¹, with a detection limit of 48 μ mol L⁻¹. Supalkova *et al.*²⁰ used square-wave voltammetry (SWV), with either a carbon paste electrode (CPE) or a graphite pencil as working electrode, to indirectly determine ASA, after its hydrolysis to SA and AA at 90 °C and pH 12 for 60 min, since ASA does not yield any SWV signal on the surface of CPE or graphite pencil. Torriero *et al.*²¹ employed a glassy-carbon electrode for SA determination in solid pharmaceutical formulations containing ASA as majority compound and observed that the SA in a Britton-Robinson buffer solution (pH 2.37) yields a single irreversible oxidation wave, at 1.088 V vs. Ag/AgCl. This method presented a linear analytical curve over the SA concentration range 1 μ g mL⁻¹ to 60 μ g mL⁻¹, with a detection limit of 0.09 μ g mL⁻¹, but it requires cold methanol for extraction of ASA from the samples. Despite the very good results found by these authors, as discussed above, their methods are time-consuming because of the need of a previous alkaline hydrolysis of ASA.

SWV is one of the most sensitive and direct analytical techniques, which has been used for the sensitive and quick determination of a wide range of organic molecules, with a low nonfaradaic current and high sensitivity. In most instances this method presents further advantages: no need for sample pretreatment and less sensitivity to matrix effects than other analytical techniques.²²⁻²⁵

Natural diamond is an insulator, but when doped with boron this material presents either semiconducting or semimetallic electronic properties, depending on the doping level. Recently, thin films of boron-doped diamond (BDD) emerged as a unique electrode material for several electrochemical applications, especially in electroanalysis.²⁶⁻³³ BDD electrodes possess very attractive properties, which are significantly different from those of other conventional electrodes, e.g. glassy-carbon or platinum electrodes. BDD, besides being corrosion stable in very aggressive media, presents a very low and stable background current, an extreme electrochemical stability in both alkaline and acidic media, a high response sensitivity, and a very wide working potential window, which can be larger than 3.5 V.³⁴⁻³⁷

In this paper the development and optimization of a square-wave (SW) voltammetric method exploiting the unique properties of BDD for the direct determination of ASA in pharmaceutical formulations for adults and children is described. This method is an attractive alternative because of its high sensitivity, relatively low cost, simplicity, and speed.

Experimental

Reagents and solutions

All reagents were of analytical grade, and the solutions were prepared using water ($> 18 \text{ M}\Omega \text{ cm}$) from a Milli-Q (Millipore®) purification system.

ASA was obtained from Sigma and sulfuric acid from Merck; potassium nitrate, sodium hydroxide, boric acid, acetic acid, and orthophosphoric acid were obtained from Synth. The pharmaceutical ASA samples were purchased from a local drugstore. Potassium nitrate, sodium hydroxide, sulfuric acid, and Britton-Robinson buffer (0.04 mol L^{-1} in acetic, orthophosphoric, and boric acids adjusted to the pH 9.01 with 0.2 mol L^{-1} sodium hydroxide) solutions were prepared for supporting-electrolyte studies.

A standard 0.01 mol L^{-1} ASA solution was prepared before use in a $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ solution and sonicated for 10 min to achieve complete dissolution. Appropriate dilutions were made from this solution.

Construction of the analytical curve

After optimizing the experimental parameters for the proposed method, the analytical curve was constructed by addition of aliquots of a standard solution of $1.0 \times 10^{-3} \text{ mol L}^{-1}$ ASA in $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ into the measurement cell containing a $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ solution. SW voltammograms were obtained after each aliquot addition.

Analysis of pharmaceutical samples

To prepare solutions of the ASA commercial samples, a representative number of ASA tablets (5 or 10) of each pharmaceutical sample were reduced to a homogeneous fine powder in a mortar. Then, an accurate mass of the powder was transferred to a 10 mL volumetric flask and the volume was made up with $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$; the resulting solution was sonicated for 10 min. Non-dissolved solids were filtered using a filter paper; after, $60 \mu\text{L}$ of each sample solution were directly transferred to the electrochemical cell containing 10 mL of $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ and the SW voltammograms were obtained. The ASA concentration in each sample solution was determined by interpolation in an analytical curve previously obtained with reference (standard) solutions.

Reference method

In order to compare the results obtained with the proposed SWV method, a back-titration method of the British

Pharmacopoeia⁶ was employed with a minor modification.¹⁰ An accurate representative amount of ASA powder from each sample was treated with 25 mL of a 0.492 mol L^{-1} NaOH solution and heated in a domestic microwave oven to ensure the complete ASA hydrolysis, avoiding boiling the solution. After cooling the solution to $25 \text{ }^\circ\text{C}$, excess NaOH was titrated with a standardized 0.508 mol L^{-1} HCl solution, using phenolphthalein as indicator.

Apparatus

Electrochemical experiments were conducted in a single-compartment glass cell provided with three electrodes. An Ag/AgCl (3.0 mol L^{-1} KCl) electrode was used as reference and the counter electrode was a Pt wire. The working electrode (0.33 cm^2 exposed area) was a boron-doped (8000 ppm) diamond film on a silicon wafer from Centre Suisse de Electronique et de Microtechnique SA (CSEM), Neuchâtel, Switzerland.³⁷ Details on the preparation of these diamond films were reported by Gandini *et al.*³⁸ Prior to the experiments, the BDD electrode was firstly cleaned with ethanol; then it was electrochemically pre-treated in a $0.5 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ solution by applying 1.0 A for 60 s, followed by -1.0 A for 120 s; this way, the BDD surface was made predominantly H-terminated.²⁹ The voltammetric measurements were carried out using an EGG-PAR potentiostat/galvanostat, model 273A. The pH was measured at $25 \pm 1 \text{ }^\circ\text{C}$ using an Orion pH-meter, Expandable Ion Analyser, model EA-940, employing a combined glass electrode with an Ag/AgCl (3.0 mol L^{-1} KCl) external reference electrode. The OriginPro 6.0 software was used for the data treatment.

Results and Discussion

The development of the here reported electroanalytical procedure involved a systematic study for the optimization of the experimental parameters that affect the SW-voltammetric response: supporting-electrolyte concentration and SW frequency (f), pulse amplitude (a), and scan increment (ΔE_s).

Firstly, preliminary cyclic-voltammetric (50 mV s^{-1}) studies on the electrochemical oxidation of ASA ($1.0 \times 10^{-3} \text{ mol L}^{-1}$) at the BDD electrode employing different supporting electrolytes were performed: (a) Britton-Robinson buffer (pH 9.01); (b) $0.5 \text{ mol L}^{-1} \text{ KNO}_3$; (c) $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$; (d) 0.1 mol L^{-1} NaOH (see Figure 2). Since the response in the H_2SO_4 solution is characterized by a well-defined oxidation peak (see Figure 2B), this solution was chosen as the suitable medium (supporting electrolyte) for further experiments.

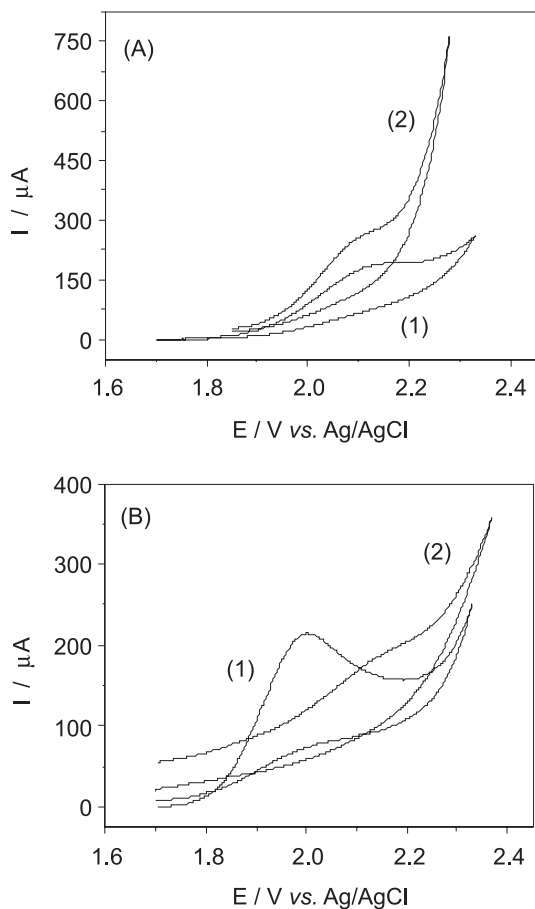


Figure 2. Cyclic voltammograms (50 mV s^{-1}) obtained using the BDD electrode as working electrode for $1.0 \times 10^{-3} \text{ mol L}^{-1}$ ASA in different supporting electrolytes; (A): (1) Britton-Robinson buffer (pH 9.01); (2) $0.5 \text{ mol L}^{-1} \text{ KNO}_3$; (B): (1) $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$; (2) $0.1 \text{ mol L}^{-1} \text{ NaOH}$.

The effect of the H_2SO_4 concentration was also investigated from $1.0 \times 10^{-3} \text{ mol L}^{-1}$ to 0.5 mol L^{-1} , for $1.0 \times 10^{-3} \text{ mol L}^{-1}$ ASA. The obtained results showed that the peak current presents a maximum value for $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$. Thus, the subsequent ASA analytical determinations were carried out using a $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ solution as the supporting electrolyte. Under these conditions, the method is selective for ASA, avoiding its hydrolysis because in the pH range 2 to 3 the ASA hydrolysis kinetics is very slow.^{5,20,21} To confirm this, the stability of the $1.0 \times 10^{-3} \text{ mol L}^{-1}$ ASA stock solution in $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ was studied during a 8 h period at $25 \text{ }^\circ\text{C}$ by monitoring the ASA concentration by cyclic voltammetry. The obtained results presented no significant differences within a relative standard deviation of 1.6%, indicating that the ASA hydrolysis was negligible.

The SW voltammograms obtained using the BDD electrode presented features similar to those initially obtained by cyclic voltammetry. A single irreversible oxidation peak at $1.97 \text{ V vs. Ag/AgCl}$ ($3.0 \text{ mol L}^{-1} \text{ KCl}$) was obtained

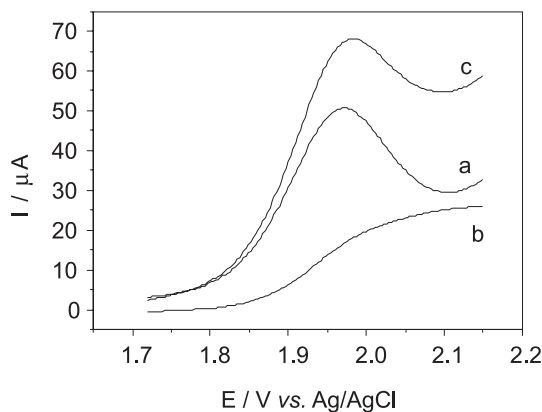


Figure 3. SW-voltammetric response of the BDD electrode for a $1.05 \times 10^{-4} \text{ mol L}^{-1}$ ASA solution (in $0.01 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$; $a = 40 \text{ mV}$, $\Delta E_s = 3 \text{ mV}$, $f = 50 \text{ Hz}$): (a) direct current; (b) reverse current; (c) total current.

using both electroanalytical techniques, confirming the irreversible nature of the observed ASA electrooxidation in the potential window investigated. Figure 3 presents the SW-voltammetric results obtained using the BDD electrode for a $1.05 \times 10^{-4} \text{ mol L}^{-1}$ ASA solution, at the conditions described in the legend of this figure.

Optimization of the experimental parameters

The SW frequency (f) is of extreme importance in SWV since it determines the intensity of the analytical signal and, consequently, the sensitivity of the technique. In this study, the peak current was found to be a linear function of f , with a peak potential displacement towards more positive values. According to accepted SWV theory,³⁹ this behavior corresponds to a totally irreversible system controlled by adsorption of the species on the electrode surface. A baseline increase was also observed when f was increased. Therefore, for the subsequent analytical applications, f was fixed at 50 Hz because a stable baseline and a good voltammogram definition were thus obtained. The SW pulse amplitude (a) is another parameter that strongly influences the values of the peak current in SWV, hence also determining the sensitivity of this technique. The obtained results showed that the peak current varies linearly for values of a up to 10 mV and reaches a plateau for $a \geq 40 \text{ mV}$. Therefore, a was set at 40 mV for the subsequent analytical application.

The effective rate of potential variation in SWV is the product between f and ΔE_s . In addition, the latter parameter also increases the analytical signal and sensitivity of this technique. However, a widening of the peak may occur for large values of ΔE_s , thus diminishing the analysis resolution.^{22,23} In this study, the peak current increased significantly for ΔE_s values up to about 4 mV, then leveling

off. Hence, for the analytical applications, ΔE_s was set at 3 mV.

Analytical Curve for ASA

Figure 4 shows the SW voltammograms obtained for ASA reference solutions at different concentrations (from 0 to 1.05×10^{-4} mol L⁻¹) in 0.01 mol L⁻¹ H₂SO₄, after optimization of the experimental parameters (for their values, see legend of Figure 4). The insert in Figure 4 corresponds to the analytical curve obtained, whose corresponding regression equation (correlation coefficient of 0.9995) is:

$$I_{ap}/\mu\text{A} = 0.488 + 4.12 \times 10^5 [c/(\text{mol L}^{-1})]$$

where I_{ap} is the anodic peak current and c the concentration of acetylsalicylic acid. The calculated value of the detection limit (three times the standard deviation of the blank solution/slope of the analytical curve) was 2.0×10^{-6} mol L⁻¹. The repeatability was determined by successive measurements of 4.5×10^{-5} mol L⁻¹ ASA solutions ($n = 10$), with a relative standard deviation of 1.4%.

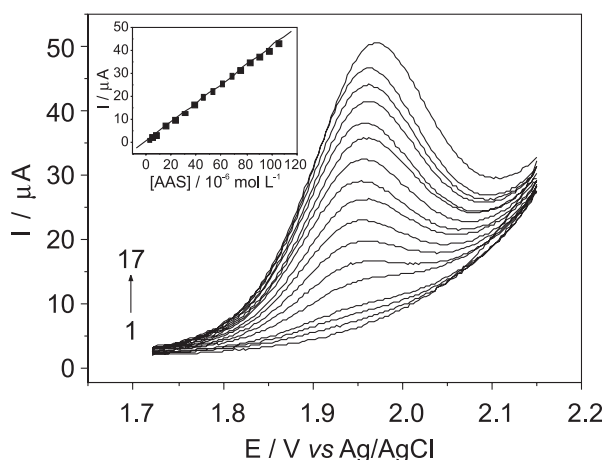


Figure 4. SW-voltammetric response (direct current) of the BDD electrode for different ASA concentrations in 0.01 mol L⁻¹ H₂SO₄: (1) 0; (2) 2.50×10^{-6} ; (3) 5.00×10^{-6} ; (4) 7.50×10^{-6} ; (5) 1.50×10^{-5} ; (6) 2.25×10^{-5} ; (7) 3.00×10^{-5} ; (8) 3.75×10^{-5} ; (9) 4.50×10^{-5} ; (10) 5.25×10^{-5} ; (11) 6.00×10^{-5} ; (12) 6.75×10^{-5} ; (13) 7.50×10^{-5} ; (14) 8.25×10^{-5} ; (15) 9.00×10^{-5} ; (16) 9.75×10^{-5} ; (17) 1.05×10^{-4} mol L⁻¹. Insert: Analytical curve for the ASA oxidation process.

Possible interferents

The selectivity of the proposed method was evaluated by the concomitant addition of possible interferents (saccharin, manitol, sucrose, fructose, glucose, starch, yellow sunset (CI 15985), and erythrosin red (CI 45430)) to 5.0×10^{-5} mol L⁻¹ ASA solutions at the concentration

ratios 1:1, 1:10, and 10:1. The responses were compared with that obtained using only the ASA standard solution. The obtained responses showed that these compounds do not interfere with the determination of ASA at the used working conditions. Only starch, in high concentration, promoted an increase and a shift of the ASA oxidation peak to more positive potential values. Nevertheless, in the analyzed samples the starch concentration is lower than those studied in this work, thus not interfering in the determination of ASA, as it was confirmed with the addition and recovery study.

Addition and recovery study

An addition and recovery study was performed by adding known amounts of standard solutions to a given sample followed by analysis using the proposed SWV method. The results presented in Table 1 show that the ASA recoveries range from 93.3% to 107% for the commercial tablets, indicating absence of a matrix effect.

Table 1. Addition and recovery of acetylsalicylic acid (ASA) from pharmaceutical products by the proposed SWV method using a boron-doped diamond electrode

Samples	ASA / (10 ⁻⁵ mol L ⁻¹)		Recovery ^a / (%)
	Added	Found	
Adult ASA			
	1.50	1.40	93.3
A	3.00	2.99	99.7
	4.50	4.53	101
B	1.50	1.53	102
	3.00	3.17	106
	4.50	4.80	107
Children ASA			
	1.50	1.51	101
A	3.00	2.99	99.7
	4.50	4.59	102
B	1.50	1.42	94.7
	3.00	3.07	102
	4.50	4.63	103

^aAverage of 3 measurements.

Analytical application

Table 2 presents the ASA amounts determined in several commercial ASA tablets employing the proposed SWV method and a standard method of the British Pharmacopoeia.⁶ Three determinations were done for each sample, and the standard deviations were calculated. The

Table 2. Determination of acetylsalicylic acid (ASA) in pharmaceutical products by a British Pharmacopoeia (reference) and the proposed SWV methods using a boron-doped diamond electrode

Samples	Label value	ASA tablet / mg		Relative error / (%)	
		Reference method ^a	SWV method ^a	RE ₁ ^b	RE ₂ ^c
Adult ASA tablets					
A	500	498 ± 3	498 ± 1	-0.4	0
B	500	501 ± 1	503 ± 2	0.6	0.4
Children ASA tablets					
A	100	99 ± 1	97 ± 1	-3	-2
B	85	85 ± 1	86 ± 1	1	1

^aAverage of 3 measurements. ^bRE₁ = 100 × (voltammetric value – label value) / label value. ^cRE₂ = 100 × (voltammetric value – reference method) / reference method.

amount of the ASA in each sample solution was determined by interpolation in an analytical curve previously obtained with reference (standard) solutions. The paired *t*-test⁴⁰ was applied to the results obtained using both methods; the fact that the calculated *t* value (2.644) is smaller than the critical value (3.182, $\alpha = 0.05$) indicates that there is no difference (at a confidence level of 95%) between the obtained results.

Conclusions

The present study shows that SWV using a BDD electrode can be used to determine the ASA content in several pharmaceutical products, with ASA dissolved in a 0.01 mol L⁻¹ H₂SO₄ solution. Optimization of the experimental parameters yielded a detection limit for ASA of 2.0 μmol L⁻¹ and a relative standard deviation smaller than 1.4% for 45 μmol L⁻¹ ASA solutions (n = 10). Addition-recovery tests were satisfactory, with values similar to those obtained using an official method of the British Pharmacopoeia⁶ and to the label values. In addition, the reported results demonstrate that the combination of SWV with a BDD electrode is a feasible alternative for the analytical determination of ASA in commercial adult and children tablets without previous hydrolysis of the analyte as required by other methods^{6,7,10,11,15-20} or the necessity of expensive apparatus along with time-consuming sample preparations.^{13,14}

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