Towards developing a screening strategy for ecstasy : revealing the electrochemical profile

ARTICLE

Towards developing a screening strategy for ecstasy: revealing the electrochemical profile

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Abstract: This article describes the development of an electrochemical screening strategy for 3,4-methylenedioxymethamphetamine (MDMA), the regular psychoactive compound in ecstasy (XTC) pills. We have investigated the specific electrochemical profile of MDMA and its electro-oxidation mechanisms at disposable graphite screen-printed electrodes. We have proved that the formation of a radical cation and subsequent reactions are indeed responsible for the electrode surface passivation, evidenced by using electron paramagnetic resonance spectroscopy and electrochemistry. Thereafter, pure cutting agents and MDMA as well as simulated binary mixtures of compounds with MDMA were subjected to square wave voltammetry at pH 7 to understand the characteristic electrochemical profile. An additional measurement at pH 12 was able to resolve false positives and negatives occurring in pH 7. Finally, validation of the screening strategy was done by measuring a set of ecstasy street samples. Overall, our proposed electrochemical screening strategy has demonstrated a rapid, sensitive and selective detection of MDMA resolving most of the false positives and negatives given by the traditional Marquis colour tests, thus exhibiting remarkable promises for the on-site screening of MDMA.

Introduction

Amphetamine-type stimulants (ATS) are globally prevalent in the field of synthetic drugs with an international pattern of supply and demand.[1] Amphetamine (AMP), methamphetamine and "ecstasy" (XTC) belong to the class of ATS, with the XTC market becoming increasingly multifaceted.[2,3] The main psychoactive compound in XTC tablets is 3,4-methylenedioxymethamphetamine (MDMA). According to the 2021 world drug report, three main types of XTC are available: ecstasy tablets with a high content of MDMA, tablets containing little or no MDMA, and XTC sold in crystals/powder form under different street names.[4] Until 2010, the XTC drugs were frequently adulterated by various cutting agents and/or cheaper chemicals such as caffeine, amphetamine, procaine, paracetamol, ketamine, dextromethorphan (DXM) and bath salts,[5–8] essentially to mimic the stimulant effects. Besides the psychoactive adulterants, XTC tablets might also contain excipients such as diluents, binders, lubricants disintegrators, dyes or flavourings and sometimes prescription drugs.[9] According to the latest world drug report 2021, the content of MDMA in XTC tablets increases yearly, particularly in the European market, with an estimate of 50-70 million tablets produced in the European Union and EU-member states yearly.[4] Adding to the trend of increasing the purity of MDMA pills, there has also been an increase in the size of some of the tablets available.[2] With this ever-changing vogue of drug product compositions, it is of crucial importance to develop a detection strategy for increasingly diversified ecstasy products.

The prevention of drug trafficking benefits from a highly selective detection of drugs of abuse in the field. A two-fold approach is traditionally followed to identify the drug in seized samples: (i) screening with presumptive tests, and (ii) confirmatory lab analysis. Presumptive tests enable an on-site screening of suspicious samples. Law enforcement agencies (LEAs) use portable rapid testing kits, typically colour tests, with the Marquis colour test kit being the test of choice for ecstasy identification.[10] A Marquis colour test is based on the formation of a purple to a black coloured complex containing two carbenium ions when MDMA reacts with the sulfuric acid in the presence of formaldehyde.[11] However, selectivity issues are reported showing false positives or false negatives with the use of presumptive colour tests.[12–14] Moreover, uncertainty in the qualitative analysis is given by subjective judgements due to individual perceptions of colours. Therefore, the forensic analyst must confirm a positive colour test with additional laboratory tests for any legally controlled compound.[13] Alternative analytical methods such as Fourier Transform Infra-Red (FTIR) spectroscopy and Raman spectroscopy have been employed as an initial screening approach for ATS followed by GC/LC-MS (Gas Chromatography/Liquid Chromatography-Mass Spectrometry) (qualitative), GC-FID (Gas Chromatography-Flame Ionization Detection) (quantitative) as confirmatory analysis.[15] Indeed, portable Raman, FTIR and mass spectrometers have been proposed for use in airports/customs and at crime scenes for the rapid analysis of illicit drugs.[16,17] However, this instrumentation is rather expensive, bulky and mostly requires specialized

personnel to interpret the results. Thus, there is a great interest in developing fast, easy-to-use, portable and reliable screening methods to on-site detect ecstasy drugs by law enforcement personnel.

Due to its sheer simplicity, affordability, portability and fast analysis, electrochemical detection is an inviting approach for the selective detection of target compounds in many fields. Electrochemical sensors have broadly been used for forensic analysis,[18] and particularly in the detection of drugs of abuse.[19,20] For example, screen printed electrodes (SPEs) have been used to determine amphetamine[21] and heroin[22] in confiscated samples. Moreover, few studies report on the electrochemical detection of MDMA and related substances.[23–32] For example, Garrido et al. explored the electrochemical oxidation of amphetamine-like drugs and its application to the electroanalysis of ecstasy in human serum.[28] In 2014, Tadini et al. developed chemically modified electrodes to detect MDMA by voltammetry.[29] A year later, Cumba et al. used SPEs for the simultaneous detection of MDMA and para-methoxyamphetamine (PMA).[31] Recently, the oxidation mechanism was demonstrated on boron-doped diamond electrode with differential pulse voltammetry by Teófilo et al.[32] However, an extensive study on the influence of a wide range of cutting agents and excipients on the electrochemical detection of XTC products has not yet been conducted. Moreover, the electrode mechanism behind MDMA oxidation at graphite screen printed electrodes (G-SPEs) has not been explored in detail.

In this work, we report the development of an electrochemical sensor using G-SPEs to detect MDMA in ecstasy pills, adulterated powder or crystal forms, based on the electrochemical profile of MDMA at pH 7. An additional test, based on the electrochemical profile of MDMA at pH12, is also developed to boost the accuracy of the methodology if necessary. First, the electrode processes observed at G-SPEs are investigated in aqueous buffer solutions. During the electrochemical oxidation, the formation of a highly unstable radical cation was hypothesized and supported by electron paramagnetic resonance (EPR) experiments using *N-tert*-butyl-*α*-phenylnitrone (PBN) as the spin-trap. Spin-traps react with the highly unstable radical cation and convert them into more stable radical species that are detectable by EPR. Subsequently, the passivation of the electrode surface during the electrochemical screening of MDMA, caused by these oxidized products, is studied with electrochemistry. Second, the effect of cutting agents on the electrochemical signal of MDMA is investigated. The cutting agents of interest are psychoactive adulterants, excipients and other related compounds. Both the sensitivity and specificity of this approach are compared with the results obtained from the Marquis colour tests and commercially available XTC colour test kits to demonstrate the strength of the approach. Finally, the results from the electrochemical analysis of confiscated MDMA street samples are validated with laboratory standard methods (i.e., GC-MS and GC-FID). Herein, the electrochemical screening method allows the rapid and low-cost profiling of MDMA in seized samples, which ultimately will allow easy on-site discrimination of ecstasy samples by LEAs.

Results and Discussion

Electro-oxidation of MDMA at G-SPE

To define the electrochemical profile of MDMA in ecstasy (XTC) samples, the voltammetric behaviour of MDMA at G-SPEs was first explored in phosphate-buffered saline (PBS) using three electrochemical techniques, i.e. cyclic voltammetry (CV), linear sweep voltammetry (LSV) and square wave voltammetry (SWV). Initially, CV was performed at pH 7 as a first screening method to understand the redox behaviour of MDMA in the measuring conditions. The resulting cyclic voltammogram presented an irreversible oxidation peak at +1.06 V and an anodic feature at +1.26 V (**Figure 1a**). Linear sweep voltammograms of 1 mM MDMA in pH 7 buffer solution with varying scan rates (**Figure S1 and S2**) confirmed that the electrode process of the main oxidation peak is diffusion controlled. Subsequently, SWV was employed as the electroanalytical technique to enhance the peak resolution and sensitivity of MDMA analysis. Moreover, a baseline-corrected SWV indicated the characteristic but more pronounced oxidation peak at +1.04 V (peak P1) (**Figure 1b**). The peak current P1 increases with MDMA concentration (**Figure S3),** with a linear range of 0.005 – 1 mM and a slope of 35.4 μA mM^{-1}, and importantly, without any critical change in the electrochemical profile. Besides, excellent reproducibility was displayed for 0.4 mM (RSD=0.4%, N=3). The limits of detection (LOD) and quantification (LOQ) of MDMA at pH 7 were found to be 15 μM and 52 μM, respectively. This makes SWV with a single scan (from negative to positive potentials) the preferred voltammetric technique for this study.

Following the choice of the voltammetric technique, it is important to comprehend the influence of the pH on the oxidation process and to define the electrochemical profile of MDMA at different pH conditions. For this purpose, SWV was performed for 1 mM MDMA in PBS over a broad pH range (pH 1-13) (**Figure S4**). The characteristic peak (P1) was observed throughout the entire pH range, slightly shifting to more negative potentials as the pH increases (**Figure 1c**). Besides, an anodic shoulder appears at +1.26 V starting from pH 7 (P2) leading to a well-defined peak at alkaline conditions, as well as another oxidation shoulder appeared at lower potentials at basic pH (P3). These peaks define the profile of MDMA at different measuring pH conditions. To better understand the process of MDMA oxidation behind these peaks, as a first step, structurally similar compounds (i.e. MDEA, PMK and BZX) were subjected to SWV at pH 7 (**Figure 1d**) and pH 12 (**Figure 1e**).

Comparing the resulting voltammograms to chemical functionalities present in the molecules and the electrochemical studies reported previously for MDMA on glassy carbon electrode and boron doped diamond electrodes, we can attribute the voltammetric peaks to certain oxidation processes.[28,32] The peak P1 can be linked to the oxidation of the methylenedioxy-functionality present on the aromatic ring (similar to MDEA, PMK and BZX). Moreover, this peak has been ascribed to the oxidation of the aromatic nuclei of the molecule to form radical cations. Thus, the anodic shoulder peak P2 (+1.26 V) starting from pH7, clearly visible at pH 12 **(Figure 1e)** for MDMA, MDEA, PMK and BZX can evidently be attributed to the further oxidation of the polymeric species from the radical cation generated earlier. The additional peak (P3) appears at +0.77 V as a shoulder of P1 when the pH is greater than the pKa value of MDMA (pKa= 9.9, strongly basic)[33] in MDMA and MDEA, whereas they are absent for PMK, BZX and AMP. This suggests that P3 is related to the oxidation of the secondary amine linked to the aromatic ring with methylenedioxy-

functionality. Future research, involving mass spectrometry, will clarify the existence of these peaks and the underlying oxidation mechanism. From the above experiments, it is also clear that the electrochemical profile of MDMA can be enriched by changing from pH 4 (one redox process), to pH 9 (two redox processes) and further to pH 12 (three redox processes).

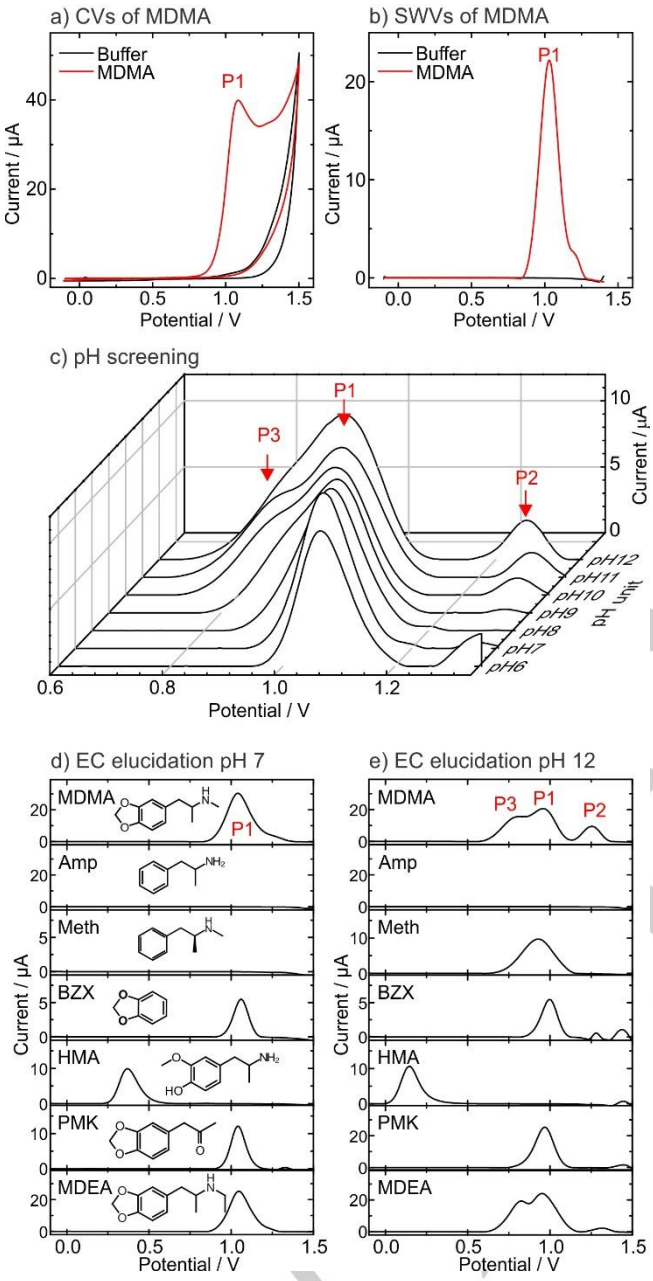

***Figure 1**. (a) Cyclic voltammogram and (b) baseline-corrected square wave voltammogram of 1 mM MDMA in PBS solution (pH 7) at a G-SPE, scan rate 50 mV/s (black), (c) baseline-corrected SWVs of 1 mM MDMA in PBS solutions at different pHs (6-12), and (d) baseline-corrected SWVs of 1mM MDMA and structurally related compounds at pH 7 and (e) pH 12 (right) PBS solution. The chemical structures of the respective compounds are indicated at the side of the voltammograms. The dotted lines show the typical oxidation peak of MDMA appearing at both pH 7 and pH 12.*

To evidence the formation of a radical cation in the oxidation of MDMA, EPR was employed. EPR spectroscopy being a valuable tool to detect and identify free radical cations formed at the electrode, provides vital information about the mechanism of MDMA oxidation. We have utilized this technique by analysing the samples after performing a short *ex-situ* electrolysis at G-SPEs using PBS. Due to the fact that the methylenedioxy-functionality is both oxidized in pH 7 and 12, an EPR study was only performed in pH7 solution. 1 mM MDMA was subjected to amperometry at +0.98 V for 30 minutes using G-SPE at ambient conditions. The solution (25 µL) was then transferred to a small capillary tube for EPR measurements. No detectable signals were seen (**Figure 2**) possibly due to the high instability of radical cations formed in the aqueous solution. To characterise the radical cations, an excess amount of PBN was added to the solution acting as a spin-trap, which reacts with the reactive radical and forms a more stable radical detectable by EPR. **Figure 2** shows the X-band cw EPR spectra (in black, accumulated over 40 scans) of MDMA with excess PBN. The spectra appear as a triplet of doublets, which agrees with nitroxide radicals formed by the reaction of PBN with radicals.[34] Besides, the spectra of control experiments measured using similar parameters showed insignificant EPR signals.

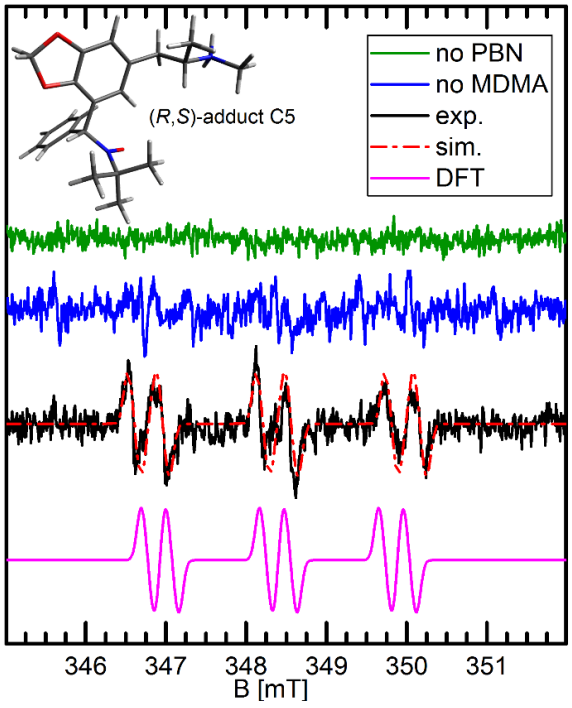

***Figure 2.** X-band cw EPR spectra of 1 mM MDMA.HCl with 50 mM PBN after controlled potential electrolysis in PB solution at pH 7 (black), the corresponding control experiments in the absence of MDMA (green) or PBN (blue), simulation of the experimental spectra (red), and the spectra of (R,S)-adduct C5 derived from density functional theory (DFT) calculations (magenta). The parameters for the simulations were g_{iso} =2.0055, A_N =45.0 MHz, $A_{H\alpha}$ =9.8 MHz, and the calculations were g_{iso} =2.0054, A_N =41.5 MHz, $A_{H\alpha}$ =8.5 MHz. A geometry optimised molecular model of (R,S)-adduct C5 is also depicted.*

Parameters such as isotropic g value (g_{iso}) and the hyperfine interactions (A_N, $A_{H\alpha}$) between the unpaired electron and the

adjacent magnetic nuclei were obtained from the simulation of the spectra. The values of isotropic ^{14}N and ^{1}H hyperfine coupling constants ($A_{N=}$ 45.0 MHz, $A_{H\alpha}$= 9.8 MHz), are suggestive of carbon-centred radicals that have been trapped by PBN.

A series of molecular models of the possible PBN-MDMA radical adducts (**Figure S5**) were constructed and their geometries were optimised by density functional theory (DFT). The choice of basis sets and functionals were based on the previously published method.[35] The series included adducts formed by spin-trapping at the three different unsubstituted carbons (C2, C5 or C6) on the proposed aromatic radical cation, as well as diastereomers formed by the spin-trapping reaction on racemic MDMA. The models were also protonated ($pK_{a(MDMA)}$ ~ 9.9 [36]). The corresponding EPR parameters were calculated for each optimised structure as well as thermally allowed conformers, which were then averaged (**Table S1** and **S2**). From these calculations, the EPR parameters of the PBN-MDMA radical adducts where the spin-trapping occurred at the C5 ((*R*,*R*)-adduct C5 and (*R*,*S*)-adduct C5) were in close agreement with the experimental data (**Figure 2**, **Table S2**), suggesting these to be the most likely products, providing strong evidence of radical cation formation in the MDMA oxidation process.

Radical adducts with PBN at C5 suggest that unstable radical cation, in the absence of any spin trap, would undergo rapid dimerization or polymerisation and/or passivate the working electrode surface in an electrochemical setup. To prove this, repetitive SWV scans of 1 mM MDMA at the same electrode were carried out. Indeed, it was observed that the current of the oxidation peak decreases consecutively (**Figure S6**) indicating passivation of the electrodes by the oxidation products of MDMA formed during the previous scan. It has been shown that binding of any organic layer to the electrode's surface decreases the electron transfer rate of the oxidation (hence the decrease in peak intensity) and shifts the peak potential toward more positive potentials [37,38] as is also observed in **Figure S6**. Those observations provide evidence of the formation of a passivation layer by the radical formation after the SWV, which correlate with our EPR findings. The formation of a passivation layer following the electron transfer step and also further oxidation of the formed products at a higher potential was further confirmed by cyclic voltammetry measurements using a well-known redox system, potassium ferrocyanide ($K_4[Fe(CN)_6]\cdot 3H_2O$) (**Figure S7**).[39,40] Future research with mass spectrometry will help to further understand the underlying oxidation mechanism and add more insights into this fundamental study.

Electrochemical analysis of XTC related compounds

Following the mechanistic elucidation, it is also essential to assess the analytical capability of the proposed screening method. We evaluated the efficiency of the technique to detect MDMA in the presence of common adulterants and cutting agents found in street samples.[2,5–8] Therefore, the voltammetric behaviour was firstly investigated in binary mixtures to detect possible overlaying peaks in the electrochemical profile (EP) region of MDMA in PBS at pH 7 by SWV. A positive result for MDMA was considered if the peak potential of the signal fits into the profile region of MDMA (E_p value between 1.053 V - 1.034 V). Firstly, common excipients or cutting agents (i.e. lactose, glucose, myo-inositol, starch, cellulose, mg stearate and tabletting mixture) were electrochemically analysed in PBS pH 7 (**Figure S8a**) and with the binary mixture of MDMA (i.e., 1 mM equimolar concentrations) (**Figure S8b**). As expected, the cutting agents without any electroactive moiety did not exhibit any interference in the MDMA EP.

As the peak potential depends strongly on the potential of the reference electrode, when using a disposable electrode with quasi Ag reference. Any presence of chloride in the excipients might affect the position of the peak potential thus leading to false negatives. To address this issue, we tested the influence of having NaCl as an excipient in measuring MDMA (**Figure S8c**). It can be seen that despite having an addition of 1 mg mL^{-1} NaCl in the measuring buffer, there was no noticeable peak shift in the voltammogram.

A series of psychoactive compounds (encountered in XTC tablets) was analysed and compared with the profile of MDMA for interference assessment (**Figure 3**). For this reason, pure compounds and binary mixtures of MDMA with potential interferent (equimolar concentration at 1 mM) were interrogated in PBS pH 7 by SWV. Most of the investigated compounds did not pose problems for MDMA detection displaying peaks outside the profile region of MDMA. For example, potentially dangerous designer drugs such as PMA and PMMA showed an oxidation peak at 1.25 V, while new psychoactive substances such as mCPP and pCPP (piperazines) showed an oxidation peak in a more negative potential region than MDMA (at 0.83 V). Nonetheless, methylenedioxy-related (MDx) compounds, such as MDEA and MBDB, share a methylenedioxy functionality with MDMA, and thus oxidise at the same potential, resulting in false positives. Bath salts such as butylone, methylone and MDPV, exhibiting close peak potentials to MDMA, overlap with MDMA EP in the binary mixture, thus resulting in a true positive for MDMA. However, mephedrone can become a false positive as the oxidation peak is allocated at the same potentials as MDMA. Opioids such as morphine and heroin show peaks near the profile region of MDMA, although exhibiting a prominent peak for MDMA when present. A challenge is raised by cocaine, which oxidizes at 1.038 V, overlapping with the signal of MDMA and thus resulting in a false positive in the absence of MDMA. Interestingly, mCPP shifted the MDMA oxidation peak towards a more positive potential, leading to false negatives. Adulterants (i.e., caffeine and DXM) did not hinder the determination of MDMA, although DXM might produce a suppression effect in MDMA signal. The peak potentials of these compounds and MDMA mixtures at pH 7 are presented in **Table S4**. To overcome the issues of false positives and negatives, the effect of pH 12 on the electrochemical response of these compounds and mixtures was further investigated.

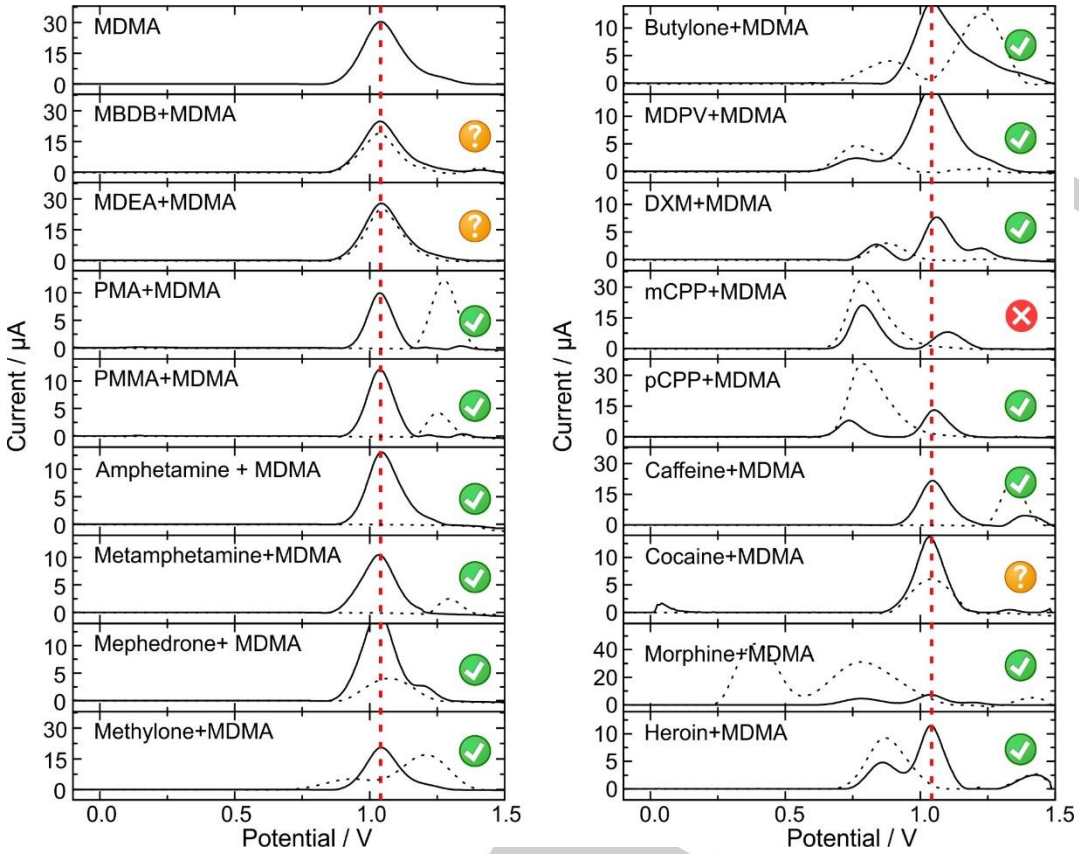

***Figure 3**. SWVs for MDMA related psychoactive compounds in 1mM standard solutions (dotted curves) and as binary mixtures with 1 mM MDMA (full curves) in PBS pH 7 at G-SPEs. The question mark indicates overlapping signals of the compounds with MDMA signal, resulting in a false positive. Cross indicates peak shift and/or suppression of the MDMA signal in binary mixtures, resulting in a false negative.*

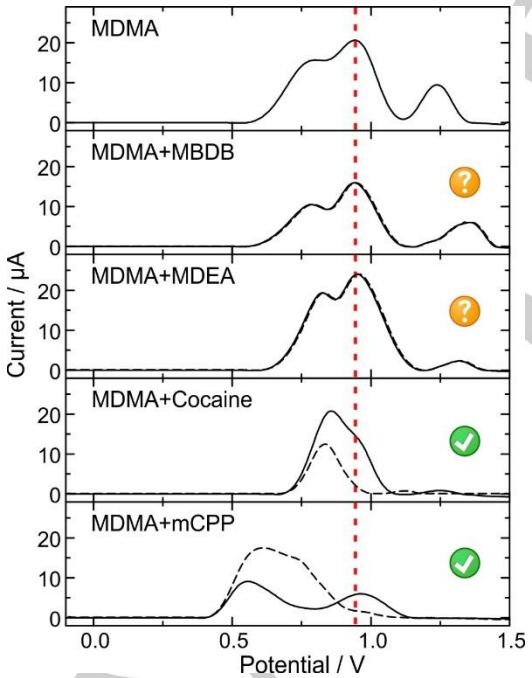

***Figure 4**. SWVs for MDMA related psychoactive compounds as 1 mM standard solutions and as binary mixtures with 1 mM MDMA in PBS pH 12 at G-SPEs.*

Presumptive colour tests versus electrochemical strategy for MDMA analysis

To define the potential of electrochemistry for the on-site sensing of ecstasy, the SWV results of the XTC related compounds and mixtures were compared with regular on-site tests (i.e., presumptive colour test) obtained with both laboratory-made Marquis reagent and commercially available Marquis test kits. **Table 1** compares the qualitative results of the presumptive colour tests (tested in powder form) with the respective results from SWV method. A more detailed description of the tests either pure compounds and binary mixtures are listed in **Table S4**. For both the in-lab Marquis test and EZ test, a positive result is considered when a discolouration into dark purple occurs.

As expected, apart from MDMA, the colour of the other solutions containing the methylenedioxy type (MDx) compounds such as MBDB and MDEA turned into dark purple giving a false positive (FP) result. Besides the MDx compounds, DXM, cocaine, codeine, heroin and morphine also gave a dark purple result (FP) due to the complexation with formaldehyde in the presence of the strong acid. The remaining compounds without the characteristic colour change resulted in negative (N) results. The colour tests gave a positive result for MDMA in binary mixtures with other MDx compounds, piperazines (mCPP, pCPP), PMA and PMMA. For MDMA mixed with mephedrone, butylone, methylone, MDPV, amphetamine and methamphetamine, a false negative test result was obtained indicating that a correct interpretation of mixtures is

more hallenging compared to performing a colour test on single compounds.

The sensitivity of the colour test, electrochemical approach at pH 7 and electrochemical approach at pH 12 for the binary mixtures and pure compounds was determined to be 62.5%, 87.5% and 93.75% respectively. Whereas the specificity was determined to be 63.16%, 78.95% and 89.47%, respectively. This indicates that the electrochemical approach is highly sensitive in detecting the presence of MDMA in mixtures, especially at pH 12, in comparison to presumptive colour tests. However, in future work, the ideal approach would be to have an electrochemical methodology that differentiates MDMA from other drugs as well with only one test.

Table 1. Presumptive colour tests versus SWV results.

Sample content	Colour tests result		SWV result	
	Marquis test	EZ test	pH 7	pH 12
MDMA (M)	TP	TP	TP	TP
MBDB	**FP**	**FP**	**FP**	**FP**
MBDB + M	TP	TP	TP	TP
MDEA	**FP**	**FP**	**FP**	**FP**
MDEA + M	TP	TP	TP	TP
PMMA	TN	TN	TN	TN
PMMA + M	TP	TP	TP	TP
PMA	TN	TN	TN	TN
PMA + M	TP	TP	TP	TP
Amphetamine	TN	TN	TN	TN
Amphetamine + M	**FN**	**FN**	TP	TP
Methamphetamine	TN	TN	TN	TN
Methamphetamine + M	**FN**	**FN**	TP	TP
Mephedrone	TN	TN	TN	TN
Mephedrone + M	**FN**	**FN**	TP	TP
Butylone	TN	TN	TN	TN
Butylone + M	**FN**	**FN**	TP	TP
Methylone	TN	TN	TN	TN
Methylone + M	**FN**	**FN**	TP	TP
MDPV	TN	TN	TN	TN
MDPV + M	**FN**	**FN**	TP	TP
DXM	**FP**	**FP**	TN	TN
DXM + M	TP	TP	TP	TP
mCPP	TN	TN	TN	TN
mCPP + M	TP	TP	**FN**	TP
pCPP	TN	TN	TN	TN
pCPP + M	TP	TP	TP	TP
Caffeine	TN	TN	TN	TN
Caffeine + M	TP	TP	TP	TP
Morphine	**FP**	**FP**	TN	TN
Morphine + M	TP	TP	TP	**FN**
Cocaine	**FP**	**FP**	**FP**	TN
Codeine	**FP**	**FP**	**FP**	TN
Heroin	**FP**	**FP**	TN	TN
Ketamine	TN	TN	TN	TN

TN, true negative; TP, true positive; FN, false negative; FP, false positive

Electrochemical analysis of street samples

The electrochemical detection of MDMA in 21 random ecstasy street samples was carried out at pH 7. The additional test in pH 12 was not executed here because the additional accuracy it could provide, does not outmatch the added time and effort for this set of ecstasy street samples. This is especially because the cases that are resolved by pH 12, i.e. cocaine and mCPP, are unlikely to be encountered in this street sample set (which were all XTC pills). Cocaine's appearance is different from that of ecstasy, and mCPP has lost its popularity over recent years. It has not been named in any EMCDDA drug report in the past decade, a trend attributed to bad user experiences.[41] The results were compared with confirmatory tests from regular laboratory methods (i.e., GC-MS and GC-FID). **Table 2** shows the qualitative output of the electrochemical measurement and composition of these samples validated by standard techniques. It can be observed that all the MDMA containing samples gave the desired "Positive" result. Sample 10 with MDEA also displayed a "Positive" result (false positive, FP) due to MDEA having the same oxidation potential as that of MDMA. However, this does not represent a major problem as MDEA is also an illicit substance. Remarkably, samples containing illicit drugs such as ketamine, amphetamine and even mCPP were correctly identified as "Negative". Additionally, the methamphetamine precursor APAAN was also subjected to analysis and correctly identified as "Negative". For the 21 random street samples tested, the accuracy of the electrochemical method was 95%, showing high promises for the use of the proposed method in the field.

Table 2. Electroanalysis of MDMA in random street samples at pH 7.

Sample number	SWV pH 7	Sample composition[a]
1	TP	tablets containing 39.7 % MDMA
2	TN	powder containing amphetamine (87.1%) and ketamine (0.9%)
3	TP	47.0 % MDMA
4	TP	MDMA (% unknown)
5	TN	white powder containing starch and no psychoactive compounds
6	TN	beige powder sample containing APAAN (% Unknown)
7	TN	white tablet containing mCPP (% Unknown)
8	TN	red tablet with no psychoactive compounds
9	TN	yellow tablet with no psychoactive compounds
10	FP	powder containing MDEA (% unknown)
11	TN	chunks of yellow paste containing amphetamine (26.1%) and caffeine (50%)
12	TP	white tablet containing MDMA (% Unknown)
13	TP	red tablet containing MDMA (% Unknown)
14	TP	powder containing MDMA (% Unknown)
15	TP	white powder containing MDMA (10%)
16	TP	white powder containing MDMA (15%)

17	TP	yellow tablet containing MDMA (26.7%)
18	TP	blue tablet containing MDMA (25.6%)
19	TN	amphetamine sulfate, caffeine powder
20	TN	38.5% amphetamine sulfate, 27.8 caffeine, 24.9% 3-FA powder
21	TN	68.1% amphetamine, 29% caffeine, 0.3% 3-FA powder

[a]data obtained from NICC (confirmed with GC-MS and GC-FID)

Conclusion

We have demonstrated a rapid and selective electrochemical screening to detect MDMA in XTC pills and powder in confiscated samples by using unmodified G-SPE. Moreover, the MDMA oxidation at G-SPEs has been explored by EPR and SWV, unravelling the oxidation processes occurring during the electrochemical interrogation. Subsequently, the determination of the EP of MDMA and XTC related compounds provides all necessary analytical information to detect MDMA in the presence of other substances or to identify tablets sold as XTC that do not contain MDMA. Remarkably, our proposed strategy of electrochemical screening by performing SWV at pH 7 has demonstrated to be more sensitive and selective detection of MDMA either in pure or binary mixtures when compared to other on-site methods such as the Marquis colour tests. Besides, a secondary electrochemical test at pH 12 can resolve some potential false positives that might occur in the method at pH 7. Finally, the electrochemical strategy has been validated with 21 street samples of different compositions and compared with laboratory standard methods (GC-MS and GC-FID) showing outstanding accuracy for a rapid on-site determination of MDMA in seizures and/or cargos. The focus of future work will be to further explore the oxidation processes using mass spectrometry. In addition, a novel screening strategy will be attempted that preserves the accuracy of the proposed method (pH7 + pH12 analysis), but integrated into a single test to maximize the user-friendliness of the approach. Overall, the developed electrochemical strategy represents progress for the portable detection of illicit drugs in the field which will set the next army of tools to hinder drug trafficking and drug consumption among society.

Experimental Section

Reagents and sampling

Psychoactive standards such as d,l-MDMA·HCl, 3,4-methylenedioxy-N-ethylamphetamine (d,l-MDEA·HCl), d,l-MBDB·HCl, d-amphetamine·HCl, methamphetamine·HCl, para-methoxymethapmphetamine (d,l-PMMA·HCl), d,l-PMA·HCl, ketamine·HCl, mephedrone·HCl, 1-(3-chlorophenyl)-piperazine·HCl (mCPP·HCl), 1-(4-chlorophenyl)-piperazine·HCl (pCPP·HCl), butylone·HCl, methylone·HCl, cocaine·HCl, heroin and 3,4-methylendioxypyrovalerone·HCl (MDPV·HCl) with purity >98.5% were purchased from Lipomed (Arlesheim, Switzerland). DXM was purchased from Sigma-Aldrich (Diegem, Belgium). PMK (piperonyl methyl ketone) was provided by the National Institute for Criminalistics and Criminology (NICC) in Belgium. Caffeine and excipients such as lactose, glucose, myo-inositol, starch, cellulose, and magnesium stearate were purchased from VWR Chemicals (Leuven, Belgium). 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. 1,3-Benzodioxole (BZX) was purchased from Sigma-Aldrich (Belgium); 1,3-Benzodioxole (BZX) was purchased from Sigma-Aldrich (Belgium); sulfuric acid and formaldehyde (37%) used in the colour tests were purchased from Merck and Sigma-Aldrich (Belgium), respectively. 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). The pH of these buffer solutions was adjusted with KOH and H_3PO_4 solutions to reach the desired pH (pH 2 – pH 13). All aqueous solutions were prepared using Milli-Q water (R > 18 MΩcm). The spin-trap PBN (> 98%) was purchased from TCI Europe N.V.

The XTC related compounds were subjected to both colour tests and electrochemical analysis as individual compounds and binary mixtures with MDMA (1:1). For real samples analysis, such as ecstasy pills, tablets were crushed or scrapped with a spatula for collecting the sample (approximately 1 mg) and dissolved in 1 mL PBS pH 7 in a 1.5 mL tube.

Electrochemistry

Electrochemical measurements were performed using an Autolab PGSTAT101 potentiostat with NOVA software. Disposable graphite screen-printed electrodes (G-SPEs) were purchased from ItalSens or PalmSens (Utrecht, The Netherlands). The G-SPEs consist of a graphite working electrode (geometric area of 7.07 mm^2), a carbon counter electrode and a silver reference electrode on a flexible polyester support. Measurements were performed in a 50 µL drop placed on the G-SPE. Voltammetric techniques such as linear sweep voltammetry (LSV) and cyclic voltammetry (CV) were carried out for a better understanding of the electrochemical behaviour of MDMA on G-SPEs. Both CV and LSV of MDMA were performed in the potential window of -0.1 V to 1.5 V vs Ag/AgCl at a scan rate of 50 mV/s and the CV of the redox probe, potassium ferrocyanide ($K_4[Fe(CN)_6]\cdot 3H_2O$) was carried out in the potential window of -0.4 V – 0.6 V at 50 mV/s. Square wave voltammetry (SWV) was used for unravelling the electrochemical profile of all substances owing to its high sensitivity. SWVs were corrected for the background current by a moving average principle, integrated into the NOVA 1.11 software. All electrochemical measurements were performed at room temperature. The SWV parameters were optimized by studying the variation of the peak currents with the square wave frequency, pulse amplitude and step potential. The optimized parameters are: frequency 10 Hz, amplitude 25 mV and step potential 5 mV. The potential was swept from -0.1 V to 1.5 V vs Ag/AgCl.

A detailed protocol to prepare sample and analyse street samples is now mentioned in the supplementary information Section S8.

Electron paramagnetic resonance spectroscopy (EPR)

EPR measurements were carried out with a 0.9 mm inner diameter capillary tube containing the electrolysed solution positioned in a TE102 cavity in a Bruker E580 Elexys spectrometer. The EPR spectra are measured at X-band in continuous-wave (CW) mode (~9.7 GHz) with a microwave power of 5 mW, 0.1 mT modulation amplitude and 100 kHz modulation frequency. The measurements were carried out at room temperature. The EPR spectra were simulated with Matlab2017a using the EasySpin-5.1.11 module.[42] DFT calculations were performed using the ORCA package [43] adapting the method described by D. Pauwels et al.[35] (see supplementary information).

Colour tests

In-lab Marquis colour test kits with concentrated sulphuric acid and 37% formaldehyde were prepared in the laboratory and the tests were conducted according to the United Nations recommended guidelines.[13] Commercial Marquis colour tests for ecstasy were purchased from EZ Test (Amsterdam, The Netherlands). Colour tests were performed according to the producer's instructions, by adding little sample material (i.e., in powder form), about the size of a pinhead to the test vial, mixing and observing the colour change visually.

The sensitivity and specificity of the Marquis colour tests and electrochemical detection were evaluated based on the following formulae;

$$sensitivity = \left(\frac{n(P)}{n(P)+n(FN)}\right) * 100 \quad \text{(eq. 1)}$$

$$specificity = \left(\frac{n(N)}{n(N)+n(FP)}\right) * 100 \quad \text{(eq. 2)}$$

Where n(*P*), n(*F*), n(*FP*) and n(*FN*) indicates the number of true positives, true negatives, false positives and false negatives, respectively. As a strategy for sampling ecstasy pills, tablets were crushed or scrapped with a spatula for collecting the sample and dissolving in a 1 mL PBS in an 1.5 mL tube.

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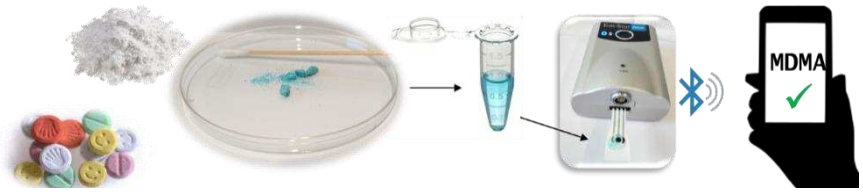

Understanding the electrochemical oxidation mechanism of methylenedioxymethamphetamine (MDMA) and developing a screening strategy for street samples led to electrochemical profiling of MDMA.

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