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ABSTRACT: Herein, a straightforward electrochemical approach for the determination of ketamine in street samples and seizures is presented by employing screen-printed electrodes (SPE). Square wave voltammetry (SWV) is used to study the electrochemical behavior of the illicit drug, thus profiling the different oxidation states of the substance at different pHs. Besides, the oxidation pathway of ketamine on SPE is investigated for the first time with liquid chromatography-high resolution mass spectrometry. Under the optimized conditions, the calibration curve of ketamine at buffer solution (pH 12) exhibits a sensitivity of 8.2 μA μM^{-1}, a linear relationship between 50-2500 μM with excellent reproducibility (RSD= 2.2%, at 500 μM, n=7), and a limit of detection (LOD) of 11.7 μM. Subsequently, binary mixtures of ketamine with adulterants and illicit drugs are analyzed with SWV to investigate the electrochemical fingerprint. Moreover, the profile overlapping between different substances is addressed by the introduction of an electrode pretreatment, and the integration of a tailor-made script for data treatment. Finally, the approach is tested on street samples from forensic seizures. Overall, this system allows for the on-site identification of ketamine by the law enforcement agents in an easy-to-use and rapid manner on cargos and seizures, thereby disrupting the distribution channel and avoiding the illicit drug to reach the end-user.

INTRODUCTION

Ketamine (2-(2-chlorophenyl)-2-(methylamino)cyclohexan-1-one) **(Figure S1, supporting information)** is a medical agent widely used for the induction and maintenance of anesthesia,[1] as well as for pain management.[2] Moreover, clinical studies have reported on the rapid and sustained antidepressant effects of sub-anesthetic doses of the drug.[3] Limiting factors in the more widespread use of ketamine for these purposes include dissociative and hallucinogenic effects experienced by patients.[2,4] Exactly those limiting factors have made ketamine an appealing drug for recreational use.[2,4] At large doses, it induces a strong state of dissociation, wherein users experience an intense detachment from reality.[2,5] Additionally, research has indicated that both the acute and chronic use of ketamine impact human memory, leading to impaired verbal fluency, cognitive processing speed, verbal learning and more.[6,7]

On the illicit drug market, ketamine is primarily found as a powder and is generally administered by nasal insufflation or inhalation.[2,5] Other routes of administration include intramuscular injection and oral ingestion in the form of a tablet.[2,5] Unfortunately, due to its colorless, odorless and tasteless characteristics and rapid onset, ketamine has been used as "club drug" to facilitate sexual assault.[8]

Illicit drug samples often contain a wide range of other substances that are added to increase bulk, enhance or mimic pharmacological effects or facilitate drug delivery. These are often legal substances such as caffeine, procaine, paracetamol and sugars.[9] Although illicit ketamine samples tend to be unadulterated (i.e., 78% of the analyzed ketamine samples are without adulterants[10]), the presence of a variety of adulterants (e.g. caffeine, creatine, paracetamol, benzocaine) and other illicit drugs (e.g. cocaine, MDMA, amphetamine, mephedrone) in these samples has been reported.[10–12] Ketamine is also frequently found as adulterant in ecstasy, cocaine and methamphetamine samples.[10–12]

Even though ketamine is largely diverted from the pharmaceutical market and imported from China and India, clandestine laboratories for its illicit manufacturing are now also found in Europe.[13] There is therefore an increasing interest in the development of on-site screening methods for the detection of illicit drugs in suspicious powders and seizures, to aid for example law enforcement agencies (LEAs) in preventing these drugs from reaching the market.

Different approaches for the analysis of ketamine have been proposed, ranging from bulky laboratory-based equipment to portable paper-based sensors for on-site measurements (state-of-the art of current methods, **Table S1**). Traditionally, the combination of chromatography (LC/GC) and mass spectrometry (MS) are seen as the gold standard in drug analysis and are used in forensic laboratories to validate seizures.[14,15] These techniques offer excellent specificity and sensitivity but their high cost, laborious measurements and low portability make them unsuitable for on-site analysis. Because of their simplicity, colorimetric tests are widely used as

presumptive tests for drug screening.[16] Several reagents have been proposed for the detection of ketamine, including a modified version of the cobalt(II)thiocyanate test commonly used for cocaine.[17] Musile et al. used this reagent in their microfluidic paper-based analytical devices (μPADs) for the multiplexed determination of different illicit drugs, including ketamine.[18] Moreover, Yehia et al. recently reported on a trimodal paper-based system including colorimetric, fluorometric and potentiometric detection zones for the determination of ketamine in beverages.[19]

In the last 25 years, electrochemical methods have increasingly been employed for on-site detection due to their simplicity and low-cost analysis, as well as due to their outstanding analytical performance in terms of sensitivity and specificity for the determination of ions,[20] biomolecules,[21] and pathogens.[22] Their portability was enhanced by the introduction of screen-printed electrodes (SPE) and the miniaturization of the electrochemical devices.[23] In recent years, electrochemical methods have been used for the detection of illicit drugs[24,25] such as cocaine,[26,27] MDMA,[28] methamphetamine,[29] and heroin.[30] Previous reports on the electrochemical analysis of ketamine (**Table S1**) have mainly utilized ion-selective electrodes (ISEs),[19] differential pulse voltammetry (DPV),[31] and even electrochemical impedance spectroscopy (EIS).[32] However, these strategies require long incubation times (whether antibodies or DNA strands being used) or experience selectivity issues against other illicit drugs. Recently, molecular imprinted polymers (MIPs) have been employed for the determination of ketamine using DPV[33] and square wave voltammetry (SWV).[34] This approach increases the selectivity of the analysis but also adds extra complexity to the electrode, thus increasing the cost of the sensor.

Herein, we present for the first time an electrochemical ketamine sensor for the fast analysis of seized samples without the use of any complex electrode modifications. First, the characteristic electrochemical behavior of ketamine at different pH-values and concentrations was studied to explore its oxidative fingerprint. In parallel, the oxidation pathway of ketamine was studied for the first time by analyzing partially electrolyzed samples with LC/MS aiming to understand the redox processes at the SPE. Subsequently, the influence of common adulterants, cutting agents and other illicit drugs on the electrochemical behavior of ketamine was studied by SWV. Any suppression or overlapping effects caused by other electroactive substances, which could potentially lead to false positive or false negative results, were overcome by the introduction of additional detection strategies (i.e., electrode pretreatment). Furthermore, a tailor-made data treatment approach is implemented with the aim of enhancing peak separation and facilitating identification. Finally, the optimized strategies were employed for the analysis of real street samples and validated against standard methods from forensic laboratories. Overall, the electrochemical fingerprinting proposed in this work allows the rapid and low-cost profiling of ketamine in cargos and seizure samples, which ultimately will allow for the discrimination of illicit drugs by LEAs in a decentralized manner.

EXPERIMENTAL SECTION

Reagents and Samples. Ketamine.HCl, cocaine.HCl, d,l-amphetamine.HCl, methamphetamine.HCl, MDMA.HCl, and mephedrone.HCl were purchased from Lipomed, Switzerland. Paracetamol, lidocaine, benzocaine were purchased from Sigma-Aldrich, Belgium, a standard of caffeine was purchased from VWR Chemicals, Belgium and creatine monohydrate was purchased from J&K Scientific (Lommel, Belgium). Ketamine street samples were provided by the National Institute for Criminalistics and Criminology (NICC, Belgium) and Customs laboratory (Belastingdienst, the Netherlands). Qualitative and quantitative analysis of the street samples were performed by NICC using gas chromatography/mass spectrometry (GC-MS) and gas chromatography-flame ionization detection (GC-FID), respectively.

Analytical grade salts of potassium chloride, potassium phosphate and boric acid, as well as potassium hydroxide, were purchased from Sigma-Aldrich (Overijse, Belgium). All solutions were prepared in 18.2 MΩ cm^{-1} doubly deionized water (Milli-Q water systems, Merck Millipore). The pH was measured using a CyberScan 510 pH-meter from Eutech Instruments (Landsmeer, The Netherlands) connected to a HI-1131 glass bodied pH electrode from Hanna Instruments (Bedfordshire, United Kingdom). Adjustment of the pH was performed using a 100 mM KOH solution.

Instrumentation and Apparatus. All SWV measurements were performed using a MultiPalmSens4 or EmStat Blue potentiostats (PalmSens, The Netherlands) with PSTrace/MultiTrace or PStouch software, respectively. Disposable ItalSens IS-C graphite screen-printed electrodes (SPE) (provided by PalmSens, the Netherlands), containing a graphite working electrode (Ø = 3 mm), a carbon counter electrode, and a 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 iterative background correction" (peak width = 1) tool in the PSTrace software.

Electrochemical measurements were performed in buffer at 20 mM ionic strength with 100 mM KCl (i.e., phosphate and borate buffer) by applying 50 μL of the buffer onto the SPE. Electrochemical pretreatment was performed by applying -0.8V during 360 s on the target sample in PBS pH 7.

Portable devices for Raman spectroscopy (Bruker Bravo, UK) and ATR-FTIR spectroscopy (Bruker Alpha 2, UK) were used to analyze real samples in powder for comparative purpose.

A custom-made script (Matlab R2018b, MathWorks, USA) is used after the analysis by SWVs to enhance peak separation and identify the compounds found in the suspicious powder.

The chromatography-mass spectrometry experiments were performed on an liquid chromatograph coupled to a quadrupole time-of-flight mass spectrometer (LC-QTOF-MS) using electrospray ionization (ESI) in positive 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). Further information on the LC-QTOF-MS conditions in the supporting information.

RESULTS AND DISCUSSION

Electrochemical behavior of ketamine on SPE. Preliminary experiments were performed to explore the electrochemical behavior of ketamine using cyclic voltammetry (CV) (Figure S2). **Figure S2a** shows the non-reversible behavior of ketamine at SPE at pH 7, 9 and 12. **Figure S2b** shows the oxidation

behavior of ketamine at different concentrations using PBS buffer pH 12 clearly proving a non-reversible process. Subsequently, the electrochemical fingerprint of ketamine was explored at different pHs (7-12) using SWV (**Figure 1a**). SWV exhibits an improved resolution of the non-reversible oxidation peaks. Two oxidation processes were observed, i.e. P1 and P2 (ca. 0.96 V and ca. 1.05 V, respectively at pH 12). As displayed in **Figure S3a**, there is barely a shift in the peak potential of P1 when varying the pH. It is suggested that the peak potential of P1 is maintained in the pH range 7-12 because the secondary amine responsible for the oxidation peak is deprotonated (pKa 7.5). Indeed, ketamine starts to exhibit a characteristic electrochemical fingerprint (EF) at pH 7 with the highest peak currents at pH 12 (**Figure S3b**). Below pH 7, ketamine is not redox active in the given potential window. Concerning P2 (ca. 1.3 V), this process is clearly related to ketamine being present in the solution, however, its exact nature is not yet clear (**Figure S4**, blank SWV with background electrolyte). Given the close proximity of the boundary of the potential window exhibiting high background current at these potential range, this oxidation process is less reproducible in its current response (**Figure S4**).

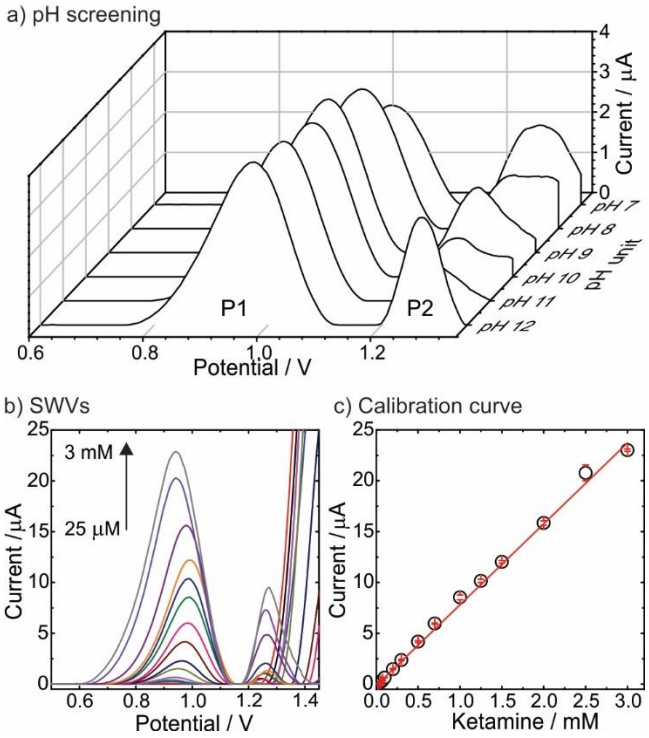


Figure 1. Electrochemical behavior of ketamine: a) Square wave voltammograms of a 0.5 mM ketamine solution in buffer solutions with 100 mM KCl from pH 7-12 at SPE. b) SWVs of increasing concentration of ketamine, and c) calibration curve (P1) in PBS pH 12 from 25 µM to 3 mM at SPE (N=3). P1 = peak 1, P2 = peak 2.

The analytical performance was evaluated for the determination of different concentrations of ketamine at pH 12, given its high reproducibility of P1 at this pH. **Figure 1b** shows the SWVs at increasing concentrations of ketamine (25–3000 µM) and the corresponding linear dependency for P1. The first peak at 0.95 V showed a linear relationship upon increasing concentration of ketamine (**Figure 1c**) leading to a slope of 8.2 µA mM^{-1}, from 50-2500 µM and a limit of detection (LOD) of 11.7 µM. Besides, **Figure S4** showed excellent reproducibility for P1 (RSD= 2.2%, at 500 µM, N=7).

A stability study of ketamine was carried out at pH 12 to evaluate whether the compound degrades over time in the alkaline solution (**Figure S5**). Hence, different measures from 1 min to 120 min after preparation were performed, showing high reproducibility: Ip=3.7±0.1 µA (RSD= 3%, n=9) at Ep=0.95 V. Thus, negligible degradation of ketamine through time in pH 12 was observed so that no risks are associated with the on-site detections at pH 12.

Elucidation of the oxidation pathway of ketamine. Understanding the oxidation processes taking place during the voltammetric scans can play an important role in the development of efficient detection strategies. Asghary et al. previously proposed an oxidation mechanism for ketamine, based on electrochemical data, in which the secondary amine is oxidized and subsequently undergoes dimerization.[35] However, to the best of our knowledge, an analysis focussing on the identification of oxidation products has not yet been reported. Hence, in order to gain an insight into the oxidation processes, ketamine solutions were electrolysed on SPE and subsequently analysed using LC-QTOF-MS. After a 60 min electrolysis, the samples (200 µM) were diluted to 20 ng $µL^{-1}$ with ultrapure water and directly injected. To assess the influence of pH and electrolysis potential (EP), solutions were prepared in pH 7 and 12, and the electrolysis was performed at potentials coinciding with both the first (P1, pH 7: 1.05 V, pH 12: 0.96 V) and second oxidation peak (P2, pH 7: 1.25 V) observed for ketamine.

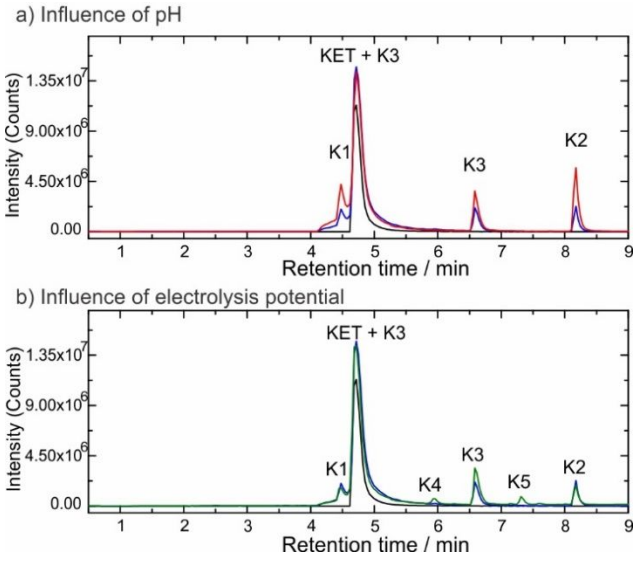
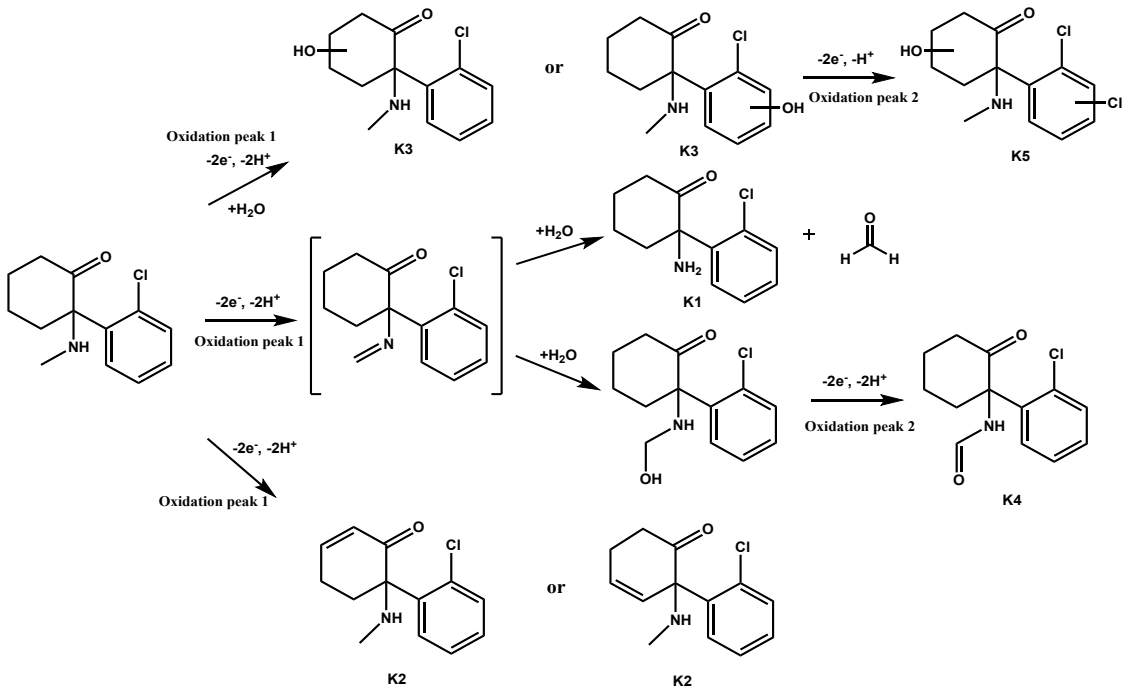


Figure 2. LC-QTOF-MS study. a) Influence of pH on electrolysis products. Total ion chromatograms of 20 ng $µL^{-1}$ solutions of ketamine (black) and ketamine electrolysis samples in pH 7 (blue) and pH 12 (red). EP pH 7: 1.05 V; EP pH 12: 0.96 V. b) Influence of EP on electrolysis products at pH 7. Total ion chromatograms of 20 ng $µL^{-1}$ solutions of ketamine (black) and ketamine electrolysis samples in pH 7 with an EP of 1.05 V (blue) and 1.25 V (green). Structure and additional information on oxidation products K1–5 is included in **Table S2**.



Scheme 1. Observed oxidation products in the electrochemical oxidation of ketamine.

Scheme 1 and **Table S2** provides an overview of all the oxidation products identified with their corresponding structure and additional information. **Figure 2** shows that three oxidation products (K1-3) were observed for the electrolysis samples at P1 oxidation potential (i.e., EP pH 7: 1.05 V and EP pH 12: 0.96 V). A first oxidation product K1 (*m/z* 224.0829, $C_{12}H_{14}ClNO$) elutes at 4.48 min just before the remaining ketamine (4.72 min, *m/z* 238.1002, $C_{13}H_{16}ClNO$). After comparing the $[M+H]^+$ ion and fragmentation pattern of K1 with relevant literature, K1 can be linked to norketamine, the primary amine analogue of ketamine.[36,37] Norketamine is the product of a demethylation reaction occurring after oxidation of the secondary amine, resulting in the corresponding primary amine and formaldehyde as by-product.[38,39] A second product K2 (*m/z* 236.0828, $C_{13}H_{14}ClNO$) elutes at 8.17 min and has an m/z-value (-2 compared to ketamine) that indicates the formation of a double bond in the structure (dehydroketamine). Although the exact location of the double bond is uncertain, the fragmentation pattern shows that the fragments with an *m/z* difference of 2 compared to ketamine all contain (part of) the cyclohexanone ring. The third product K3 (*m/z* 254.0936, $C_{13}H_{16}ClNO_2$) elutes at both 4.65 min and 6.58 min and can be attributed to the hydroxylation of ketamine. The strongly differing MSMS spectra for *m/z* 254.0936 (**Figure S6**) at the separate elution times indicate the formation of both the alcoholic and phenolic products, which is analogous to the metabolic pathway of ketamine.[40] This is further evidenced by the absence of the *m/z* 125.0127 fragment, which is attributed to the chloromethylbenzene fragment and features in the MSMS spectra of all the other products, in the second elution peak (**Figure S6**).[36,37,40] No additional products were observed for the electrolysis in pH 12. The quantitative increase in the formation of the three products K1-3 was expected since proton loss and hydroxylation reactions are facilitated in alkaline environment.

Analysis of the sample electrolysed in pH 7 at a more positive potential (P2) (1.25 V) revealed the presence of two more oxidation products. K4 (*m/z* 252.0790, $C_{13}H_{14}ClNO_2$) elutes at 5.93 min and it is proposed that this is the N-formyl derivative of ketamine. Apart from the previously mentioned demethylation reaction to form norketamine, the imine formed at the first oxidation peak can also react with water to form the corresponding alcoholic compound (**Scheme 1**). This compound is subsequently oxidised at the second oxidation peak to form the N-formyl derivative. Lastly, K5 (*m/z* 288.0545, $C_{13}H_{15}Cl_2NO_2$) elutes at 7.31 min and contains a chlorine isotope pattern that indicates the introduction of a second chlorine atom in the structure. It is proposed that this product is the result of chlorination, occurring at higher potentials, of one of the hydroxyketamine compounds (**Table S2, Scheme 1**). Both K4 and K5 are formed after the oxidation of products during the first oxidation peak, which are still in the vicinity of the electrode surface. This explains the irreproducible nature of this second peak and its lower intensity compared to the first peak. As shown in **Figure 2a** and in the electrochemical pH screening (**Figure 1a**), **s**imilar results are expected on the products of the electrolysis at P2 potentials for pH 12, albeit expecting higher products concentration at pH 12.

Electrochemical screening of ketamine in binary mixtures at different pH. The aim of the proposed method is to distinguish ketamine among other substances and to identify possible overlapping and suppression effects which could lead to false positive and false negative results, respectively. Therefore, the first step is to analyze binary mixtures between ketamine and other substances commonly found in real samples. The mixing agents were selected based on seized sample data in literature and forensic reports (**Table S3**). Ketamine samples are generally found with high purity, although caffeine and creatine are used as adulterant and cutting agent, respectively.[10,11,41–43] Besides, ketamine has been used as an adulterant for other drugs of abuse, such as MDMA.[6,43]

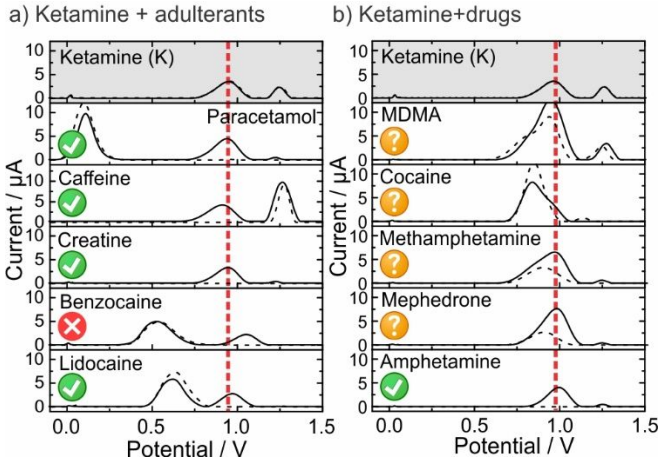


Figure 3. Electrochemical fingerprint of ketamine in different binary mixtures in PBS 100 mM KCl pH 12 at SPE: a) SWVs of 0.5 mM ketamine with 0.5 mM adulterants. b) SWVs of 0.5 mM ketamine with 0.5 mM illicit drugs. The dashed red line indicates where the first peak of ketamine is located. The dashed SWVs display the EF of the pure compounds.

In order to determine the optimal detection strategy, a screening of ketamine in binary mixtures at different pH (7, 9 and 12) was performed. First, the screening of common adulterants and cutting agents was carried out in PBS pH 7: pure compounds (**Figure S7a**), and binary mixture at 0.5 mM each compound (**Figure S7b**). Besides, the screening of common illicit drugs (pure compounds, **Figure S7c**) and their binary mixtures (**Figure S7d**) were also evaluated at the same ratio. At pH 7, some compounds complicated the detection of ketamine in their mixtures: (i) benzocaine suppressed the signal from ketamine; (ii) MDMA completely overlaps the oxidation peak of ketamine; (iii) cocaine oxidation peak also overlaps with ketamine, although broadening the peak; and (iv) mephedrone which exhibits a small peak is overlapped by ketamine, although this mixture would still allow the detection of ketamine. Subsequently, borate buffer at pH 9 was employed in the screening. Similarly, pure compounds of adulterants and cutting agents (**Figure S8a**), their binary mixtures with ketamine (1:1, 0.5 mM each) (**Figure S8b**), pure illicit drugs (**Figure S8c**) and corresponding binary mixtures with ketamine (**Figure S8d**) were assessed. At pH 9, the detection of ketamine presented difficulties in: (i) the presence of benzocaine, the oxidation peak of ketamine displays/undergoes a substantial shift in/on peak potential (+95 mV); (ii) the mixture with MDMA, where the presence of ketamine and MDMA causes one broad and intense peak; and (iii) mephedrone exhibits an oxidation peak at same peak potential, thus being impossible to distinguish between substances. Interestingly, cocaine shows a clear shoulder corresponding to the ketamine oxidation, which allows for a potential discrimination between compounds. Finally, the mixing agents and their corresponding binary mixtures at same compositions were explored in PBS pH 12. **Figure 3a** displays the electrochemical profile of binary mixtures of ketamine with common adulterants (solid line) as well as the pure compounds (dashed line), all 0.5 mM. In addition, **Figure 3b** contains the SWVs of the binary mixtures with common illicit drugs with corresponding SWVs of pure compounds (dashed lines). Still, the determination of ketamine in some mixtures represents a challenge: (i) benzocaine still produces a shift of the peak potential of ketamine (+103 mV); and (ii) MDMA, similarly to pH 9, results in one broad and intense peak. In contrast, in the binary mixture with cocaine, the shoulder corresponding to ketamine is still present in the profile. Similarly, the oxidation peak of methamphetamine forms a shoulder on the left of the ketamine signal. Finally, ketamine peak potential overlaps the low signal produced by the oxidation of mephedrone, thus showing no issue to detect ketamine. Overall, pH 12 provides enriched EFs from binary mixtures of illicit drugs in comparison with pH 7 and 9, and thus it was chosen as the best strategy for a preliminary test to screen for ketamine. As previously described, there is still signal overlap for some compounds (i.e., MDMA, cocaine, methamphetamine, mephedrone) and peak shift (i.e., benzocaine) to reliably ascertain ketamine in samples. In an attempt to overcome these issues, our group reported a cathodic pretreatment to electrochemically determine cocaine in cocaine/levamisole mixtures.[27] Following a similar strategy, an additional test was carried out by using a pretreatment step before the electrochemical measurement. Interestingly, the peak shift of ketamine resulting from benzocaine interaction was successfully addressed with the cathodic pretreatment. Furthermore, a data analysis step consisting of a tailor-made Matlab script was integrated to enhance peak separation and recognition of overlapping signals from conflict mixtures.

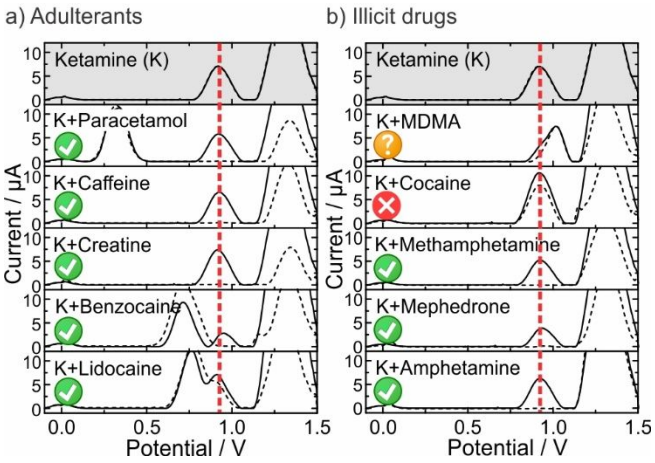


Figure 4. SWVs of ketamine in binary mixtures after cathodic pretreatment in PBS 100 mM KCl pH 7: a) SWVs of ketamine with cutting agents at 0.5 mM. b) SWVs of ketamine with illicit drugs at 0.5 mM. The dotted red line indicates where the signal of ketamine is located. The dashed SWVs indicates the EF of the pure adulterants or illicit drugs.

Pretreatment step to selectively detect ketamine in the presence of benzocaine. First, a preliminary study on the effect of the duration of a cathodic pretreatment (i.e., -0.8 V) on the EF of ketamine was performed both on blank solutions (**Figure S9a**) and on 0.5 mM solutions of ketamine (**Figure S9b**) using PBS pH 7. The results showed a shift in the Ep of ca. 100 mV towards lower potentials. Interestingly, a ca. 3-fold increase in the current was obtained after 360 s of pretreatment. Therefore, the cathodic pretreatment showed promise in increasing the sensitivity and the selectivity of ketamine detection using a SPE. Subsequently, a screening of the common adulterants (**Figure 4a**) and illicit drugs (**Figure 4b**) with corresponding binary mixtures was carried out with this approach. The plots show the SWV of the pure compound (dashed line) and the corresponding binary mixture (straight line) at 0.5 mM. In this

case, ketamine and benzocaine were simultaneously detected with minimal suppression or shifts compared to the previous analysis at pH 7-9-12 without pretreatment. Besides, ketamine is separately detectable as a shoulder on the peak of MDMA, which only has one characteristic signal after the cathodic pretreatment. The only challenge remaining is cocaine, which still completely overlaps with ketamine. The following step was the utilization of the data treatment to assist in the peak identification when peak overlap takes place (e.g., shoulder in the ketamine-MDMA mixture).

Data treatment towards an enhanced peak analysis. A Matlab script was designed to identify all the oxidation peaks in the EF, particularly for the cases in which the overlapping of signals creates shoulders and tails in the peak. In addition, the script can provide an automatic identification of the compounds found in the suspicious powder according to the parameters of the peak potential characteristic of each compound previously analyzed (**Figure S10**). **Figure 5** displays the comparison between SWVs obtained from the potentiostat software (i.e., moving average correction), and after employing data analysis (i.e., tailor-made script) of critical binary mixtures of ketamine and cocaine, methamphetamine, MDMA and mephedrone). **Figure 5a** shows SWVs of mixtures that clearly exhibit shoulders on oxidation peaks corresponding to partially overlaying signals of different compounds. After data analysis (**Figure 5b**), an improved peak separation was accomplished, allowing an easy identification of each compound present in the sample. It is worth mentioning that the treated signal after the script does not correspond to the current intensity of the SWVs, thus producing signals for a qualitative analysis. Hence, the script successfully allows to distinguish the oxidation peaks which is the purpose for the on-site determination of illicit drugs in suspicious samples. All in all, the tailor-made script for data treatment improves peak separation in critical mixtures with significant signal overlay, consequently allowing an effective determination of ketamine in buffer solution.

Protocol for the determination of ketamine. At this point, a two-step protocol is described for the determination of ketamine: i) a preliminary screening at pH 12 to obtain an enriched EF of the suspicious sample, and ii) a confirmatory test, either at pH 9 or by employing a cathodic pretreatment step in PBS pH 7, to discriminate against critical compounds (e.g., cocaine and benzocaine, MDMA, or methamphetamine, respectively) that overlap the oxidation signal with ketamine. Particularly, borate buffer pH 9 (**Figure S8d**) would allow for the discrimination between cocaine and ketamine. Moreover, pretreatment at pH 7 would be necessary when the peak of benzocaine is present in the EF as well as to confirm that ketamine is present when MDMA is detected at pH 12.

Electrochemical screening of ketamine in complex samples. Electrochemical profiling of complex samples was performed using plausible compositions of street samples, based on data found in literature (**Table S3**). Accordingly, complex mixtures of different substances were prepared (composition found in **Table S4**) and analyzed using the proposed electrochemical method. The aim of this analysis is to simulate real samples and evaluate all the forensic cases before validating the methodology in the real scenario. **Figure 5c** presents the SWVs analysis of the complex samples from best to worst possible scenario (from 1 to 6) in terms of selectivity (i.e., mixtures of compounds with overlapping signal at different concentrations). Lidocaine, benzocaine and MDMA presented some difficulties after employing the electrochemical analysis at pH 12 and the pretreatment at pH 7, respectively. However, **Figure 5d** displays the output signal after the use of the script over the SWVs of the complex samples allowing to a favorable identification of the compounds found in the mixtures. The most critical mixtures encountered were: (i) complex sample 3, which contains benzocaine, although barely found in confiscated samples. Fortunately, it is resolved with the script application. (ii) Complex sample 6 which contains MDMA (ketamine can be used as an adulterant in ecstasy). The latter case should not present a trouble for LEAs, as the false positive of ketamine sample for MDMA presence also indicates the identification of an illicit drug.

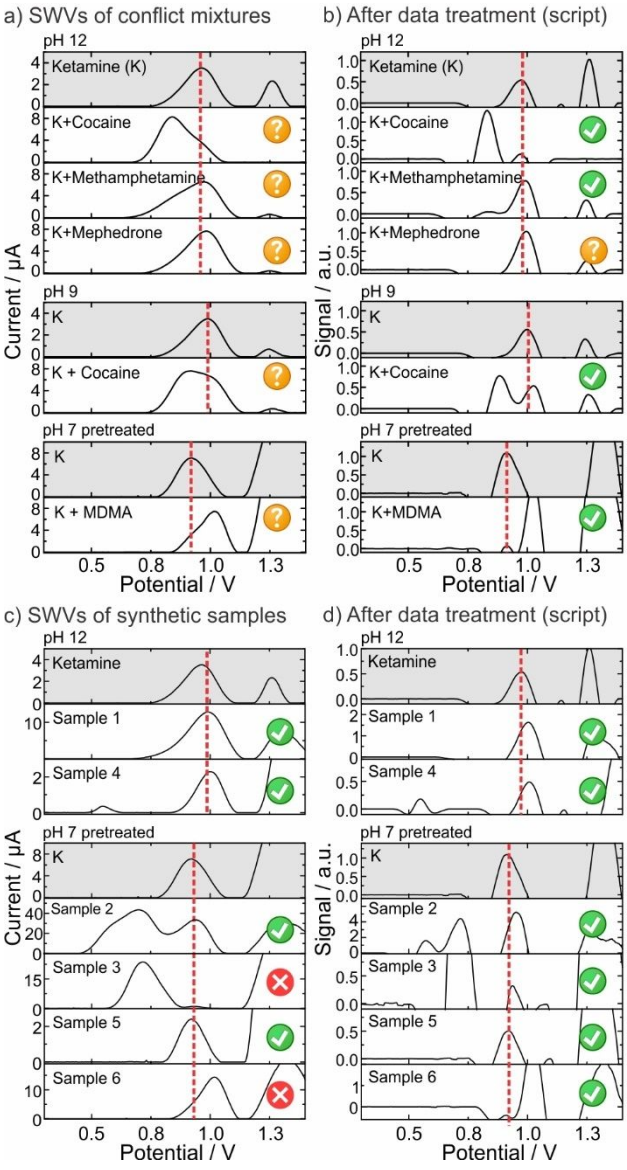


Figure 5. Use of data treatment to improve peak identification. a) SWVs of critical mixtures where peak overlap exists, b) output signal after the application of the script. Complex mixtures: c) SWVs, d) output signal after the application of the script. Composition of samples 1-6 are described in **Table S4**. Screening with PBS pH 12 and PBS 7 using pretreatment at SPE. The dotted red line indicates where the signal of ketamine is located.

Determination of ketamine in seized samples. The aim of the presented method is the fast and accurate detection of ketamine in seizures and street samples. Therefore, feasibility tests were performed at LEAs from European countries (i.e., NICC, Belgium, and the Dutch customs laboratory, the Netherlands), to analyze the presence of ketamine in confiscated samples by using a portable potentiostat connected to a laptop for real-time data readout. Moreover, the street samples were analyzed in parallel with the standard methodology of the forensic laboratory (i.e., LC-MS and GC-FID). For the on-site analysis, ca. 1 mg of the suspicious powder was dissolved in 1 mL of PBS pH 12, thoroughly mixed for 30 s, and placed at the SPE surface for the subsequent analysis by SWV. **Figure 6a** displays the electrochemical profile (SWVs with moving average data treatment) of each sample revealing a prominent presence of ketamine in suspicious samples 1, 3 and 4. In contrast, sample 2 exhibited a profile in which ketamine was not detected. Fortunately, the script was able to determine the oxidation peak of ketamine, thus avoiding a false positive for sample 2 (**Figure 6b**). Indeed, **Table S5**, which contains the composition of the analyzed samples after GC-FID analysis, shows that sample 2 only contains traces of ketamine and that it is the most difficult sample to successfully detect the drug in. In addition, real samples were analyzed with other portable devices (i.e., FTIR spectroscopy, **Figure S11** and Raman spectroscopy, **Figure S12**) to compare the qualitative test (**Table S5)**. Similarly, samples 1, 3 and 4 were positive for ketamine in both devices. In contrast, ketamine was not detected in sample 2 by employing spectroscopic techniques. Therefore, the electrochemical methodology presented in this work improved the results of on-site methods with comparable outcomes with lab-bench analysis. Overall, the electrochemical devices shows promise to replace regular methods of analysis in the field yielding to an improved screening by LEAs.

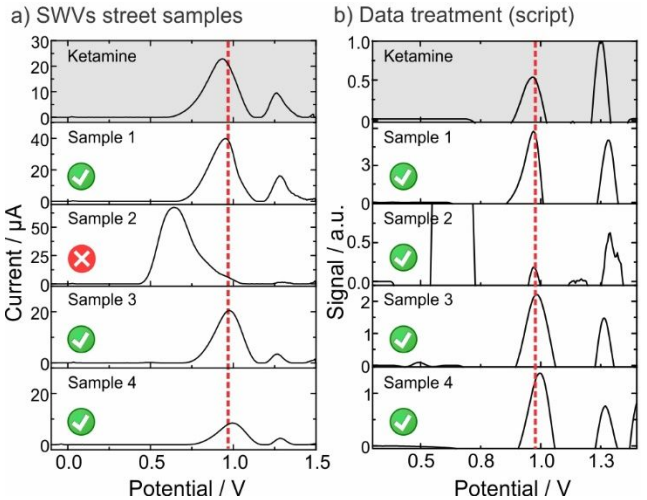


Figure 6. Electrochemical profile of real samples in PBS pH 12 at SPE: a) SWVs raw data, b) output signal after the application of the script. Samples from 1-4. The dotted line indicates where the signal of ketamine is located.

CONCLUSIONS

We have demonstrated, for the first time, the electrochemical detection of ketamine on unmodified SPE via rapid voltammetric detection strategies in complex and real samples. Importantly, we have unraveled for the first time the oxidation pathways of ketamine on unmodified SPE. Furthermore, the electrochemical profiling of ketamine was advantageously resolved over common cutting agents, adulterants, and illicit drugs. A successful strategy for the determination of ketamine was described by a two-step protocol: i) a preliminary screening at pH 12 to obtain an enriched EF of the sample, and ii) a confirmatory test, either at pH 9 or by employing a cathodic pretreatment step in PBS pH 7, to discriminate against critical compounds that overlap the oxidation signal of ketamine. In addition, an innovative data treatment was designed to enhance peak separation between overlaying signals by the integration of a script in the protocol. Finally, the methodology was validated with real samples from forensic laboratories and compared with lab-bench standard methods. Besides, the results were compared to commonly used portable spectroscopic devices, showing similar, or even, enhanced performance over currently used on-site methods. Overall, the potential of electrochemical methods for providing rapid and reliable illicit drugs screening during on-site testing was demonstrated. The advances presented in this article will pave the way for a new set of electrochemical sensors that will constitute the next generation of portable devices used by LEAs for screening illicit drugs in the field.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

PDF file including: experimental details; Tables: state-of-the-art, composition of samples; Figures: chemical structure of ketamine, reproducibility and stability studies, MS data, ECF of mixing agents at pH 7 and pH 9, effect of pretreatment, raw output signal and optimization from the script, ATR-FTIR and Raman spectra.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the grant agreement No 833787, BorderSens. The authors acknowledge financial support from the Fund for Scientific Research (FWO) Flanders, Grants 1SB8120N and 1S3765817N and from University of Antwerp (IOF).

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