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Portable voltammetric determination of acetaminophen using DropSens-based screen-printed carbon electrodes for clinical and pharmaceutical applications

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The increasing demand for portable, low-cost, and sustainable analytical methodologies has motivated the development of miniaturized electrochemical systems for pharmaceutical and clinical monitoring. In this work, an electrochemical method based on screen-printed carbon electrodes and nickel oxide-modified SPCE was optimized for the voltammetric determination of acetaminophen in pharmaceutical formulations and human urine using the DropSens configuration. Square-wave voltammetry, selected for its sensitivity and suitability for on-site analysis, enabled reliable quantification under alkaline conditions (pH 9.5). Both electrode types provided detection limits in the low-ppm range and broad linearity. Precision values varied depending on the matrix and electrode type, with RSDs generally below 20%. Accuracy ranged from 87 to 108% in drug formulations and exceeded 90% in urine samples. The DropSens platform produced analytical results comparable to those obtained with a conventional electrochemical cell while reducing sample and reagent consumption. Method validation for urine analysis was performed using in-tube solid-phase microextraction coupled to nano liquid chromatography with diode-array detection, which confirmed the reliability of the voltammetric method. Excretion profiles obtained by the proposed electrodes closely matched chromatographic data, with differences below 1% at the main excretion maxima (2 and 6 h after administration). Overall, the proposed miniaturized electrochemical approach provides a rapid, accurate, and sustainable alternative for the determination of acetaminophen in complex biological matrices, offering significant advantages in terms of portability, operational simplicity, and alignment with green analytical chemistry principles. The HEXAGON tool was used to support this last statement.

1 Introduction

Acetaminophen (AC), also known as paracetamol, is one of the most widely used analgesic and antipyretic drugs worldwide due to its efficacy and availability.[1,2] Despite its widespread use, concerns about its toxicity persist,[3,4] particularly regarding *p*-aminophenol (*p*-AP), its main degradation or synthesis impurity, which may be present in pharmaceutical formulations and is actively investigated due to its nephrotoxic and hepatotoxic effects.[5,6] Given these health implications, the accurate and simultaneous detection of AC and its toxic metabolite in pharmaceutical preparations is therefore critical for public health and pharmaceutical quality control.

Electrochemical sensing methods have emerged as powerful analytical tools for such applications due to their inherent advantages of high sensitivity, low cost, rapid response, and portability.[7,8] In particular, screen-printed electrodes have gained significant attention as disposable sensing platforms that enable miniaturization and facile surface modification. Various carbon-based screen-printed electrodes (SPCEs)—including graphite, carbon nanotubes, and graphene composites—have been successfully deposited onto screen-printed platforms, which are typically fabricated on ceramic or plastic substrates, allowing for easy customization through commercially available inks for the working, reference, and counter electrodes.[8,9]

Recent advances have focused on enhancing sensitivity and selectivity through the modification of electrodes surfaces with nanostructured materials. For example, surface modification of screen-printed graphite electrodes with a hybrid of molybdenum disulfide nanosheets and nickel-based metal–organic frameworks (MoS_2/Ni-MOF) has enabled ultrasensitive

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detection of AC with successful application to pharmaceutical and environmental samples.[10] In addition, the decoration of electrodes with platinum–nickel microstructures (Pt–Ni/SPE) has been reported to simultaneously quantify AC, *p*-AP, ascorbic acid, and zinc ions with excellent reproducibility and stability.[11] This platform demonstrates detection limits in the submicromolar range for AC, facilitating applications in pharmaceuticals and human plasma. Thus, nickel-based screen-printed electrodes have attracted significant attention as promising material for analytical sensing platforms,[12,13] enhancing selectivity in AC detection, as demonstrated by square wave voltammetry (SWV) measurements.[14–16] Among the wide variety of commercially available modified electrodes, nickel oxide-modified screen-printed carbon electrodes (NiO-SPCE) were specifically selected for this study. The bulk modification of screen-printed electrodes with nickel oxide (NiO) has been previously reported for sensing applications, such as hydroxide detection.[17] Furthermore, NiO nanoparticles are important transition metal oxides with a wide range of applications, including electrochemical supercapacitors, secondary batteries, electrochromic smart windows, and photoluminescence, and have proven highly effective for ultrasensitive glucose sensing.[18] In the context of AC and *p*-AP detection, NiO modification was chosen aiming to enhance both the sensitivity—by amplifying the faradaic currents—and the selectivity. Since NiO has been successfully employed in previous studies for the analysis of acetaminophen in paracetamol tablets and human blood serum samples,[19] it emerges as an ideal candidate for analytical screening in other complex biological matrices, such as human urine, before resorting to more complex laboratory modifications. The electrochemical oxidation of AC originates from its electroactive hydroxyl and acetamido groups.

Electrochemical determination of AC in human urine has also been addressed.[20] As a non-invasive biological matrix, urine provides a convenient sample for timely monitoring of AC in clinical settings, where early detection is crucial for effective treatment and ensuring patient safety. The matrix complexity and potential interfering substances in urine have promoted solid-phase extraction (SPE) as pre-extraction and pre-concentration sample preparation steps to overcome these limitations.[21]

In this work, we propose a comprehensive and portable analytical strategy that goes beyond simple electrode characterization by focusing on its clinical utility for real-time monitoring. While NiO-modified electrodes have been previously explored, this study focuses on the integration of the DropSens configuration—a drop-on-electrode approach that minimizes reagent consumption—into a clinical workflow. The primary novelty lies in the successful application of this portable platform to track human urinary excretion profiles after oral administration, providing critical pharmacokinetic data. Furthermore, to ensure the highest degree of reliability, the results obtained with this decentralized electrochemical method were rigorously cross-validated against a sophisticated laboratory-based technique: in-tube solid-phase microextraction coupled to nano-liquid chromatography with diode-array detection (IT-SPME-nanoLC-DAD).

2 Materials and methods

2.1 Materials, stock solutions and instrumentation

Acetaminophen (4′-hydroxyacetanilide, 99%) and *para*-aminophenol (≥98%) was purchased from Merck (Darmstadt, Germany). Ammonia buffer (pH 9.5) was prepared by dissolving ammonium chloride (NH_4Cl) from Probus (Badalona, Spain) in ultrapure water and adding ammonia solution (NH_3) from Scharlau (Barcelona, Spain) to obtain final concentrations of 1.0 M NH_4Cl and 2.0 M NH_3. The pH was adjusted to 9.5 using NaOH or HCl, as required. Sulfuric acid was purchased from Sigma (St. Louis, MO, USA). All aqueous solutions were prepared with ultrapure water obtained from a Barnstead Nanopure II system (Sybron, MS, USA). Methanol (HPLC grade) was obtained from Probus. Solid-phase extraction was performed using Strata C18-U silica cartridges (55 µm, 70 Å, 100 mg/1 mL) from Phenomenex (Torrance, CA, USA).

To obtain a 0.01 M stock solution, the required mass of AC was accurately weighed and dissolved in 100 mL of ultrapure water. A 0.01 M *p*-aminophenol (*p*-AP) stock solution was prepared by dissolving the appropriate amount of solid in 10 mL of 0.5 M sulfuric acid and diluting to a final volume of 100 mL with ultrapure water. Both stock solutions were stored in amber glass bottles at room temperature, where they remained stable for one month without noticeable degradation. Working standard solutions were freshly prepared by appropriate dilution of the stock solutions.

Voltammetric measurements were carried out using a 910 PSTAT mini potentiostat (Metrohm, Herisau, Switzerland), a compact USB-powered instrument connected to a PC. Screen-printed carbon electrodes (SPCE) and nickel oxide-modified SPCEs (NiO-SPCE) were supplied by Metrohm DropSens (Oviedo, Spain). Each strip integrates a three-electrode configuration consisting of a carbon or NiO-modified working electrode, a silver quasi-reference electrode, and a carbon counter electrode, all printed on a ceramic substrate. The electrodes feature a circular working area of 4 mm in diameter and strip dimensions of 3.4 × 1.0 × 0.05 cm, with silver electrical contacts. Data acquisition and analysis were performed using PSTAT software version 1.1 (Build 120217). The optimized instrumental and experimental conditions for the square-wave

Table 1 Optimized instrumental parameters for the SWV-DropSens operation

Parameter	Optimized value/description
Supporting electrolyte	Ammonia buffer (1.0 M NH_4Cl/2.0 M NH_3)
Solution pH	9.5
Conditioning potential	0 V
Conditioning time	5 s
Pulse amplitude	50 mV
Step potential	10 mV
Frequency	5 Hz
Sample volume	40 µL (direct deposition)

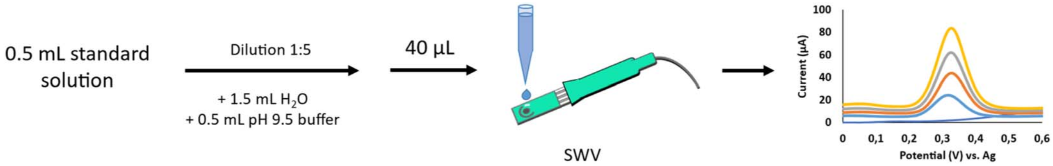

Fig. 1 Experimental procedure for AC determination in standard and pharmaceutical solutions using SPCE and NiO-SPCE electrodes under the DropSens operation mode, including voltammetric signal acquisition. The screen-printed electrodes consist of a ceramic substrate with a carbon auxiliary electrode and a silver quasi-reference electrode.

voltammetric (SWV) determination of acetaminophen using the DropSens configuration are summarized in Table 1. These parameters were selected to ensure maximum sensitivity and stability for samples analysis during the portable, smartphone-based operation.

Voltammetric responses were recorded in single-scan mode by mixing 0.5 mL of the standard or sample solution with 1.5 mL of ultrapure water and 0.5 mL of ammonia buffer (pH 9.5), as illustrated in Fig. 1. Between successive measurements, the electrode was rinsed with nanopure water. All electrochemical experiments were carried out at ambient laboratory temperature (22 ± 2 °C).

For method comparison, a nanoLC Agilent 1200 series LC coupled online to IT-SPME was used. The capillary employed for IT-SPME consisted of a segment of untreated fused silica capillary (0.075 × 100 mm and 440 nL of internal volume), which was coated with a tetraethoxysilane and methyltriethoxysilane polymer doped with SiO_2 nanoparticles, following the procedure proposed by Serra-Mora *et al.*[22] and the configuration of two valves given in ref. 23 for nano-LC. The analytical separation was conducted using an Agilent ZORBAX 300SB-C18 (0.1 mm × 150 mm, 3.5 µm particle diameter) analytical column. The mobile phase consisted of a mixture of water and acetonitrile in a gradient elution, starting with 2% acetonitrile for 5 minutes, increasing to 70% in 0.5 minutes, holding for 1.5 minutes, and finally returning to 2% at 8 minutes, where it was maintained for an additional 10 minutes. The IT-SPME volume processed was 100 µL and the injection volume was 440 nL. The signal was recorded in the 200–500 nm range, with 243 nm selected for quantification. All data were processed using Agilent HPLC ChemStation software.

2.2 Sample treatment of pharmaceutical formulations

Two commercial pharmaceuticals, namely effervescent and non-effervescent tablets, with 1 g AC content were analysed. To this end, tablet formulations were weighted and pulverized. Sample solutions were prepared by diluting with ultrapure water, ultrasonication for 30 minutes and filtration through 0.45 mm nylon membrane. The filtrate was taken to

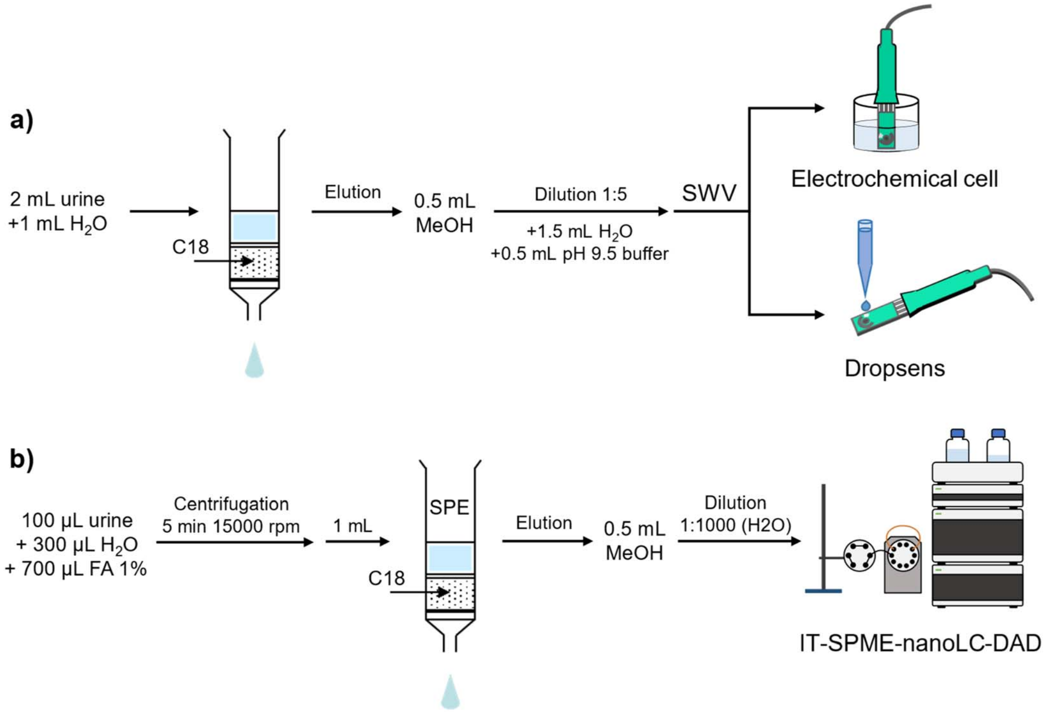

Fig. 2 Schematic representation of the urine pretreatment procedure for: (a) SWV with electrochemical cell and DropSens configuration and (b) IT-SPME-nanoLC-DAD analyses.

a volumetric flask with ultrapure water to prepare stock sample solutions (sample 1 and sample 2). For electroanalytical purposes, intermediate solutions were prepared, and 0.5 mL were subsequently diluted with ammonia buffer following the procedure outlined in Fig. 1. From this mixture, a 40 μL aliquot was deposited onto the working area of the DropSens electrode, and the voltammetric response was recorded in single-scan mode. The analytical performance of the proposed DropSens configuration was then compared with that obtained using a conventional electrochemical cell, in which the electrodes were directly immersed in the solution.

2.3 Sample treatment of human urine

Human urine samples ($n = 3$) were collected from healthy volunteers in accordance with voluntary and informed consent requirements. All participants were fully informed of the experimental purpose and procedures prior to their involvement in the study. Each urine sample was analyzed before and after the oral administration of AC from commercial tablets over a 10 hour period. Samples were stored at 4 °C under refrigeration until analysis.

For the sample treatment step, 2 mL urine sample were diluted with 1 mL ultrapure water, followed by SPE procedure using a C18-U silica cartridge. Elution was performed with 0.5 mL methanol; then, the eluate was further diluted as illustrated in Fig. 2a. Voltammetric measurements were performed following the DropSens configuration. All experiments were conducted at room temperature.

For the IT-SPME-nanoLC-DAD analysis, samples were first deproteinised mixing 100 μL of sample, 300 μL of formic acid 1% (v/v) and 700 μL of water, followed by centrifugation for 5 min at 15 000 rpm. Then, 1 mL of the supernatant was processed through a Strata C18-U SPE cartridge, pre-conditioned with 1 mL each of methanol and water. The cartridge was then washed with 1 mL of ultrapure water, and the AC was eluted with 500 μL of methanol. Finally, the methanolic extracts were diluted 1 : 1000 prior to its injection in the nanoLC (see Fig. 2b).

3 Results and discussion

3.1 Electrochemical characterization of acetaminophen and *p*-aminophenol

AC is a biologically active pharmaceutical compound capable of undergoing redox reactions due to the presence of electroactive hydroxyl and acetamido functional groups.[24] Its primary metabolite and degradation product, *p*-aminophenol (*p*-AP), also exhibits redox activity attributed to hydroxyl and amino groups.[25] The chemical structures of both compounds are shown in Fig. 3a, and their electrochemical behavior was subsequently characterized using cyclic voltammetry (CV) on bare (Fig. 3b) and NiO-modified electrodes (Fig. 3c) in ammonium buffer at pH 9.5, an alkaline medium previously reported as optimal for carbon-based electrodes.[26,27] Under these conditions, voltammograms for AC and *p*-AP were recorded at a scan rate of 100 mV s^{-1}, scanning the potential between −1 V and 1 V with a step size of 10 mV, as shown in Fig. 3b and c. Anodic (A_1) and cathodic (C_1) peaks for AC were observed, corresponding, as reported in the literature, to a quasi-reversible two-electron, two-proton process converting AC to *N*-acetyl-*p*-benzoquinone imine.[28] Comparison of the responses of the two carbon-based screen-printed electrodes revealed that the oxidation and reduction peak potentials of AC on the bare SPCE (0.30 V and 0.20 V, respectively) are shifted to more positive values relative

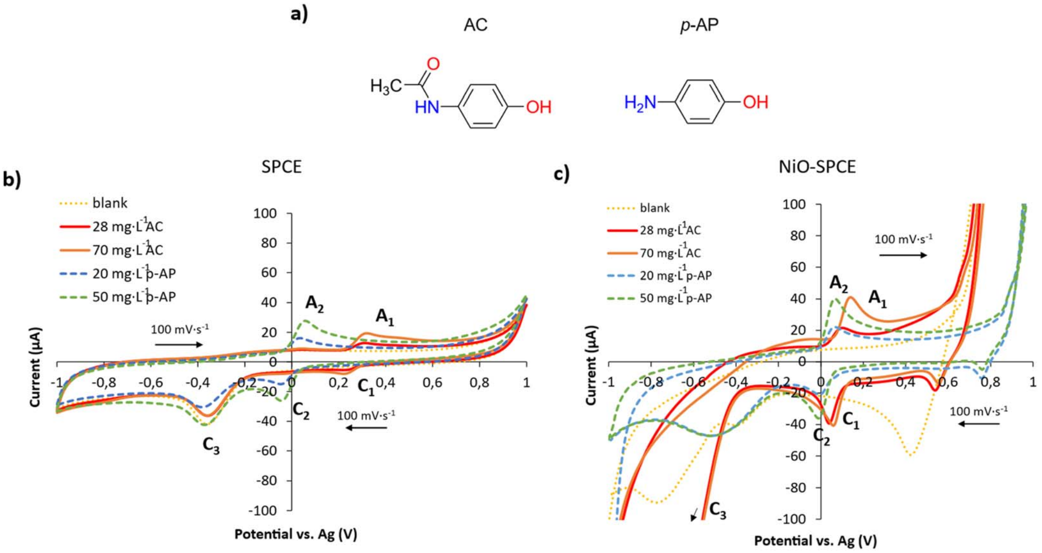

Fig. 3 (a) Chemical structures of acetaminophen (AC) and *p*-aminophenol (*p*-AP), with the electroactive hydroxyl, acetamido, and amino functional groups highlighted. Cyclic voltammograms recorded in ammonia buffer solution (pH 9.5) at a scan rate of 100 mV s^{-1} with a potential step of 10 mV (single-scan mode), using a silver quasi-reference electrode; (b) bare screen-printed carbon electrode (SPCE) and (c) NiO-modified SPCE. Voltammograms correspond to blank buffer (yellow, dotted line), AC at 28 and 70 mg L^{-1} (red and orange solid lines, respectively), and *p*-AP at 20 and 50 mg L^{-1} (blue and green dotted lines, respectively).

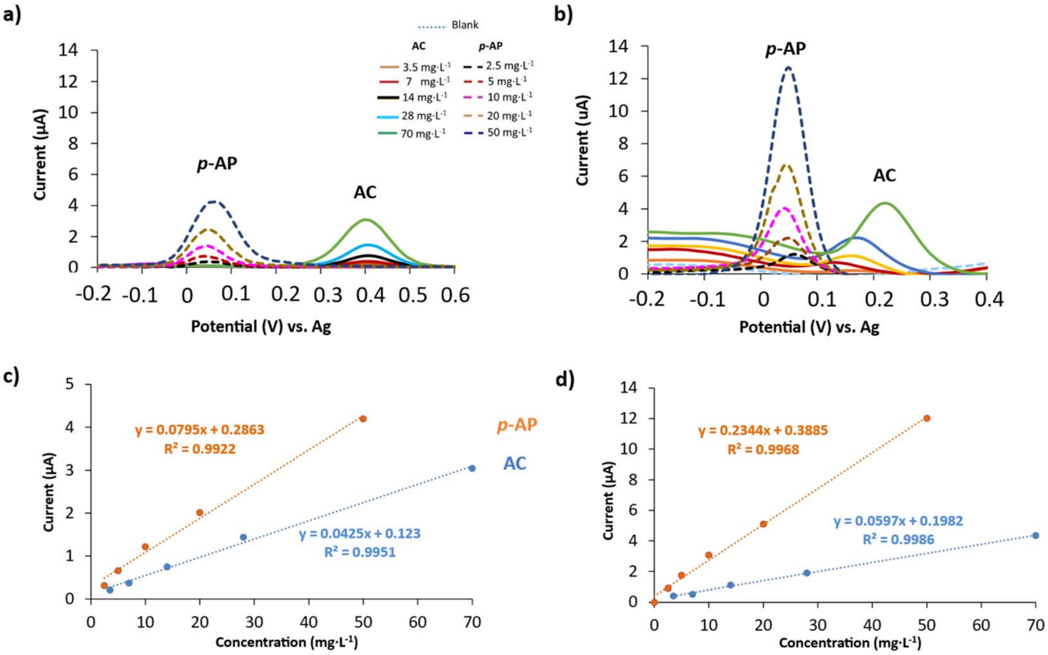

Fig. 4 Differential pulse voltammograms obtained for determination of acetaminophen (AC, solid lines) and *p*-aminophenol (*p*-AP, dashed lines) in ammonia buffer (pH 9.5) using: (a) bare SPCE, (b) NiO-modified SPCE. Experimental parameters: pulse height 10 mV, pulse width 5 mV, pulse time 25 ms, scan rate 0.1 V s^{-1}, potential range −1.0 to 1.0 V. Calibration plots for AC (3.5–70 mg L^{-1}) and *p*-AP (2.5–50 mg L^{-1}) are shown in: (c) SPCE, (d) NiO-SPCE.

to those observed on the NiO-modified SPCE (0.17 V and 0.08 V, respectively). The anodic peak A_2 in the forward scan and the cathodic peak C_2 in the reverse scan are attributed to the redox behavior of *p*-AP, which has been reported to undergo hydrolysis reaction to yield *p*-benzoquinone.[29] The NiO-modified electrode exhibits *p*-AP peak potentials (0.15 V anodic and 0.08 V cathodic) closer to those of AC, whereas the SPCE shows more negative potentials (0.07 V anodic and −0.04 V cathodic). At potentials above +1 V, an increase in current is observed, mainly associated with the oxidation of the carbon-based electrode surface.[30] In the cathodic region, a pronounced peak C_3 appears at −0.40 V and −0.75 V for SPCE and NiO-SPCE, respectively (see Fig. 3b and c). Given that all measurements were performed in aqueous media, this peak is likely due to the reduction of dissolved oxygen to hydrogen peroxide.[31,32]

The CV profiles obtained provide valuable insight into the interfacial behavior of the SPCE and NiO-SPCE. Under the experimental conditions defined in Fig. 3, the anodic–cathodic peak potential separation ($\Delta E_p = E_{pa} - E_{pc}$) for AC was 100 mV and 110 mV for *p*-AP when using the bare SPCE. In addition, the anodic peak current (A_1) of AC was significantly higher than the corresponding cathodic peak (C_1), yielding a peak current ratio (I_{pc}/I_{pa}) of 0.6. This suggests that the oxidation product of AC was not fully reduced at the electrode surface, likely due to kinetic limitations influenced by the scan rate.[28] In contrast, the current ratio for *p*-AP was closer to unity, indicating a more reversible redox process under the same experimental conditions. For the NiO-modified SPCE, the peak potential separations were slightly lower: 90 mV for AC and 70 mV for *p*-AP, suggesting improved electron transfer kinetics. Notably, the I_{pc}/I_{pa} ratio for AC increased markedly to 3, while remaining close to 1 for *p*-AP, confirming enhanced electrocatalytic activity of the NiO-modified surface toward AC oxidation. These observations are consistent with the higher sensitivity of the NiO-modified electrode, as evidenced by the slopes of the calibration curves obtained from the peak heights of the CV profiles: 0.310 ± 0.020 µA L mg^{-1} for AC and 0.552 ± 0.007 µA L mg^{-1} for *p*-AP, compared to 0.119 ± 0.003 µA L mg^{-1} (AC) and 0.294 ± 0.007 µA L mg^{-1} (*p*-AP) for the bare SPCE.

3.2 Calibration curves and practicability of carbon-based electrodes

Differential pulse voltammetry (DPV) has been successfully applied to the analysis of diverse samples containing AC, including human urine and pharmaceutical formulations.[20,33,34] Since voltammetric techniques are known to be more selective, less costly, and faster, DPV stands out as a reliable and efficient technique for the routine determination of AC in both pharmaceutical preparations and biological matrices. Initially, quantification of AC and its degradation product *p*-AP was carried out using standard addition calibration with prepared standard solutions of both analytes, applying DPV on both bare and NiO-modified SPCEs under the experimental conditions detailed in Fig. 4.

Voltammograms showed well-defined anodic peaks, with oxidation potentials at 0.40 V for AC and 0.05 V for *p*-AP on the bare SPCE as represented in Fig. 4a. In both cases, the anodic peak current (I_{pa}) increased linearly with analyte concentration. For AC, a linear response was observed in the concentration range of 3.5–70 mg L^{-1}, with a calibration slope of 0.0425 µA L

Table 2 Analytical parameters for the determination of acetaminophen in standard solution using DPV and SWV methods by standard addition calibration

	DPV		SWV	
Parameter	SPCE	NiO-SPCE	SPCE	NiO-SPCE
Linear range (mg L^{-1})	3.5–70	3.5–70	20–80	20–80
Slope (μA L mg^{-1})	0.043 ± 0.005	0.060 ± 0.004	0.835 ± 0.020	0.810 ± 0.050
Intercept (μA)	0.1 ± 0.2	0.2 ± 0.1	2 ± 1	-1 ± 2
Correlation coeff. (R^2)	0.995	0.999	0.998	0.984
LOD (mg L^{-1})	6.5	3.6	4	4

mg^{-1} and a correlation coefficient (R^2) of 0.9951 (see Fig. 4c). For *p*-AP, the linear range was 2.5–50 mg L^{-1}, with a slope of 0.0795 μA L mg^{-1} and $R^2 = 0.9922$. The limits of detection (LODs), calculated as $3 \cdot s_{yx}/b$ (where s_{yx} is the standard deviation of the linear regression and b the slope), were 6.5 mg L^{-1} for AC and 6 mg L^{-1} for *p*-AP.

When using the NiO-modified SPCE, improved sensitivity was observed as shown in Fig. 4b. The calibration slope for AC slightly increased to 0.0597 μA L mg^{-1} with a LOD of 3.6 mg L^{-1}, while *p*-AP exhibited a substantially steeper slope of 0.2299 μA L mg^{-1}, with a LOD of 2.4 mg L^{-1} (see Fig. 4d). Additionally, the oxidation potentials of both analytes appeared within a narrower potential window compared to the bare SPCE, with the AC oxidation peak shifted to a lower value (0.23 V) whereas the *p*-AP remained at 0.05 V. Overall, both SPCE and NiO-SPCE demonstrated selective and sensitive detection of AC and *p*-AP within appropriate linear concentration ranges and distinguishable oxidation potentials.

It is important to note that the electrocatalytic effect provided by the NiO modification significantly reduces the oxidation overpotential of AC, shifting its peak potential towards lower values. Consequently, the potential separation between AC and *p*-AP is reduced to approximately 180 mV on the NiO-SPCE, compared to the 350 mV observed on the bare SPCE. As seen in Fig. 4b, at higher concentrations of AC, a slight increase in the background current in the *p*-AP potential region may occur due to this closer proximity. However, this behavior did not affect the accuracy of the methodology for real-sample analysis, as *p*-AP was not detected in any of the pharmaceutical or clinical matrices studied. The expected analytical gain of the NiO modification lies primarily in its electrocatalytic properties. As demonstrated by the CV studies, the NiO-SPCE provides a substantial thermodynamic advantage by lowering the oxidation overpotential of AC by 130 mV (from 0.30 V to 0.17 V). Furthermore, it enhances electron transfer kinetics and significantly increases the sensitivity (calibration slope), particularly for *p*-AP detection, which increases nearly four-fold.

To evaluate the analytical performance of the proposed methods, calibration curves were constructed for both DPV and Square-Wave Voltammetry (SWV). The comparative figures of merit for these electrochemical techniques—including linear ranges, regression equations, sensitivity (slopes), and limits of detection (LOD)—are summarized in Table 2. Although the sensitivities (slopes) obtained by SWV were comparable for both bare and NiO-modified SPCEs, this behavior can be attributed to the inherent nature of the square-wave technique, where pulse parameters are specifically optimized to minimize charging current, potentially equalizing the peak current response despite improved electron transfer kinetics. Nevertheless, the use of NiO-SPCE is fully justified by the marked electrocatalytic effect observed, characterized by a 130 mV reduction in the oxidation overpotential (from 0.30 V to 0.17 V) and enhanced peak resolution, which significantly improve the selectivity and stability of the analytical method. By consolidating these parameters into a single table, the superior sensitivity and lower LOD achieved *via* the SWV method become more readily apparent compared to the DPV results previously shown in Fig. 4.

The stability and precision of both types of carbon-based electrodes were further evaluated using SWV. While DPV was initially employed for the fundamental assessment of the analytes' electrochemical behavior due to its high resolution, SWV was ultimately selected as the definitive method for sample analysis using the proposed portable DropSens-based platform. This choice is justified by its well-established superior sensitivity and faster scan rates, yielding sharp and well-defined peaks, and has been successfully applied in the literature to obtain electroanalytical calibration curves for urine, environmental water, and pharmaceutical tablets.[15,16,24,35] To this end, key instrumental parameters affecting the current response—such as conditioning period, pulse amplitude, step potential, and frequency—were optimized within a potential window ranging from −1.0 to +1.5 V. These measurements were performed using the DropSens configuration (Fig. 1) by depositing a 40 μL aliquot of AC standard solution in ammonia buffer (pH 9.5) onto the carbon-based electrode surface. As *p*-AP was not detected in the samples, it was excluded from further analyses. To evaluate the analytical performance of the system before

Table 3 Comparison of SWV electroanalytical curves obtained using external calibration and standard addition methods for acetaminophen determination following the DropSens experimental setup

		Linearity ($y = b_0 + b_1x$)		
Calibration method	Electrode	$b_1 \pm s_{b_1}$	$b_0 \pm s_{b_0}$	R^2
External	SPCE	0.590 ± 0.060	2 ± 3	0.981
	NiO-SPCE	0.730 ± 0.040	-2 ± 2	0.995
Standard addition	SPCE	0.835 ± 0.020	2 ± 1	0.998
	NiO-SPCE	0.810 ± 0.050	-1 ± 2	0.984

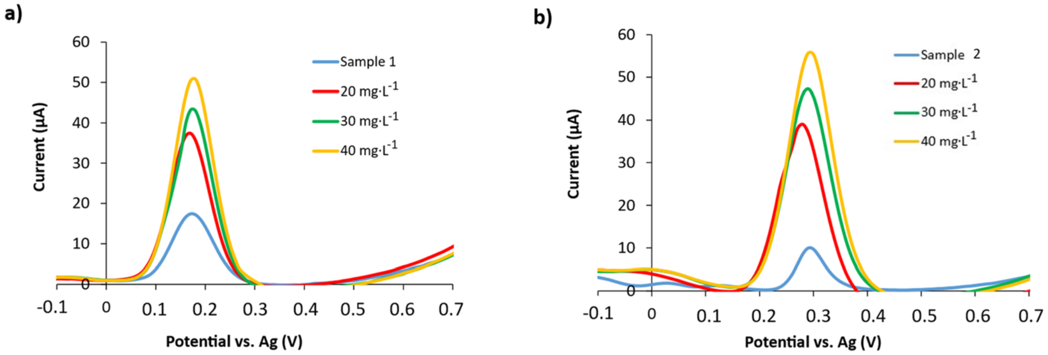

Fig. 5 Square-wave voltammograms of commercial tablet samples 1 (a) and 2 (b) (blue) recorded in ammonia buffer (pH 9.5) using SPCE under optimized voltammetric conditions. Spiked samples with AC concentrations of 20 mg L^{-1} (red), 30 mg L^{-1} (green), and 40 mg L^{-1} (yellow) were analysed ($n = 3$).

clinical application, both external calibration and standard addition methods from 20 up to 80 mg L^{-1} were evaluated using standard solutions, and the resulting electroanalytical curves were compared (Table 3). Due to the presence of significant matrix effects in urine samples, the standard addition method was selected for samples analysis.

The stability and precision of the commercially available electrodes were rigorously evaluated *via* SWV using paracetamol standard solution. Regarding the peak potential for paracetamol oxidation, the sensors exhibited excellent interelectrode reproducibility, with Relative Standard Deviation (RSD) values of 2% and 5% for the bare SPCE and NiO-SPCE, respectively ($n = 3$). Analytical precision was further validated at a paracetamol concentration of 20 mg L^{-1}, yielding peak current RSDs of 9% for the SPCE and 12% for the NiO-modified electrode when measuring by triplicate. These results underscore the robustness of both carbon-based platforms for electrochemical sensing. Furthermore, electrode reusability was assessed based on a 20% RSD threshold, a benchmark commonly accepted for decentralized screening. The NiO-SPCE maintained acceptable performance for up to 12 cycles, whereas the bare SPCE remained stable for 20 cycles. The higher variability observed for the NiO-modified sensor likely stems from the gradual leaching of NiO nanoparticles or surface passivation by adsorbed oxidation products in the alkaline medium. Consequently, this 12-cycle limit establishes a practical operational threshold to ensure analytical reliability during field-testing and on-site monitoring.

3.3 Electroanalysis of pharmaceutical tablets

Effervescent (sample 1) and non-effervescent (sample 2) tablets containing 1 g of AC were analyzed by means of SWV, and the results were compared against the declared values. Working sample solutions were prepared according to the procedure detailed in Fig. 1. SW voltammetric measurements were conducted under the optimized conditions described in Section 2.2. Bare SPCE was selected as the preferred electrode-type for this kind of sample because of its improved stability and precision with respect to NiO-modified electrode in view of a significant impact in patients health.

Sample solutions spiked in the 20–40 mg L^{-1} concentration range were prepared using the standard addition method, and the corresponding square-wave voltammograms were recorded in triplicate. As shown in Fig. 5, a single peak corresponding to AC was observed, while no signal attributable to *p*-AP was detected, indicating the high quality of the pharmaceutical formulations. The accuracy of the proposed method was evaluated by analyzing two types of commercial tablets. As summarized in Table 4, AC recovery values ranged from 87% to 108%. These results are in good agreement with recovery ranges reported in the literature for the validation of portable electrochemical sensors in pharmaceutical analysis,[16] confirming the reliability of the DropSens-based SPCE platform.

3.4 Voltammetric determination of acetaminophen in urine samples

Analysis of human urine was performed following oral administration of commercially AC tablets. Typically, an adult dose of 1 g AC is prescribed for pain or fever relief, with analgesic effects beginning approximately 30 minutes post-administration and lasting up to 4 hours.[36] Accordingly, urine samples were collected over a 0 to 10 hour period after AC intake to monitor its concentration profile in the biological matrix. The AC content excreted was evaluated.

Table 4 Determination of AC in commercial pharmaceutical tablets using SPCE under optimized SWV conditions ($n = 3$)

Sample	Declared AC (mg per tablet)	Found AC (mg per tablet)	Recovery (%)	Relative error (%)
Sample 1 (effervescent)	1000	960–1080	96–108	6
Sample 2 (non-effervescent)	1000	870–1050	87–105	14

Table 5 Analytical parameters for quantification of acetaminophen in urine samples using SPCE and NiO-SPCE in ammonia buffer (pH 9.5) by square-wave voltammetry

		Linearity ($y = b_0 + b_1x$)			
Experimental setup	Electrode	$b_1 \pm s_{b_1}$ (μA L mg^{-1})	$b_0 \pm s_{b_0}$ (μA)	R^2	LOD (mg L^{-1})
Electrochemical cell	SPCE	0.840 ± 0.020	2 ± 1	0.999	4
	NiO-SPCE	0.840 ± 0.080	−3 ± 4	0.984	4
DropSens	SPCE	0.620 ± 0.020	−4 ± 1	0.997	4.5
	NiO-SPCE	0.410 ± 0.010	−3 ± 1	0.999	3

Blank urine samples (0 hour) were analyzed by SWV without any pretreatment using the standard addition method under both, a conventional electrochemical cell setup and the proposed DropSens configuration. It was observed that electrochemical cell measurements impose single-use electrodes for each sample. In addition to this, no linear relationship was found between concentration and current (μA). In this regard, the drop-based configuration previously described for pharmaceuticals analysis was tested. Particular attention was given to obtaining sharp, well-defined peaks with appropriate widths and sufficient peak-to-peak separation. Although an enhancement of peak morphology was observed, further sample treatment was needed to improve electrode sensitivity and lifespan. Hence, the experimental procedure outlined in Fig. 2a was performed to minimize interferences and pre-concentrate analytes. Typically, solid-phase extraction (SPE) has been demonstrated as an effective pretreatment method for AC-containing urine samples, achieving recoveries exceeding 90%.[20] SWV calibration curves obtained for both screen-printed electrodes under the two experimental setups tested (electrochemical cell and DropSens) are listed in Table 5. Comparable and well-resolved peaks were registered around 0.3–0.4 V using the standard addition method for a blank urine sample spiked from 20 up to 80 mg L^{-1}. Thus, the DropSens configuration was selected as preferred operation mode. According to this, two blank urine samples spiked with 40 mg L^{-1} AC yielded the corresponding SW voltammograms illustrated in Fig. 6.

Following oral administration of AC by healthy volunteers ($n = 3$), urine samples at 2, 6, and 7 hours post-administration selected as representative time points to illustrate AC excretion kinetics. The DropSens experimental setup was carried out with NiO-SPCE electrodes and the corresponding SWV profiles were

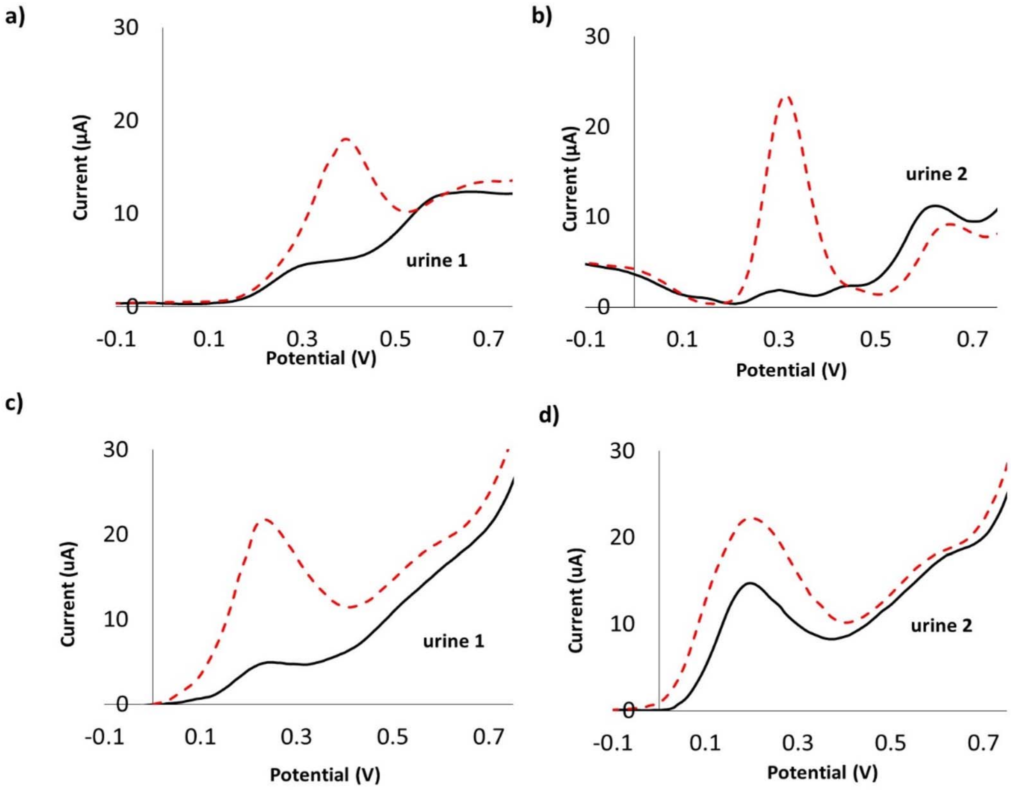

Fig. 6 Square-wave voltammograms recorded using SPCE (a and b) and NiO-SPCE (c and d) electrodes under the DropSens configuration for two urine samples collected prior to acetaminophen administration (black solid lines). Standard addition of 40 mg L^{-1} AC was performed for both urine samples (red dotted lines). Potentials are reported *versus* a silver quasi-reference electrode.

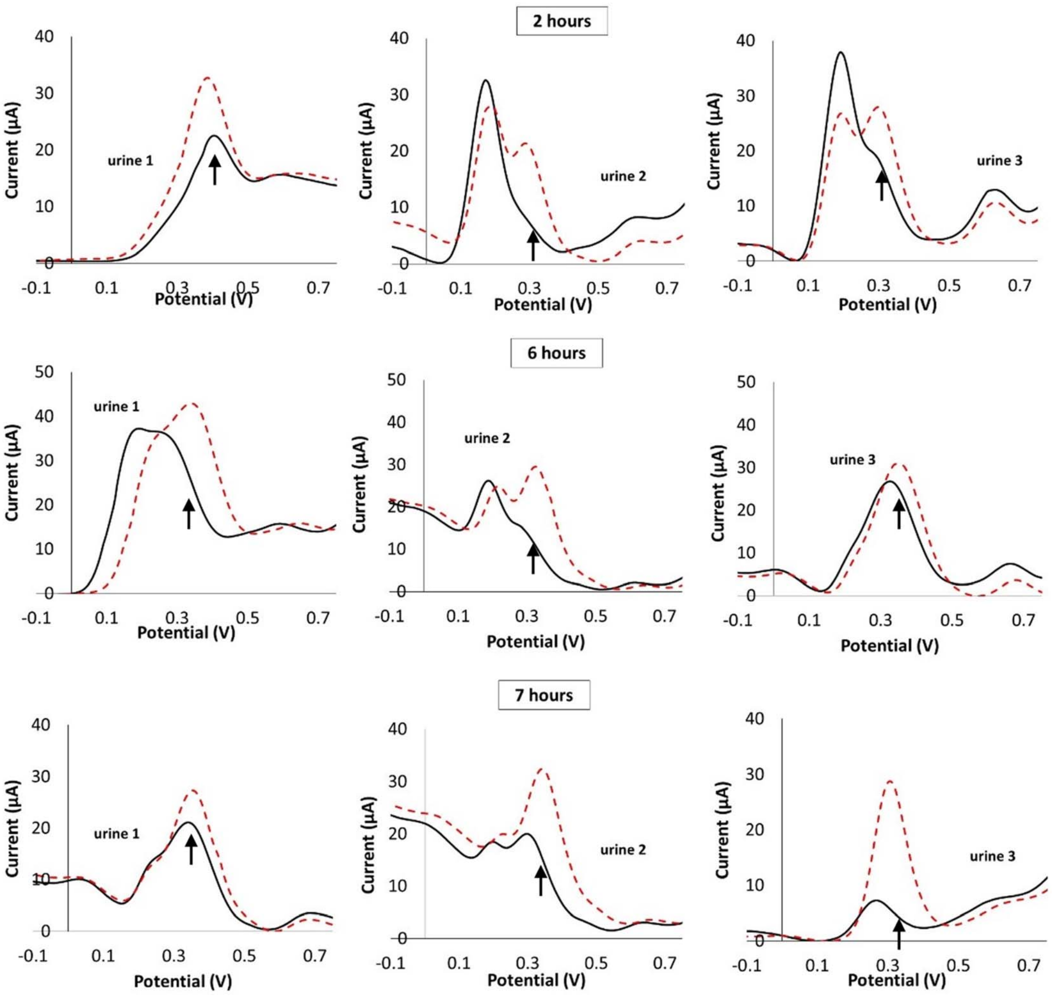

Fig. 7 SW voltammograms of three urine samples collected at 2, 6, and 7 hours after acetaminophen intake (black solid lines), recorded using SPCE electrode under the DropSens configuration. Standard addition of 40 mg L^{-1} AC was applied to all samples (red dotted lines). Potentials are reported *versus* a silver quasi-reference electrode. Black arrows indicate the potential within the 0.3–0.4 V window at which the AC oxidation peak current was measured.

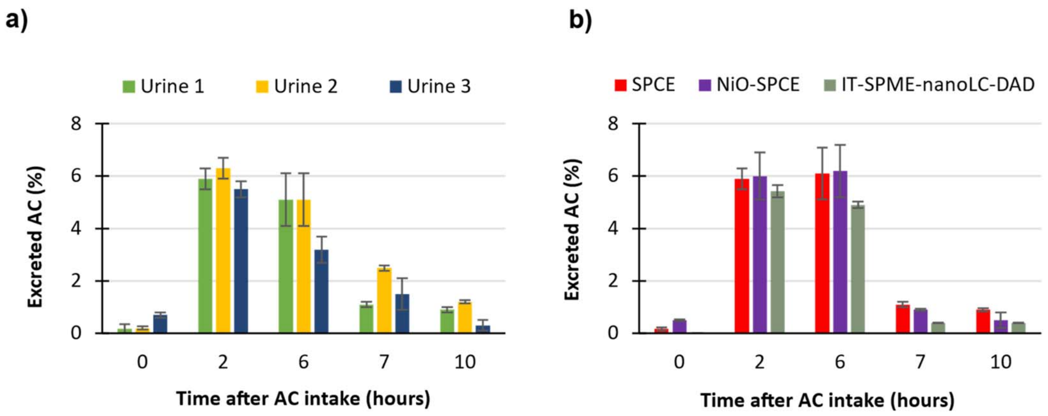

Fig. 8 Percentage of acetaminophen excreted in urine as a function of time following oral administration of 1 g AC from commercial tablets. (a) Results obtained using SPCE. (b) Comparative excreted AC content in urine sample 1 measured with SPCE, NiO-SPCE electrodes and IT-SPME-nanoLC-DAD.

Table 6 Standard addition calibration curve parameters: slope (b) and its standard deviation (s_b), y-intercept (a) and its standard deviation (s_a), and coefficient of determination (R^2). The working range was 15–100 μg L^{-1} ($n = 5$)

	Linearity ($y = b_0 + b_1x$)		
Sample	$b_1 \pm s_{b_1}$ (mg L)$^{-1}$	$b_0 \pm s_{b_0}$	R^2
Water	2.57 ± 0.06	−10 ± 3	0.998
0 h urine	2.10 ± 0.04	3 ± 2	0.999
2 h urine	2.11 ± 0.07	53 ± 4	0.997
6 h urine	2.47 ± 0.04	48 ± 2	0.999
7 h urine	2.18 ± 0.06	19 ± 2	0.999
10 h urine	2.23 ± 0.10	12 ± 5	0.997

recorded using the multiple standard addition calibration method. As depicted in Fig. 7, the evolution of the voltammetric profiles over time, revealing a linear correlation between oxidation peak currents and AC concentration. Additionally, spiked urine samples with 40 mg L^{-1} AC standard were analyzed to assess variations in the SWV response for each time point. Based on the SWV data, no significant matrix effects were observed during standard additions. The AC concentration in urine was found to peak between 2 and 7 hours after ingestion.

For the estimation of AC content in urine samples, spiked urines within the linear working concentration range of 20–80 mg L^{-1} were analyzed. Assuming an average urinary bladder volume of 250 mL, the total mass of excreted AC was calculated for each collection time. These values were then normalized to the initial administered dose (1 g) to express the results as a percentage of excreted AC, as illustrated in Fig. 8a. This representation was chosen over simple concentration units (mg mL^{-1}) to facilitate a standardized comparison with pharmacokinetic literature and to provide a clearer assessment of the drug's metabolic fate, effectively mitigating the impact of individual variations in urine volume or dilution. The highest percentage of excreted AC was detected around 2 hours post-ingestion for SPCE. For comparative purposes, the estimated AC content in urine sample 1 was evaluated using both SPCE and NiO-SPCE, with the results presented in Fig. 8b. Both electrode types yielded similar estimations of excreted AC, with maximum excretion observed within the 2 to 6 hour timeframe. These findings indicate that both SPCE and NiO-SPCE are suitable electrochemical sensors for the detection and quantification of AC in urine samples.

3.5 Cross-method comparison and sustainability assessment for urine sample analysis

The accuracy of the electrochemical method for the quantification of AC in urine was evaluated by comparing the results with those obtained by IT-SPME-nanoLC-DAD (see Table 6). The chromatographic analysis was carried out under optimized gradient conditions with a re-equilibration time of 10 min to ensure consistent retention times (RSD <1%). The chromatograms obtained for AC determination in the urine samples at different time points after intake are shown in Fig. 9. The instrumental LOD was 0.45 μg L^{-1}, and no significant matrix effect was observed.

The excretion profiles obtained by SPCE and NiO-SPCE were largely consistent with the chromatographic results, with deviations of 0.5–1% at 2 and 6 h post-dose that fall well within the experimental precision of the sensors (RSD 9–12%). Consequently, these differences were considered statistically negligible and did not warrant further formal assessment. While the electrochemical methods exhibited higher LODs (~4 mg L^{-1}), this sensitivity was adequate for the concentration range studied.

To objectively evaluate the greenness and sustainability of the proposed electrochemical method for paracetamol determination in urine, the HEXAGON tool was applied. This metric assesses analytical procedures based on six criteria represented in a regular hexagonal diagram: economic cost, two sets of figures of merit (sample treatment/calibration and quality

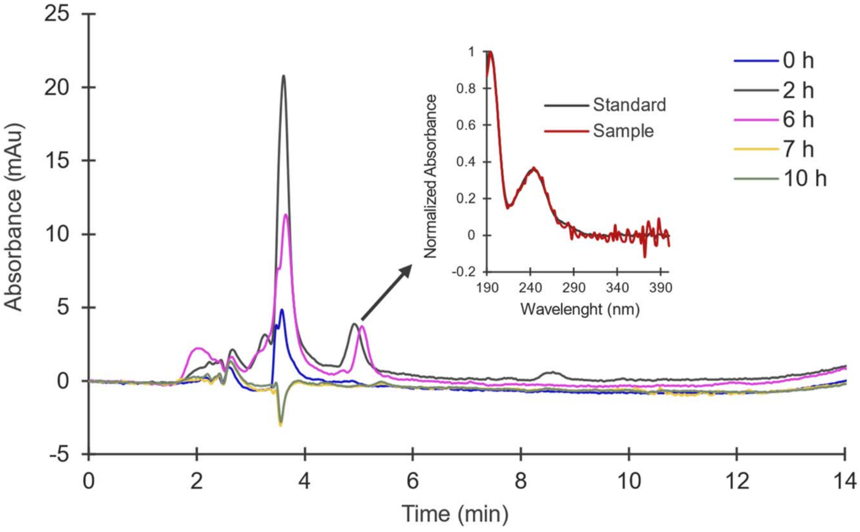

Fig. 9 Urine sample chromatograms collected at 0, 2, 6, 7 and 10 h after 1 g of AC intake. Normalized UV spectra of the AC chromatographic peak in the samples and the standard are also shown.

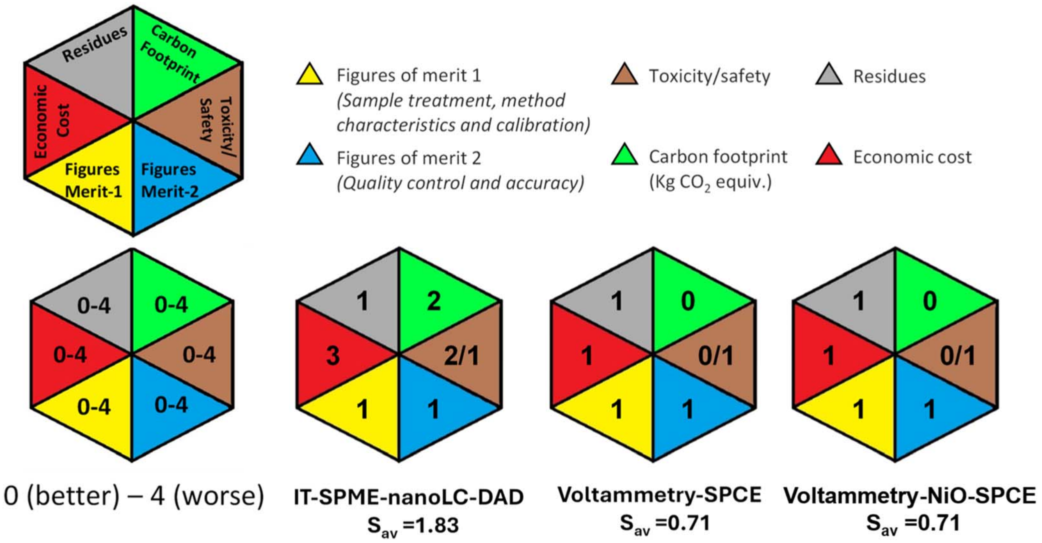

Fig. 10 Evaluation of the analytical eco-scale using the HEXAGON tool for the IT-SPME-nanoLC-DAD and the proposed voltametric methods based on SPCE electrodes. S_{av} values closer to 0 denote a more environmentally friendly method. For more explanation see text.

control/accuracy), toxicity/safety, carbon footprint, and residues. As depicted in Fig. 10, each criterion is rated on a penalty scale from 0 (better) to 4 (worse), meaning a lower score indicates a better adaptation to sustainability principles without compromising analytical reliability.

When comparing the methodologies, the reference chromatographic method (IT-SPME-nanoLC-DAD) yielded an average score (S_{av}) of 1.83. In contrast, both developed voltammetric methods (using SPCE and NiO-SPCE) demonstrated significantly lower penalties, achieving an excellent S_{av} of 0.71. The electrochemical approaches showed notable improvements particularly in the "carbon footprint" (score reduced from 2 to 0) and "economic cost" (score reduced from 3 to 1) categories, as well as lower toxicity. These results highlight the inherent advantages of the proposed screen-printed electrodes, which minimize energy consumption, reduce hazardous waste, and lower operational costs compared to complex liquid chromatography systems, all while maintaining equivalent scores in the analytical performance categories (figures of merit 1 and 2). Detailed guidelines on calculating these penalty points, carbon footprint, and annual costs can be found in ref. 37–39.

4 Conclusions

In this work the electrochemical detection of AC in pharmaceutical tablets and urine samples using voltammetric measurements with a portable potentiostat has been proposed. The results demonstrate that the proposed bare and NiO-modified SPCEs exhibit good selectivity and offer a promising alternative as compact, low-cost electrochemical sensors for direct analysis with minimal sample consumption employing a DropSens experimental setup. This configuration improved electrode durability not only in pharmaceutical formulations but also in complex biological matrices such as human urine up to 12 cycles. The NiO-modified electrode can provide an advantage when sensitivity and selectivity is low on the bare SPCE, highlighting their potential for practical applications in pharmaceutical quality control and clinical monitoring. In urine, the excretion profiles were in line with those observed by the chromatographic reference method, with deviations in the measured AC percentages within expected experimental variability. The proposed voltammetric approach is of sustainable nature, which is primarily attributed to the use of screen-printed electrodes, the requirement of microvolume samples, and the minimization of complex sample pretreatment steps. These features consolidate the method as a robust, cost-effective, and eco-friendly alternative to conventional chromatographic techniques for the monitoring of acetaminophen in both clinical and pharmaceutical settings.

In conclusion, the developed methodology demonstrates that miniaturized and portable electrochemical platforms, when properly optimized under the DropSens configuration, can provide clinical information equivalent to high-end laboratory systems. The high degree of agreement (differences below 1%) between the portable SPCE method and the advanced IT-SPME-nanoLC-DAD technique validates this approach for decentralized clinical monitoring. Beyond the electrode modification, the ability to accurately track urinary excretion profiles highlights the potential of this cost-effective tool for personalized medicine and point-of-care pharmacokinetics, bridging the gap between material science and practical clinical applications.

Conflicts of interest

There are no conflicts to declare.

Data availability

All data supporting this study are provided in full in the Results section.

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