**Electrochemical profiling and LC-MS characterization of synthetic cathinones: from methodology to detection in forensic samples**

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**Abstract**

The emergence of new psychoactive drugs in the market demands rapid and accurate tools for the on-site classification of illegal and legal compounds with similar structures. Herein, a novel method for the classification of synthetic cathinones (SC) is presented based on their electrochemical profile. First, the electrochemical profile of five common SC (i.e., mephedrone, ethcathinone, methylone, butylone and 4-chloro-alpha-pyrrolidinovalerophenone) is collected to build calibration curves using square wave voltammetry on graphite screen-printed electrodes (SPE). Second, the elucidation of the oxidation pathways, obtained by liquid chromatography-high resolution mass spectrometry, allows the pairing of the oxidation products to the SC electrochemical profile, providing a selective and robust classification. Additionally, the effect of common adulterants and illicit drugs on the electrochemical profile of the SC is explored. Interestingly, a cathodic pretreatment of the SPE allows the selective
detection of each SC in presence of electroactive adulterants. Finally, the
electrochemical approach is validated with gas-chromatography-mass spectrometry
by analyzing 26 confiscated samples from seizures and illegal webshops. Overall, the
electrochemical method exhibits a successful classification of SC including structural
derivatives, a crucial attribute in an ever-diversifying drug market.

Keywords
Electrochemical profile, synthetic cathinones, drug seizures, redox pathways, screen-
printed electrodes, forensic analysis

1. Introduction
Synthetic cathinones (SC) are a group of derivatives of the natural stimulant cathinone,
found in the Khat plant (Catha edulis, found in East Africa and the Arabian
peninsula).\textsuperscript{[1,2]} These synthetic compounds are classified as “new psychoactive
substances” (NPS), mimicking the effects of established illicit drugs, and typically not
controlled by legislation (often purposely designed to circumvent the law), earning
them the nickname “legal highs”. SC are strongly related to amphetamine-type
stimulants (ATS) in their structure and effects and, therefore, are seen as cheaper
alternatives for ATS.\textsuperscript{[2]} SC can be easily found online, where they are sold under
different names such as “research chemicals”, “plant food” and “bath salts” to bypass
controls. Due to this strong variation in appearance, SC can occur as powders,
crystals, pills, capsules, liquids and other forms.\textsuperscript{[3,4]} Usually, SC samples have higher
purity (especially mephedrone, MEP >95 %) compared to other stimulant drugs such
as 3,4-methylenedioxymethamphetamine (MDMA) and cocaine.\textsuperscript{[3,4]} However,
recently, samples with lower purity (40-69%) have also been encountered.\textsuperscript{[5,6]} SC can
also be mixed with other recreational drugs (e.g., MDMA, amphetamine) \textsuperscript{[3,4,7]} or
adulterants commonly used to cut the traditional drugs (e.g. caffeine, creatine,
lidocaine) (Table S1).\textsuperscript{[3,5,8]}

Due to their rapid rise in popularity, often unknown composition and largely unstudied
effects, these drugs are considered a potential danger to public health.\textsuperscript{[1,2]} Therefore,
many countries have started to amend existing legislation to control NPS, leading to
over 15000 seizures in Europe alone in 2017 (24% of all European NPS seizures).\textsuperscript{[9]}
However, the NPS market is highly dynamic, making it difficult for authorities to keep
up with the pace of appearance of new compounds.\cite{10} It is therefore important for law enforcement agencies to possess analytical tools to effectively monitor cargo, luggage and individual samples of these compounds in the field.

In drug analysis, the combination of gas or liquid chromatography (GC/LC) and mass spectrometry (MS) is regarded as the gold standard in lab settings due to its excellent sensitivity and selectivity. A wide range of GC/LC-MS methods has been reported for the identification and quantification of SC in powders, biological samples (e.g. blood, urine, hair) and wastewater.\cite{11-13} However, these laboratory techniques are not suitable for the use by law enforcement agencies in the field for fast screenings due to their high cost, low portability and lengthy analysis times.

Colorimetric tests offer high simplicity and low cost per analysis, making them the traditional choice for the on-site screening of suspicious samples.\cite{14} In the case of SC, the Marquis and Liebermann reagents are capable of detecting methylenedioxy substituents (e.g. methylone, MET) and methcathinone analogues (e.g. MEP) respectively, while the United Nations Office on Drugs and Crime (UNODC) recommends the Zimmermann reagent, which targets all $\beta$-amino ketones.\cite{15} However, these tests suffer from significant drawbacks such as a lack of specificity and are prone to misinterpretation.\cite{14} In an attempt to overcome these issues, Yen et al. developed a colorimetric sensor based on carbon dots functionalized paper for the sensitive and selective detection of 4-chloroethcathinone and analogues,\cite{16} while Luo et al. reported a multimodule split aptamer construct for the naked-eye detection of methylenedioxypyrovalerone (MDPV).\cite{17}

On-site alternative methods are based on the use of handheld spectroscopic techniques such as attenuated total reflectance Fourier transform-infrared (ATR-FTIR),\cite{18} near-infrared (NIR)\cite{19} and Raman spectroscopy.\cite{20} These methods are also commonly used by law enforcement agencies due to the rapid and non-destructive nature of the sampling method.\cite{21} However, these techniques still present some drawbacks. For example, FTIR is capable of providing structural information for the SC, although requiring high drug purity.\cite{22} Meanwhile, Raman spectroscopy might have interference from fluorescence in the case of colored samples. Besides, spectroscopic devices are expensive which limits the spread of multiple devices within law enforcement officers.
In the last years, electrochemical techniques have become increasingly appealing for the development of portable sensors as they combine rapidness with high selectivity and sensitivity.\textsuperscript{[23,24]} Especially in forensic science, electrochemical techniques have found their application in the detection of explosives,\textsuperscript{[25]} gunshot residues\textsuperscript{[26]} and illicit drugs.\textsuperscript{[27–29]} Particularly, Table S2 provides an overview of previous reports on the electrochemical detection of SC. Interestingly, Banks' group pioneered in the use of voltammetry for the detection of methcathinone analogues by oxidation\textsuperscript{[30]} and by reduction\textsuperscript{[31]} as well as mephedrone metabolites in biological samples using screen-printed electrodes (SPEs).\textsuperscript{[32]} However, (i) a deep study on the effect of adulterants commonly found in seizures to the voltammetric responses, (ii) approaches to overcome the interferences, (iii) strategies for the detection and classification of different SC structures, and (iv) further validation in real samples from seizures have been not addressed yet and are extremely relevant for the on-site testing.

![Figure 1. Classification of synthetic cathinones by oxidizable groups.](image)

Here we present electrochemical strategies for the rapid on-site detection of three classes of SC (Figure 1, Figure S1) in seized drug samples using graphite SPEs. Five representative compounds of SC were selected based on their relevance and divided into three classes according to their chemical structure: MEP and ethcathinone (ETC) are N-alkylated cathinones and represent Class I (SC-I), MET and butylone (BUT) are 3,4-methylenedioxy-N-alkylated cathinones and form Class II (SC-II), while 4-chloro-alpha-pyrrolidinovalerophenone (Cl-PVP) as an N-pyrrolidine cathinone represents Class III (SC-III). First, the voltammetric behaviour of these drugs is studied in various pH's and concentrations to identify their electrochemical profile. This is the particular electrochemical signal or pattern of a certain molecule in a specific analytical context.\textsuperscript{[33]} Second, the oxidative pathways are unravelled by liquid chromatography-high resolution mass spectrometry analysis of the oxidation products formed at
specific potentials. Subsequently, mixtures of SC and adulterants or illicit drugs are explored. Remarkably, the introduction of a cathodic pretreatment overcomes potential interference from adulterants. Ultimately, the proposed strategies are applied to real seized samples from forensic laboratories with data validated with standard methods, containing not only the SC discussed in this work but also structural derivatives to demonstrate the robustness of the approach, a crucial attribute in an ever diversifying drug market.

2. Materials and methods

2.1. Reagents and Samples.

Standards of mephedrone·HCl, ethcathinone·HCl, methylone·HCl, butylone·HCl, 4-Cl-alpha-PVP·HCl, cocaine·HCl, d,l-amphetamine·HCl, methamphetamine·HCl, MDMA·HCl, heroin·HCl were purchased from Lipomed, Switzerland. Paracetamol, lidocaine, benzocaine, phenacetin, procaine, caffeine standards and SC street samples were provided by the National Institute for Criminalistics and Criminology (NICC, Belgium) and the Netherlands Forensic Institute (NFI, the Netherlands). Analytical grade salts of potassium chloride, potassium phosphate and potassium hydroxide were purchased from Sigma-Aldrich (Overijse, Belgium). All solutions were prepared in 18.2 $\Omega$ cm$^{-1}$ doubly deionized water (Merck Millipore). The pH was measured using a pH-meter (914 pH/Conductometer, 2.914.0020, Metrohm, Switzerland).

2.2. Instrumentation and Apparatus.

Square wave voltammograms (SWV) were recorded using a MultiPalmSens4 (PalmSens, The Netherlands) with PSTrace/MultiTrace software. Disposable ItalSens screen-printed electrodes (SPE) (PalmSens, the Netherlands), containing a graphite working electrode ($\varnothing = 3$ mm), a carbon counter electrode, and a (pseudo) silver reference electrode were used for all measurements. The SWV parameters that were used: potential range of -0.1 to 1.5 V, frequency 10 Hz, 25 mV amplitude and 5 mV step potential. All the voltammograms are background corrected using the “moving average correction” (peak width = 1) tool in the PSTrace software.

Electrochemical tests were performed in 20 mM phosphate buffer solutions (PBS) with 100 mM KCl by applying 50 µL of the solution onto the SPE. Every test was performed
with a new SPE. Electrochemical pretreatment was carried out by applying -0.8 V during 300 s to the sample in PBS pH 12, before launching the SWV method in the same solution. During the analysis of the seized samples, a portable potentiostat (EmStat Blue potentiostat, PalmSens, The Netherlands) connected to a laptop or tablet with PSTouch application was used. Approximately 1 mg of the suspicious powder was dissolved in 3 mL of PBS pH 12 to a concentration of 0.3 mg mL\(^{-1}\), thoroughly mixed for 30 s, and subsequently, a drop is placed at the SPE surface for the analysis by SWV.

The composition of the street samples was previously analyzed in the forensic laboratory at NICC with standard methods in order to subsequently validate the electrochemical approach. The qualitative analysis of the street samples were performed using gas chromatography-mass spectrometry (GC-MS).\(^{[34]}\) The applied chromatographic methods are ISO17025 accredited and are continuously evaluated through participation to international quality control programmes (United Nations Office on Drugs and Crime – UNODC, and European Network of Forensic Science Institutes – ENFSI).

The liquid chromatography-mass spectrometry experiments were performed using liquid chromatography (LC) coupled to a quadrupole time-of-flight mass spectrometer (QTOF-MS) using electrospray ionization (ESI) in positive ionisation mode. The apparatus consisted of a 1290 Infinity LC (Agilent Technologies, Wilmington, DE, United States) connected to a 6530 Accurate-Mass QTOF-MS (Agilent Technologies) with a heated-ESI source (JetStream ESI). Chromatographic separation was performed on a Kinetex Biphyl column (150 × 2.1 mm, particle size 2.6 μm, and pore size 100 Å) (Phenomenex, Inc., USA), maintained at room temperature, and using a mobile phase composed of 0.04% of formic acid in ultrapure water (A) and acetonitrile/ultrapure water (80/20, v/v) with 0.04% formic acid (B), in gradient. The flow rate and the injection volume were set at 0.3 mL/min and 1 μL, respectively. The instrument was operated in the 2 GHz (extended dynamic range) mode, which provides a full width at half-maximum (FWHM) resolution of approximately 4700 at m/z 118 and 10 000 at m/z 922. Positive polarity ESI mode was used under the following specific conditions: gas temperature 300 °C; gas flow 8 L/min; nebulizer pressure 40 psi; sheath gas temperature 350 °C; sheath gas flow 11 L/min. Capillary and
fragmentor voltages were set to 4000 and 135 V, respectively. A reference LC/MS calibration standard for ESI-TOF was continuously sprayed into the ESI source of the QTOF-MS system. The reference LC/MS calibration standard for ESI-TOF based on acetonitrile (90%) and deionized water (10%) (Part number G1969-85001, provided by Agilent Technologies) consists of 5 mM purine, 100 mM ammonium trifluoroacetate, and 2.5 mM hexakis(1H, 1H, 3H-tetrafluoropropoxy)phosphazine. The ions selected for recalibrating the mass axis, ensuring the mass accuracy throughout the run was m/z 121.0508 and 922.0097 for positive mode. The QTOF-MS device was acquiring from m/z 50 to 1000 in MS mode. Data-dependent acquisition mode (auto-MS/MS) was applied using two different collision energies (10 and 20 eV) for the fragmentation of the selected parent ions. The maximum number of precursors per MS cycle was set to 4 with minimal abundance of 2500 counts. In addition, precursor ions were excluded after every spectrum and released after 0.2 min.

3. Results and discussion

3.1. Electrochemical performance of SC on SPE.

First, solutions of MEP, ETC, MET, BUT and Cl-PVP were interrogated under SWV on SPE at different pH's to investigate their characteristic electrochemical profile. Figure 2 displays the pH screening for each SC at 0.5 mM concentration. The electrochemical profile of MEP and ETC (categorized as SC-I, Figure 1) show a redox peak suggesting the oxidation of their secondary amine moieties from pH 7 to pH 12 (Figure 2a and 2b, respectively). MET and BUT profiles (categorized as SC-II, Figure 1) exhibit two redox peaks suggesting the oxidation of the secondary amine group (P1) and the oxidation of the methylenedioxy group (P2) from pH 7 to pH 12 (Figure 2c and 2d, respectively). Lastly, Cl-PVP (categorized as SC-III, Figure 1) shows a different behavior in its electrochemical profile suggesting an oxidation of its pyrrolidine moiety at P1 from pH 4 to pH 12, as well as a subsequent oxidation of one of its oxidation products P2 from pH 7 to pH 12 (Figure 2e). The corresponding oxidation peaks of the aforementioned SC are confirmed by LC/ESI-MS studies in the following section. Besides, Figure 2f summarizes the peak potential shift according to the pH variation, moving towards lower potentials when the SWV analysis is performed in more alkaline pH's, suggesting the involvement of protons in the oxidation process. The peak potential ($E_p$) of MEP and ETC follow the linear relationship $E_p$ (V) = -0.032 pH + 1.29
and $E_p (V) = -0.034 \text{pH} + 1.34$, respectively, showing half of Nernstian slope (i.e. 0.059 V pH$^{-1}$ at 298 K), which suggest the transfer of a proton and two electrons in the electrochemical oxidation process. The $E_p$ of MET and BUT show a relationship for P1 of $E_p (V) = -0.055 \text{pH} + 1.54$ and $E_p (V) = -0.058 \text{pH} + 1.54$, respectively, suggesting the transfer of equal amount of protons and electrons ($2e^- / 2H^+$). Regarding P2 of MET and BUT, the relationship follows $E_p (V) = -0.053 \text{pH} + 1.81$ and $E_p (V) = -0.053 \text{pH} + 1.82$, respectively, the same oxidation behavior as for P1. The $E_p$ of Cl-PVP exhibit a linear relationship $E_p (V) = -0.031 \text{pH} + 0.96$ for P1 from pH 6 to pH 12, indicating the transfer of a proton and two electrons in the process. Concerning the optimal parameters for the analysis of SC, pH 12 was chosen as the optimal pH as it exhibits higher current, and lower oxidation potentials, making it easier to distinguish them from the background current which increases rapidly at high potentials (+ 1 V).

The analytical performance of the SPE was evaluated at pH 12 by varying the concentrations of the SC from 10 to 1000 µM. Figure S2a and Figure S2b show the SW voltammograms and corresponding calibration curves for five SC, respectively. Further details are described in the supplementary material. Besides, Figure S3 shows excellent intraday reproducibility for the peak current ($I_p$) using different SPE in pH 12 for MEP (RSD= 2.2%, at 500 µM, N=4), ETC (RSD= 4.6%, at 500 µM, N=4), P1 of MET (RSD= 11.8%, at 500 µM, N=4), P1 of BUT (RSD= 6.2%, at 500 µM, N=4), and P1 of Cl-PVP (RSD= 5.1%, at 500 µM, N=4). Finally, a stability study of the SC showed high stability of SC in pH 12 over time in the alkaline solution (Figure S4).
Figure 2. Electrochemical screening of 0.5 mM SC in different pH at SPE by SWV: a) MEP (pH 7 – 12), b) ETC (pH 7 – 12), c) MET (pH 7 – 12), d) BUT (pH 7 – 12), e) 4-Cl-alpha-PVP (pH 4 – 12), and f) peak potential distribution of each synthetic cathinone at different pH’s.

3.2. Elucidation of the SC oxidation pathway.
Linking the observed electrochemical responses to the corresponding redox processes, and thereby unravelling the oxidation mechanism occurring on the SPE, provides valuable insights in the development of selective detection strategies. To the best of our knowledge, an analysis of the oxidation products of SC formed during a voltammetric scan at pH 7 and pH 12 (commonly used pH for electrochemical detection) has not yet been reported.

**Figure 3.** LC/ESI-MS study. Total ion chromatograms of a) 20 ng µL⁻¹ solutions of mephedrone (MEP) (red), nor-mephedrone (green), electrolyzed MEP sample in PBS pH 7/1.10V (blue) and PBS pH 12/0.96V (black); b) 10 ng µL⁻¹ standard of methylone (MET) (red) and 20 ng µL⁻¹ electrolyzed MET samples in PBS pH 7/1.10V (blue), pH 7/1.32V (green) and PBS pH 12/0.96V (black), pH12/1.27V (orange); c) 20 ng µL⁻¹ standard of Cl-PVP (red) and 20 ng µL⁻¹ Cl-PVP electrolyzed samples in PBS pH 7/0.73V (blue), pH7/1.10V (green) and in PBS pH 12/0.59V (black), pH12/0.96V (orange).
The analysis at both pH’s can reveal the influence of pH over the products formed by electrochemical oxidation. First, MEP solutions (200 µM) were prepared in both pH’s, and electrolyzed at potentials coinciding with the single oxidation peaks observed for MEP: 1.10 V/pH 7 and 0.96 V/pH 12. The chromatograms of the electrolyzed samples are compared to a 20 ng µL⁻¹ standard in Figure 3a. Table S3 provides an overview of the identified oxidation products, their corresponding structures and additional information. One main oxidation product M1 (m/z 164.1068, C₁₀H₁₃NO) was formed during the electrolysis in both pH 7 and 12, eluting at 5.48 min shortly before the remaining non-oxidized mephedrone at 5.79 min (m/z 178.1226, C₁₁H₁₅NO). Based on this information, it is expected that M1 is nor-mephedrone, the demethylated analogue of MEP and one of its main phase-1 metabolites. This was confirmed by comparing the elution time, MS and MS/MS spectrum (Figure S5) of product M1 to a standard of nor-mephedrone (m/z 164.1063, C₁₀H₁₃NO). This product is formed after oxidation of the secondary amine and subsequent hydrolysis of the imine intermediate (Figure 4a), which is analogous to the mechanism previously reported for methamphetamine. Additionally, a minor product M2 (m/z 150.0910, C₉H₁₁NO) was identified in both electrolysis samples, eluting at 6.73 min. In previous studies on the degradation of MEP in alkaline environment, this m/z-value was attributed to N,4-dimethylbenzamide, one of the observed degradation products. As a control experiment, a solution of MEP in 1 M NaOH analyzed after 30 min. Figure S6 shows a product with a corresponding m/z-value formed in the degradation sample with the same elution time, and thus showing that M2 most likely is N,4-dimethylbenzamide. The fact that M2 is also observed in the electrolysis sample in pH 7 indicates that the electrochemical oxidation also leads to the formation of a second imine, which subsequently reacts with the aqueous solution to form the proposed product (Figure 4a).

Next, MET solutions (200 µM) were electrolyzed at potentials coinciding with their two redox signals in pH 7 (1.10 V and 1.32 V) and pH 12 (0.96 V and 1.27 V). The chromatograms of the electrolysis samples are compared to a 10 ng µL⁻¹ standard of MET in Figure 3b. An overview of the observed products is provided in Table S4. In both of the samples electrolyzed at the first peak potentials, one main product T1 (m/z 194.0812, C₁₀H₁₁NO₃) elutes at 3.75 min, shortly before the remaining MET (m/z 208.0981, C₁₁H₁₅NO₃) at 3.99 min. After comparing the [M+H]⁺ ion and fragmentation
pattern (Figure S7) with the relevant literature, T1 can be linked to normethylone, which is, analogous to MEP, the result of a demethylation reaction after oxidation of the secondary amine.\textsuperscript{[35,38]} The presence of minor product T2 (m/z 180.0651, C\textsubscript{9}H\textsubscript{9}NO\textsubscript{3}), which elutes at 4.75 min, is also analogous to the MEP observations, as this is the corresponding benzamide formed through the same mechanism proposed in Figure 4a. Therefore, it can be concluded that the first redox signal observed for MET is caused by the oxidation of the secondary amine.

Previous studies on the electrochemical behavior of MDMA derivatives have attributed one of the main redox signals observed to the oxidation of the aromatic nucleus.\textsuperscript{[36,39]} Furthermore, it has been suggested that the resulting radical cation undergoes dimerization and is subsequently oxidized again.\textsuperscript{[36]} However, some of the previously mentioned studies also demonstrated how the presence of electron-donating or electron-withdrawing substituents influences the stability of the radical cation formed, and consequently also affects the oxidation potential.\textsuperscript{[36,39]} Therefore, it is expected that the electron-withdrawing β-keto moiety in MET will shift this signal to higher potentials and could possibly also affect the reaction mechanism. In the LC/ESI-MS analysis of the samples electrolyzed at potentials coinciding with the second redox signal (pH 7: 1.32 V, pH 12: 1.27 V), one additional product was identified (Figure 3b). T3 (m/z 196.0966, C\textsubscript{10}H\textsubscript{13}NO\textsubscript{3}) elutes at 2.16 min and after comparing its fragmentation with that of MET and T1 (Figure S7), it can be concluded that the secondary amine is still present in the structure and it is therefore proposed that this product is 3,4-dihydroxymethcathinone (Table S4), the result of O-demethylation of MET. No indications of dimerization were found in this analysis. The complete oxidation mechanism for MET is depicted in Figure 4b.

Lastly, Cl-PVP solutions (200 µM) were electrolyzed in pH 7 and 12. For the former, a potential in between the two features of the redox signal (0.73 V) and one after (1.10 V) were selected. For the latter, one potential on the single peak (0.59 V) and one behind (0.96 V) were chosen as it is expected that the signal is the result of two peaks overlapping. The chromatograms of these four electrolysis samples are compared to a 20 ng µL\textsuperscript{-1} standard in Figure 3c. A total of four oxidation products was detected in various amounts, depending on the electrolysis conditions. Table S5 contains an overview of the formed products with their corresponding information, Figure S8
compares the intensities of each product in the different conditions and Figure S9 displays the MS/MS spectra of all products. Due to the structural similarities between electrochemical oxidation products of drugs and their metabolites, the literature on LC-MS analyses of pyrrolidinophenone metabolites is used as a reference. Product P1 (m/z 212.0832, C_{11}H_{14}ClNO) results from the transformation of the pyrrolidine ring into a primary amine, likely via a ring-opened intermediate. This conversion requires high potentials since it is only present in large amounts at the higher potentials in both pH 7 and 12 (Figure S8). P2 (m/z 264.1143, C_{15}H_{18}ClNO) is the most important product formed for both pH's at lower potentials and is the result of electron abstraction from the nitrogen lone pair and subsequent formation of a double bond. Due to the observation of the same fragments that were attributed to the valerophenone structure for PVP and P1 (m/z 195.0553, m/z 138.9938 and m/z 125.0124), it is proposed that the double bond is formed in the pyrrolidine ring (although the exact location is uncertain). At higher potentials, although less prominent, P2 is still an important product. P3 (m/z 282.1244, C_{15}H_{20}ClNO_2) is the result of hydroxylation of the pyrrolidine ring, with subsequent oxidation of this hydroxy-group resulting in product P4 (m/z 280.1089, C_{15}H_{18}ClNO_2). Similarly to P2, these reactions are proposed to have occurred in the pyrrolidine ring due to the presence of the same previously observed fragments attributed to the valerophenone core of Cl-PVP. The formation of P3 is enhanced by alkaline environment due to the abundance of hydroxyl ions, while the subsequent oxidation to form P4 mainly takes place at the higher potentials (pH 7: 1.10 V, pH 12: 0.96 V). Based on these findings, the mechanism depicted in Figure 4c is proposed. The oxidation of Cl-PVP at the electrode surface forms an intermediate after the exchange of two electrons and one proton, yielding the first oxidation peak. This is followed by a chemical step, e.g. hydroxylation (P3) or double bond formation (P2). When the potential is further increased, additional electrode reactions occur to form P1 and P4, resulting in a second oxidation peak. In pH 12, the two contributions form one broader peak.
Figure 4. Proposed oxidation mechanism of three main classes of SC. a) mephedrone; b) methylone, and c) Cl-PVP. In the latter case, the black arrows represent redox processes mainly taking place at the lower oxidation potential (peak 1, pH 7/0.73 V, pH 12/0.59 V). The red arrows represent the redox processes taking place at more positive potential (peak 2 in pH 7/1.10 V, pH 12/0.96 V).

3.3. Electrochemical screening of adulterated SC.

SC can be adulterated to enhance the psychoactive response, to avoid undesired effects, and to increase drug traffickers’ profits while maintaining the drug’s weight.
These adulterants are often electroactive, which could hinder the electrochemical detection of SC.\textsuperscript{[42]} Therefore, it is essential to evaluate the electrochemical profile of binary mixtures of SC and adulterants to properly assess the presence of SC in real samples.

First, binary mixtures between SC and adulterants commonly found in seizures were analyzed (Table S1). Figure S10 displays the electrochemical profile of binary mixtures (equimolar 0.5 mM) between SC and adulterants (i.e., paracetamol, caffeine, procaine, lidocaine, benzocaine and phenacetin) in pH 12. Unfortunately, procaine, phenacetin and benzocaine exhibited a suppression on the oxidation peak signal of the SC as well as a peak potential shift, thus hindering the selective detection of SC. Particularly, benzocaine showed the strongest effect over SC-I (MEP Figure S10a, ETC Figure S10b) and SC-II (MET Figure S10c and BUT Figure S10d) SC. Besides, SC-III (Cl-PVP Figure S10e) presented overlap signal for lidocaine and the aforementioned adulterants.

Electrochemical pretreatments have recently presented as a rapid solution to overcome the interferences from cutting agents (e.g., paracetamol, levamisole, etc.).\textsuperscript{[29,43]} In this work, a cathodic pretreatment (CP) at pH 12 with the sample was introduced following a reported protocol.\textsuperscript{[28,44]} The protocol overcomes the suppression effect from cutting agents such as benzocaine on the electrochemical profile of illicit drugs, thus unravelling the characteristic profile. Figure S11 shows the effect of the CP (i.e., apply -0.8 V) through time (from 10-600 s). Interestingly, the current intensity of Cl-PVP and lidocaine were dramatically enhanced upon increasing time of the CP. In contrast, MEP and BUT did not show any clear pattern upon increasing CP times. Hence, 300 s was fixed as the CP time for further experiments as it produces a narrower peak for benzocaine, one of the main problematic adulterants. A reproducibility study was also performed using the optimal CP pH12 strategy (Figure S12): (a) MEP I_p=3.0±0.1 μA (RSD= 4.6%, N=4) at E_p=0.85 V; (b) ETC I_p=4.8±0.1 μA (RSD= 2.3%, N=4) at E_p=0.88 V; (c) MET I_p=0.3±0.0 μA (RSD= 12.3%, N=4) at E_p=0.80 V; (d) BUT I_p=2.8±0.3 μA (RSD= 12.0%, N=4) at E_p=0.81 V; and (e) Cl-PVP I_p=58.4±2.3 μA (RSD= 3.9%, N=4) at E_p=0.63 V.
Figure 5. SWV of binary mixtures (equimolar concentrations, 0.5 mM) with common adulterants employing a cathodic pretreatment in 20 mM PBS 100 mM KCl pH 12 at SPE: a) mephedrone (M), b) ethcathinone (E), c) methylone (My), d) butylone (B), and e) 4-Cl-alpha-PVP (P). The dotted SWVs display the electrochemical profile of the pure compounds. The dashed line indicates where the oxidation peak signal of SC is located. Par=paracetamol; Caf=caffeine; Pro=procaine; Lid=lidocaine; Ben=benzocaine; Phe=phenacetin.

Figure 5 displays the electrochemical profile of equimolar binary mixtures (0.5 mM) of SC and adulterants after applying CP conditions. Interestingly, the oxidation peaks corresponding to SC were positioned at the same peak potential as the pure compounds, thus allowing for a selective determination of the SC in the presence of conflict adulterants. However, the CP impedes the detection of SC when lidocaine is found in the mixture because of overlapping signals. Moreover, the Cl-PVP exhibited high current allowing to discriminate even in the presence of adulterants. Overall, the CP allows to determine different classes of SC in the presence of the majority of adulterants encountered in street samples. When lidocaine is present in the sample, a quick confirmatory test in pH 12 (without CP) will uncover the characteristic oxidation peak for SC-I and -II. Concerning SC-III, a confirmatory test at pH 7 can unravel a characteristic electrochemical profile for Cl-PVP exhibiting a second oxidation peak at 0.91 V (Figure S13).

3.4. Electrochemical screening of SC with other illicit drugs.

SC can be mixed with other illicit drugs to enhance its psychotropic effects.[4,7] For this reason, the electrochemical analysis of binary mixtures (equimolar 0.5 mM) of SC with common illicit drugs was evaluated in pH 12 at SPE (Figure S14) and using CP
(Figure 6). First, mixtures of multiple SC were evaluated under CP to demonstrate the ability of the electrochemical profile to discriminate between the proposed classes of SC. Interestingly, SC-I (e.g., MEP or ETC), which exhibit a single oxidation peak (0.86 V), overlap with P1 oxidation peak (0.82 V) of SC-II (e.g., MET and BUT). However, P2 (1.22 V) of SC-II allows the discrimination between both classes. SC-III shows an oxidation peak at 0.62 V, which is clearly out of the potential range of the other classes of SC. Overall, the electrochemical profile allows for the selective detection of SC at SPE in pH 12 (Figure S14a) and with the CP pH 12 (Figure 6a). Second, MEP, BUT and Cl-PVP as representatives of the SC classes were mixed with common illicit drugs (i.e., cocaine, MDMA, heroin, amphetamine and methamphetamine) and electrochemically analyzed in pH 12 (Figure S14) and with CP pH12 (Figure 6). Figure 6b shows difficulties to discriminate SC-I (e.g. MEP) over common illicit drugs due to the overlap oxidation peak from the structurally similar amine group. Figure 6c exhibits the same issue for SC-II (e.g., BUT) than for MEP in the P1 region. However, P2 allows to distinguish among heroin, cocaine and methamphetamine. Figure 6d displays a successful determination of SC-III (e.g., Cl-PVP) in mixtures because the P1 window of Cl-PVP is out of the common potential zone of oxidation peaks of other illicit drugs. It is worth mentioning that amphetamine does not exhibit any electroactivity in the potential window of the graphite SPE. In contrast, MDMA exhibits issues for the selective detection of SC-I and SC-II because of the similar structure. Overall, similar results were obtained for both conditions (with and without CP). The main difference among the conditions belongs to the detection of Cl-PVP and heroin using CP in which the intensity of Cl-PVP increases and allows for its selective detection.

The use of the electrochemical profile in pH 12 or CP in pH 12 has proven to be a reliable method for the electrochemical profiling of SC in binary mixtures with other electroactive molecules. Importantly, when potential adulterated samples are found, CP should be employed to overcome suppressing and peak potential shifts, thus increasing the specificity of the method and avoiding false negative. Concerning the discrimination of SC among illicit drugs, the electrochemical profile might indicate the presence of another illicit drug encountered in the cargo which overlaps of the SC, thus generating a false positive. However, the confiscation of the seizure would not represent a problem for the law enforcement agencies as the overlapping molecules
(i.e. cocaine, MDMA, heroin and methamphetamine) are also declared illegal by the authorities.

![SWV analysis of binary mixtures](image)

**Figure 6.** SWV analysis of binary mixtures (equimolar concentrations, 0.5 mM) with illicit drugs using a cathodic pretreatment in 20 mM PBS 100 mM KCl pH 12 at SPE: a) SC, b) mephedrone, c) Butylone, and d) 4-Cl-alpha-PVP. The dashed SWVs display the electrochemical profile of pure compounds. The dotted line indicates the oxidation peak potential of SC. Amp=amphetamine; Coc=cocaine; Her=heroin; Meth=methamphetamine.

3.5. Detection and validation of the electrochemical method in seized samples.

The rapid and accurate determination of licit and illicit drugs in the field is essential during regular decision-making processes of law-enforcement officers. Currently, handheld devices are the optimal choice in comparison to presumptive color tests as they exhibit higher versatility and specificity.\[^{45}\] Portable Raman spectroscopy has proven an accurate method to detect controlled substances\[^{46}\] as well as a wide variety of drugs.\[^{47}\] Recently, the combination of NIR-based devices with chemometric tools is offering a reliable solution for the decentralization of the forensic analysis of illicit drugs.\[^{48,49}\] Moreover, electrochemical devices are becoming a solution as they offer excellent sensitivity, miniaturization and affordable analysis.\[^{45}\]

The applicability of the electrochemical profile method to detect SC in seized samples was compared with a reference standard method (i.e., GC-MS\[^{34}\]) using seized samples provided by forensic institutes (i.e., NICC and NFI). During the analysis of the seized samples, a portable potentiostat connected to a laptop or tablet was used.
Approximately 1 mg of the suspicious powder was dissolved in 3 mL of PBS pH 12 to a concentration of 0.3 mg mL\(^{-1}\), thoroughly mixed for 30 s, and subsequently, a drop is placed at the SPE surface for the analysis by SWV.

Street samples of SC were previously analyzed by GC-MS at the forensic laboratory, and subsequently provided for the validation with the electrochemical analysis. SC samples were obtained from confiscated street samples and illegal webshops. The nature of the samples were fine powders, crystal powders and pills. Powder was directly used for preparing the samples, while pills were first crushed and then dissolved in the buffer. Concerning the electrochemical profiling, both strategies at pH 12 (i.e., with and without CP) were successfully tested. Figure 7a shows the electrochemical profile of 10 confiscated samples obtained during SWV interrogation in pH 12 at bare SPE. As a result, nine of the samples displayed characteristic peaks for the three classes of SC (marked in red for SC-I, purple for SC-II, and blue for SC-III, see detailed information of the structures in Figure S1). For the purpose of comparison, the dotted SW voltammogram of each class standard (i.e., MEP, BUT and CI-PVP) is added. The only unclassified sample was Cs10, which was identified as cathinone itself and does not contain any electroactive group in the potential window of graphite SPE. Hence, cathinone is categorized as Class 0 (Figure S1). In order to prove the reliability of the CP, 16 different confiscated samples from seizures (Figure 7b) and webshop products (Figure 7c) were analyzed under CP pH 12. Similar to Figure 7a, SC street samples are successfully categorized into the three main classes indicated with a colored dashed line at the peak potential of the representative SC (displayed in a dotted SWVs). Table S6 shows the compounds identified by the GC-MS analysis and the comparison with the electrochemical profile method, showing a positive detection of each class of SC. Besides, high reproducibility was obtained showing negligible effect of the street sample compositions on the approach (Figure S15). Hence, these findings indicate the applicability of the electrochemical method to detect the main classes of SC in field tests. Only, Cs15 corresponding to 2-methylaminoindane (2-MAI) presents a false positive result as a shoulder on its electrochemical profile at the peak potential of SC-I is shown. Nevertheless, 2-MAI is registered as an illicit drug, consequently it might not represent an issue for customs officers during the screening test. Finally, confiscated samples from SC-III were also tested in pH 7 as a confirmatory test to show the characteristic double oxidation peak
Overall, a selective method to determine illicit drugs, particularly SC, over common adulterants has been explored. The electrochemical profile approach shows promise for integration in a miniaturized device for rapid analysis in the field as a qualitative detection and classification of synthetic cathinones in cargos at border customs.

**Figure 7.** Electrochemical profiles of real samples at SPE in PBS pH 12: a) confiscated samples at SPE (Cs1-Cs10); b) website samples analyzed at CP SPE (Ws1-Ws9); c) confiscated samples analyzed at CP SPE (Cs11-Cs17). The profile in dotted line indicates where the signal of standard SC is located. Colored dotted line shows the peak potential of the standard SC, red-SC I, purple-SC II and blue- SC III. Table S6 contains a list of the identified compounds in the real samples by GC-MS.

4. Conclusions
We have established, for the first time, the electrochemical profiling of several classes of SC on unmodified SPEs employing SWV measurements in confiscated samples from forensic laboratories. The electrochemical profile allows to categorize SC compounds into three main classes based on their oxidation mechanism (SC-I based on oxidation of secondary amine; SC-II on secondary amine and aromatic nucleus; SC-III on pyrrolidine moiety). Moreover, we pioneered in unravelling the oxidation pathways of three SC representatives (i.e., MEP, MET and Cl-PVP) on unmodified SPE. The oxidation pathway provides the understanding of the electrochemical signal upon SWV interrogation, thus showing a trustworthy classification of SC. Furthermore, the electrochemical profile of five different SC were studied with mixtures of regularly found adulterants and illicit drugs in seized samples. Interestingly, a cathodic pretreatment was successfully applied to overcome suppression and peak shifts of SC in the presence of benzocaine, phenacetin and procaine. Lastly, the electrochemical method was validated with confiscated samples previously analyzed with GC-MS by forensic laboratories. The characteristic electrochemical profile of each class of SC yielded a successful categorization of the confiscated samples with a rapid and easy-to-use sampling process. Overall, forensic electrochemistry proved to be a reliable method for the detection of illicit drugs among adulterants, and particularly for the classification of SC. This new system will provide useful information to identify new designer drugs, and ultimately, to assist law enforcement agencies in preventing NPS from reaching the market.

Supplementary Material

Supplementary data to this article can be found online at doi: PDF file including: Experimental details; Tables: state-of-the-art, composition of samples, LC-MS products; Figures : chemical structure of SC, reproducibility and stability studies, MS data, CP studies, electrochemical profiles of mixing agents at pH 12, output signal from script.

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Notes

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