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ABSTRACT: Identifying and quantifying 3,4-methylenedioxymethamphetamine (MDMA) on-site in suspected illicit drug samples, whether it be at recreational settings or manufacturing sites, is a major challenge for law enforcement agencies (LEAs). Various analytical techniques exist to fulfil this goal, e.g. colourimetry and portable spectroscopic techniques, each having its specific limitations (e.g. low accuracy, fluorescence, no quantification) and strengths (e.g. fast, easy to use). In this work, for the first time, an electrochemical MDMA sensor is presented to become a detection tool that can realistically be used on-site. More specifically, the use of a single buffer solution and an unmodified screen-printed electrode, along with the integration of a data analysis algorithm and mobile application permits the straightforward on-site identification and quantification of MDMA in suspicious samples. Multiple studies investigating different parameters, including pH, concentration, reproducibility, temperature and binary mixture analyses, were executed. To fully understand all the occurring redox processes, liquid chromatography coupled with high-resolution mass spectrometry analysis of partially electrolyzed MDMA samples was performed unravelling oxidation of the methylenedioxy group. Validation of the methodology was executed on 15 MDMA street samples analysed by gas chromatography coupled with mass spectrometry and compared with the performance of a commercial portable Raman and Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) device. The novel methodology outperformed the spectroscopic techniques, correctly identifying all 15 street samples. Additionally, the electrochemical sensor predicted the purity of the tablets with a mean absolute error of 2.3%. Overall, this new, electrochemical detection strategy provides LEAs the rapid, low-cost, on-site detection and quantification of MDMA in suspicious samples, without requiring specialized training.

Keywords: Electrochemical sensors; Qualitative detection; Quantitative detection; Smartphone application; MDMA detection

1. INTRODUCTION

MDMA (3,4-methylenedioxymethamphetamine) was first synthesized in the 1910's by an employee of the pharmaceutical company Merck as a precursor to circumvent the patents of their rival Bayer.[1] Its structural similarities with hallucinogens, such as amphetamine and mescaline, attracted research interest over the next decades, but it was not until the 1970s that the compound really came to the forefront.[2,3] Influential psychotherapist Leo Zeff, and others in his wake, started promoting the compound as an empathogen, praising the seeming increased communication and empathy a user gains after administration.[4–6] These effects made MDMA popular amongst another group of people as well, that is college students. The compound quickly claimed a prominent role as a recreational party drug on college campuses in the United States and Europe.[7] Nevertheless, multiple side effects are associated with MDMA use, including brain damage, hypertension, depersonalization and nausea.[8–11] Unsurprisingly, MDMA quickly became a Schedule I drug under the Controlled Substance Act (which it still is today), meaning that no medical use of the compound is allowed and that it has a high potential for abuse.[12,13]

MDMA has various street names (molly, XTC, X) on the illegal drug market, of which ecstasy is the most well-known. However, MDMA and ecstasy are no synonyms. In the majority of cases (90% in 2019), ecstasy pills do contain MDMA as the sole active compound that causes the psychostimulant effects that the user is seeking. Nevertheless, other psychostimulant compounds can be found in ecstasy pills as well (5-10% in 2019), often mimicking or slightly altering the effects of MDMA.[12] Some other established compounds found in ecstasy are 4-bromo-2,5-dimethoxyphenethylamine (2C-B), 4-chloro-*alpha*-pyrrolidinovalerophenone (4-Cl-*alpha*-PVP), *para*-methoxyamphetamine (PMA), *para*-methoxy-N-methylamphetamine (PMMA), 5,6-methylenedioxy-2-aminoindane (MDAI), 3-fluoroamphetamine (3-FA) and methylone.[14–17] The unexpected presence of a compound different from MDMA might expose the user to unforeseen, undesired effects. Additionally, if the effects of the unexpected compound have a delayed start compared to MDMA, the user might be tempted to take an additional dose, greatly increasing the risk of overdosing.[18]

MDMA usually comes in the form of (ecstasy) pills with a distinct colour and logo, but other forms of appearance such as, crystal and powder, are encountered as well.[19,20] In 2020, the average purity of MDMA powder amounted to 79%.[13] Approximately 90% of MDMA powders submitted to pill testing services in Europe, contain solely MDMA as expected substance, with 7% containing MDMA together with cutting agent(s) (i.e. agents added to the pill to alter or intensify the effect of MDMA). For MDMA pills, these percentages are 94% and 3%, respectively. MDMA is thus not commonly cut, and if it is observed, the cutting agent is mostly caffeine.[12] It occurs that other psychostimulant agents are present besides MDMA, but this is rather rare. Specifically for ecstasy pills, some binders and colouring agents are included as well to manufacture the pill. Ecstasy pills contain on average between 125 and 200 mg of MDMA per pill and are mainly manufactured in Western Europe.[13] The MDMA content in ecstasy pills increases year after year, which in turn increases the risk of (unexpected) overdosing.[13]

Even though MDMA is a highly regulated drug, statistics show that the drug has a very large audience (estimated 20 million users worldwide in 2019).[12] This enormous illicit MDMA consumption calls for specialized tools that can aid law enforcement in their fight against illicit MDMA use. An important set of tools in the repertoire of law enforcement are the identification tools, i.e. tools that can screen suspicious samples for the presence of MDMA. More specifically, the illicit manufacturing in clandestine laboratories, in combination with the use in specific party settings such as festivals and clubs, call for specialized *on-site* identification tools. Importantly, these tools also need to be able to quantify the amount of MDMA in suspicious samples. Overall, these tools are necessary to aid law enforcement agencies (LEAs) speed up their decision-making process on-site.

Currently, a wide variety of illicit drug testing methodologies is available, with bulky laboratory-based equipment on one side of the spectrum, and light, portable technologies on the other side of the spectrum. Typically, laboratory-based identification technologies such as gas or liquid chromatography-mass spectrometry (GC-MS or LC-MS) have high selectivity and sensitivity but have as drawbacks low portability, high cost and low usability by non-experts.[21,22] The light, portable technologies on the other hand, with colour tests (Marquis field test for MDMA) as a primary example, show the opposite pattern.[23] They are portable, low-cost and controllable by non-experts, but are low in sensitivity and selectivity. Recently, a trend emerged in which portable versions are made of spectroscopic techniques such as Fourier transform infrared spectroscopy (FTIR) and Raman.[24–29] Although showing promising results for on-site drug detection, these devices have a relatively high price tag (20 000+ euros), and the more portable Raman devices tend to struggle with coloured samples (ecstasy pills are often coloured) due to fluorescence. This is why LEAs typically use a combination of two techniques. The first step is to employ an indicative on-site test with a portable device. If this test raises suspicion, a second, confirmatory test is executed in the lab with more accurate laboratory-based equipment.

Electrochemical sensors have recently proven a suitable candidate for illicit drug detection, fusing the advantages of technologies at both ends of the spectrum.[30–32] They are low-cost, offer fast analysis times, are portable due to advances in miniaturization and, to an extent, have high selectivity and sensitivity. Recent advances in data analysis approaches have opened up the technology to non-experts by taking care of all required data treatment steps.[33] During previous years, electrochemical illicit drug sensors have emerged for the detection of various illicit drugs, including cocaine, heroin, ketamine and MDMA.[31,34–36] Initially, electrochemical MDMA sensors employed all sorts of modified (boron-doped diamond, zinc oxide nanorods, graphene, multi-walled carbon nanotubes) electrodes to reach a desired sensitivity and selectivity.[37–39] However, these modifications make it cumbersome to deploy the technology on-site, since they require long incubation times, add complexity to the manufacturing process and thus increase the cost. Cumba *et al.* reported in 2017 a methodology to detect MDMA and PMA at unmodified screen printed electrodes (SPEs) using differential pulse voltammetry (DPV).[17] Their method demonstrated the opportunities of an electrochemical MDMA sensor, reaching low detection limits (0.04 $\mu g\ mL^{-1}$), and showing an improved speed and cost over other analytical techniques such as Raman and HPLC. They, however, did not validate their method on street samples, nor did they include data analysis software in their methodology to open up the technology for non-experts. In 2021, Alves *et al.* reported an electrochemical method to detect MDMA at unmodified SPEs employing linear sweep voltammetry (LSV).[40] Their method was successfully validated on four MDMA street samples. The major drawback of their method, apart from the small validation set, is that it still relied on interpretation by experts in electrochemistry, thereby limiting its potential use by law enforcement personnel. Also in 2021, Shanmugama *et al.* reported an electrochemical approach for the detection of MDMA, using a combined PBS pH 7 and PBS pH 12 approach employing square wave voltammetry (SWV) at unmodified SPEs.[41] The two buffers were selected by the authors since MDMA has a rich electrochemical profile (EP) in them, allowing multiple signals to work with for identification. Even though the combined pH method proves to be very efficient in MDMA identification, it will be time-consuming to use two electrodes and buffers for each on-site analysis. Besides, this methodology also fails to integrate a data analysis algorithm and has no quantification module.

In this work, we present an electrochemical approach for MDMA detection and quantification that overcomes the major drawbacks associated with previously developed electrochemical MDMA sensors. The approach does not rely on electrode modifications but instead employs a single, unmodified SPE and one buffer solution. This study includes the elucidation of the oxidation pathway of MDMA by LC-MS of partially electrolyzed samples, to fully understand the redox processes occurring at the electrode surface. Based on this knowledge, a detection strategy was proposed and further improved by binary mixture analysis. The latter analysis is performed to identify potential false negatives and false positives resulting from cutting agents, adulterants or other drugs. Furthermore, a data analysis approach was tailored towards the detection strategy to open up the sensor to non-expert end-users. Uniquely, the final MDMA detection strategy was then integrated into a smartphone application with a user-friendly interface, bringing the sensor as close as possible to the market. Finally, the novel detection strategy was validated on a set of 15 street samples, both

qualitatively and quantitatively, and compared with the performance of a commercial portable Raman and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) on that same set of street samples. Overall, this new, electrochemical detection strategy provides LEAs with the rapid, low-cost, on-site detection of MDMA, without requiring specialized training. As such, it offers a valuable tool in the fight against illicit drugs.

2. MATERIALS AND METHODS

2.1 Reagents and sampling

Standards of d,l-MDMA·HCl, d-amphetamine·HCl, methamphetamine·HCl, d,l-PMMA·HCl, d,l-PMA·HCl, ketamine·HCl, butylone·HCl, methylone·HCl, cocaine·HCl, 3-FA·HCl, 3,4-Methylenedioxy-*N*-ethylamphetamine·HCl (MDEA) and 1,3-Benzodioxolyl-*N*-methylbutanamine·HCl (MBDB) with purity >98.5% were purchased from Chiron AS (Norway). Dextromethorphan (DXM), paracetamol, phenylethylamine·HCl, phenacetin, piracetam and lidocaine were purchased from Sigma-Aldrich (Diegem, Belgium). Caffeine was purchased from VWR Chemicals (Leuven, Belgium). Creatine monohydrate was purchased from J&K Scientific (Lommel, Belgium). A 2C-B standard, MDAI standard and 4-Cl-alpha-PVP standard, as well as the ecstasy street samples were provided by the NICC in Belgium. The street samples were analyzed by GC-MS (qualitatively) and GC-FID (quantitatively) to define their chemical composition. The applied chromatographic methods are ISO17025 accredited and are continuously evaluated through participation in international quality control programs (UNODC and European Network of Forensic Science Institutes—ENFSI).

Phosphate buffer saline (PBS) solutions were prepared for the electrochemical measurements, containing 20 mM KH_2PO_4 and 100 mM KCl, purchased from Sigma-Aldrich (Belgium). Acetate buffer (ACE) solution was prepared containing 20 mM CH_3COONa and 100 mM KCl, purchased from Sigma-Aldrich (Belgium). The pH of these buffer solutions was adjusted with KOH and HCl solutions to reach the desired pH. All aqueous solutions were prepared using Milli-Q water (R > 18 MΩcm). The pH was measured using a pH-meter (914 pH/Conductometer, 2.914.0020, Metrohm, Herisau, Switzerland).

The ecstasy related compounds were subjected to electrochemical analysis as individual compounds and binary mixtures with MDMA (1:1). For real samples analysis, tablets were crushed or scrapped with a spatula for collecting the sample (approximately 3 mg) and dissolved in 1 mL milli-Q water in a 1.5 mL tube to obtain a stock solution. Prior to measurement, these stock solutions were diluted ten times in ACE pH 5 buffer. The final concentration of 0.3 mg/mL allows purity determination via a calibration curve obtained through the concentration study.

2.2 Instrumentation and Apparatus

All SWV measurements were performed using MultiPalmSens4 or EmStat Blue potentiostats (PalmSens, The Netherlands) with PSTrace/MultiTrace or PStouch software, respectively. Disposable ItalSens IS-C graphite 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 (no pre-conditioning or pre-treatment required). The SWV parameters that were used were the following: potential range of −0.1 to 1.5 V vs Ag/AgCl, frequency 10 Hz, 25 mV amplitude, and 5 mV step potential. These parameters were optimized in previous research.[31] 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[31,34–36,41,42] (i.e., phosphate and acetate buffer) by applying 50 µL of the buffer onto the SPE. 100 mM KCl is sufficient for a fix concentration of chloride ions and mainatin a constant potential using the pseudoreference electrode based on Ag/AgCl.

Temperature experiments were performed using a Mistral oven heater (Spark Holland B.V., the Netherlands) for exact and reproducible temperature control. An SPE connector cable (PalmSens, Houten, The Netherlands) was fixed inside the oven and connected to a portable EmStat Blue potentiostat (PalmSens, Houten, The Netherlands) located outside the oven. The steel probe of a digital thermometer (VWR, Leuven, Belgium) was fixed in the proximity of the SPE to obtain an accurate indication of the temperature. When the temperature in the oven had reached the desired temperature, the SPE was inserted. Subsequently, the solution was prepared and applied to the SPE.

A Bruker Bravo Handheld Raman spectrometer (Bruker Optik GmbH, Ettlingen, Germany) was used for all Raman measurements. The instrument uses a dual laser excitation feature with two laser diodes (wavelengths: 785 nm and 852 nm). Spectra were recorded from 170 cm^{-1} to 3200 cm^{-1}. OPUS 8.2.28 (Bruker Optik GmbH, Ettlingen, Germany) software was used for data acquisition and analysis. All seized samples were processed into powdered form and stored in transparent plastic bags. All measurements were performed by placing the plastic bag containing the sample on the measuring tip. Identification was performed using the TICTAC Drug Library (TICTAC Communications Ltd., London, United Kingdom). Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) (Bruker Alpha II spectrometer, Bruker Optik GmbH, Ettlingen, Germany) was used for the analysis of the confiscated samples, employing a diamond crystal. For each measurement, a small amount of sample was placed directly on the crystal. The spectra were recorded from 4000 cm^{-1} to 400 cm^{-1} with a spectral resolution of 4 cm−1 and consisted of 128 co-added scans (analysis time: ca. 170 s). A background scan (128 scans) was run against air before the measurements commenced. Data acquisition and analysis were also performed using OPUS 8.2.28 software. The TICTAC Drug Library (for ATR spectra) was used for identification.

A custom-made script (Matlab R2018b, MathWorks, U.S.A.) is used after the analysis by SWV to enhance peak separation and identify the compounds found in the suspicious powder. The script was integrated in a smartphone app developed in C#/.NET (PalmSens, The Netherlands).

The liquid chromatography−mass spectrometry experiments were performed on a 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). Solutions of 200 µM MDMA were electrolyzed in both PBS pH 7 (0.92 V and 1.26 V) and pH 12 (0.81 V and 1.10 V). After 60 minutes the electrolyzed samples were diluted to 20 ng/µL with ultrapure water and injected directly. Chromatographic separation was performed on a Kinetex Biphenyl 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, and 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 is based on acetonitrile (90%) and deionized water (10%) (Part number G1969-85001, provided by Agilent Technologies) and 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, were *m/z* 121.0508 and 922.0097 for positive mode. The QTOF-MS device was acquired 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 the minimal abundance of 2500 counts. In addition, precursor ions were excluded after every spectrum and released after 0.2 min.

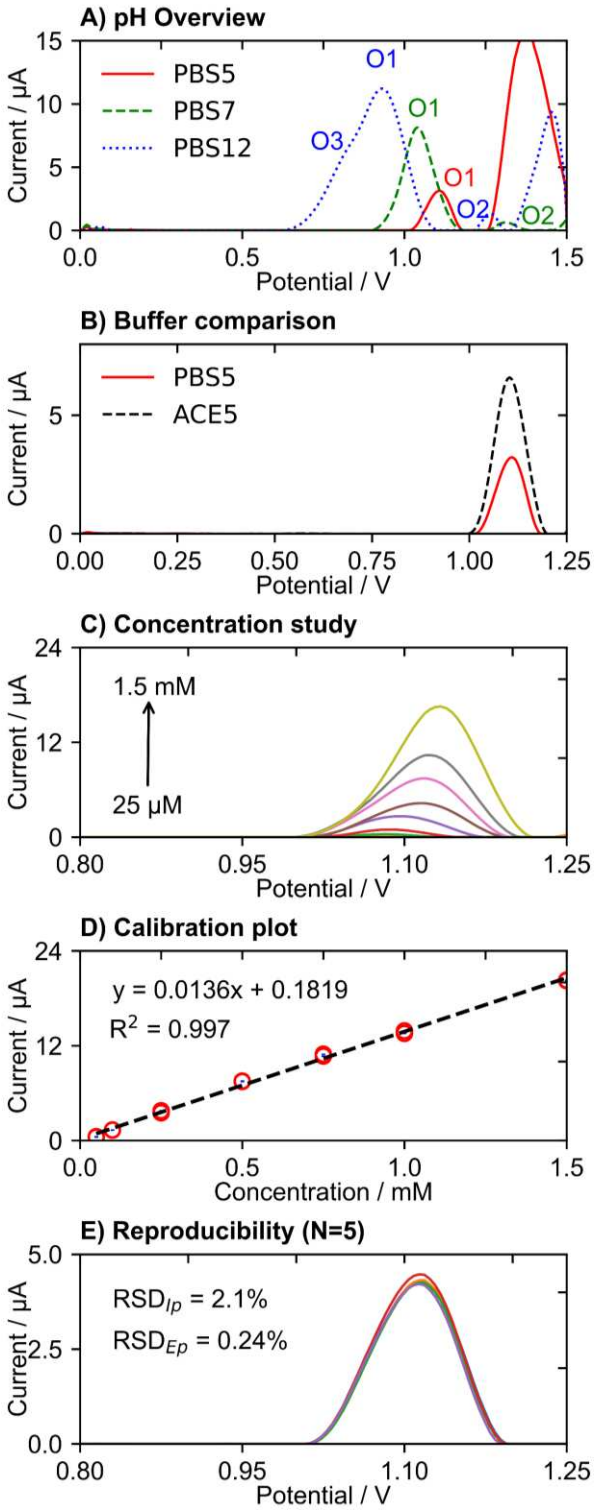

Figure 1. Electrochemical behaviour of MDMA: A) Square wave voltammograms (SWVs) of 0.5 mM MDMA solution in PBS buffer solutions at pH 5, 7 and 12 at SPE. Oxidation peak 1, 2, and 3 are abbreviated by O1, O2 and O3, respectively. B) SWVs of 0.5 mM MDMA solution in PBS pH 5 vs ACE pH 5. C) SWVs of increasing concentration of MDMA in ACE pH 5 from 25 µM to 1.5 mM at SPE. D) Calibration curve of MDMA in ACE pH 5 from 25 µM to 1.5 mM at SPE, and E) Reproducibility study at 500 µM in ACE pH 5.

3 RESULTS AND DISCUSSION

In order to develop an electrochemical approach to detect MDMA (**Figure S1**) in one measurement within 30 seconds, SWV is the technique of choice grace to its high sensitivity and previous success in electrochemical illicit drug sensors. Crucial in the development of an electrochemical detection approach is the buffer selection. Previous research has focused on PBS pH 7 and PBS pH 12 for MDMA detection, due to the richness of the EP in those conditions (**Figures 1A and S2**).[41] PBS pH 7 allows the use of oxidation peaks O1 and O2, with PBS pH 12 additionally allowing use of O3. O1 is linked to the oxidation of the benzodioxole functionality, whereas O2 and O3 can be linked to the secondary amine.[41] Therefore, it comes as no surprise that other compounds (sharing the secondary amine-moiety) have rich EPs as well in pH 7 and pH 12, making the identification of MDMA more challenging. This is why, in this work, we will focus on a pH 5 buffer (**Figure 1A**), since the oxidation peaks O2 and O3 are not visible in the considered potential range when employing a graphite SPE. The signal at 1.35V in pH 5 is not related to the oxidation of MDMA. It is present in blank measurements, and can be related to the oxidation of water or the oxidation of a material in the electrode (**Figure S3**). It is signal O1, linked to the benzodioxole moiety and still visible around 1.11 V in pH 5, that will be used as the diagnostic signal. The benzodioxole moiety is rare among illicit drugs, especially in comparison to secondary amines, and focussing on the corresponding signal will therefore result in a reduced amount of false positives, which in turn will improve the accuracy.

An acetate pH 5 buffer (ACE pH 5) is selected over a PBS pH 5 buffer due the latter's lower buffer capacity at this pH, having the pK_a of 4.76 and 7.21, respectively (**Figure 1B**). Increasing the concentration slightly shifts the diagnostic signal to more positive peak potentials (**Figure 1C**). The temperature on the other hand has only a very minor influence on the peak potential (E_p=1.114 V, RSD_{Ep}=0.57%) of the diagnostic signal (**Figures S4 and S5**). The shifts caused by changes in concentration and temperature will be accounted for in the data analysis software by using a peak interval located around the diagnostic peak for identification, rather than a fixed value (1.05V-1.15 V). This is important since ecstasy pills are known for having various levels of purity, and a realistic on-site detection technology needs to detect MDMA at various concentrations.[12,13] A detection window of 1.05V to 1.15V therefore allows detection of MDMA in both low and high purity ecstasy samples. Furthermore, a peak current/concentration calibration plot is obtained, using oxidation peak O1 in ACE5 buffer (**Figure 1D**). The linear relationship is described by the following equation: I_{O1} (µA) = 0.0136 (± 0.0002) * [MDMA] (µM) + 0.1819 (± 0.1412). A theoretical limit of detection (LOD) and limit of quantification (LOQ) were subsequently calculated using the formulas LOD = (3*σ)/m, and LOQ = (10*σ)/m, with σ being the standard deviation of the blank (N = 10), and m being the slope of the linear equation. The calculated LOD and LOQ of the methodology are 4.03 µM and 12.2 µM, respectively, which are in the same order of magnitude as the LODs and LOQs found by Teofilo *et al*. (0.3 µM and 1.0 µM), Shanmugam *et al*. (15 and 52 µM), de Faria *et al*. (0.6 µM, no LOQ) and Alves *et al*. (1.83 µM and 6.11 µM).[37,40,41,43] Zhang *et al.* reach remarkably lower LODs (0.018 µM) by using Pt nanoparticles/carbon nanohorns.[44]The linear relationship between peak current and concentration, together with the excellent LOD and LOQ, allows the addition of a quantification module, which is highly relevant for a MDMA sensor. Note that a LOQ in the low µM-range is more than sufficient for quantification of MDMA in real scenarios. As an illustration, sampling 1 mg of an MDMA-containing ecstasy pill with 10% purity in 1 mL of buffer, results in an MDMA concentration of 435 µM, which is well above the LOQ of this method. The quantification module will be discussed more in-depth in a separate paragraph later on. Good reproducibility of the diagnostic signal was obtained at 500 µM (N=5, new SPE for each measurement) with an RSD_{Ip} of 2.1% and RSD_{Ep} of 0.24% (**Figure 1E**). Finally, a scan rate study confirmed that the mass transport mechanism is governed by diffusion of the MDMA towards the SPE (**Figure S6**), which is in line with the findings of Shanmugam *et al.*[41]

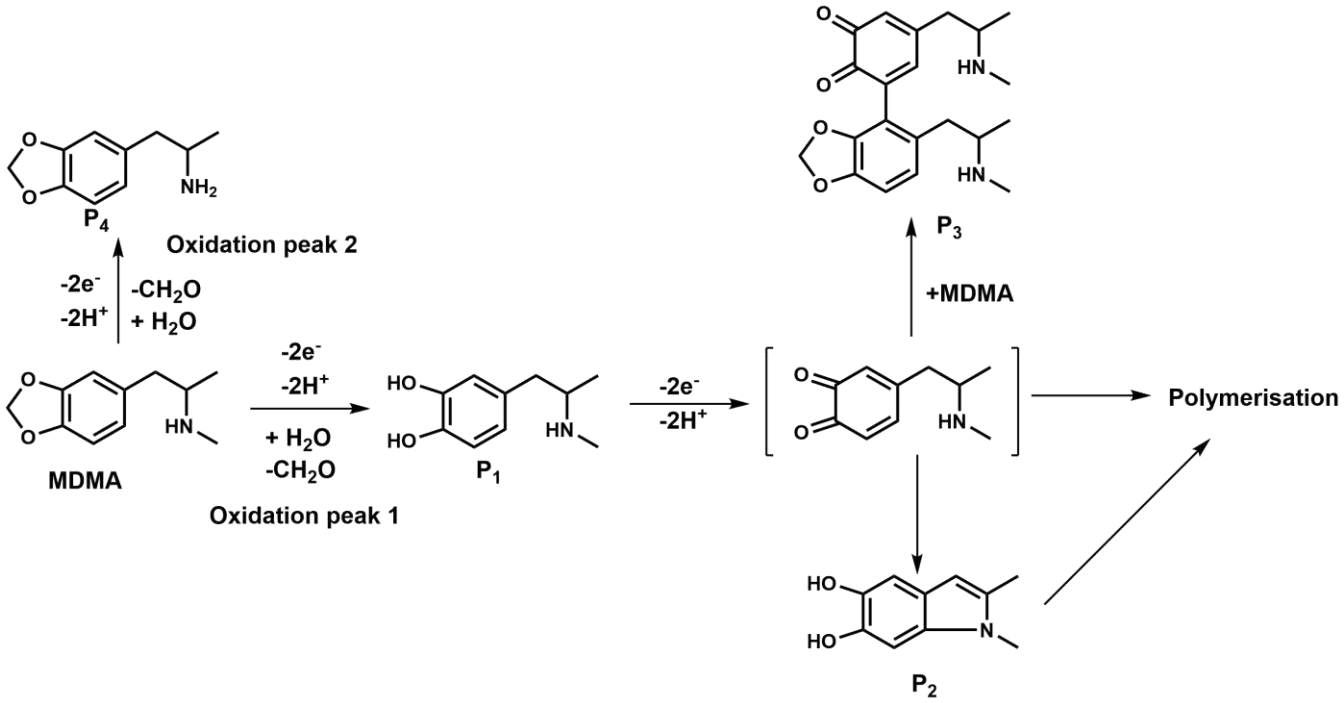

Figure 2. Proposed oxidation pathway of MDMA as elucidated by LC-MS.

3.1 Elucidation of the oxidation pathway of MDMA

When developing an electrochemical sensor, it is important to understand the oxidation processes that take place to comprehend the origin of the signals in the EF. Previous research on MDMA, employing several techniques (cyclic voltammetry, electron paramagnetic resonance), led to two propositions about the oxidation mechanism of MDMA.[41] It was hypothesized that the latter involves the formation of a radical cation, and that polymerization takes place at the electrode surface. However, to fully elucidate the oxidation pathway, a more thorough analysis is necessary. Therefore, for the first time to our knowledge, an LC-QTOFMS analysis on the partially electrolyzed solutions of MDMA was performed to identify possible oxidation products. In total four oxidation products were found. The obtained oxidation products (P1-P4) are listed in **Table S1**.

Interestingly, three out of four products were identified in the electrolysis samples at the more negative potential in both pH 7 (0.92 V) and pH 12 (0.81 V), indicating a complex oxidation mechanism during the first voltammetric peak. We hypothetised that the product from pH 5 (1.11 V) is the same as found in the electrolysis at pH 7 (0.92V). On the contrary, P4 at 3.99 min (*m/z* 180.1016, $C_{10}H_{13}NO_2$) is the only product resulting from the second oxidation peak. Based on the *m/z*-value of P4 and its MS/MS fragmentation spectrum (**Figure S7**), it is concluded to be the product resulting from an oxidative demethylation reaction of the secondary amine (**Figure 2**). An identical reaction was reported for the oxidation of the secondary amine of ketamine.[34] In total, four products were found after electrolysis. P1 and P4 are products that are also formed during the metabolic oxidation of MDMA.[45] P4 is due to the oxidation of the secondary amine at high potentials (oxidation peak 2) as was also shown by comparison of the EPs of MDMA and methamphetamine.[46] However, the oxidation products formed during the first oxidation peak must be related to the oxidation of the benzodioxole group in MDMA. Shanmugam *et al.* already demonstrated the redox activity of this group in the MDMA structure. Moreover, they described a complex underlying mechanism which resulted in a polymer formation on the surface of the working electrode which could explain the generation of the four different products observed by LC-QTOF analysis.[41] In the electrolysed samples, the first product (P1) at 2.47 min was identified as 3,4-dihydroxy methamphetamine based on its *m/z*-value (*m/z* 182.1171) and MS/MS-spectrum (**Figure S7**). Therefore, the initial step revealed a complex oxidation mechanism. The first step in the oxidation is O-demethylation of the benzodioxole group in MDMA into a catechol-group (**Figure 2**), similar to the metabolic pathway of MDMA, resulting in 3,4-dihydroxy methamphetamine, which *m/z* and MS/MS spectrum fits P1 (*m/z* 182.1171, $C_{10}H_{15}NO_2$) at 2.47 min (**Figure S8**).[45]

Moreover, this formed oxidation product P1 strongly resembles dopamine in its structure. The electrochemical mechanism and behaviour of dopamine are described in depth in literature and are known to lead to follow up reactions after its oxidation.[47,48] Therefore, it is likely that product P1 exhibits similar behaviour and consequently, a solution of dopamine was electrolysed and analysed by LC-QTOFMS (**Figure S9**). The resulting main product D1 (*m/z* 150.0550) at 3.75 min is known from literature to be the final product after oxidation of the catechol-group into its keto analogue which undergoes a further cyclisation reaction to form 5,6-dihydroxyindole as its final.[48] Starting from product P1, which is similar to dopamine, a similar mechanism can be followed and would result in the formation of 1,2-dimethyl-1H-indole-5,6-diol which corresponds exactly to m/z-value P2 (*m/z* 178.0857, $C_{10}H_{11}NO_2$). Furthermore, it has been reported that these kinds of molecules undergo auto polymerization resulting in the formation of brown, insoluble polymers.[47] It should be noted that also the

MS/MS-fragmentation of P3 (*m/z* 371.1984, $C_{21}H_{26}N_2O_4$) differs from the other found products (**Figure S7**). P3 is the result of the dimerization reaction of the keto-analogue and a MDMA molecule which eventually allowed us to propose the full oxidation mechanism of MDMA in **Figure 2**, which fits the EPs as well as the polymerisation observed by Shanmugam et al.[41]

3.2 Electrochemical screening of MDMA in binary mixtures

A binary mixture analysis is a crucial tool in the development of a novel screening method for MDMA. It facilitates a thorough evaluation of potential false positives and false negatives that might be caused by cutting agents, other illicit drugs, substances that might be confused with MDMA or agents that are used to make a(n) (ecstasy) pill. Indeed, it is not sufficient to develop a method that can detect MDMA in pure MDMA samples, since suspected illicit drug street samples (i) might contain cutting/mixing agents and (ii) might contain an illicit drug different from MDMA or even a licit compound. It is therefore essential to evaluate that (i) these compounds (cutting agents/other drugs) do not shift or mask the diagnostic signal of MDMA (potential false negative) and (ii) these compounds do not exhibit a signal at the same potential as the diagnostic signal of MDMA (potential false positive).

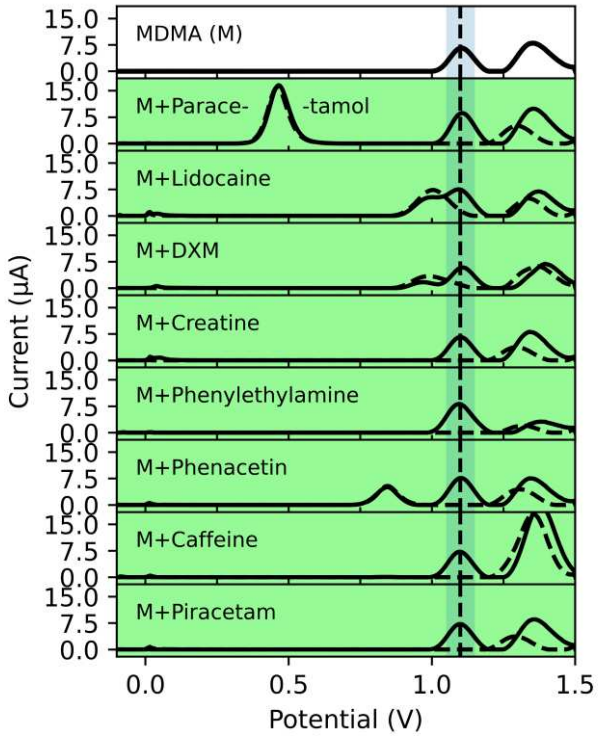

Figure 3. Electrochemical profile (EP) of MDMA binary mixtures in ACE pH 5 at SPE: SWVs of 0.5 mM MDMA with 0.5 mM cutting agents. The dashed line indicates where the signal of MDMA is located. The dashed SWVs indicate the EPs of the respective pure compounds. The detection window (1.05V-1.15V) is highlighted.

In **Figures 3 and 4**, the binary mixture analysis for MDMA is shown. A selection of relevant compounds was selected, i.e. eight common cutting agents and fourteen illicit drugs that are likely to be confused with MDMA (appearance and/or effects). The white voltammogram on top shows the characteristic EP of MDMA in ACE pH 5, with the diagnostic peak around 1.11V. Listed below the EP of MDMA, with the dotted lines, are the EPs of pure cutting agents (**Figure 3**) and pure illicit drugs (**Figure 4**). The equimolar binary mixtures of MDMA with these respective cutting agents and illicit drugs are shown in full lines. For the binary mixtures (full lines), it was checked that the diagnostic signal of MDMA (1.11 V) was not shifted or masked by the cutting agent or illicit drug. As can be seen, none of the investigated compounds exhibits this behaviour, indicating that likely none of the investigated compounds will cause false negatives. When evaluating the pure cutting agents and drugs (dotted lines), it is verified that these compounds do not have a signal in the detection window of MDMA (1.05V-1.15V). Lidocaine and DXM have an oxidation signal at 0.99V and 0.98V, respectively, which is close to, but outside of, the MDMA detection window, and will therefore not cause false positives. 2C-B, a psychoactive compound sometimes found in ecstasy pills, has a signal in the detection window, and might therefore cause a false positive for MDMA. Since 2C-B is an illegal compound, this potential false positive is not a substantial drawback of the novel method.

However, further research has been conducted on this topic within our research group to provide LEA's with the option to diversify between MDMA and 2C-B. A separate strategy was developed to overcome the potential false positive on 2C-B, and has been reported in Van Echelpoel *et al.*[42] Furthermore, multiple (illicit) compounds with similar structure to MDMA were analyzed as well to assess the limitations of the methodology. It is remarkable that several illicit drugs with a very similar structure to MDMA, e.g. MDAI, methylone, PMA and PMMA, do not exhibit a signal that overlaps with the diagnostic signal of MDMA. This is somewhat expected for PMA and PMMA, since these compounds do not share the methylenedioxy-moiety that is linked to the diagnostic signal. However, MDAI and methylone do have this moiety. MDAI has a signal close to the diagnostic signal, probably due to oxidation of the dimethoxy-moiety, but at a slightly different potential due to the difference in structure. A potential reason for this shift is that the ring-closure that is observed for MDMA, is not possible for MDAI since the latter already has this ring in its structure. It is hypothesized that the absence of a signal for methylone might have a similar origin. Schram *et al.* showed that no ring-closure is observed in the oxidation mechanism of methylone.[49] Another possibility is that inductive effects of the keto-moiety of methylone play a role in the absence of a signal in the EP of methylone. Following this reasoning, it is not surprising that MDEA and MBDB do have a signal in the detection window. The sole structure differences between these two compounds and MDMA, are the respective lengths of the alkyl chains connected to the amine-moiety. Since the diagnostic signal is related to oxidation of the methylenedioxy-moiety, and not the amine-moiety, it makes sense that similar electrochemical profiles are observed. However, since MDEA and MBDB are both illicit compounds, and rarely observed, potential false positives for these two compounds do not pose major drawbacks for the methodology. Overall, the binary mixture analysis strengthens us in our buffer choice, as indeed the majority of illicit drugs and cutting agents have few to no signals in the selected buffer.

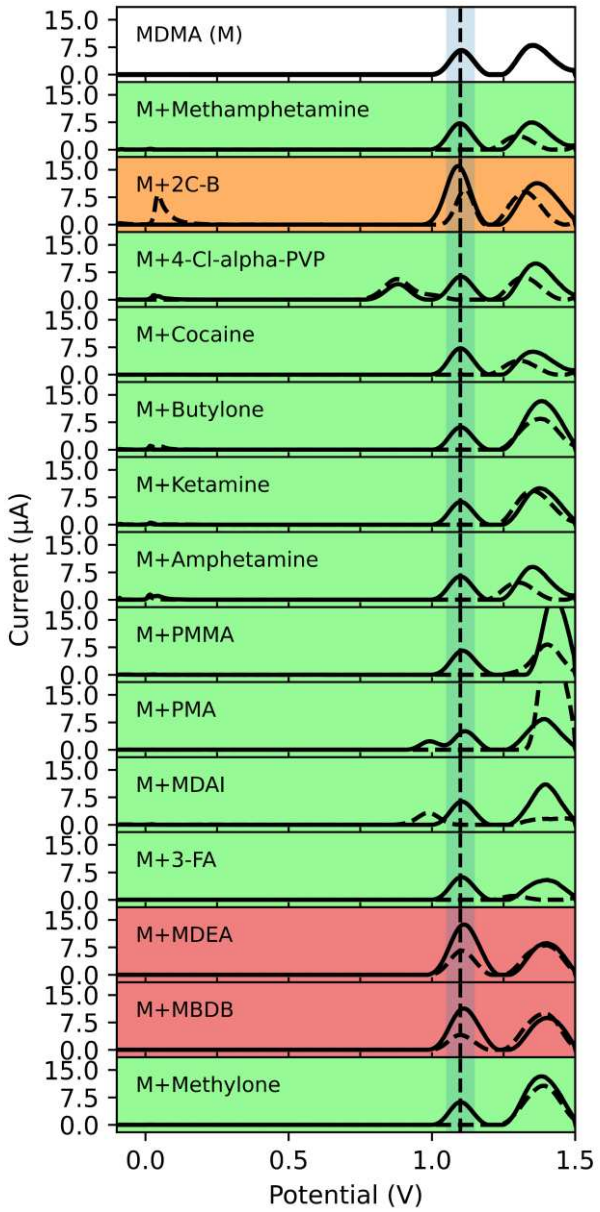

Figure 4. EP of MDMA binary mixtures in ACE pH 5 at SPE: SWVs of 0.5 mM MDMA with 0.5 mM illicit drugs. The dashed line indicates where the signal of MDMA is located. The dashed SWVs indicate the EPs of the respective pure compounds. The detection window (1.05V-1.15V) is highlighted.

3.3 Electrochemical quantification of MDMA in ecstasy samples

Quantification of MDMA in ecstasy samples is imperative. Underestimation of the MDMA content in ecstasy samples can lead to overdosing, and thus even death.[50] It is possible to integrate a quantification module in the electrochemical sensor by employing linear regression. That is, the peak current of the diagnostic MDMA peak can be linked to the MDMA concentration, thereby allowing the determination of purity and thus also absolute MDMA content in an analysed sample. Important for the accuracy of the predicted values is that the weight of the analysed pill and the dissolved part, are carefully measured.

The calibration plot shown in **Figure 1D**, together with the equation of the trend line, i.e. $y = 0.0136 * x + 0.1819$, is used for the quantification module. To quantify a novel sample, the SWV is recorded, baseline-corrected and the peak current of the diagnostic peak is extracted. Via the equation of the trend line, this current is converted to a concentration (Equation 1). The maximum potential concentration is calculated via the weight of the dissolved sample (Equation 2), and compared to the calculated concentration, allowing the determination of the purity (Equation 3). This purity also allows the determination of the absolute MDMA content present in the analysed sample.

$$c_{sample} = \frac{(I_{peak} - 0.1819)}{0.0136} \text{ (Equation1)}$$

$$c_{100} = \frac{m_{sample}}{V * M(MDMA \cdot HCl)} \text{ (Equation 2)}$$

$$Purity = \frac{c_{sample}}{c_{100}} * 100 \text{ (Equation 3)}$$

The temperature has a (minor) influence on the observed peak currents, and thus on the quantification (**Figure S4**). The best results are therefore observed by measuring close to the temperature that was present when the calibration curve was constructed, i.e. room temperature (20 °C – 25 °C).

3.4 Integration of novel methodology in software and mobile app, making the step towards on-site application

An essential part of a novel sensing methodology is that it is operatable to its target audience, here law enforcement personnel. Although highly skilled, members of LEAs are usually not trained in electrochemistry. Therefore, we integrated the novel methodology in the peak recognition approach of Van Echelpoel *et al.*, as such taking away the data analysis from the end-users.[33] The approach performs all steps of the data analysis, from raw electrochemical output, over data processing (baseline correction and digital top hat filter), to eventually a clear indication of the presence/absence of MDMA in the analyzed sample. A detailed description of the peak recognition approach can be found in the previous reference. For this application, the EP of MDMA was added to the internal database. More specifically, an interval (1.05 V-1.15 V) was defined around the diagnostic peak of MDMA at 1.11 V. If a peak is encountered in that specific interval, the sample is said to contain MDMA. The use of an interval allows for the correct identification of the diagnostic peak, even if it has slightly shifted to more positive or negative peak potentials due to changes in concentration or temperature. The width of the interval is defined based on the SWVs of pure MDMA in ACE pH 5 at various concentrations and temperatures, as well as on the SWVs obtained during the binary mixture analysis.

Uniquely, the methodology was subsequently also integrated into a mobile application, guiding the end-user through all steps of the measurement and analysis. The peak recognition algorithm is also integrated in the application, ensuring that no prior knowledge of electrochemistry or even science is required to employ the MDMA sensor. **Figure 5** shows a screenshot of the final output screen, indicating that the analyzed sample contains MDMA. More impressions of the application are shown in **Figure S10**. Furthermore, a video (**Video 1**) was made to demonstrate that the developed MDMA sensor is truly ready for on-site use. The video shows: i) the preparation steps, ii) the sampling method, and iii) the measurement itself.

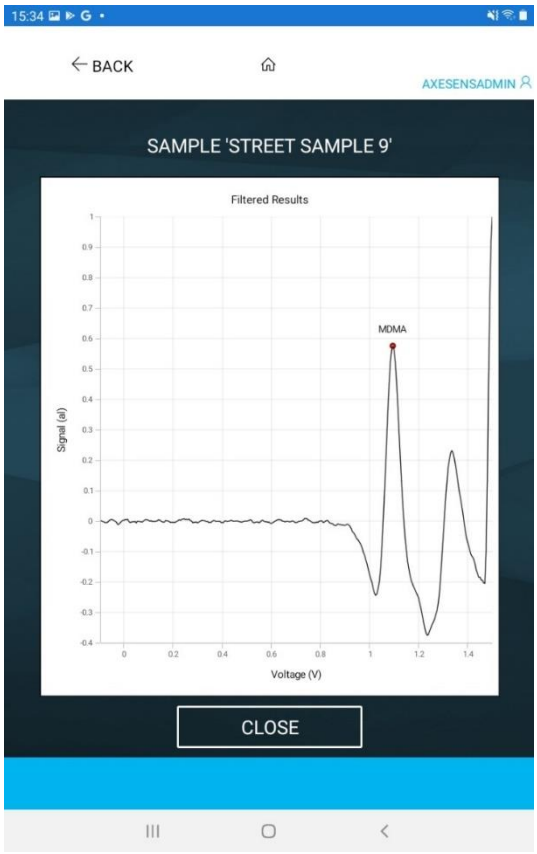

Figure 5. Screenshot of the processed voltammogram of street sample 9 measured in ACE pH 5 buffer, as shown in the mobile application. The diagnostic peak of MDMA around 1.11 V is clearly visible. Other screenshots of the mobile application are shown in **Figure S10**.

3.5 Comparison of novel electrochemical methodology vs portable spectroscopic techniques on 15 street samples

Finally, the novel methodology (with the peak recognition approach and quantification module) was validated on 15 street samples containing MDMA (**Figure 6**). This set of street samples is representative of the illicit ecstasy market, showing colour variations (white, green, red,…), MDMA concentration (19.4%-96.6%) and appearance (powder, crystal and tablet) (**Table S2**). The results of the electrochemical sensor on these street samples are shown in **Table 1**.

It can be seen that each of the 15 street samples was correctly identified as MDMA, demonstrating the great potential of electrochemistry in on-site drug detection. Two of the major benefits of electrochemical sensors, i.e. high sensitivity and indifference to colour or appearance, are underlined by the results. Indeed, low concentration samples pose no difficulties, nor do the variations in appearance between the different samples (**Table S2**). Grace to the integrated software, the data output is automatically processed and transformed into a clear label, easily interpretable by non-experts.

Additionally to the good performance on qualitative analysis, **Table 1** also illustrates an excellent performance on the quantitative analysis part. The electrochemical sensor has a mean absolute error (MAE) of 2.3%, compared to the quantitative results obtained via the golden standard technique, GC-FID. The maximum deviation amounts to 6.6%, and is observed for sample 6. Overall, the addition of the electrochemical quantification module is an asset for the novel methodology. A realistic side note here is that this quantification methodology is currently lab-based, thus not on-site ready, requiring carefull weiging and employing 0.3mg/mL solutions.

Besides the analysis with the electrochemical methodology, the 15 street samples were investigated as well with the current state-of-the-art spectroscopic on-site detection devices: portable Raman and portable ATR-FTIR (**Figures S11 and S12**). Although the latter is indeed portable, it cannot be considered handheld, as it still requires a benchtop for its operation.

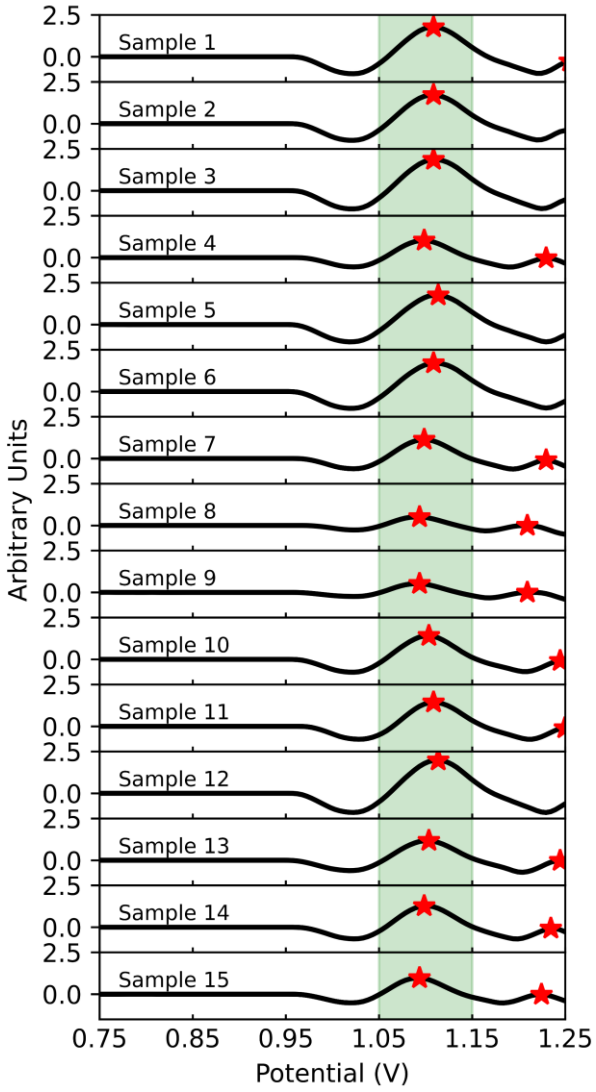

Figure 6. Processed SWVs (black) of MDMA on 15 street sample mixtures in ACE pH 5 at SPE. The red stars indicate the peaks detected by the software application, the green area represents the MDMA interval (1.05 V-1.15 V). If a peak is detected in the selected interval, the analyzed sample is set to contain MDMA. **Figure S13** shows the baseline-corrected SWVs of the street samples.

Contrary to the electrochemical methodology, not all samples are correctly identified as MDMA by the portable Raman device. In fact, only 9 out of 15 samples are correctly identified. Four of the six wrongly identified samples were surprisingly

identified as 2-amino propane, a primary amine. Sample 8, a blue tablet, was wrongfully identified as Phtalo blue, a blue pigment. There seems to be no clear correlation between the false identifications by the Raman device and the appearance/concentration. The portable ATR-FTIR device performs better on the street sample set than the portable Raman device, as it identifies 13 out of 15 samples correctly. Sample 8 is a problem for the ATR-FTIR device as well, identifying the blue ecstasy pill as containing only cornflour and sucrose. A possible explanation for the false identification of sample 8, is its low purity (19.4%). Furthermore, sample 15 is falsely said to contain the illegal drug 5-MeO-DMT.

It must be noted that both the portable Raman and FTIR make use of a library approach, targetting a very broad range of compounds present in the employed library, in contrast to the electrochemical sensor that only targets MDMA. On the other hand, the employed spectroscopic devices do not allow direct quantification of MDMA, something the electrochemical sensor does. Overall, this street sample comparison shows that the electrochemical sensor is a worthy competitor or addition to the portable spectroscopic devices for on-site MDMA detection.

4 CONCLUSIONS

This work presents a novel electrochemical methodology for the on-site detection and quantification of the illicit drug MDMA, commonly found in ecstasy pills. The novel methodology is an improvement over existing electrochemical MDMA sensors since it does not require specifically modified electrodes, nor does it require the use of more than one buffer. Additionally, a data interpretation algorithm performing all data analysis steps is integrated in the methodology, thereby opening up the methodology to non-expert end-users.

After integrating the data analysis algorithm and developing a mobile application, the performance of the methodology was assessed by means of 15 MDMA street samples. Despite a large variation in concentration (19.4%-96.6%) and appearance among these street samples, the electrochemical methodology correctly identified MDMA in all 15 instances. Additionally, the purity of the 15 street samples was calculated with a MAE of 2.3% by employing the quantification module in lab setting. As such, it outperforms two competitive devices, a portable Raman device and a portable ATR-FTIR device, on this same set of street samples (9/15 and 13/15 respectively), especially considering these devices do not provide any direct quantitative information.

These excellent results, combined with the ease of use and integrated data analysis algorithm and mobile app, make the novel electrochemical highly suited for law enforcement personnel, facilitating the decentralisation of forensic analysis.

Table 1. 15 real street samples with various MDMA content and appearance were analyzed with the novel electrochemical methodology (qualitatively and quantitatively), a portable Raman device (Bruker Bravo) and a portable IR device (Bruker Alpha 2).

Sample Name	Sample Content (w/w%)	Appearance	Electrochemical Sensor	Calculated purity (%) (absolute error)	Bruker Bravo (Raman)	Bruker Alpha 2 (FT-IR)
1	MDMA (93.7)	Powder, white	MDMA	89.2 (-4.5)	MDMA crystals	MDMA
2	MDMA (92.6)	Powder, white	MDMA	89.3 (-3.3)	2-amino propane	MDMA
3	MDMA (97.0)	Powder, white	MDMA	96.4 (-0.6)	2-amino propane	MDMA
4	MDMA (41.2)	Tablet, green	MDMA	41.1 (-0.1)	Unknown	Cornflour/MDMA
5	MDMA (95.1)	Powder, brown	MDMA	95.9 (+0.8)	MDMA crystals	Crystal MDMA
6	MDMA (88.0)	Crystals, white	MDMA	94.6 (+6.6)	MDMA crystals	Crystal MDMA
7	MDMA (40.4)	Tablet, rose	MDMA	40.2 (-0.2)	MDMA tablet	Crystal MDMA
8	MDMA (19.4)	Tablet, blue	MDMA	18.6 (-0.8)	Phtalo blue	Cornflour/Sucrose
9	MDMA (24.7)	Tablet, grey	MDMA	18.6 (-6.1)	2-amino propane	Sorbitol/Crystal MDMA
10	MDMA (57.2)	Tablet, turquoise	MDMA	57.4 (+0.2)	MDMA crystals	Crystal MDMA
11	MDMA (54.1)	Tablet, grey	MDMA	52.8 (-1.3)	2-amino propane	Crystal MDMA
12	MDMA (96.8)	Crystals, white	MDMA	98.4 (+1.6)	MDMA crystals	Crystal MDMA
13	MDMA (39.5)	Tablet, orange	MDMA	35.9 (-3.6)	MDMA crystals	Crystal MDMA
14	MDMA (54.4)	Tablet, yellow	MDMA	50.0 (-4.4)	MDMA crystals	Crystal MDMA
15	MDMA (35.3)	Tablet, red	MDMA	35.5 (+0.2)	MDMA crystals	Cornflour/5-MeO-DMT

ASSOCIATED CONTENT

Supporting Information

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All authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

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