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The opportunity of 6-monoacetylmorphine to selectively detect heroin at preanodized screen printed electrodes

Noelia Felipe Montiel,^{a,b,‡} Marc Parrilla,^{a,b,‡} Victoria Beltrán,^{a,b} Gert Nuyts,^{a,b} Filip Van Durme,^c Karolien De Wael^{*,a,b}

*Corresponding author: Karolien De Wael (karolien.dewael@uantwerpen.be)

^a AXES Research Group, Department of Bioscience Engineering, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium

^b NANOlab Center of Excellence, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium.

^c Drugs and Toxicology Department, National Institute for Criminalistics and Criminology (NICC), Vilvoordsesteenweg 100, 1120, Brussels, Belgium.

Abstract

The illicit consumption of heroin is an increasing concern in our society. For this reason, rapid analytical methods to seize heroin samples in the field are of paramount importance to hinder drug trafficking, and thus prevent the availability of heroin in the drug market. The present work reports on the enriched electrochemical fingerprint of heroin, allowing its selective detection in street samples, based on the use of electrochemical pretreated screen printed electrodes (p-SPE). The voltammetric identification is built on two oxidation peaks of both heroin and its degradation product 6-monoacetylmorphine (6-MAM), generated in alkaline conditions. Interestingly, an anodic pretreatment of the screen printed electrodes (SPE) shifts the peak potential of paracetamol (the most encountered cutting agent in heroin seizures), allowing the detection of 6-MAM peak, overlapping with the paracetamol signal in the case of untreated SPE. Subsequently, the characterization of the p-SPE with scanning electron microscopy, cyclic voltammetry, electrochemical impedance spectroscopy, Raman and Fourier transform infrared (FTIR) spectroscopy is provided to demonstrate local changes on the surface of the electrode. From an analytical perspective, p-SPE provide higher sensitivity ($0.019 \mu\text{A} \mu\text{M}^{-1}$), excellent reproducibility (6-MAM, RSD = 2.85%, and heroin RSD = 0.91%, $n = 5$) and lower limits of detection (LOD) ($5.2 \mu\text{M}$) in comparison to untreated SPE. The proposed protocol which integrates a tailor-made script is interrogated against common cutting agents, and finally, validated with the

screening of 14 street samples, also analyzed by standard methods. Besides, a comparison with portable spectroscopic techniques on the confiscated samples shows the better performance of the electrochemical strategy. Overall, this sensing approach offers promising results for the rapid on-site profiling of suspicious heroin samples, also in the presence of paracetamol.

Keywords

Electrochemical fingerprint, pretreated screen-printed electrodes, heroin, 6-monoacetylmorphine, cutting agents, forensics

1. Introduction

Trafficking and illegal consumption of drugs of abuse are a serious threat to our society [1]. For example, the current COVID-19 crisis could trigger an increase in harmful drug use among the population as a result of the economic downturn [2]. Besides, this alarming trend has been boosted by the growing illicit opium poppy cultivation. Current data estimates that, in 2018, 80% of opioid production was converted into heroin [3]. Heroin (3,6-diacetylmorphine, diamorphine, **Fig. 1a**) may be defined as a powerful semi-synthetic derivative obtained during the acetylation process of morphine. From a societal perspective, heroin can lead to harmful diseases [4] and, in a worst case scenario, drug-induced death (opioid overdose rose 82% in 2020) [5].

Although the retail market of heroin tends to be fairly stable, new formulations and a great variety of uses are becoming more popular [6]. The composition of heroin seizures has been reported only in a few scientific papers and it is also limited in forensic literature as it is classified as sensitive information [7]. Current reports describe that heroin seizures are barely found as a pure compound, but mainly found after adulteration (**Table S1** and **S2**) [8,9].

Many analytical methods have been reported for the detection of heroin [10]. Gas chromatography or high performance liquid chromatography coupled with mass spectrometry are often the first choice for the analysis in laboratories [11,12]. When it concerns on-site analysis, both chemical color tests and sophisticated, more expensive instrumentation such as portable Raman, infrared or ion mobility spectroscopy are regularly used [13–15].

In the last years, special attention has been given to electrochemistry for the analysis of illicit drugs due to its outstanding features (e.g., affordable, rapid and user-friendly tests) [16,17]. For example, current advances have been made for the electrochemical detection of cocaine [18], ketamine [19] and fentanyl [20], though, few articles are related to heroin detection. In 1990, Barreira-Rodríguez et al. performed a complete study of heroin oxidation and its mechanism at carbon paste electrode [21]. Later, Garrido et al. explored the electrochemical behavior of heroin and its metabolites in a wide range of pHs to prove a new oxidative mechanism by using glassy carbon electrodes and differential pulse voltammetry [22].

Recently, the use of nanomaterials has been employed to enhance the sensitivity and improve the limit of detection (LOD) of electrochemical sensors [23,24]. It is, however, important to notice that these nanomaterials-based strategies can be time-consuming and costly in terms of electrode preparation [25]. As a way to overcome those issues, our group developed a method to detect heroin in street samples by using a dual pH strategy on graphite screen printed electrodes (SPE) [26]. Nevertheless, the method was still time-consuming and exhibited some false positives when validating in the field due to signal overlapping from common adulterants (e.g., codeine, noscapine and lidocaine) or illicit drugs (e.g., cocaine and MDMA). Moreover, the masking phenomena between paracetamol and 6-monoacetylmorphine (6-MAM) in both pHs was first identified. For these reasons, an enhanced approach is necessary to overcome this selectivity issue and allocate electrochemical methods as an alternative on-site screening solution.

Herein, we report an improved method for the selective profiling with square-wave voltammetry (SWV) of heroin seizures by using anodically pretreated screen-printed electrodes (p-SPE). The principle is based on the selective electrochemical fingerprint (EF) of heroin obtained from the SWV pattern that exhibits two oxidation peaks (i.e. the tertiary amine of heroin and the phenol group of 6-MAM) (**Fig. 1a**). The phenol oxidation peak appears upon the degradation of heroin under alkaline conditions, which leads to the formation of 6-MAM. However, the oxidation peak of 6-MAM is overlapped with the oxidation peak of paracetamol at untreated SPE [26], thus hindering its potential use for the determination of heroin in complex samples. As paracetamol is present in more than 90% of heroin seizures in Europe [27], a solution for this overlapping issue is essential. Here it is proposed a rapid and smart anodic

pretreatment of the electrode surface. Hence, the use of p-SPE presented in this work allows the discrimination of the oxidation signal of 6-MAM upon paracetamol signal by altering the electrocatalytic activity on the SPE. This pretreatment poses an enhanced peak separation overcoming the potential masking phenomena faced at the untreated SPE. In order to characterize the electrode's surface after the modification with the anodic pretreatment, scanning electron microscopy (SEM), cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), Raman and Fourier transform infrared (FTIR) spectroscopy were used.

The analytical performance is demonstrated by the screening of common adulterants and illicit drugs with heroin in binary mixtures as well as in complex samples employing SWV. Importantly, the integration of a data analysis script enhances the peak separation and thus the identification of substances when multiple compounds are present in the sample and the related peaks may be overlapped. Finally, the electrochemical method is validated with standard methods (i.e. gas chromatography-mass spectrometry, GC/MS) and current on-site methods (i.e. FTIR) by testing confiscated samples from forensic laboratories. Overall, this new approach presents a one-step analysis to enlighten the full composition of heroin samples aiming to increase the accuracy of the existing screening tests, and ultimately, facilitating law enforcement duties.

2. Material and methods

2.1. Materials

All reagents used were of analytical grade and used without supplementary purification. Standards of heroin.HCl.H₂O and cocaine.HCl were purchased from Chiron Chemicals, Australia. Standards of ketamine.HCl, d,l-amphetamine.HCl, methamphetamine.HCl, MDMA.HCl, mephedrone.HCl, MDVP.HCl and ethcathinone.HCl were provided by the National Institute of Criminalistics and Criminology (NICC) of Belgium. Cutting agents such as paracetamol, caffeine, diazepam, dextromethorphan (DXM), lidocaine, procaine, griseofulvin, papaverine, quinine, codeine, levamisole and noscaphine were also provided by NICC.

Aqueous solutions were prepared using doubly deionized water by a Millipore Milli-Q System (R >18.2 MΩ cm). Potassium phosphate monobasic, potassium dihydrogen phosphate and potassium chloride were acquired from Sigma Aldrich (Overijse,

Belgium). Phosphate buffer saline (PBS) used as buffer solution and supporting electrolyte was prepared with potassium phosphate monobasic and dibasic in concentration of 20 mM and with 100 mM KCl. The pH was adjusted to the desired value by using a 100 mM KOH solution using a Conductometer 914 pH-meter from Metrohm (The Netherlands). 10 mM of drug stock solutions were first prepared and stored at a temperature of -20 °C. When required, the fraction was defrosted and used.

Seized street samples were provided by NICC of Belgium. Prior the electrochemical measurements, NICC analyzed seized samples qualitatively and quantitatively by GC/MS and gas chromatography/flame ionization detection (GC/FID) in order to establish their chemical composition.

2.2. Methods

Voltammetric measurements were performed using a PalmSens 3 potentiostat (PalmSens, Houten, The Netherlands) coupled to a Multiplexer 8 R2 with data management by PSTrace software. For EIS measurements, MultiPalmSens4 (PalmSens, Houten, The Netherlands) was used. EIS signals were analyzed based on Randles equivalent circuit $R_s - ([R_{ct}W]C_{dl})$, where R_s is the solution-phase resistance, R_{ct} is the charge-transfer resistance, C_{dl} is the double-layer capacitance and W is the Warburg impedance that is related to the mass transfer effect. The parameters were obtained by PSTrace software fitting the points to a semicircle.

Disposable ItalSens graphite screen printed electrodes (SPE) (PalmSens, the Netherlands) containing a three-electrode cell was used to perform SWV measurements: a carbon counter electrode, a pseudo Ag reference electrode and graphite working electrode ($\varnothing = 3$ mm).

The following procedure for drug detection was developed according to previous optimized experimental and instrumental parameters: (i) Electrochemical activation of the SPE was done in 2 steps. Briefly, the general procedure consists of an anodic treatment at 1.5 V for 60 s in PBS solution at pH 7 by drop casting 60 μ L on the SPE. The electrode was then exposed to a SWV measurement (potential range -0.1 V to 1.5 V, frequency 10 Hz, 25 mV amplitude and 5 mV step potential) and a stable baseline curve was obtained; (ii) SWV measurement, at the same conditions abovementioned, was performed with a freshly analyte solution at PBS pH 12. All voltammograms obtained were baseline corrected using a mathematical algorithm

“moving average” (peak width=1) in PSTRace software to improve the resolution of the peaks over the background.

A custom-made script (Matlab R2018b, MathWorks, U.S.A.) is used after the SWV analysis to enhance peak separation and identify the compounds found in the sample. In brief, the script removes the background signal and applies a top-hat filter that provides an enhanced separation of overlapped peaks which permits a successful identification of the substances.

For the morphological characterization of the surface of SPE and p-SPE, Quanta Scanning Electron Microscope 250 was used. For the chemical characterization of the surface of SPE and p-SPE, FTIR spectroscopy and Raman spectroscopy were used. FTIR spectra were collected using a ThermoFischer Nicolet 5700 FTIR spectrometer attached to a Continuum XL microscope. The microscope was equipped with a 32x objective and a MCT detector (650 cm^{-1} cut-off). Samples were analyzed in transmission mode accumulating 256 scans at 4 cm^{-1} spectral resolution, with a wavenumber range of 4000–700 cm^{-1} , the spot size was 40x40 μm^2 . Raman spectra were collected with a DXR 3xi Raman Imaging Microscope from Termofischer equipped with a 785nm laser and a x100 objective. Spectra were collected from 3800 to 80 cm^{-1} , using 150 mW energy and 10 seconds exposure during 5 accumulations.

Street samples electrochemical analyses were performed in a solution of 10 mg mL^{-1} of sample, which was later diluted to achieve a concentration range of 1-0.5 mM (depending on the purity of the samples) in PBS pH 12. A portable Attenuated Total Reflectance (ATR) FTIR spectrometer (Bruker Alpha 2, UK with a diamond crystal) was used to analyze the street samples in powder for comparative purpose. For each spectrum, 128 scans were accumulated at 4 cm^{-1} spectral resolution, the wavenumber range was between 4000 to 475 cm^{-1} . The spectra have been baseline corrected, no additional treatments have been applied. The corrections and the analysis of several components have been done using the OPUS 8.2 software.

3. Results and discussion

3.1. Electrochemical behavior of heroin: the 6-MAM opportunity

Heroin is an electroactive compound which can be electrochemically oxidized at graphite SPE. Hence, the EF of heroin was firstly interrogated by SWV at SPE in PBS

pH 7 and pH 12. As shown in **Fig. 1b**, heroin shows a single oxidation peak by 0.88 V at pH 7 due to the oxidation of its tertiary amine. At pH 12, two well-defined signals at 0.15 V and 0.82 V are displayed. The first peak can be ascribed to the oxidation of the phenolic group (P1) of one of its active metabolites (6-MAM, **Fig. 1a**) and the second one to the tertiary amine (P2) present in heroin and 6-MAM [22,26]. Remarkably, 6-MAM is present in the majority of heroin seizures as a product of the incomplete synthesis or spontaneous deacetylation of heroin (see also **Table S3** and **S4**) [28]. In addition, it was observed that strong alkaline conditions (i.e. pH 12) can induce fast conversion of heroin into 6-MAM. Indeed, a time study of heroin in PBS pH 12 is displayed in **Fig. 1c**, showing the conversion to 6-MAM within less than one minute after heroin is mixed with buffer pH 12. After ca. 5 minutes, 6-MAM and heroin oxidation peaks reached a plateau (**Fig. 1d**). Since law enforcement agents (LEAs) should perform the on-site measurement in few minutes, two minutes were selected for mixing street samples in PBS pH 12. Importantly, the oxidation peak of 6-MAM (P1) enriches the EF of heroin at pH 12, allowing a selective discrimination of heroin in complex mixtures as the majority of critical compounds does not overlap with P1 in PBS pH 12 (**Fig. S1a** and **S1b** for cutting agents and illicit drugs, respectively). Therefore, this novel strategy based on two identification peaks assures a selective detection of heroin.

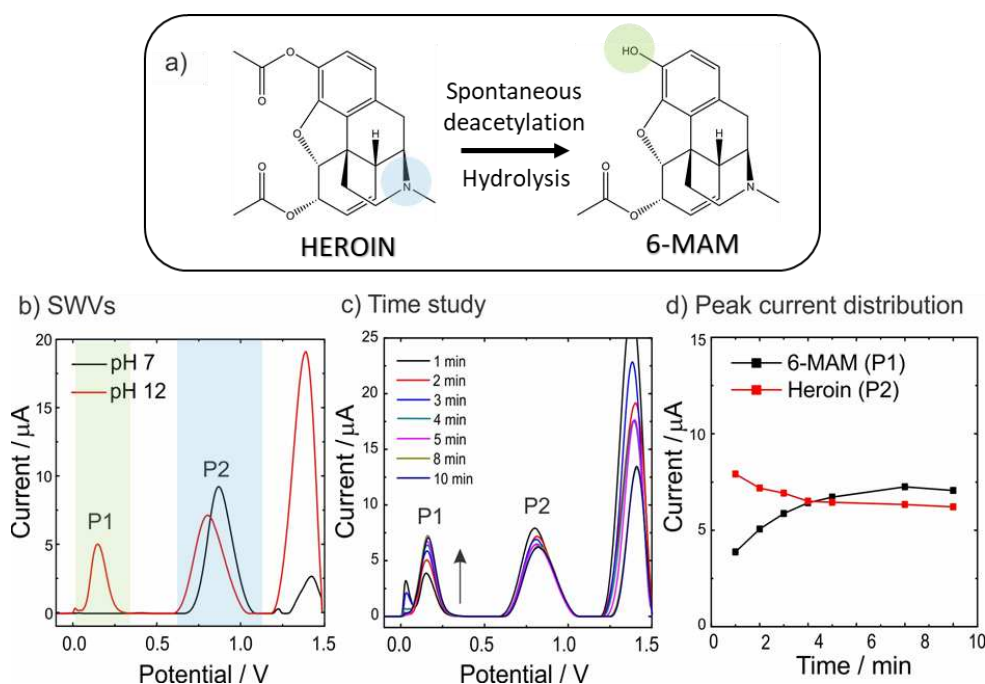


Fig. 1. a) Hydrolysis reaction of heroin and conversion to 6-MAM. Degradation study of 0.5 mM heroin at SPE by SWV: b) pH screening at PBS pH 7 and pH 12, c) time study (1 -10 min)

and d) peak current Vs. time. P1 corresponds to the oxidation of the phenolic group (light green area), and P2 corresponds to the oxidation of the tertiary amine (light blue area). The oxidation peak around 1.5 V corresponds to the background signal of PBS pH 12 at SPE. The oxidation peak at 0.05 V corresponds to the oxidation of remaining silver particles from the pseudo-reference electrode.

One of the most used adulterants in heroin street samples is paracetamol. However, its oxidation signal occurs at 0.1 V in PBS pH 12 which is similar to the oxidation peak of 6-MAM (P1). Therefore, when an equimolar mixture of heroin and paracetamol is analyzed by SWV at pH 12, a unique peak occurs, hindering the potential use of 6-MAM (P1) for qualitative analysis of heroin (**Fig. 2a**) [26]. Since this work aims to use the 6-MAM peak as a complementary indicator for heroin detection in street samples, we investigated an anodic pretreatment [29] to overcome this overlapping phenomenon.

3.2. Toward paracetamol and 6-MAM (heroin) discrimination

Preliminary investigations were performed for the optimization of the anodic pretreatment in heroin paracetamol mixtures toward an enhanced peak separation in PBS pH 12. First, SPE were pretreated at different potentials in a wide range (i.e. 0.8-1.5 V) for 1 min, and subsequently, SWV measurements of the mixtures were conducted (**Fig. S2**). It was observed that potentials above 1.5 V decreased the analytical performance of the graphite SPE suggesting a damage on the electrode's surface [30]. The results showed that p-SPE at imposed potentials in the region between 1.2 and 1.5 V increased the peak intensity of paracetamol and thus improved the discrimination of the two peaks, i.e. paracetamol and P1. Overall, 1.5 V and 60 s were selected as the optimal conditions in following assays (a compromise between the peak resolution and the time of analysis).

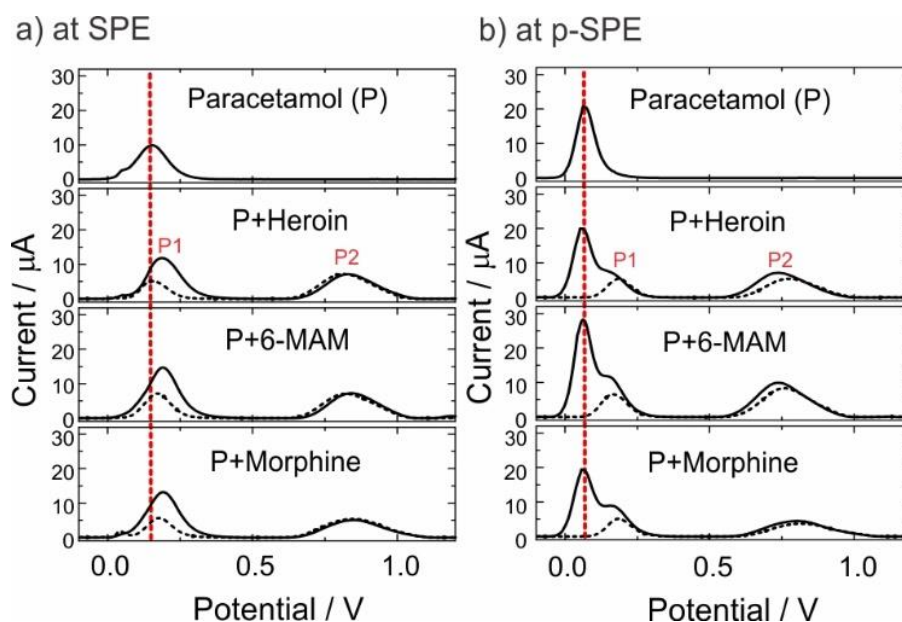


Fig. 2. SWVs of 0.5 mM paracetamol and corresponding 1:1 binary mixtures with heroin, 6-MAM and morphine in PBS pH 12 at: a) untreated SPE and b) p-SPE (previous treatment: 60s at 1.5 V). The dotted red (vertical) line indicates where the signal of paracetamol is located. The dashed SWVs indicates the EF of the additional pure compounds besides paracetamol.

Using the optimized conditions, the electrochemical behavior of paracetamol, heroin and 6-MAM was explored by CV to fully understand the electrochemical mechanism at p-SPE and compared it to untreated SPE (**Fig. S3**). **Fig. S3** depicts the non-reversible nature of the oxidation processes of 6-MAM and heroin on both electrodes (SPE and p-SPE). In contrast, paracetamol shows a quasi-reversible behavior on the SPE and an improved reversible behavior on the p-SPE. Thereafter, SWV was used to investigate paracetamol mixtures with heroin, 6-MAM and morphine at p-SPE. These compounds, which share a common phenolic group in their structure, were chosen to demonstrate the effect on the oxidation peak (P1) (**Fig. 1a**) over the paracetamol signal. All in all, **Fig. 2** displays the comparison between untreated SPE and p-SPE showing only one oxidation peak for the SPE and two overlapped oxidation peaks for the p-SPE at about 0.06 and 0.17 V, respectively. In the p-SPE, P1 shifts slightly towards positive values meanwhile the potential of paracetamol signal is more negative. This leads to a better peak-to-peak identification with a signal separation in mixture of about 90 ± 4 mV ($n = 5$). Overall, these findings show that p-SPE permit the separation between the oxidation peaks of 6-MAM and paracetamol.

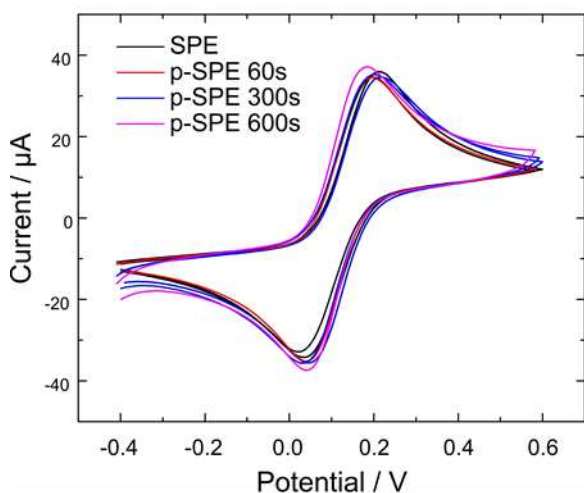
3.3. Characterization of the SPE surface after the anodic pretreatment

First, a morphological characterization was performed with SEM. **Fig. S4a** and **S4b** depict typical scanning electron micrographs, at 50k X magnifications, of the surface of SPE and p-SPE, respectively. Both images show aggregated flake-like graphite micro- and nanoparticles over the polymeric binder material which produces a rough surface with a webbed aspect. Moreover, **Fig. S4c** and **S4d** show a homogenous surface in both conditions at 10k X magnifications. The results are in agreement with conventional SPE based on graphite ink [31,32]. Therefore, the anodic pretreatment apparently does not modify the structure of the SPE surface. Indeed, Su et al. reported a disruption of the surface morphologies after the application of higher potentials (> 1.6 V) [30].

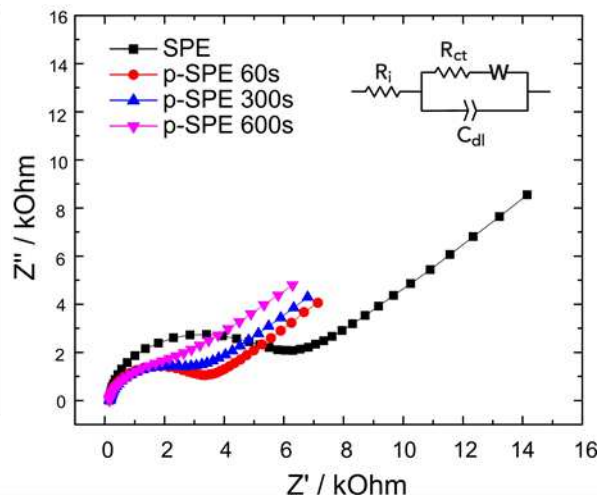
Second, an electrochemical characterization of the electron transfer capacity of p-SPE and SPE was carried out. Attention was turned on the electrochemical properties of the p-SPE by observing the redox behavior of a widely known redox probe, potassium ferricyanide. **Fig. 3a** displays the observed cyclic voltammograms of SPE and the p-SPE (pretreatment times of 60, 300, 600 s). The analysis of the voltammetric profiles for the electrochemical response of 2 mM $K_3[Fe(CN)_6]$ at the untreated SPE revealed a slow electron transfer rate exhibiting large peak-to-peak separation ($\Delta E_p=170$ mV). On the other hand, the p-SPE showed slightly lower values ($\Delta E_p=140$ mV) after 600 s of preanodization, thus showing an influence on the electron transfer rate.

Subsequently, EIS was also conducted to evaluate potential changes after the electrochemical pretreatment. **Fig. 3b** depicts the Nyquist plots of the untreated SPE and the p-SPE at different times in the presence of 2 mM $K_3[Fe(CN)_6]$. EIS responses were analyzed based on a Randles equivalent circuit (inset **Fig. 3b**). All the electrodes showed similar solution resistances (162 Ω , RSD = 1.8 %, n = 4), although the double-layer capacitance (C_{dl}) was lower at SPE and consecutively increased over pretreatment time ($C_{dl\ SPE} = 0.673$ μF ; $C_{dl\ p-SPE\ 60s} = 1.242$ μF ; $C_{dl\ p-SPE\ 300s} = 3.694$ μF ; $C_{dl\ p-SPE\ 600s} = 10.65$ μF). Interestingly, the activation of the SPE by the anodic pretreatment reduced the charge transfer resistance values to 2672, 2165 and 1525 Ω for the p-SPE at 60, 300 and 600 s, respectively, compared to the 5092 Ω of the SPE. Therefore, these observations revealed that the electrode surface was considerably affected with an enhancement of the kinetic rate.

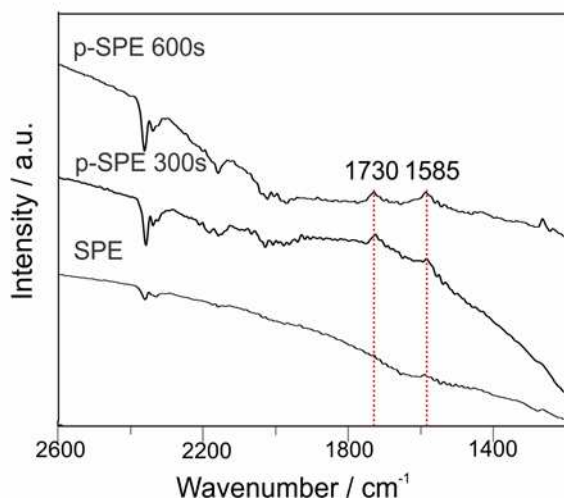
a) CV study



b) EIS study



c) FTIR



d) Raman

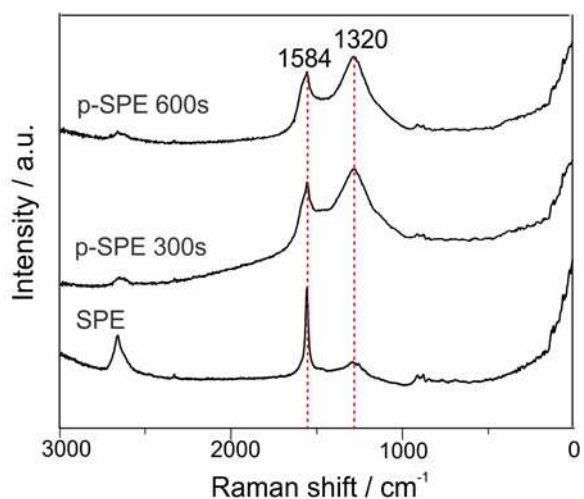


Fig. 3. Electrochemical characterization of the anodic pretreatment: a) CV measurements of SPE and p-SPE at 60, 300 and 600 s. CV was performed at 0.1 V s^{-1} . b) EIS measurements of SPE and p-SPE at 60, 300 and 600 s. EIS was performed under the following parameters: $E_{dc} = 0.2 \text{ V}$, $E_{ac} = 5 \text{ mV}$, frequencies = 0.1 Hz - 100 KHz . All electrochemical measurements were performed in PBS with $2 \text{ mM K}_3[\text{Fe}(\text{CN})_6]$ pH 7.4. Chemical characterization of the anodic pretreatment: c) FTIR spectra of p-SPE compared to SPE d) Raman spectra of p-SPE compared to SPE.

Finally, a chemical analysis of the SPE surface was evaluated by FTIR and Raman spectroscopy. The FTIR spectra (**Fig. 3c**) reported the formation of oxygen-containing groups (bands at 1730 and 1585 cm^{-1} , e.g., carbonyl groups) upon anodic pretreatment [30,33], exhibiting acid-base properties [34,35]. Similarly, anodic

pretreatments have shown the increment of the electrocatalytic activity toward other molecules [34,36]. The Raman spectra suggested the generation of more edge-plane graphite sites on the electrodes' preanodized surface (**Fig. 3d**). This can be seen in the increasing ratio between the E_{2g} band at 1584 cm^{-1} (basal plane) and the D band at 1320 cm^{-1} (edge plane) in the preanodized surfaces [33,34].

3.4. Analytical performance of p-SPE

The lower oxidation potential and the higher current response clearly indicate that p-SPE have excellent electrocatalytic activity toward paracetamol. Moreover, a considerable enhancement in the analytical performance toward heroin was noticed when applying the activation step in comparison to untreated SPE [26]. **Fig. S5a** and **S5b** display the plot corresponding to increasing concentration of heroin (P2) and the calibration curve, respectively, at p-SPE. The linear dependence between the SWV signal and heroin concentration was obtained in the range of 10-1000 μM , exhibiting a slope of $0.019\text{ }\mu\text{A }\mu\text{M}^{-1}$ and $5.2\text{ }\mu\text{M}$ LOD, and an excellent reproducibility at 0.5 mM, $\text{RSD}_{\text{heroin}} = 6.9\%$ ($n = 5$). Besides, the electrochemical profile of paracetamol-heroin mixture exhibited outstanding oxidation peak reproducibility ($\text{RSD}_{\text{heroin}} = 0.91\%$; $\text{RSD}_{\text{paracetamol}} = 6.2\%$; $\text{RSD}_{6\text{-MAM}} = 2.85\%$ at 0.5 mM, $n = 5$) (**Fig. S6**).

Finally, the p-SPE shelf-life was evaluated at room temperature and 4°C . The assay consisted of performing the anodic pretreatment at SPE and store it for 7 days. Hence, **Fig. S7** exhibited reproducible peak potentials ($\text{RSD}_{\text{RT-paracetamol}} = 8.30\%$; $\text{RSD}_{\text{RT-6-MAM}} = 4.29\%$; $\text{RSD}_{\text{RT-heroin}} = 1.15\%$; $\text{RSD}_{4^{\circ}\text{C-paracetamol}} = 5.30\%$; $\text{RSD}_{4^{\circ}\text{C-6-MAM}} = 2.24\%$; $\text{RSD}_{4^{\circ}\text{C-heroin}} = 1.03\%$ at 0.5 mM, $n = 5$). Overall, the electrochemical activation proved to be an excellent and highly-stable strategy to overcome the lack of selectivity of SPE without involving sophisticated materials, thus allowing a selective detection of heroin based on two oxidation peaks.

3.5. Electrochemical screening of heroin in binary mixtures at p-SPE

After proving the excellent capability of the p-SPE to separate the oxidation peaks of paracetamol and 6-MAM (from heroin hydrolysis), the following step was to screen the influence of common mixing agents and other illicit drugs on the EF of heroin at p-SPE. **Fig. 4** displays the EF of 1:1 (0.5 mM) binary mixtures (straight SWV) compared with pure compounds (dashed SWV). The results showed that some cutting agents (i.e. caffeine, griseofulvin, diazepam) do not have influence on the oxidation signal of

heroin (**Fig. 4a**). However, the identification of the oxidation peak of 6-MAM (P1) from heroin was of paramount necessity in presence of codeine, quinine and lidocaine for the selective discrimination of heroin in these binary mixtures (**Fig. 4a**). Particularly, quinine and lidocaine exhibit an oxidation signal that totally overlaps the heroin signal at 0.77 V, thus interfering with heroin identification based on P2. Besides, the anodic pretreatment also improved the determination of heroin in noscapine mixtures in comparison to untreated SPE (**Fig. S1a**), glimpsing a third peak corresponding to P2. Heroin can be combined with a wide variety of drug-of-abuse, including methamphetamine, cocaine, amphetamine and MDMA, mainly to enhance the pharmacologic effects [37]. Therefore, 1:1 (0.5 mM) binary mixtures of heroin with different illicit drugs were interrogated by SWV on p-SPE at PBS pH 12. **Fig. 4b** exhibited the screening of pure drugs (dashed SWV) and corresponding binary mixtures of heroin (straight SWV). Most of the oxidation peaks between illicit drugs overlapped due to the common structural amine group (secondary or tertiary amines). Therefore, simultaneous detection of heroin and the aforementioned illicit drugs remained a challenge if only P2 is used. In contrast, the oxidation of the phenolic group of 6-MAM successfully allowed the identification of heroin in all the binary mixtures (**Fig. 4b**), proving an innovative strategy for the selective heroin detection in illicit drugs mixtures.

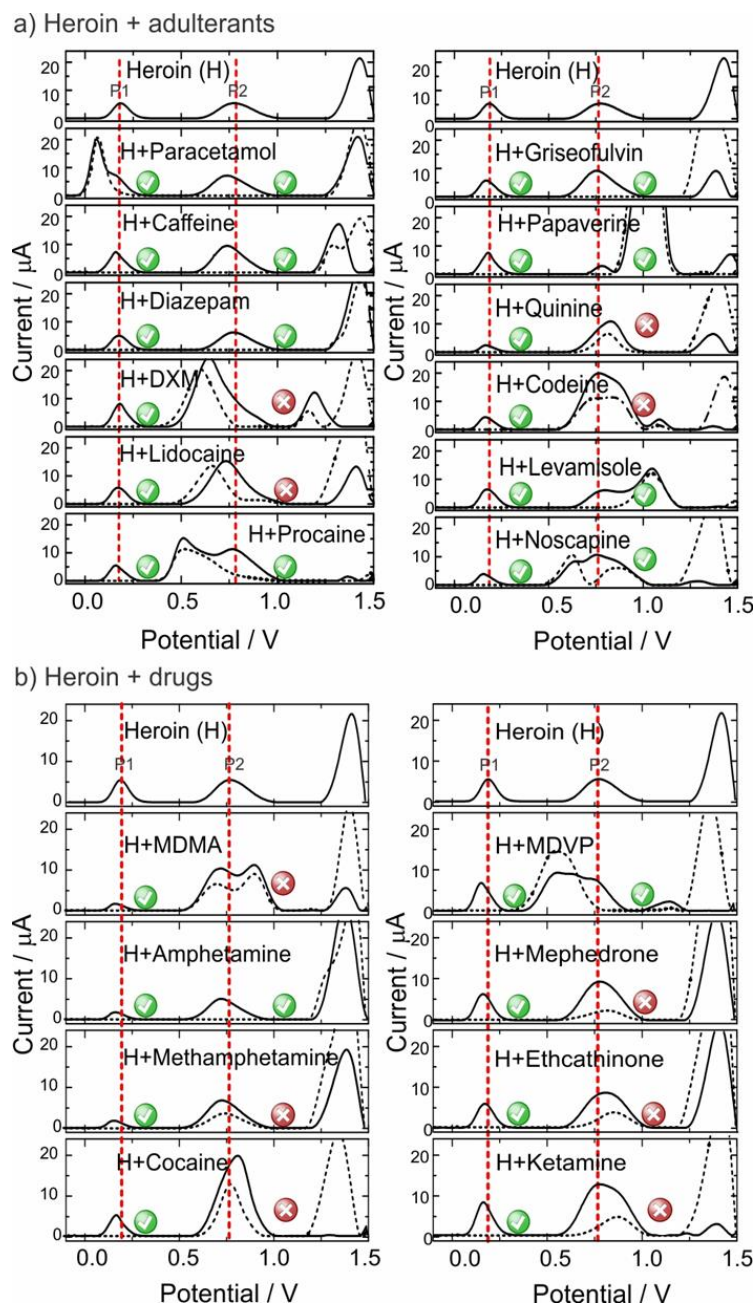


Fig. 4. SWVs of heroin on different 0.5 mM binary mixtures in PBS pH 12 at p-SPE: a) cutting agents, b) illicit drugs. The dotted red (vertical) line indicates the signal of 6-MAM (P1) and heroin (P2), respectively. The dashed SWVs indicates the EF of the pure adulterants or illicit drugs. A green tick indicates a correct identification of the target (6-MAM or heroin) while a red cross indicates a wrong one due to masking or overlapping phenomena.

3.6. Use of data analysis to enhance peak separation

Data analysis is currently used as a method to increase the signal obtained experimentally. A Matlab script was developed and integrated to increase the peak-

to-peak separation when overlapping signals are encountered, and thus enhance the compound identification in the SWVs output. Particularly, the aim of the script is to improve the discrimination of the oxidation peaks from paracetamol and the phenolic group of 6-MAM, thus facilitating the identification of heroin in street samples, even with low heroin amount. In this way, the shoulder that appears next to the oxidation peak of paracetamol (**Fig. S8a**) becomes a resolved peak (**Fig. S8b**). Moreover, the overlapped peaks on P2 region (e.g. noscapine and heroin mixture) (**Fig. S8a**) could be also discriminated, thus providing a full profiling of heroin seizures (**Fig. S8b**).

3.7. Electrochemical screening of synthetic mixtures at p-SPE

Before testing the method in street samples, an electrochemical profiling of complex mixtures was performed following compositions reported in literature (**Table S1** and **S2**). This step is essential to evaluate the limits of the approach to determine heroin in complex samples that include paracetamol (see composition in **Table S3**).

Fig. 5a displays the analysis by SWVs on p-SPE and **Fig. 5b** shows the output signal after employing the script. The script was able to separate the oxidation peak of 6-MAM (P1) from the paracetamol signal in all complex samples. Complex samples 3 and 4 were the most challenging since the concentration of heroin (i.e. 0.65 and 0.32 mM, respectively) and, particularly, 6-MAM (i.e. 0.09 mM) were extremely low. In these cases, the 6-MAM peak was displayed as a shoulder next to the paracetamol oxidation peak, still allowing the identification of heroin by P1 and P2. It is important to mention that the induction of the spontaneous deacetylation of heroin to 6-MAM by increasing the time on pH 12 should improve the peak resolution. Nevertheless, the detection of heroin is still possible through P2, which as a result of the pretreatment, is not overlapped with the signal of noscapine. Therefore, the integration of the script is essential to discriminate heroin in complex mixtures, thus avoiding false positives. In addition, the same samples were interrogated with an untreated SPE to demonstrate the need of using p-SPE for a reliable detection of heroin (**Fig. S9**). As expected, untreated SPE showed an overlapping signal for P1 with paracetamol as well as a masking effect in P2 with noscapine signal.

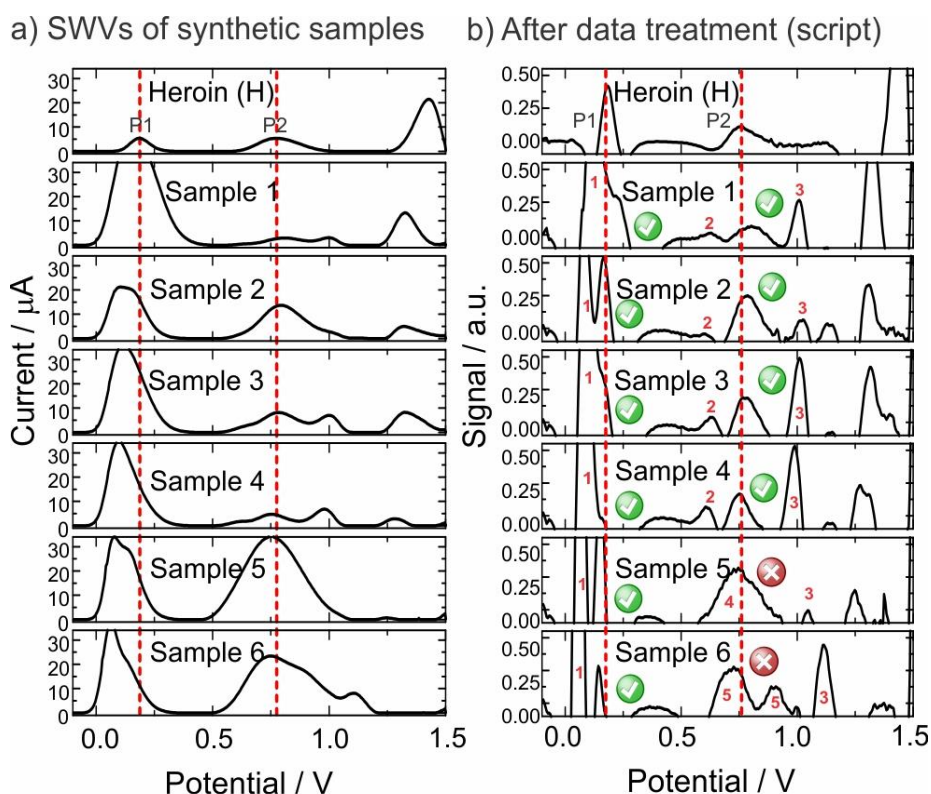


Fig. 5. Electrochemical fingerprints of complex samples in PBS pH 12 at p-SPE: a) raw SWVs; b) Output from the script. The composition of samples 1-6 is described in Table S3. The dotted red (vertical) line indicates the signal of 6-MAM (P1) and heroin (P2), respectively. Numbers 1-5 indicates the presence of paracetamol (1), noscapine (2), papaverine (3), lidocaine (4) and codeine (5). A green tick indicates a correct identification of the target (6-MAM or heroin) while a red cross indicates a wrong one due to masking or overlapping phenomena.

3.8. Street samples profiling

To validate the approach, street samples from a forensic laboratory (i.e. National Institute for Criminalistics and Criminology, NICC, Belgium) were tested by SWVs with p-SPE and analyzed with the tailored script. Besides, these samples were qualitative and quantitative analyzed using GC/MS and GC-FID. Accordingly, **Table S4** shows the corresponding composition of the 14 street samples studied. **Fig. 6** displays the output signal after applying the script.

Remarkably, the script allows for a profiling of the different compounds found in the confiscated samples within 30 seconds. In contrast, **Fig. S10** shows the SWVs of the confiscated samples without data treatment which does not allow the identification of all the compounds in the sample, proving the need of the script. The results showed an accuracy of 85% for P2 and 93% for P1 for heroin detection according to the

standard methods (**Table S4**). It is worthy to remark that heroin is still detected in Sample 5 (with <9% heroin) due to the discrimination between paracetamol and 6-MAM. A more complex sample is Sample 10 that combines heroin with another opioid with a similar structure, morphine. In this case, the similarities in their electrochemical profiles can hinder the detection of heroin when morphine is present. Hence, further studies are needed to assess the effect of morphine on heroin EF in p-SPE. New strategies will be studied to find out a potential method to discriminate both compounds (e.g., dual pH screening).

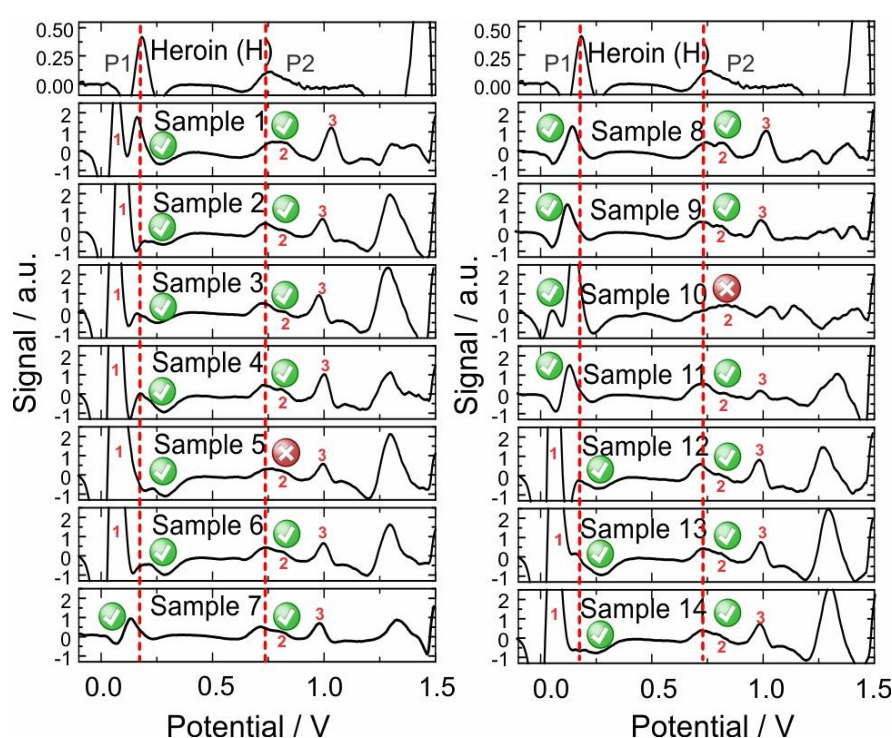


Fig. 6. Electrochemical fingerprints of street samples in PBS pH 12 at p-SPE after the script output. The composition of samples 1-14 is described in Table S4. The dotted red (vertical) line indicates the signal of 6-MAM (P1) and heroin (P2), respectively. Numbers 1-3 indicates the presence of paracetamol (1), noscapine (2) and papaverine (3). A green tick indicates a correct identification of the target (6-MAM or heroin) while a red cross indicates a wrong one due to masking or overlapping phenomena.

To prove the validity of the approach for the on-site screening of heroin, a comparison with current portable technologies for illicit drugs detection was performed. Despite of Raman spectroscopy being one of the most popular techniques, it is not used in this work due to the interfering fluorescence phenomena of colored heroin samples when

using low-power lasers [38]. High-power lasers could be used to avoid fluorescence, however, this type of lasers might produce a radiation damage in the organic substances which can lead to wrong interpretation of the results. Hence, street samples were interrogated with a portable ATR-FTIR device (**Fig. S11**). An analysis of several components was conducted where the spectroscopic method showed an accuracy of 40%. **Table S4** also depicts the comparison between current on-site techniques and the proposed method, demonstrating the enhanced performance for the EF method.

4. Conclusions

In conclusion, the voltammetric interrogation of street samples on p-SPE with integrated data analysis allows a selective detection of heroin based on its electrochemical profiling. This novel strategy uses two oxidation peaks: the one from heroin and the one from its degradation product in alkaline conditions, 6-MAM, to selectively determine heroin in complex mixtures. Remarkably, the pretreatment allows to differentiate 6-MAM in mixtures with the most used cutting agent (i.e. paracetamol), which was the main challenge for the electrochemical detection of heroin based on two-peak oxidation processes.

The characterization of the SPE surface suggested an enhancement of the electron transfer rate after the anodic pretreatment. This is explained by the potential introduction of oxygen-containing groups such as carbonyl groups and the increasing formation of edge-planes graphite sites on the surface. A deep study of heroin mixtures with cutting agents and common illicit drugs has successfully demonstrated outstanding sensor performance to discriminate heroin.

Finally, the integration of a script allows the successful profiling of the street samples, showing improved analytical performance than current portable spectroscopic methods. Overall, this new approach paves the way to a rapid, user-friendly and low-cost on-site detection of heroin in real scenarios by LEAs: (i) analysis of suspicious powders in the street; and (ii) rapid screening of cargos in border settings (e.g., airports, harbors).

CRedit authorship contribution statement

Noelia Felipe Montiel and Marc Parrilla: Conceptualization, methodology, electrochemical analysis, writing original draft. **Victoria Beltrán:** chemical

characterization of p-SPE and analysis of street samples by spectroscopic techniques.

Gert Nuyts: morphological characterization of p-SPE by SEM. **Filip Van Durme:** analysis of confiscated samples with standard methods. **Karolien De Wael:** Investigation, review and editing.

Declaration of Competing Interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version.

PDF file including: Tables: composition of samples; Figures: EF of mixing agents, complex samples and street samples at SPE, CV analysis, SEM characterization, anodic pretreatment optimization, reproducibility and stability studies, output signal from the script, and ATR-FTIR spectra of street samples.

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Graphical abstract

