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Electrochemical detection of MDMA and 2C-B in ecstasy tablets using a selectivity enhancement strategy by *in-situ* derivatization

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ABSTRACT

Forensic drug laboratories are confronted with increasing amounts of drugs and a demand for faster results that are directly available on-site. In addition, the drug market is getting more complex with hundreds of new psychoactive substances (NPS) entering the market in recent years. Rapid and on-scene presumptive drug testing therefore faces a shift from manual colorimetric tests towards approaches that can detect a wider range of components and process results automatically. Electrochemical detection offers these desired characteristics, making it a suitable candidate for on-site drug detection. In this study, a two-step electrochemical sensor is introduced for the detection of MDMA and 2C-B. Firstly, a direct electrochemical analysis was performed to detect MDMA. Validation experiments on over 70 substances revealed that 2C-B was the only frequently encountered drug that gave a false positive result for MDMA in this first analysis. A second step using *in-situ* derivatization was subsequently introduced. To this end, formaldehyde was used for *N*-methylation of 2C-B thereby enhancing its electrochemical profile. The enriched electrochemical fingerprint in the second step allowed for clear differentiation between MDMA and 2C-B. The applicability of this approach was demonstrated with 71 ecstasy tablets seized by the Amsterdam Police. The MDMA/2C-B sensor correctly identified all 39 MDMA-containing tablets and 10 out of 11 tablets containing 2C-B. Most notably, correct results were also obtained for dark colored tablets in which both spectroscopic analysis and colorimetric tests failed due to obscured signals.

Introduction

From 2014 onwards, a rising trend is reported for seized amounts of amphetamine-type stimulants, ecstasy and cocaine both globally and in Europe [1–2]. In addition, the drug market also further diversified with over 1000 different new psychoactive substances (NPS) emerging in the last decade [2–3]. This increase in both size and complexity of the drugs-of-abuse market necessitated the need for detection methods that produce results directly on-site and that can deal with a wider range of substances. Reliable and rapid detection provides opportunities for prompt decisions such as an arrest involving pre-detention, the issue of a search warrant or the start of advanced investigative measures. This may therefore help to more effectively combat the illicit-drug market.

The current common strategy for presumptive testing of ecstasy tablets is a colorimetric spot test with Marquis reagent [4–5]. This reagent comprises of formaldehyde in concentrated sulphuric acid and yields a dark purple to black color with MDMA-containing samples. Although easy to use, inexpensive and readily available, this test has several drawbacks. Firstly, concentrated sulphuric acid holds a safety risk for the investigative officers and Marquis tests are therefore often sold as single use pouches or ampoules containing a small amount of reagent. This both increases the cost per sample and the amount of waste that is produced. Secondly, the Marquis test is not very specific and many other drugs (both licit and illicit) also produce a colored reaction

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product that may lead to false positive results [6]. NPS may also be missed or misidentified since many of these produce a yellow or orange color. These colors also correspond to several generic substances (*i.e.* sugar, ibuprofen) [7]. Thirdly, the result of the color test may be obscured or misinterpreted by strongly colored samples as may be the case in smuggling scenarios [8] or esthetically designed tablets. Another general drawback of chemical tests, such as all colorimetric spot tests, is their subjective result as the color needs to be assessed, interpreted and registered by a human operator. This limits the possibilities of this technique to be implemented in a digital remote forensics setting with automated processing, validation and reporting. Such an approach may speed-up the total forensic process and ultimately eliminate the need for inefficient sample transport and logistics [9].

Spectroscopic techniques such as attenuated total reflectance Fourier transform infrared (ATR-FTIR) [10–11], Near InfraRed (NIR) [11–13] and Raman spectroscopy [14–16] are also suitable for on-site presumptive drug-testing, and various handheld devices specifically designed for this purpose are currently available. Both FTIR and Raman spectra are highly diagnostic for organic compounds, making the technique applicable for field testing of a broad range of substances [17]. However, limitations arise when samples have a dark color or are composed of multiple substances that show overlapping spectral signals [8,11,16]. In general, limits of detection between 10 and 40 wt% are reported for these direct spectroscopic techniques [12–13,16] where the higher limits of detection may be explained by strong signals from excipients partly obscuring the signal from the main active ingredient [16]. A limitation specific for Raman spectroscopy is interference by fluorescence. Both impurities, excipients and active ingredients (*e.g.* MDMA) may produce strong fluorescent signals that obscure the less abundant Raman signals and hamper detection and identification [15]. Contrary to cocaine samples that typically have a white powder-like appearance, most ecstasy tablets have intense and vivid colors that may influence direct spectroscopic detection.

Electrochemical sensors are not affected by the spectroscopy-associated issues since they are based on redox characteristics instead of light absorbance or emittance of the material analyzed. The applicability of portable electrochemical sensors in the forensic drug testing field is demonstrated for several common drugs of abuse such as cocaine [18–19], heroin [20] and ketamine [21]. Specific electrochemical profiles can also be established for NPS, either as individual compounds [22–23] or for specific classes such as cathinones [24]. Since electrochemical screenings are rapid and may be applied simultaneously using an array of electrodes, selectivity issues from identical electrochemical profiles can be overcome by application of subsequent electrochemical strategies. De Jong *et al.* [25] for instance applied such an approach to differentiate the frequently encountered cutting agent levamisole from cocaine.

A limitation of electrochemical sensors is their inability to detect primary amines, since these are not electrochemically active within the potential range used in these devices. Parrilla *et al.* [26] introduced a derivatization approach to convert electrochemically inactive amphetamine into a detectable secondary amine by derivatization with 1,2-naphthoquinone-4-sulfonate. Another approach to enrich the electrochemical fingerprint (EF) of primary amines was introduced by Schram *et al.* [27] In this study, those functionalities were converted into their redox active *N*-methylated and *N*-dimethylated species by reaction with formaldehyde. This strategy has also been used in this work to discriminate between MDMA and 2C-B. Indeed, in this study, among 70 NPS and other drug-related substances, 2C-B was found to yield an electrochemical response comparable to MDMA and therefore poses a risk of misidentification. Since both MDMA and 2C-B are among the most abundant active substances found in ecstasy tablets, a dedicated approach was developed to differentiate these compounds. To this end, the EF of the primary amine 2C-B was enhanced by *in-situ N*-methylation with formaldehyde. The effectiveness of this approach was demonstrated on a set of 71 seized tablets of various shape, color and composition that were confiscated and analyzed by the Amsterdam Police in 2020. Comparison with NIR and Raman spectra showed the advantages of electrochemical detection especially for the more intensely colored tablets.

Materials and methods

Chemicals

Water (purified, Ph.Eur.), formaldehyde 35 wt% in H_2O (formalin) and sodium acetate were obtained from Sigma-Aldrich (Overijse, Belgium). 3,4-Methylenedioxymethamphetamine (MDMA), 4-bromo-2,5-dimethoxyphenethylamine (2C-B) and a wide range of 72 other drugs-of-abuse, pharmaceuticals, excipients and adulterants mentioned in Table S1 were provided by the Police Laboratory and originated from either high purity casework samples which identity was confirmed by the laboratories validated GC–MS and FTIR methods or from commercial reference materials.

Disposable carbon ItalSens IS-C Screen Printed Electrodes (SPE) were purchased from PalmSens (Utrecht, The Netherlands) and were used during all electrochemical measurements. The SPEs contain an internal silver pseudo reference electrode and a carbon counter electrode. A 0.1 M acetate pH5 buffer was used for all electrochemical measurements. The set of 71 tablets originated from different forensic casework samples that were seized by the Amsterdam Police in 2020. A picture of all tablets is shown in Fig. S1 of the Supplemental Information. Tablet colors and individual tablet weight can be found in Table S2. Note that sample numbers #15 and #103 were omitted due to duplicates in the original set. In total, 39 samples were MDMA-containing and 32 samples did not contain MDMA, but another synthetic drug (either controlled or uncontrolled). Tablets were crushed using a spoon and the resulting powder was used for testing. The active ingredient identities were established using the Amsterdam Police laboratory's validated GC–MS methods reported elsewhere [28–29]. It must be noted that the ratio of MDMA-containing and non-MDMA-containing tablets does not reflect the actual ratio in casework. In 2020, over 94% of the tablets analyzed by the Police Laboratory contained MDMA. The number of non-MDMA-containing tablets was deliberately increased to provide insight in the possible false positives in ecstasy tablets. The composition of the tablets in the set was as follows: 39x MDMA, 11× 2C-B, 4× 2-bromo-4,5-DiMethoxyPhenEthylAmine (2-Br-4,5-DMPEA), 7× FluoroAmphetamine (FA), 5x FluoroMethAmphetamine (FMA), 1x mephedrone, 1× pentylone, 1x 2C-B-fly, 1x *meta*-ChloroPhenylPiperazine (mCPP), 1× 6-AminoPropylBenzofurane (6-APB). It should be noted that mixtures of multiple active ingredients were not represented in this study. Although hardly observed in seized tablets, these samples may occur in forensic illicit-drug related casework. [30] The set of ecstasy tablets comprised of over 60 different designs (*i.e.* color, shape, imprint).

Instruments and settings

Electrochemical measurements, more specifically square wave voltammetric analyses, were carried out using a PalmSens4 potentiostat with PSTrace 5.7 software (Utrecht, The Netherlands). The optimized SWV 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 versus Ag/AgCl. Since no reverse scan is executed in SWV, only the oxidation of the analytes is considered in this work. A tailored-made peak recognition script was used to process the raw data generated with the potentiostat into a clear-cut interpretation and representation thereof. The script contains a database of compounds, only those compounds included in this database are targeted by the sensor. Comparative NIR and Raman scans were acquired using a 900–1675 nm range microNIR spectrometer (VIAVI Solutions, San Jose, CA, USA) and a TruNarc Handheld Narcotic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a 785-nm laser and detecting the Raman shift in the 300–1800 cm^{-1}

wavenumber range. Preprocessing for NIR and Raman data was an inversed 2nd derivative with a Savitzky-Golay filter.

Methods

Original MDMA method (MDMA sensor)

The sample set was tested using an electrochemical MDMA sensor developed at the De Wael research lab. The electrochemical sensor employs square wave voltammetry (SWV), as its core scientific technology. In voltammetry, a varying potential is imposed on a sample solution, after which the current resulting from oxidation/reduction processes in the sample solution, is measured. Specifically for SWV, a combined square wave and staircase potential is employed, resulting in an increased sensitivity over other voltammetric techniques. The sensor subsequently employs an in-house developed script to process the SWV output [31]. This script is a vital part of the sensor, as it interprets the SWV output signal and provides a clear-cut interpretation and representation thereof, interpretable by non-experts. Overall, the sensor is tailor-made towards MDMA detection, and accordingly confirms the presence or absence of MDMA in the analyzed sample.

Fig. 1 depicts the procedure that was followed for each measurement. First the sample was dissolved in a 0.1 M acetate buffer pH5 at a 0.3–0.4 mg/mL concentration (*e.g.* between 1.5 and 2 mg of sample in 5 mL buffer solution), the latter contains supporting electrolytes and ensures a constant pH. Subsequently, a droplet (~0.05 mL) of the resulting solution is placed on the screen printed electrode (SPE) surface, covering all three electrodes. The SPE is connected with a potentiostat, which in turn is connected (wired or via Bluetooth) to a measuring device (laptop, smartphone or tablet). A software application is installed on this measuring device, integrating the control over the electrochemical measurement with the in-house analysis script. The analysis software applies two steps. Firstly, a preprocessing tandem, including a moving average baseline correction and a digital top hat filter transformation, improves peak separation. Secondly, the script identifies the peaks present, and compares the identified peaks with an internal database. The detection of one or more compounds is based on the presence of associated diagnostic peaks in the EF. Details of this data analysis process are described in the article of Van Echelpoel *et al.* [31]. Since the sensor in this application is tailored towards MDMA detection, only MDMA is included in this database. The EF of MDMA contains one single peak around 1.11 V, thus if the EF of the measured sample after preprocessing contains a peak around this potential, the sample is said to contain MDMA.

To summarize, after sampling ~ 2 mg of suspicious material the measurement is initiated with a single click on the software application, the EF is recorded, interpreted by the in-house script and a final decision (MDMA/NO MDMA) is shown on the display. This whole procedure takes around one minute. Hereafter, a new measurement can be started quickly as this only involves disposing the old SPE and placing a new SPE into the potentiostat.

MDMA/2C-B-sensor

The method for MDMA/2C-B detection is similar as the method described for the MDMA sensor with the following three exceptions: 1. An increased sample concentration of ~ 2.5 mg/mL was used due to the expected lower active ingredient content in 2C-B-containing tablets. 2. directly after adding 5 mL of buffer to the sample, another 2.5 mL of formalin was added (or equivalently, if less buffer was used). The sample was vortexed for 10 s followed by a delay time of 30 s. The latter is necessary to give the formalin time to react with the sample. 3. During EF analysis of the preprocessed signal, two peaks at 0.95 V and 1.14 V were selected as diagnostic for derivatized 2C-B. The peak at 1.11 V is still used for MDMA detection.

Results and discussion

Influence of color on electrochemical and spectroscopic sensors

Synthetic drugs, such as MDMA and 2C-B, are commonly distributed and encountered in the form of illegally produced tablets. These tablets occur in a large variety of colors and shapes, typically showing imprints of logos from famous brands (*e.g.* expensive car brands, perfume or designer brands, superheroes). The diversity in ecstasy tablets is also visible in the selection of seized case tablets (Fig. S1) used in this study. The presence of intense and vivid colorants in these tablets may hamper direct spectroscopic analyses since photons from the light source may be absorbed or fluorescence from samples may obscure diagnostic signals (*i.e.* Raman spectra). Pure MDMA and all 71 tablets (crushed to powder) were subjected to handheld NIR, handheld Raman, the Marquis test and electrochemical analysis. For NIR spectra, MDMA specific spectral features were visible in the majority of tablets. However, the level of detail in the spectra varied by the color of the material. Especially darker colored tablets (black, purple) yielded less intense signals as can be seen in Fig. 2A. In the obtained Raman spectra, background fluorescence seriously hampered detection as shown by the major offset of especially the purple spectrum (originating from a purple ecstasy tablet) in Fig. 2B. For some tablets, fluorescence -either from MDMA itself or the tablet excipients- was so much severe that noisy spectra above the limits of the plot were observed. It was also noted that heat generation from the absorption of the laser light caused the powder from six of the darker colored tablets to burn, creating a black burn mark in the sample. This showed that direct spectroscopic analysis of ecstasy tablets may be cumbersome for colored formulations. It must however be noted that direct Raman analysis is often not suggested for MDMA-suspected materials because of the known fluorescent nature of MDMA. For this, devices using longer excitation wavelengths (*i.e.* a 1064 nm laser) that produce less fluorescence or dedicated Surface-enhanced Raman Spectroscopy (SERS) kits are suggested [15]. Treatment with Marquis reagent yielded a black color for all MDMA-containing tablets and a lime-green color for all 2C-B containing tablets. However, for most tablets their respective color was also to some extent reflected in the reagent masking the reaction product or producing a false-positive color. A

Fig. 1. Sampling and analysis procedure for the electrochemical MDMA sensor.

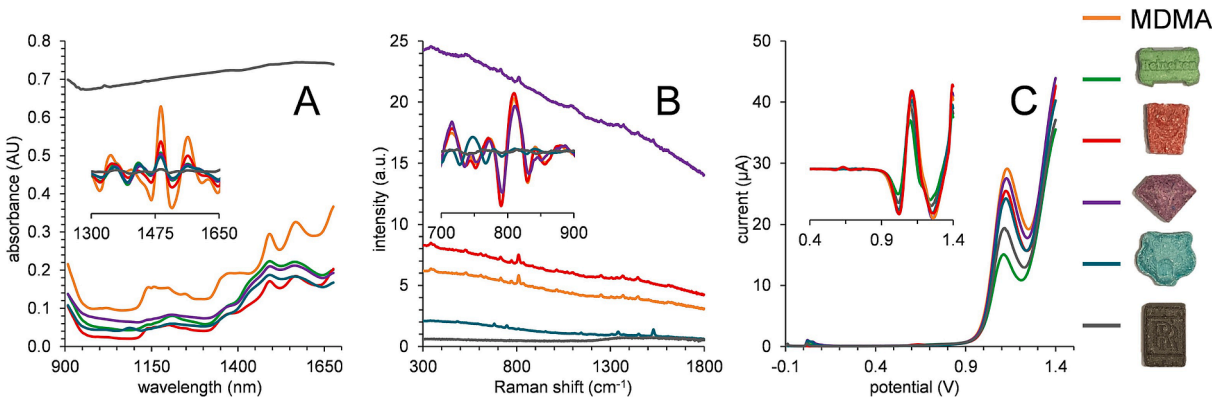

Fig. 2. Influence of sample color on MDMA detection shown for pure MDMA (orange plots) and 5 MDMA-containing ecstasy tablets. NIR (A), Raman (B) and electrochemical analysis using the MDMA sensor (C). Insets depict preprocessed data of the most diagnostic part of the spectrum to emphasize limitations for certain colors. *Note that the Raman signal for the green tablet is missing due to saturation caused by fluorescence.*

trained laboratory technician was able to correctly assign the result by also taking the rate and intensity of the color reaction into account. This nevertheless poses a risk for misidentification when less experienced staff is involved, as may be the case in on-site testing outside of the forensic laboratory. Contrary to the spectroscopic techniques, electrochemical analysis produced signals that were completely independent of the sample color (Fig. 2C) but only depended on the amount of sample taken into account. This definitely showed a benefit for electrochemical testing over colorimetric and spectroscopy-based testing for intensely colored samples. The indifference towards color is not surprising, since the methodology is based on the oxidation of the analytes in the sample, and the sensor thus is not influenced by the color of the sample.

Selectivity of the MDMA sensor

Table 1 shows the results of the electrochemical MDMA-sensor for the set of 71 tablets. The corresponding EFs can be found in Table S2 of the Supplemental Information. All 39 tablets containing MDMA were correctly identified by the sensor. Since the MDMA samples in the set are representative for the ecstasy market in 2020, it can be concluded that

Table 1
Results of both electrochemical sensors on the ecstasy tablet set containing 39 MDMA tablets (sample ID 1–40) and 32 tablets containing another active ingredient (sample ID 101–133). Results in green are true positives or true negatives, results in red are false positives for MDMA, results in orange are false positives for 2C-B.

TABLET ID	IDENTITY	COLOR	MDMA SENSOR	MDMA/2C-B SENSOR
1	MDMA	grey	MDMA	MDMA
2	MDMA	purple	MDMA	MDMA
3	MDMA	pink	MDMA	MDMA
4	MDMA	blue	MDMA	MDMA
5	MDMA	beige	MDMA	MDMA
6	MDMA	orange	MDMA	MDMA
7	MDMA	turquoise	MDMA	MDMA
8	MDMA	pink	MDMA	MDMA
9	MDMA	yellow	MDMA	MDMA
10	MDMA	white	MDMA	MDMA
11	MDMA	purple	MDMA	MDMA
12	MDMA	brown	MDMA	MDMA
13	MDMA	pink	MDMA	MDMA
14	MDMA	orange	MDMA	MDMA
16	MDMA	red	MDMA	MDMA
17	MDMA	white	MDMA	MDMA
18	MDMA	purple	MDMA	MDMA
19	MDMA	yellow	MDMA	MDMA
20	MDMA	grey	MDMA	MDMA
21	MDMA	pink	MDMA	MDMA
22	MDMA	grey	MDMA	MDMA
23	MDMA	pink	MDMA	MDMA
24	MDMA	green	MDMA	MDMA
25	MDMA	blue	MDMA	MDMA
26	MDMA	blue	MDMA	MDMA
27	MDMA	purple	MDMA	MDMA
28	MDMA	pink	MDMA	MDMA
29	MDMA	grey	MDMA	MDMA
30	MDMA	grey	MDMA	MDMA
31	MDMA	pink	MDMA	MDMA
32	MDMA	orange	MDMA	MDMA
33	MDMA	green	MDMA	MDMA
34	MDMA	yellow	MDMA	MDMA
35	MDMA	orange	MDMA	MDMA
36	MDMA	pink	MDMA	MDMA
37	MDMA	green	MDMA	MDMA
38	MDMA	beige	MDMA	MDMA
39	MDMA	green	MDMA	MDMA
40	MDMA	orange	MDMA	MDMA
101	2C-B	green	MDMA	2C-B
102	4-MMC	yellow	/	/
104	2C-B	oker	/	2C-B
105	2C-B	beige	MDMA	2C-B
106	2-Br-4,5-DMPEA	red	/	/
107	2-Br-4,5-DMPEA	pink	/	2C-B
108	FA	pink	/	/
109	FA	orange	/	/
110	2C-B	green	MDMA	MDMA
111	2C-B	green	MDMA	2C-B
112	4-FMA	yellow	/	/
113	2-Br-4,5-DMPEA	pink	/	2C-B
114	2C-B	yellow	MDMA	2C-B
115	pentylone	orange	/	2C-B
116	2C-B	green	MDMA	2C-B
117	FMA	green	/	/
118	2C-B	pink	MDMA	2C-B
119	2C-B	green	MDMA	2C-B
120	2C-B	yellow	MDMA	2C-B
121	2C-B	green	MDMA	2C-B
122	2-Br-4,5-DMPEA	red	/	/
123	FA	orange	/	/
124	FA	pink	/	/
125	FMA	brown	/	/
126	4-FA	white	/	/
127	2C-B-fly	pink	/	/
128	FMA	pink	/	/
129	FA	salmon	/	/
130	FMA	blue	/	/
131	mCPP	pink	MDMA	/
132	6-APB	pink	/	/
133	4-FA	pink	/	/

the sensor is robust to changes in color, concentration, form and adulteration. The indifference of the MDMA sensor towards variations in color again is a major advantage over the spectroscopic detection devices.

Unquestionably, the merit of a sensor is not solely defined by its true positive identifications. A correct negative result for samples that don't contain MDMA, is of equal (if not higher) importance to prevent erroneous decisions in the criminal justice system. Over seventy substances that may be encountered in forensic settings (Table S1) were selected to assess the selectivity of the MDMA sensor. These substances comprised of common drugs-of-abuse, licit pharmaceuticals, NPS and adulterants. Fig. 3 shows the EFs of the most frequently encountered substances compared to MDMA itself. The sensor results and all other EFs can be found in Table S1 in the Supplemental Information. A total of 66 out of 74 substances were detected as negative for MDMA, indicating the good selectivity of the MDMA sensor. It is clearly visible in Fig. 3B that 2C-B is the only substance with a signal at the same position as the 1.11 V peak diagnostic for MDMA. Although differences were also notable (*e.g.* the absence of a valley at 0.96 V for 2C-B) the similarities led to false positive results for this compound. This is a drawback of the MDMA-sensor since 2C-B is a compound that, just as MDMA, is commonly encountered in seized tablets. It is hypothesized that the similarity between the dimethoxy-group of MDMA and the two methoxy-groups of 2C-B generate a very similar EF, which in turn causes the script to give false identifications of the 2C-B samples. Other false positive detections were only observed for some rarely occurring MDMA analogues that also contained the 3,4-methylenedioxy-moiety and mCPP. Similar results were obtained for the seized casework tablets (Table 1), unfortunately 2C-B (10 FPs out of 11 samples) and mCPP (1 FP out of 1 sample) did cause false positives. Even though both compounds are illegal substances in most countries, the false identification of MDMA for these two compounds is undesirable. For example, in the case of an overdose it is important that the medical staff treating the patient knows which psychoactive compound has been consumed. Therefore, further steps were taken to overcome these false positives, which are described in the following paragraph.

Finally, it is noteworthy to highlight that the MDMA-sensor did not identify any of the four samples containing 2-Br-4,5-DMPEA (Table 1, tablets 106, 107, 113 and 122) as having MDMA in it. This compound, 2-Br-4,5-DMPEA, is an isomer of 2C-B, the compound responsible for several false positive outcomes. From a research point of view, this electrochemical selectivity between two isomers is fascinating. Clearly, the substitution pattern on the benzene ring has a substantial influence on the oxidation mechanism of both compounds. Moreover, from a forensic point of view, this is an exciting result as well. In some countries, including the Netherlands, 2-Br-4,5-DMPEA is a legal substance, whereas 2C-B is not. The selectivity of the electrochemical MDMA sensor could therefore offer an added value in a forensic strategy to distinguish these two isomers.

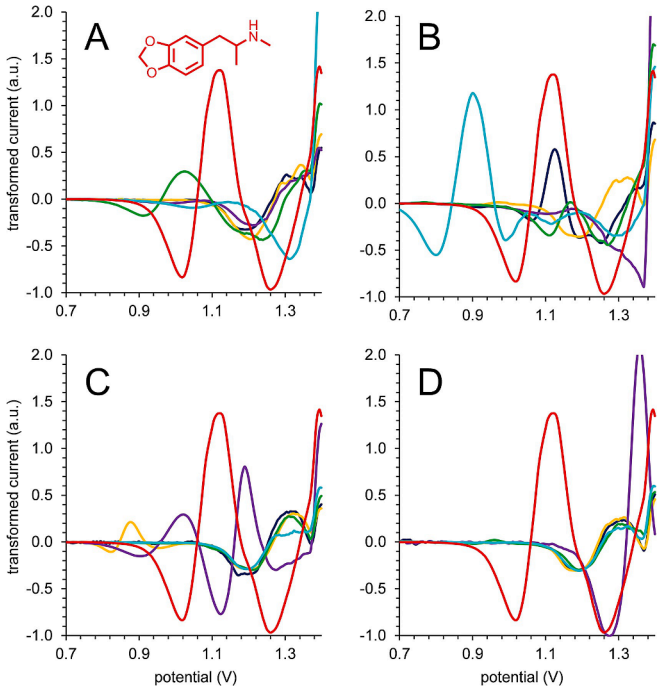

Fig. 3. Electrochemical fingerprints (EFs) following baseline correction and top hat filter transformation as preprocessing. MDMA (red trace) overlayed with common drugs (panel A): amphetamine (dark blue), methamphetamine (orange), cocaine (purple), heroin (green), ketamine (light blue); synthetic drugs (panel B): 2C-B (dark blue), 4-FA (orange), mephedrone (purple), methylone (green), alpha-PVP (light blue); regular drugs in tablets (panel C): paracetamol (dark blue), aspirin (orange), sildenafil citrate (purple), oxazepam (green), methylphenidate (light blue); common adulterants (panel D): lactose (dark blue), mannitol (orange), vitamin C (purple), flour (green) and inositol (light blue).

Electrochemical fingerprint enhancement for MDMA/2C-B differentiation

The MDMA-sensor, as described in the previous paragraph, performed very well on the sample set. However, there was one compound that did cause multiple false positives: 2C-B. Ideally, the sensor could be upgraded to an improved sensor which can correctly distinguish between these substances. To achieve this, the EF of MDMA should be diversified from the EF of 2C-B. An electrochemical pretreatment has proven to be successful in the past for similar cases [26], however a more appealing approach is an *in-situ* derivatization of one of the target compounds. Schram *et al.* recently reported the *in-situ* methylation of the primary amine of amphetamine using formalin to convert the redox inactive amphetamine into the redox active methamphetamine and dimethampetamine [27]. A similar opportunity arises here, as 2C-B contains a primary amine while MDMA does not. Indeed, *in-situ* derivatization with formalin changes the EF of 2C-B, but leaves the EF of MDMA almost unaffected (Fig. 4). Reproducibility studies show that the signals at 0.95 V and 1.14 V in the derivatized EF of 2C-B can reliably be used for identification of this compound. The EF of MDMA remains unaltered by the formalin, and the peak at 1.11 V, used by the MDMA sensor, can thus still be used for MDMA identification. The derivatization approach achieves the desired goal, *i.e.* generating a different EF for MDMA and 2C-B, thereby creating the opportunity to develop a MDMA/2C-B sensor. Integration in the identification software of the EFs of MDMA and 2C-B after derivatization realizes the new MDMA/2C-B

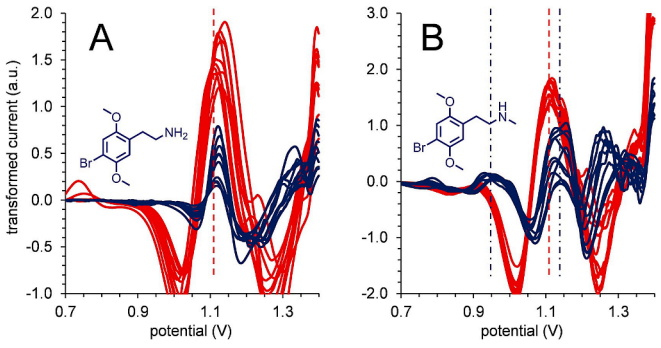

Fig 4. Electrochemical fingerprints (EFs) following baseline correction and top hat filter transformation as preprocessing. Overlay of 10 MDMA-containing tablets (red) and 10 2C-B-containing tablets (blue) analyzed by the MDMA sensor (panel A) and the MDMA/2C-B sensor following *in-situ* derivatization with formaldehyde (panel B). Red dashed line indicates the 1.11 V peak diagnostic for MDMA, blue dashed lines indicate the 0.95 V and 1.14 V peaks diagnostic for derivatized 2C-B.

sensor. Note that the selectivity towards 2C-B is achieved by requiring the presence of both the peak at 0.95 V and the peak at 1.14 V after derivatization. In line with earlier work by Schram *et al.* [27] the observed reaction was proposed to be a Eschweiler-Clarke methylation whose reaction scheme is shown in Fig. S2. Formaldehyde and formate (present in trace amounts in the formalin) react with 2C-B to form *N*-methyl-2C-B. Tertiary amine reaction products, such as dimethylated 2C-B were only formed in minor quantities. The indifference of the main 1.11 V peak in the EF for MDMA complements this finding since no effect is observed that can be attributed to the formation of the dimethylated analogue of MDMA. A more in-depth analysis of the derivatization reaction rate and products can be found in the Supporting Information, Figs. S3–S5. This new MDMA/2C-B sensor thus evaluates a sample on the presence of both MDMA and 2C-B. If neither of these two compounds are identified, the sample is said to not contain these two compounds. If both substances are present the sensor can report both substances due to the presence of all three diagnostic signals. The partial overlap of peaks may however impose a risk that the detection of one substance is obscured, especially when a single substance is present at a lower concentration compared to the other. Because mixtures of MDMA and 2C-B are seldomly encountered in seized tablets this situation is not further investigated in this study. The new sensor was tested on the seized casework tablets set to validate its applicability and compare its performance with the MDMA-sensor.

Performance on case samples

All 39 samples containing MDMA are still correctly identified by the MDMA/2C-B sensor (Table 1, meaning that the selectivity towards MDMA is maintained. Additionally, 10 out of 11 samples containing 2C-B are now correctly identified as containing 2C-B, which is a major improvement over the MDMA-sensor. Only for sample #110, the MDMA/2C-B sensor falsely identified MDMA. (EFs are shown in Fig. S6.) This particular 2C-B-containing sample exhibited peaks at a voltage slightly lower than the 0.95 V and 1.14 V used for 2C-B detection, and for the latter thus more towards the 1.11 V used for MDMA detection. The reason of the incorrect result was thus most likely related to thresholds in the software. The relatively small set of case samples in this study did not allow for additional software optimization and validation due to the risk of overfitting, however this may be an interesting opportunity for future work. Sample #131 containing mCPP, caused a false positive for the MDMA-sensor, but not for the MDMA/2C-B sensor. When looking at the EFs of both reference mCPP (#46, Table S1) and the tablet sample (#131, Table S2) a major peak is observed ~ 0.88 V, thus well outside the detection windows of both MDMA and 2C-B. However, minor signals in this range produced the erroneous results. This again shows that future optimization work on the thresholds in the software may increase the overall sensor performance. Increasing these thresholds should however be performed with caution as a decrease of false positive results may come with the price of an increase in false negatives (*e.g.* case samples with a lower concentration such as mixtures are possibly missed). Sample #115 containing pentylone exhibits the opposite behavior, creating a false positive for the MDMA/2C-B sensor as opposed to a true negative for the MDMA sensor. Remarkably, the 2C-B isomer 2-Br-4,5-DMPEA is identified as 2C-B twice by the MDMA/2C-B sensor. The false positive EFs are shown in comparison with corresponding true positives in Fig. 5. It can be seen that at both areas diagnostic for 2C-B (*i.e.* 0.95 V and 1.14 V) the false positive EFs also yield signal although their peak maxima and ratios are slightly different from the true positive EF. These false positives provide interesting leads for future optimization of the peak detection software. Although the false positive spectra in this situation may be clearly distinguished from the true positive spectra by visual comparison of the overall pattern, it must be noted that deviations in peak height and the presence of additional peaks can occur in case samples (such as mixtures or lower dosed tablets). An approach based on individual peak recognition is therefore preferred over generic chemometric pattern recognition tools. Reasons for this include facile mixture detection, database searches and the possibilities to easily apply specific inclusion or exception criteria for challenging substances in the software. [31] In general, the MDMA/2C-B sensor is an improvement over the MDMA sensor. It overcomes the false positives caused by 2C-B, being able to identify 2C-B correctly in 91% of the samples containing 2C-B. Besides, the strengths of the MDMA sensor remain largely untouched. All MDMA containing samples are still correctly identified, and solely one sample causes false positives. The sole drawback is the apparent lost selectivity between the isomers 2C-B and 2-Br-4,5-DMPEA.

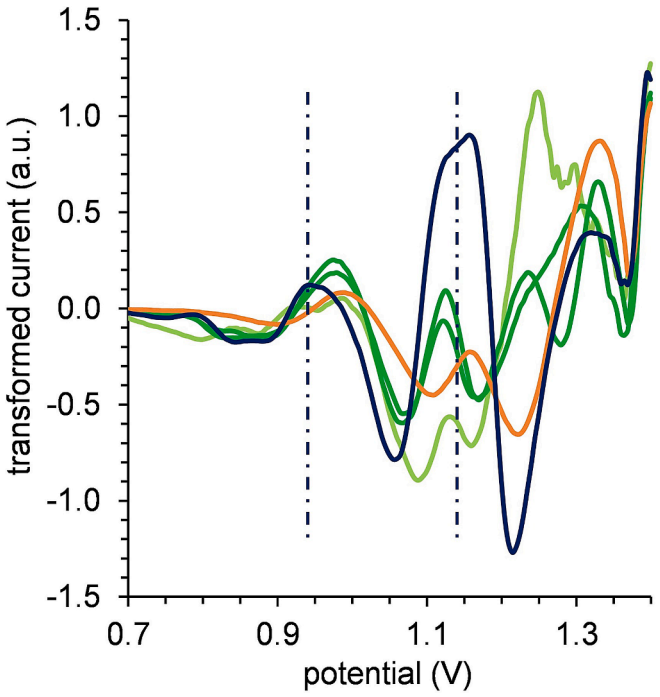

Fig. 5. Electrochemical fingerprints (EFs) following baseline correction and top hat filter transformation as preprocessing. False positive reactions on the MDMA/2C-B sensor: pentylone (orange) and 2-Br-4,5-DMPEA (dark green) with a true positive 2C-B sample (dark blue) and a true negative 2-Br-4,5-DMPEA (light green) plot for comparison. Blue dashed lines indicate the 0.95 V and 1.14 V peaks diagnostic for derivatized 2C-B.

Conclusions

Rapid presumptive detection of MDMA and 2C-B in ecstasy tablets using electrochemical detection on screen printed electrodes is feasible. Electrochemical detection was not hampered by the presence of colorants or fluorescent substances. Since both MDMA and 2C-B are typically sold as ecstasy tablets with a large variety of colors, this is an advantage over spectroscopy-based techniques. The MDMA sensor demonstrated to be specific against 66 out of 73 common drugs, pharmaceuticals, excipients and designer drugs that may be encountered in the forensic field. Unfortunately, 2C-B yielded an electrochemical fingerprint that shared the 1.11 V peak characteristic for MDMA. Since 2C-B is also frequently encountered in ecstasy tablets, a dedicated MDMA/2C-B sensor was developed to differentiate both substances. *In-situ* derivatization with formaldehyde converted the primary amine 2C-B into secondary amine analogues thereby modifying its electrochemical fingerprint. The secondary amine MDMA was not affected by formaldehyde, leaving its EF mainly unchanged. The applicability of the combined two-step sensor was demonstrated on a set of 71 ecstasy tablets seized in 2020. All 37 MDMA-containing tablets were correctly detected by both sensors. In 10 out of 11 2C-B-containing tablets the

active ingredient was correctly identified by the second sensor, leaving one erroneous result in which 2C-B was misidentified as MDMA. False positive results for 2C-B were observed for its isomeric analogue 2-Br-4,5-DMPEA and pentylone. Other synthetic drugs in ecstasy tablets were correctly detected as negative for MDMA or 2C-B.

The current drugs-of-abuse market faces a diversity of hundreds of different psychoactive substances that may be encountered in the field. Presumptive methods that can detect multiple substances in a single analysis are therefore preferred over traditional colorimetric tests that are only applicable for a small group of common drugs. The dual approach of both an EF and an enhanced EF in combination with database-aided peak detection software therefore is a promising technique for on-site drug testing. As an outlook, the EFs of relevant substances may be assessed for their selectivity and subsequently added to the database allowing their simultaneous detection. The required additional sampling in the two-step approach may be considered a drawback. An interesting future opportunity may be the development of multiple working electrodes on a single SPE. In this way, the original analysis can be performed on the first working electrode, a derivatization reagent is added to the sample solution and a second analysis may be directly performed using the second working electrode on the same SPE strip in the potentiostat. Another challenge for on-scene use is safe handling of hazardous chemicals (*i.e.* formaldehyde). The development of enclosed kits or pouches is envisioned to prevent direct contact with chemicals.

Another outlook for the on-site detection of possible illicit-drugs lies in the orthogonal nature of electrochemical detection towards spectroscopy-based detection. Results from different portable devices could be combined -either separately or by means of data fusion- to increase the overall specificity and evidential value. Ultimately, the combination of multiple tests could in certain cases lead to sufficient evidence that subsequent confirmatory analysis in the forensic laboratory is no longer necessary. This way, the overall forensic processing time can be reduced dramatically since time consuming transportation and laboratory analysis can be avoided.

CRediT authorship contribution statement

Robin Van Echelpoel: Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft. **Ruben Kranenburg:** Methodology, Investigation, Project administration, Formal analysis, Data curation, Writing - original draft. **Arian Asten:** Supervision, Writing - review & editing. **Karolien De Wael:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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