Making an impact with voltammetric illicit drug sensors

Bridging the gap between fundamental lab research and on-site application

Robin Van Echelpoel

Supervisor **prof. dr. Karolien De Wael**

Thesis submitted in fulfilment of the requirements for the degree of doctor in Bioscience engineering
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ISBN 987-90-57285-34-7
Wettelijk depot D/2022/12.293/03

Dutch title:

Impact creëren met voltammetrische sensoren voor illegale drugs

De verbinding maken tussen fundamenteel lab onderzoek en toepassing in het werkveld

"It's a magical world, Hobbes, ol' buddy... Let's go exploring!"

Bill Watterson, "Calvin and Hobbes"

Dankwoord

The past four years mark a period in which several important changes occurred in my personal life: I moved in with my girlfriend Charlotte for the first time, we bought a house together and, to top it all off, I can now also call her my fiancé. During this period, my job as a PhD researcher was a stable factor that gave me great satisfaction, it provided the right mix of challenges, both in terms of soft and hard skills, which allowed me to grow both professionally and personally. It is clear that these four years were a very happy period in my life. This is partly due to myself, for which I would like to thank myself, but largely due to all the fantastic people who are a part of my life. The writer John Barth famously said that everyone is the hero of their own story, and I can add without a doubt that my story has no shortage of fellow superheroines and superheroes. It is not every day that you get the opportunity to formally thank all these heroes, and I will therefore take the time to do so in this acknowledgment section, as they all deserve it so much.

The person I would like to thank first is someone without whom I would not have done a PhD at all. I am still incredibly grateful that my supervisor **Karolien De Wael** gave me the opportunity to go through this process. However, my thanks go much further than this, as I already mentioned, I have not had any professional headaches at any time during my PhD. This is not as straightforward as it might sound, especially for the often complicated (emotional and professional) process that is a PhD. She created an environment in which I could work and explore freely, with always the right mix between freedom and guidance, where I was challenged and taken out of my comfort zone in just the right amount to grow. It quickly became clear to me that Karolien is an excellent manager, and in recent years I have watched and studied with great admiration how she tackled the challenges that are associated with managing a small company of 20-30 employees that has to replace one fifth of its employees every year. One thing is certain, and that is that the bar for my future supervisors is very high.

On a more day-to-day basis, there are two people who I consider my mentors and who deserve a special thank you: **Marc Parrilla and Mats de Jong**. It was a privilege to work together and learn from people who are such experts in their field. They both master the art of patiently providing explanations, but at the same time also allowing me the space to further discover and learn on my own. I admire that you both 'lead by example' with your work ethic and positive attitude, which is something that I attempted to adopt and will strive to continue in the future.

In addition to Marc and Mats, there is a whole group of fantastic colleagues at the A-Sense lab whom I would like to thank. First and foremost I would like to thank **Jonas Schram** for the nice collaborations and the comic relief that was never far away. Festivals and their drugs, Arthur's seat and FC Lugano are things that will connect us for life. I would like to encourage you to believe in yourself even more because you have many qualities, and certainly don't let your dreams always remain dreams.

My trajectory strongly coincided with that of **Noelia Felipe Montiel**. I have always enjoyed sharing an office together, and the many trips to end users are experiences that I will fondly remember. We were the chosen ones to demonstrate the novel technology to end-users, right at the edge between the end-user expectations and the struggles of a brand new technology. These demonstrations are a shared ~~trauma~~ experience, and I was happy to have you as my partner in those situations.
There are several other colleagues that I closely collaborated with, that all deserve a special thank you. **Florine Joosten**, I am very happy to leave the voltammetric drug sensing technology in your capable hands. Your passion for the forensic field is admirable, and I have no doubts that the forensic field will benefit greatly from this passion. On a more personal note, I was always happy to have you as my partner at events like the Havendag and the Security Research Event. **Annemarijn Steijlen** and **Amelia Langley**, both excellent managers, both incredibly talented, and maybe more important, both very enthusiast and kind. Annemarijn, I admire your many talents and strong personality, for me you have the ideal mix between the stereotypical Dutch and the Flemish mentality, you do not shy away from telling things like they are, but you always present this in a respectful and correct manner. Amelia, I wish you all the best in your future with Jerome, a future filled with cooking delicious meals, nice Belgian beers and early bedtimes.
Devin Daems, you already moved on to new challenges, however, this doesn't mean I forgot you. Since day one, you took me under your wing, and your guidance has empowered me throughout my PhD journey, inspiring me to explore beyond the traditional scientific boundaries of a PhD. Hopefully we will see each other again in a few years at a derby, and until then you are always welcome at the Bosuil to enjoy high-quality football. Now that we have had the obligatory Antwerp/Beerschot joke, I would like to wish you all the best for you and your family, and in your professional future.
I would also like to specifically thank **Nick Sleegers**, **Olivier Voet** and **Gert Nuyts**. I greatly appreciated how you were always there for me whenever I asked for help.
Finally, I would also like to thank all the other A-Sense lab members that I have not yet personally mentioned, you are all awesome.

Over the years, I have had the opportunity to collaborate with people from different organizations. A common thread here is that I always got a lot of energy from these collaborations, and that it was a real pleasure for me to work with you. First and foremost, I would like to thank the people at the NICC, and in particular **Filip Van Durme**, **Amorn Slosse** and **Joy Eliaerts**. I frequently visited you, and I always felt very welcome. **Ruben Kranenburg**, you also deserve a special thank you for our very nice collaboration on the MDMA sensor. You will really have to introduce me to your twin brother at some point, otherwise I cannot explain how you manage your incredible workload, which is all done with so much care, attention and positivity. **Erik Deconinck, Margot Balcaen and Maarten Degreef** from Sciensano, I want to thank you as well for a very nice collaboration. The projects on MDMA and heroin really pushed our sensors to a higher level.
A special group of people who deserve a special mention here are the technical partners within the BorderSens consortium. Together we have created an electrochemical multidrug sensor in a short period of time, complete with online platform and customized sampling procedure. This is something we can be proud of, especially considering the fact that the project ran entirely during the Covid-19 pandemic, and we all worked within different organizations spread accross Europe. In random order, these are **Joren Van Loon, Xavier Ceto Alseda, Ana Maria Dragan, Julie De Groot, Alvaro Garcia Cruz, Christopher Zaleski, Raquel Garcia, Maria Begonia Gonzales, Daniel Izquierdo-Bote, Gema Maria Parreno Piqueras, Jaime Perez Ordieres, Daniel Ruiz Santamaria and**

Mihaela Tertis. Finally, I would like to thank **Marcel Heerschop, Ger Koomen, Stefan Bijvoets and Sharon Sap** from Dutch Customs, **Norbert Somers, Pieter Lescouhier, Werner Van Der Cruyssen and Mark De Wilde** from FOD Financiën, and **Ralph Kohn** from Luxembourg police for their hospitality, feedback and insights in the world of law enforcement and illicit drugs.

A final thanks is reserved to my jury members: **Els Du Bois, Gert De Boeck, Cecilia Cristea, Arian van Asten and Alexander L. N. Van Nuijs**. I greatly appreciate the time and effort you put into being part of my jury, as well as the enthusiasm you showed in our correspondance and interactions.

In addition to my gratitude for my professional relationships, this is also the perfect place to celebrate my family and friends. As we say so beautifully in Flemish: 'I seriously fell with my bum in the butter.'. It's difficult to express what you mean to me, and I hope this acknowledgment can contribute a bit to conveying these feelings.

Often, the best is saved for last, but here, I want to begin with the most important person, **Charlotte**. My fellow language camp participant, classmate, friend, partner, fiancée, and soon-to-be wife. Honestly, when we first met at eleven years old, I never saw it coming that things would turn out this way. We naturally grew closer to each other, simple, because it's just so wonderful to spend time together. I owe a significant part of who I am to you, and I wouldn't have it any other way. We've spent a decade together, and I'd love to add a century or better yet, a millennium to that. There are so many things I want to say, no shortage of inspiration, but I'll save those for my vows. Perhaps one last thing: 'Thank you,' and not just an ordinary thank you like you'd say to a baker or a butcher, but a thank you that encompasses all the gratitude, all the love and all the happiness you bring me.

Mama, **papa**, I can never thank you enough for what you've done for me and continue to do. No amount of words will ever be able to express my gratitude for everything you did for me. The start of my PhD coincided with the period when I moved out of the house, perhaps a year earlier than expected. Someday, I might realize the full impact of such a decision. Nevertheless, you've always supported me, shown interest, provided guidance, and we sure have a lot of fun when we are together. I am proud and grateful to be your son. Thank you, thank you, thank you!

I want to thank **my family**, especially my grandparents, it is very reassuring to know you are always in my corner. Finally, I would also like to express my gratitude to the **Verlinden-Melis family**. From day one, I was warmly welcomed with open arms, and it genuinely feels like my second family.

Last but not least, I would also like to extend my gratitude to my group of **friends**. We go way back, and over the years several partners have joined us, each one an invaluable addition to the group. I'll always work to live life and not the other way around, and all of you are a significant part of my 'life' part.

This acknowledgement section has been extensive, and I hope that as a reader, you still have the energy to delve into the thesis itself. Nevertheless, I found it worthwhile to approach this part of the thesis in such detail because the people mentioned here truly deserve it, and let's be honest, this section will probably be the most frequently read part of the thesis. Finally, I'd like to wish you much enjoyment with the rest of the thesis.

Abstract

Illicit drugs are harmful substances, posing a threat to the health and safety of society. Each year, over half a million people die because of drug overdoses, or in other words, each year, over half a million funerals are held for lives that ended too early, because of illicit drugs. The violence associated with the illicit drug trade disrupts communities across the globe, there is no region in the inhabited world that is spared from it. Policies, such as supply reduction and harm reduction, are in place to combat the illicit drug problem. Science can play a substantial role in this fight, by providing tools that enable these policies to be successfully enforced. One example are on-site detection tools, i.e. sensors that allow the on-site identification of an illicit drug in a sample of interest. Several technologies, such as color tests and portable spectroscopic techniques, are currently employed for this goal. Although these are valuable techniques, there is an opportunity for voltammetry, an electrochemical technique, to make an impactful addition to this repertoire of on-site detection tools.

Despite its attractive features (low-cost, portable, short analysis time, indifference to color,...), voltammetric illicit drug sensor have failed to make an impact in real scenarios. The work outlined in this PhD thesis aims to change this by bringing the technology from the lab to the field. Strategic choices, fueled by feedback from end-users, were made to further develop those specific aspects of the technology that previously haltered the technology to fulfill its potential. A detection algorithm was introduced that converts the voltammetric output into a clear-cut interpretation thereof, opening up the technology to end-users without prior knowledge of the technology. A sensor that allows qualitative and quantitative detection of the psychoactive drug MDMA was introduced, and importantly, validated on a large set of 212 confiscated samples. A state-of-the-art mobile application and adequate sampling methodology were developed, alongside other, often more practical studies and product developments, to evolve the technology into a product that truly creates value for end-users. Important steps towards multidrug detection were made with a festival sensor and a flowchart based on visual appearance that ties together a variety of voltammetric single sensors into a single multidrug sensing approach. Last but not least, multiple valorization aspects were researched, including a market study and an analysis to determine the optimal commercialization strategy.

Overall, this PhD thesis has facilitated the transition of the voltammetric illicit drug sensing technology from lab to on-site application. The final application creates value for end-users, and is ready to make an impact in real on-site scenarios.

Samenvatting

Illegale drugs zijn schadelijke stoffen die een bedreiging vormen voor de gezondheid en veiligheid van onze samenleving. Elk jaar sterven meer dan een half miljoen mensen aan een overdosis drugs, of met andere woorden, elk jaar worden er meer dan een half miljoen begrafenissen gehouden voor levens die te vroeg eindigden door illegale drugs. Het geweld dat gepaard gaat met de illegale drugshandel verstoort gemeenschappen over de hele wereld, geen enkele regio in de bewoonde wereld blijft ervan gespaard. Er bestaan verschillende beleidsfilosofieën om de problematiek rond illegale drugs aan te pakken. Wetenschap kan een aanzienlijke rol spelen in deze strijd door middelen te voorzien die toelaten het gekozen beleid succesvol uit te voeren. Een voorbeeld hiervan zijn sensoren die het mogelijk maken om ter plaatse een illegale drug te identificeren in een (verdacht) staal. Momenteel worden verschillende technologieën, in het bijzonder kleurtests en draagbare spectroscopische technieken, voor dit doel gebruikt. Hoewel dit waardevolle technieken zijn, biedt voltammetrie, een elektrochemische techniek, een kans om een impactvolle aanvulling te zijn op dit repertoire van huidige detectietechnologieën.

Ondanks de aantrekkelijke eigenschappen (lage kosten, draagbaarheid, korte analysetijd, ongevoeligheid voor kleur, ...), zijn voltammetrische sensoren voor illegale drugs tot nu toe niet in staat geweest om een impact te maken in het werkveld. Het werk dat in dit proefschrift wordt uiteengezet heeft als doel hier verandering in te brengen, door de technologie vanuit het laboratorium naar het veld te brengen. Strategische keuzes, gevoed door feedback van eindgebruikers, werden gemaakt om die specifieke aspecten van de technologie verder te ontwikkelen die eerder de technologie belemmerden om het potentieel ten volle te vervullen. Een detectie-algoritme werd geïntroduceerd dat de voltammetrische output omzet in een duidelijke interpretatie ervan, waardoor de technologie toegankelijk wordt voor eindgebruikers zonder voorafgaande kennis van de technologie. Een sensor die zowel kwalitatieve als kwantitatieve detectie van de psychoactieve drug MDMA mogelijk maakt, werd geïntroduceerd en gevalideerd met behulp van een grote set van 212 in beslag genomen monsters. Een mobiele applicatie en een geschikte monstername-methode werden ontwikkeld, samen met andere, vaak meer praktische studies en productontwikkelingen, om de technologie te evolueren tot een product dat werkelijk waarde creëert voor eindgebruikers. Er werden belangrijke stappen gezet naar multidrug-detectie met een festivalsensor en een stroomschema op basis van visuele verschijning, die een verscheidenheid aan voltammetrische sensoren samenvoegt tot een enkele multidrug detectiestrategie. Tot slot werden meerdere valorisatie-aspecten onderzocht, waaronder een marktonderzoek en een analyse om de optimale commercialiseringsstrategie te bepalen.

Samengevat heeft dit proefschrift de overgang gefaciliteerd voor voltammetrische illegale drugsdetectietechnologie van het laboratorium naar het werkveld. De uiteindelijke toepassing creëert een toegevoegde waarde voor eindgebruikers en is klaar om een impact te maken in echte scenario's.

Contents

Abbreviations

ACE ..... Acetate

ATR-FTIR ..... Attenuated Total Reflection - Fourier-Transform InfraRed

..... spectroscopy

ATS ..... Amphetamine-Type Stimulants

B2B ..... Business to Business

B2C ..... Business to Customer

B2G ..... Business to Government

CV ..... Cyclic Voltammetry

DIMS ..... Drugs Informatie en Monitoring Systeem

DPV ..... Differential Pulse Voltammetry

EMCDDA ..... European Monitoring Centre for Drugs and Drug Addiction

EP ..... Electrochemical Profile

ESI ..... ElectroSpray Ionization

FN ..... False Negative

FP ..... False Positive

FWHM ..... Full Width at Half Maximum

GC-FID ..... Gas Chromatography-Flame Ionization Detection

GC-MS ..... Gas Chromatography-Mass Spectrometry

GUI ..... Graphical User Interface

HPLC ..... High-Performance Liquid Chromatography

IP ..... Intellectual Property

LC-MS ..... Liquid Chromatography-Mass Spectrometry

LC-MS/MS ..... Liquid Chromatography-Mass Spectrometry/Mass Spectrometry

LC-MS-QTOF .... Liquid Chromatography-Mass Spectrometry Quadrupole Time Of

..... Flight

LDA ..... Linear Discriminant Analysis

LEA ..... Law Enforcement Agency

LNS ..... Laboratoire National de Santé (Luxembourg)

LOD ..... Limit Of Detection

LOQ ..... Limit Of Quantification

LSV ..... Linear Sweep Voltammetry

MAE ..... Mean Absolute Error

ML ..... Machine Learning

MIP ..... Molecularly Imprinted Polymer

MWCNT ..... MultiWalled Carbon NanoTube

NFI ..... Nederlands Forensisch Instituut (The Netherlands)

NICC ..... National Institute of Criminalistics and Criminology (Belgium)

NIR ..... Near-Infrared Spectroscopy

NIST ..... National Institute of Standards and Technology (United States of ..... America)

NMR ..... Nuclear Magnetic Resonance

NPS ..... Novel Psychoactive Substances

PBS ..... Phosphate-Buffered Saline

PCA ..... Principal Component Analysis

PWUD ..... People Who Use Drugs

QMS ..... Quality Management System

RSD ..... Relative Standard Deviation

SERS ..... Surface-Enhanced Raman Spectroscopy

SPE ..... Screen Printed Electrodes

SIMCA ..... Soft Independent Modelling of Class Analogy

SWOT ..... Strength, Weaknesses, Opportunities and Threats

SWV ..... Square Wave Voltammetry

TEDI ..... Trans-European Drug Information

TN ..... True Negative

TNR ..... True Negative Rate (selectivity)

TP ..... True Positive

TPR ..... True Positive Rate (sensitivity)

TRL ..... Technology Readiness Level

UAntwerp ..... University of Antwerp

UNODC ..... United Nations Office on Drugs and Crime

Compounds

2-Br-4,5-DMPEA . 2-bromo-4,5-dimethoxyphenethylamine

2C-B ..... 2-(4-bromo-2,5-dimethoxyphenyl)ethanamine

2C-B-FLY ..... 2-(4-bromo-2,3,6,7-tetrahydrofuro[2,3-f][1]benzofuran-8-yl)

..... ethanamine

3-FA 3-fluoroamphetamine

4-Cl-alpha-PVP .. 4-chloro-alpha-pyrrolidinovalerophenone

6-APB 6-aminopropylbenzofurane

DMT ..... *N,N*-dimethyltryptamine

DXM ..... dextromethorphan

FA ..... fluoroamphetamine

FMA ..... fluoromethamphetamine

MBDB 1,3-benzodioxolyl-*N*-methylbutanamine

mCPP ..... meta-chlorophenylpiperazine

MDA 3,4-methylene-dioxyamphetamine

MDAI 5,6-methylene-dioxy-2-aminoindane

MDEA 3,4-methylene-dioxyethylamphetamine

MDMA ..... *N*-methyl-1-(3,4-methylenedioxyphenyl)propan-2-amine

PMA ..... para-methoxyamphetamine

PMMA ..... para-methoxy-*N*-methylamphetamine

TNT 2,4,6-trinitrotoluene

Symbols

A	Ampere
°C	Degrees Celsius
m/z	Mass to charge ratio
M	Molarity
I_p	Peak current
τ	Period
E_p	Pulse amplitude
t_p	Pulse width
E_s	Step potential
V	Voltage

Chapter 1

Introduction

"You have to believe in the long term plan you have, but you need the short term goals to motivate and inspire you."

Roger Federer

Abstract

This chapter serves as the starting point for the thesis, providing an introduction to the key topics of this thesis, namely illicit drugs, illicit drug policy and illicit drug detection. It begins by delving into the (complex) world of illicit drugs, with an emphasis on those specific illicit drugs that play a central role in this thesis. Care has been taken to present a coherent narrative, covering various aspects of each drug, such as their societal impact, appearance, and associated risks. Additionally, an exploration is undertaken of the different governmental policies that exist to deal with the illicit drug problem.
As the focus of this thesis revolves around the (on-site) detection of illicit substances, the chapter subsequently transitions towards discussing sensing technologies for illicit drugs. Both established and emerging detection techniques are thoroughly described, providing a comprehensive understanding of the field. Lastly, the chapter concludes by providing a summary of the forthcoming chapters.

1.1 Illicit drugs

1.1.1 Illicit drugs and their place in society

Illicit drugs are harmful substances, threatening both health and safety of societies worldwide. Numerous statistics highlight the health-related risks, carefully listed each year in reports published by instances such as the United Nations Office on Drugs and Crime (UNODC) and European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)[1, 2]. Before diving into these statistics, one should be aware that these numbers describe real people: daughters, sons, fathers, mothers, friends, relatives,... Indeed, contrary to popular belief, illicit drugs affect people of all ages, genders, nationalities and

social classes[1]. In 2019, over half a million people died due to drugs worldwide, not five, not fifty, not five hundred, not five thousand, not fifty thousand, but more than five hundred thousand funerals. Drugs are a real problem.

Next to these deaths, drug use disorders resulted in 18 million years of healthy life lost in 2021[1]. It is estimated that 284 million people use drugs, of which 13.6 per cent have drug use disorders, representing 0.76 per cent of the global population aged 15-64[1]. Worryingly, statistics point out that these numbers are on the rise year after year, seemingly unaffected by the COVID-19 pandemic[3]. It is not the purpose of this introduction to repeat statistics of the UNODC and EMCDDA reports, nevertheless it is of utmost importance to realize the devastating impact illicit drugs currently have on our society.

Figure 1.1: 15 July 2021, Newspapers announcing the death of Dutch Crime Journalist Peter R. de Vries in Amsterdam.

Aside from the health risks, and sometimes overlooked, illicit drugs also bring safety hazards to our society. Think about violence between gangs that want control over the drug trade in an area, or the fact that drug use requires money, pushing people into poverty, making them more vulnerable to illicit practices[1]. In Western Europe, the upper world and under world typically live next to each other, however, more recently it seems that the underworld is no longer afraid to commit crimes in the upper world. On July 6, 2021, Dutch crime journalist Peter R. de Vries was shot in broad daylight in Amsterdam, he died 9 days later from his injuries on July 15, 2021 (Figure 1.1)[4]. The motive for the murder was his involvement as a confidante of a key witness within the Marengo trial, a major Dutch criminal case against 17 suspects who are presumably part of the Mocro Mafia, a major criminal ring that is involved, among other things, in the import of cocaine into Europe. In addition to Peter R. De Vries, the lawyer and brother

of the key witness were also murdered. Even closer to home, in my parental village Sint-Job-In-'t-Goor, two drug-related crimes happened during my PhD: one person was shot and one company building was damaged by grenades as a way of intimidation. The high demand for drugs, combined with their illegal status, creates an explosive cocktail of criminality and violence, something that is becoming the new reality, now more than ever[5].

1.1.2 Illicit drugs appear in many forms

1.1.2.1 General

In general, cannabis is the most used drug with 183 million consumers each year, followed by opioids and amphetamines with around 36 million users each year. *N*-methyl-1-(3,4-methylenedioxyphenyl)propan-2-amine (MDMA), opiates and cocaine are next in line with approximately 20 million users each worldwide[6]. Specifically for Europe, cocaine is the second most used drug. Furthermore, new drugs and drug classes appear rapidly in a constant fight to beat the current legislations. Some important classes in this 'designer drug' category, are synthetic cathinones, synthetic cannabinoids and synthetic opioids[7].

Drugs appear in many forms and colors, often different forms of appearance exist for the same illicit drug. The most common appearance forms are as resin, crystal, powder, liquid, pill or blotter. Furthermore, due to their inherent illicit nature, many illicit drug's appearance is masked or altered by criminal organizations to circumvent detection by law enforcement. Additionally, licit cutting agents and adulterants are sometimes added to illicit drugs to increase profits, mimic desired effects or counter negative side-effects[9].

The variety of effects induced by illicit drugs is enormous, and it are these effects that are sought after by the user. They can be categorized in stimulants, depressants, opioids, psychedelics, cannabinoids, dissociatives and empathogens. The so-called 'drugs wheel' gives a good overview of the drugs corresponding to each category. Some drugs have no risk of dependence, other create a mental dependence, and some even create a physical dependence within the user (Figure 1.2)[8].

It is clear that many different drugs and drug classes exist, and it is impossible to give an individual introduction on all of them, let alone a detailed description. I will therefore limit myself to an introduction of the illicit drugs that play a substantial role in this thesis: MDMA, 2-(4-bromo-2,5-dimethoxyphenyl)ethanamine (2C-B), cocaine, methamphetamine, ketamine, amphetamine and heroin (Figure 1.3). The majority of information is extracted from yearly reports by the EMCDDA and UNODC, for which the citation will be mentioned at the end of each paragraph to avoid an overload of references to the same report in each paragraph.

1.1.2.2 MDMA

MDMA plays a crucial role in this thesis, it is the central topic in Chapters 4 and 5, and plays an important role in Chapters 3 and 6. The compound has an interesting structure with several functional groups such as a secondary amine and a methylenedioxy group

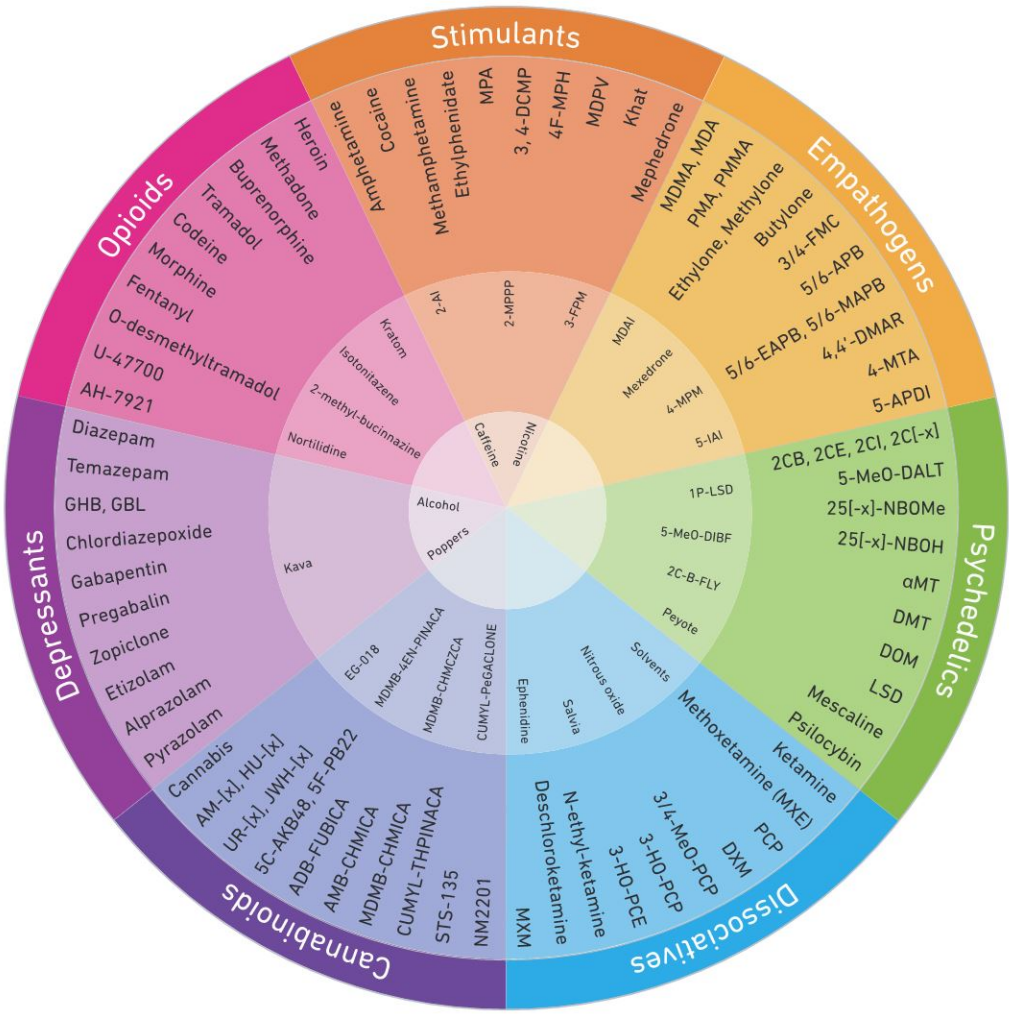


Figure 1.2: The drug wheel presents a good overview of the different potential effects of illicit drugs, together with examples for each category. The inner circle contains compounds that are not illicit, such as caffeine and alcohol. The outer circle, on the other hand, reports on compounds that are illicit[8].

attached to a phenyl moiety. How these structural features influence the electrochemical signal, and how I used that to my advantage for sensor development, are thoroughly discussed in Chapters 4 and 5. MDMA is often synonym with ecstasy, although the latter is in fact a generalised term that can cover a wide range of substances (e.g. 2C-B, 3,4-methylene-dioxyamphetamine (MDA), 3,4-methylene-dioxyethylamphetamine (MDEA) or amphetamine)[10]. MDMA is a synthetic compound that was first synthesized in 1912 by Merck chemical company. The company never marketed the compound, and it has only found (little) legal use as a therapeutic in psychiatric counselling. It is described as an empathogen by the drug wheel, meaning that it produces experiences of emotional communion, oneness, relatedness and emotional openness. It should come as no surprise that these characteristics made it popular in party settings, which it still is to this day.

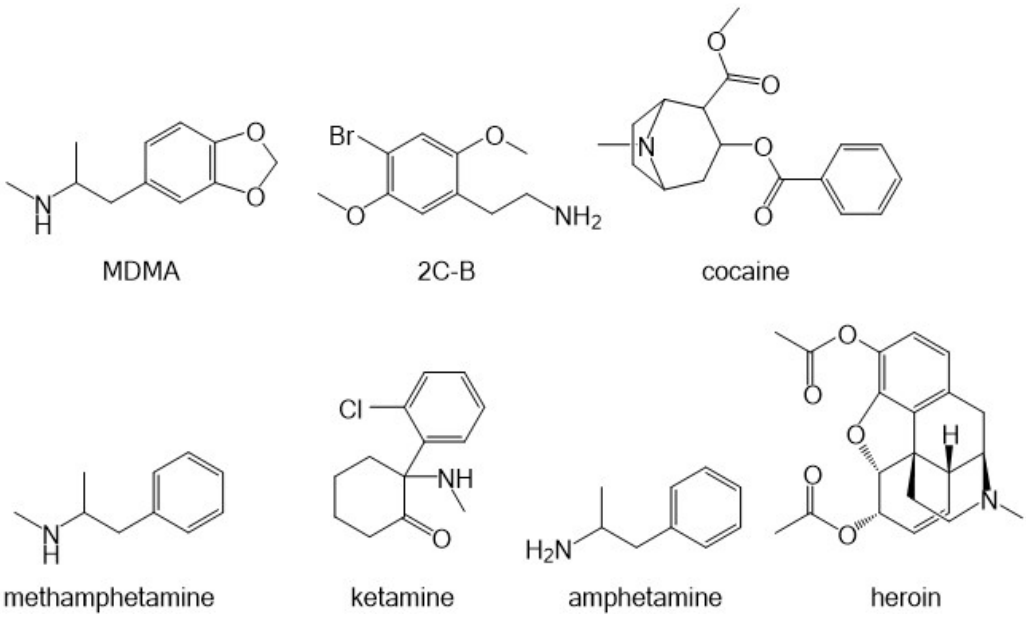


Figure 1.3: Chemical structures of the seven illicit drugs that are of particular interest in this thesis: MDMA, 2C-B, cocaine, methamphetamine, ketamine, amphetamine and heroin.

MDMA typically appears in pills, as a white or off-white powder or as water-soluble crystals. Today, an MDMA pill contains 125 to 200 milligrams of MDMA, and costs on average 5 to 10 euros (interquartile ranges). It is important to mention here that the MDMA content in ecstasy pills is on the rise, creating a risk for overdoses. It is impossible to estimate the MDMA content in a pill by visually looking at it, thus requiring chemical quantification tools to calculate the MDMA content and prevent overdoses[2, 11].

1.1.2.3 2C-B

2C-B is an illicit compound which, similar to MDMA, can be found in ecstasy pills as the psychoactive agent. Similar to MDMA, it is also a synthetic drug, however, not first synthesized by a company but by the chemist Alexander Shulgin, nicknamed the Godfather of ecstasy, in 1974. Structurally there are some similarities with MDMA as well: 2C-B too contains a benzene-moiety with two attached methoxy-groups. This overlap in structure plays a pivotal role in this thesis since it causes a similarity in electrochemical output as well. The implications of this overlap are discussed in detail in Chapters 4 and 5. 2C-B appears as a powder or pill, and requires lower doses than MDMA to achieve the desired psychoactive effects, an intermediate dosage contains in between 15 and 25 milligrams of 2C-B. MDMA and 2C-B are rarely observed together in the same sample, typically a sample contains either MDMA or 2C-B. The lower dose makes that ecstasy pills containing 2C-B are typically smaller than those that contain MDMA. The psychedelic properties in 2C-B are much more prominent than in MDMA, classifying it more as a psychedelic than as an empathogen. Online survey data have shown that drugs with psychedelic properties such as 2C-B became more popular during the COVID-19

pandemic[3]. Recent discussions, anno 2023, with stakeholders in customs and harm reduction confirmed this trend. 2C-B is probably the drug that is least well-known by the public in this list, however, this certainly does not mean that it is less relevant to research[12, 13].

1.1.2.4 Cocaine

Contrary to MDMA and 2C-B, cocaine is a natural product that is extracted from coca leaves. The latter typically grow in South America, and it is thus no surprise that most cocaine enters Europe via ship cargo coming from South American countries such as Colombia, Bolivia or Brazil. Both the base and the salt form of cocaine are encountered, the first one is smokable and better known as crack. The salt form is a white powder and typically snorted. Cocaine has a chemical structure that differs distinctly from the phenethylamine-structure that characterizes MDMA, 2C-B and (meth)amphetamine. The structure itself is dominated by a 8-azabicyclo[3.2.1]octane saturated heterocycle which is decorated with a methylester and benzoyloxyester. A user seeks the stimulant properties of cocaine, typically consumes 100 to 200 milligrams and pays 5 to 10 euros for this dose in Belgium in 2022. Since cocaine is a more expensive drug, dealers tend to increase their profits by cutting the drug, resulting in purities between 54 and 68 percent. This cutting of the drug opens up the opportunity of drug profiling, i.e. comparing the composition of confiscated samples (also called street samples) to make connections between specific drug busts and production laboratories or dealers. Cocaine is the illicit drug that has dominated the news headlines in Belgium the most during the previous decade. This can be attributed to two factors: the port of Antwerp is the second biggest port in Europe, and cocaine is rivaled only by cannabis in terms of popularity in Europe. Add to that the fact that cocaine comes a long way from South America, which is reflected in the price tag, and it is clear that it is not petty thieves, but the big boys who get involved with the import of cocaine into Europe. This has major implications for the task of customs, since the 'opponent' has access to seemingly unlimited resources. These resources are employed in elaborate schemes to smuggle cocaine into the mainland of Europe, involving bribing personnel in the port, hiding cocaine in every way one can imagine and mixing cocaine with all sorts of agents (charcoal, drinks, clothes,...) to mask its presence[2, 11, 14].

1.1.2.5 Methamphetamine

Methamphetamine became widely known to the public as crystal meth when Walter White and Jesse Pinkman synthesized it in the Netflix hit series Breaking Bad. Similar to MDMA, it is a synthetic drug with stimulant properties. This is not entirely unsurprising, since they share the same core phenethylamine-structure. In fact, the only difference is the additional methylendioxy-moiety that is present for MDMA. Interestingly, during World War II, both allies and axis powers used methamphetamine on a large scale to give soldiers energy to keep on fighting, and to suppress their hunger. Nowadays, methamphetmaine is mainly used in the United States of America and the far east, although it appears that methamphetamine is winning terrain in Europe as well. Methamphetamine appears as a racemic mixture in powder or crystal form. Methamphetamine costs 23 to 100 euros per gram (2022), and has a purity of 63 to 84 percent (interquartile ranges). Methamphetamine

is rarely heavily mixed with cutting agents, especially in Europe, and if it is, it is usually with caffeine to improve profits and mimic/improve the stimulant properties[2, 11].

1.1.2.6 Ketamine

Ketamine is a synthetic substance that was first synthesized in the sixties of the previous century. It quickly found medical application as an anesthetic in hospitals. A common side-effect are hallucinations, which, unsurprisingly, makes the compound prone to abuse. Besides these hallucinations, the users want to exploit the dissociative characteristics, seeking a dissociation of mental and physical awareness. The structure of ketamine is rather unique in the illicit drug landscape, with a saturated and unsaturated 6-ring connected to each other, decorated with a carbonyl and primary amine, and a chloride, respectively. Ketamine appears as powder, crystal or in liquid form. The effects are strongly linked to the dose, with very high doses potentially leading to a K-hole, in which the user loses (temporary) control over movement and speech. In 2019, the street price of ketamine averaged 17.10 euros per gram[2, 11].

1.1.2.7 Amphetamine

Amphetamine has, unsurprisingly when considering the chemical structure, many similarities with methamphetamine. It is a synthetic stimulant and commonly appears as a white or off-white powder. Amphetamines are typically less potent than methamphetamine, and find legal uses for patients with attention-deficit hyperactivity disorder or narcolepsy. Amphetamine costs 8 to 26 euros per gram, and has a rather low purity of 20 to 37 percent (interquartile ranges). This low purity is explained by the large amount of samples that contain a substantial amount of the legal stimulant caffeine. Indeed, a gas chromatography-mass spectrometry (GC-MS) study of Zubrycka *et al.* on 1264 confiscated amphetamine samples showed that 94 percent of those samples contained caffeine[2, 11, 15].

1.1.2.8 Heroin

Heroin is a semisynthetic compound that is synthesized through acetylation of morphine, which is obtained from the natural product opium, i.e. the dried latex of certain poppy species (e.g. *Papaver somniferum L.*). Heroin is the most commonly used opioid drug, and is the grim reaper of illicit drugs, by far the most drug-related deaths are caused by heroin. The drug is highly addictive, once you are in her grip, it becomes very hard to escape the fatal road to overdose and death. The compound typically appears as a beige/brownish powder (free base), but can also appear as a white powder if in salt form. Retail prices vary from 27 to 60 euros per gram, with a purity of 17 to 26 percent (interquartile ranges). The purity is rather low, which is similar to amphetamine, however there is a different reason for this low purity. The most commonly found cutting agent for heroin, according to the study of Zubrycka *et al.*, is again caffeine, but only with a frequency of occurrence of 29.9 percent. Since heroin is extracted from a natural product, there are agents present in confiscated heroin samples that are not added on purpose by the producer, but are present because of the manufacturing process. For heroin, these

agents include monoacetylmorphine, papaverine, noscapine and acetylcodeine which are opiate alkaloid by-products, or lead, which can end up in the final product due to the equipment that is used during manufacturing. Similar to cocaine, this pattern of very complex street samples, opens up the possibility of drug profiling[2, 11, 15–19].

1.1.3 Policies to deal with illicit drugs

It may be clear that illicit drugs are harmful, and it is no surprise that countless efforts have been done to deal with this problem. The classic approach to deal with illicit drugs, is by attempting to limit the supply. Law enforcement is typically in charge of this task, and a common phrase is 'the war on drugs'[20]. A second approach, commonly employed together with supply reduction, is demand reduction. Its goal, as the name suggests, is to discourage drug use within the public through initiatives such as information campaigns at schools or festivals[21]. A third approach, although less well known, is harm reduction[22]. Here, it is acknowledged that people will use drugs, independent of legal status or price, and the objective is to limit the health-related risks of illicit drug use[23]. I will discuss the three strategies more detailed below, with a special emphasis on supply and harm reduction since I was involved in projects that related to those two policy strategies during my PhD.

1.1.3.1 Supply reduction

Supply reduction is the most widely known approach to deal with the drug problem, and involves creating policies to avoid illicit drugs reaching the consumers[24]. Drugs of abuse receive an illicit status in legislation, allowing the government to prosecute people that produce, deal and/or use the illicit substance. The penalty (fine, prison time) in Belgium depends strongly on the committed offence, the type of drug and the judge's verdict. The judge will adjust the penalty depending on the situation of the person and the committed felony, and importantly in Belgium, focus on assistance and not punishment[25]. Furthermore, the government will often fight a war on drugs, actively searching and destroying drug producing laboratories, running big operations to unravel and subsequently prosecute drug rings, and penalizing people who use drugs (PWUD) heavily to discourage drug use[26]. Intuitively, this approach makes a lot of sense, if there are no drugs to consume, the drug problem is solved. However, history has shown that supply reduction and war on drugs are not necessarily successful[27]. Nevertheless, it is selected by top policy makers all over the world as the most effective strategy, and our role as scientists is to provide these policy makers with tools to enforce their policy. Most collaborations in this work are with partners that work in the supply reduction policy framework.

1.1.3.2 Demand reduction

Illicit drugs only become a problem when people use them, when there is a demand by the public. Discouraging the public to use drugs or other harmful substances (e.g. alcohol or tobacco) to avoid that they use those harmful substances, makes a lot of sense

as an approach to deal with the illicit drug problem. Pentz *et al.* already showed the effectiveness of this approach in 1996, proving that the approach can reduce potential drug use by 20 to 40 percent for three years or sometimes longer[21]. Integrating supply and demand reduction could increase this proportion even more to 40 to 50 percent over prolonged times. Information campaigns are the most obvious modus operandi of demand reduction, but linking high penalties to illicit substance use can also have a strong impact on the demand for illicit drugs. An appropriate anecdote here is that I myself can still vividly remember the two days in primary school where we worked on illegal drugs and, among other sessions, talked to a police officer and an ex-PWUD.

1.1.3.3 Harm reduction

Supply and demand reduction have two very clear goals: preventing drugs from reaching the public, and if they do, avoid that the public wants to use those drugs. Despite all the efforts already made in these two policy areas, it is clear that the public still uses drugs and that drug use is increasing year after year. Oddly, once a person decides to use drugs, meaning that the aforementioned policies have failed in their intent, the person is labeled a drug user, marginalized and often even dismissed as a criminal. The PWUD is strongly stigmatized as a 'drug user', there is a clear discrepancy between public opinion of the person before the first drug use and after the first drug use. Harm reduction is a policy that wants to address this discrepancy. As the name suggests, harm reduction aims at reducing the harms that are induced by drugs on the user[23]. PWUD, humans after all, are placed central and not treated as criminals, but real persons that need help and care. Importantly, studies have shown that the legal status (licit/illicit) of a drug of abuse makes no difference for PWUD, as such strongly opposing supply reduction policy, which assumes that making drugs of abuse illegal will strongly discourage drug use[28]. Two important initiatives taken by harm reduction are drug consumption rooms and pill testing services[22, 29]. Drug consumption rooms allow PWUD to use illicit drugs under supervision of a trained staff, thereby reducing the acute risks of disease transmission through unhygienic injecting, prevent drug-related overdose deaths and connect high-risk PWUDs with addiction treatment and other health and social services[30]. Pill testing services enable individual PWUDs to have their drugs chemically analyzed, providing information on the content of the samples as well as advice[29]. A country that implements harm reduction policy since 2001, is Portugal. Drug policy is often an important pawn in the political chessboard, and it is therefore not surprising that Portugal's harm reduction policy arouses controversy, resulting in overwhelmingly positive and negative articles. Hughes *et al.* elegantly compared two representative articles: one that reported on the 'disastrous failure' and a second one that reported on the 'resounding success' of Portugal's drug policy[31]. The truth probably lies somewhere in the middle, both articles used selective data, nevertheless in general it can be said that Portugal's drug policy is a breath of fresh air that shows that harm reduction can have a positive influence on key metrics such as drug use and drug-induced deaths. Harm reduction initiatives are currently present in many other countries as well, often on a smaller scale, including in Belgium (e.g. Sciensano, the national health institute). During my PhD, I have collaborated with Sciensano on two projects, of which one involved pill testing and the other involved drug consumption rooms.

To summarize, multiple policies exist to deal with the drug problem, of which supply reduction and harm reduction are two important ones. Determining the optimal policy is a task for policy makers. Nevertheless, it is clear that we as scientists can make a contribution by providing tools that allow both approaches to succeed in their goals.

1.2 Sensors for illicit drug detection

1.2.1 Current on-site detection of illicit drugs

The previous subsections described the need of supply and harm reduction policies for tools that will enable them to succeed in their objectives. A set of tools that are crucial for both, which will be the focus of this thesis, are on-site detection tools[32]. They allow a user, typically a non-expert in science, to analyze a sample on the presence of an illicit drug, in a short time frame and on-site. The latter implies that the detection tools, commonly called sensors, should work outside of the laboratory, imposing specific requirements on robustness, portability and analysis time. In supply reduction, later referred to as law enforcement, the suspicious sample can be send to a laboratory for confirmation analysis by a gold-standard technique such as GC-MS or gas chromatography-flame ionization detection (GC-FID), since the result might be necessary in court[33]. GC-MS and GC-FID are the gold standard techniques for forensic illicit drug testing as they allow trace detection of illicit drugs in suspicious samples with very low limits of detection (LODs), e.g. 4.2 nM and 5.3 μM for cocaine respectively[34, 35]. Additionally, they allow the determination of the composition of illicit drug samples, i.e. an overview of the different compounds (drugs and adulterants) present in the sample together with their distribution (in percent). GC-MS and GC-FID are employed in this thesis as validation for the analysis of confiscated samples[36]. In harm reduction, these on-site detection tools can make an impact in e.g. drug consumption rooms to analyze the quality of the drug or in pill testing services to determine the exact content of a drug sample[29]. The on-site aspect is crucial, since harm reduction initiatives don't necessarily have access to laboratories. Currently, the most frequently used on-site detection technologies are colorimetric tests and portable spectroscopic techniques such as Attenuated Total Reflection - Fourier-Transform InfraRed spectroscopy (ATR-FTIR) and Raman (Figure 1.4).

1.2.1.1 Colorimetric tests

Colorimetric tests target one specific drug or drug class. A change in color when coming into contact with the target confirms the presence of the drug (class) in the analyzed sample (Figure 1.5)[37]. A wide variety of tests exist, such as the Scott test for cocaine, Marquis test for MDMA and the Mandelin reagent for ketamine[33, 38–40]. Since MDMA will be of particular interest in this thesis, the Marquis color test is shortly discussed here. The test is based on the formation of a purple to black colored complex containing two carbenium ions when MDMA reacts with the sulfuric acid in the presence of formaldehyde. Interestingly, the Marquis test can also be used to identify amphetamine/methamphetamine and 2C-B amongst others, via different color reactions[40]. The large set of potential targets of the Marquis test makes it prone to false positives and negatives. This

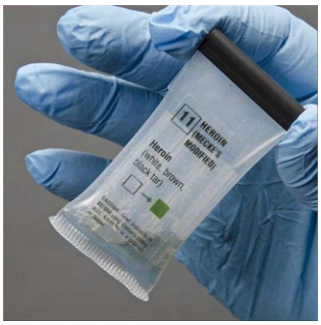


Figure 1.4: Colorimetric tests and portable spectroscopic techniques dominate the illicit drug on-site detection field. From left to right: commercial color test for heroin detection, portable ATR-FTIR device and portable Raman spectroscopy device. The latter two are used in this thesis for competitive analysis.

was proven by Shanmugam *et al.*, who reached a sensitivity of 62.5% for the Marquis test with MDMA as target, in a set of 35 samples[41].

Overall, colorimetric tests are popular, especially in law enforcement, due to their high portability, ease-of-use, short analysis time (< 1 min) and low cost (< 5 euros) (Table 1.1)[40]. Surprisingly, their accuracy, sensitivity and specificity are rather low, as shown by Shanmugam *et al.*, commonly hindered by cutting agents, adulterants and a difficult visual interpretation[41]. Another validation study was performed by de Jong *et al.* on a set of 28 street samples, describing a sensitivity of 68% for the cocaine Scott color test[43]. From academia, these low sensitivities are difficult to reconcile with the popularity of the color tests. Interestingly, it teaches two important things that should be kept in mind when developing a novel illicit drug sensing technology. First of all, the importance of ease-of-use, price and portability cannot be overestimated. Secondly, the way sensitivity and accuracy are calculated in academia, does not necessarily reflect the numbers reached in real on-site scenarios. de Jong *et al.* calculated these values based on a sample set of confiscated street samples that might not reflect a realistic sample set encountered in real life, which might consist for a large percentage out of pure cocaine samples[43]. It is therefore likely that the 'true' sensitivity and accuracy of colorimetric tests is higher than reported in academia. Nevertheless, it may be clear that colorimetric tests are prone to false positives and negatives when cutting agents or adulterants are present, which is very often the case[40].

1.2.1.2 Portable spectroscopic techniques

Portable spectroscopic techniques, such as FTIR and Raman devices, allow the recording of a spectrum which is then compared with a large internal library of spectra in order to find a match[44–46]. As such, they test a suspicious sample on the presence of a wide range of compounds. The technology is considerably more complex than color tests, which is reflected in the price tag: portable FTIR and Raman devices cost on average 25 000 - 35 000 euros and 30 000 - 60 000 euros respectively.

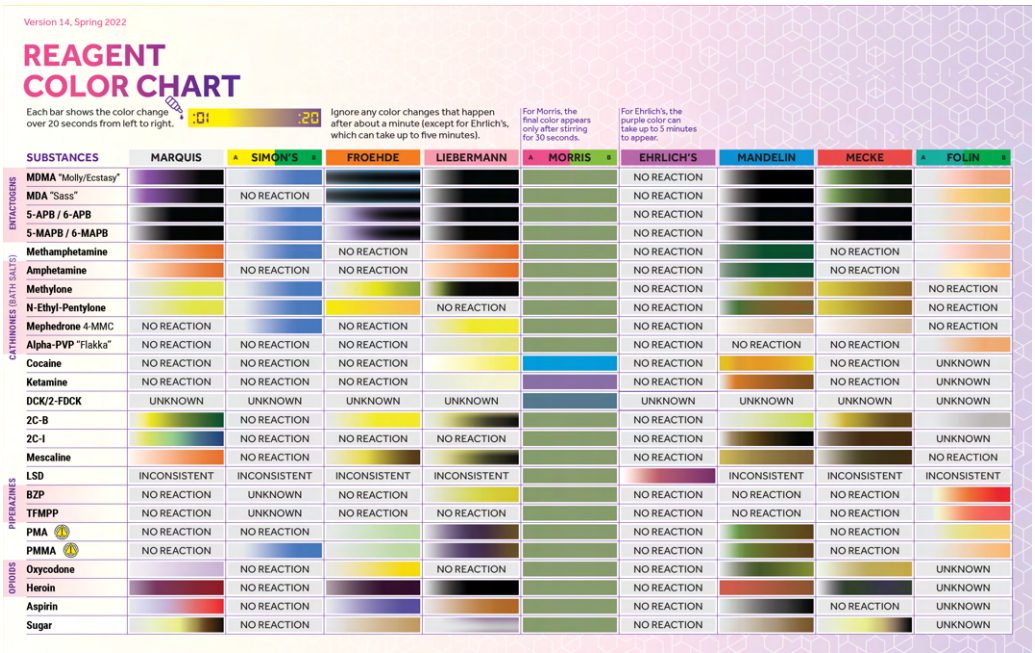


Figure 1.5: Overview of several colorimetric agents together with the color change induced by a wide range of drugs. The chart is provided by DanceSafe, an organization that is committed to harm reduction[42]

FTIR devices can be considered portable, however they remain quite bulky and their portability is hardly comparable to color tests or portable Raman devices. ATR-FTIR is commonly used, since it allows direct FTIR analysis without difficult sampling procedures, i.e. the sample (powder, liquid, crystal,...) simply needs to be placed below an ATR crystal[47]. Nevertheless, a stable benchtop is required, and preferably lab conditions, which places the technology between a pure on-site sensor and a lab-based sensor. Intrinsically, the ATR-FTIR technology has the potential to reach high accuracies, grace to a combination of good reproducibility and spectra that are unique for individual compounds due to unique spectral features. However, everything stands or falls with the detection algorithm that is used. Since this detection algorithm is commonly a matching algorithm that compares the recorded spectrum with a database of spectra, the accuracy greatly depends on the quality of the database (TICTAC library for illicit drug detection)[46, 48]. Furthermore, this task is greatly complicated in illicit drug detection by the often complex mixtures encountered in the field[49]. The state-of-the-art is moving away from library search algorithms towards machine learning based strategies to push accuracies to higher levels, but these are yet to be incorporated in commercial devices. However, this does not mean that such advanced chemometric methods are not yet used in practice. For example, an advanced identification algorithm developed by Eliaerts *et al.*, based on support vector machines, is already being implemented by the National Institute of Criminalistics and Criminology (NICC) in Belgium[50–52].

Nevertheless, it is a major advantage that the technology has the potential to target a very large set of compounds with one single search. Furthermore, the option exists to add novel compounds to the database, which is a particularly interesting feature in illicit drug

detection considering the rapid emergence of novel psychoactive substances (NPS). As previously said, the current matching algorithms are not capable to match each recorded spectrum to the correct compound, but the required information is definitely contained in the spectrum, it is only a matter of developing an algorithm that makes optimal use of this information.

Table 1.1: Comparison of color tests, ATR-FTIR and Raman on several key parameters such as price, analysis, interpretation and portability.

	Color test	ATR-FTIR	Raman
Price (euro)	<5	25 000 - 35 000	30 000 - 60 000
Analysis time (min)	<1	<4	<4
Interpretation	Visual	Software	Software
Portability	High	Medium	High

Portable Raman devices have the same operating principle as ATR-FTIR, a Raman spectrum is recorded and a library search algorithm is employed to find a match between the recorded spectrum and a spectrum in the (TICTAC) library[44, 48]. Contrary to ATR-FTIR, the portable Raman is highly portable, with a weight of approximately 1.5 kg. Additionally, the Raman device uses a laser for its spectrum recording, which allows for a non-invasive sampling. The excellent portability and non-invasive sampling are major advantages of the portable Raman technology. On the flip side, similar to ATR-FTIR, developing a good matching algorithm remains a major challenge, and additionally, there is the hindrance of fluorescence when analyzing colored samples[39]. The latter brings down the overall accuracy, since many illicit drug samples are colored, be it for commercial purposes (e.g. ecstasy pills) or smuggling purposes (e.g. cocaine or heroin samples)[14, 32].

1.2.2 Novel technologies entering the field

Colorimetric tests and portable spectroscopic techniques are popular amongst practitioners in the field, dominating the on-site illicit drug testing landscape[29, 49, 53]. Nevertheless, they are not perfect for reasons that are described in the previous paragraphs. Therefore, it is not surprising that other technologies try to overcome these shortcomings (e.g. mediocre accuracy), without making compromises for the strong assets (e.g. low cost and ease-of-use) of these technologies. Several powerful technologies are currently making a strong case to enter the on-site illicit drug detection world. These include Near-Infrared Spectroscopy (NIR), immunoassays and electrochemistry[54–56].

1.2.2.1 NIR for on-site illicit drug detection

NIR spectroscopy uses the near-infrared region of the electromagnetic spectrum, which is situated in the range of 1000 - 2500 nm. The signals in NIR spectra originate from multiple vibrational overtones and/or combination bands, and are therefore rather limited in direct chemical information compared to IR and Raman spectroscopy. Nevertheless, NIR spectroscopy is a very attractive candidate for on-site illicit drug detection since it couples

several attractive features of spectroscopic techniques, i.e. individual identification, non-destructive sampling and library searching, to other attractive features such as short analysis times and miniaturization. A current challenge of the research field is that although benchtop NIR instruments scan the whole NIR spectrum, portable NIR devices have to resort to scanning more confined parts of the NIR spectrum[57]. The powderpuck developed by the Van 't Hoff Institute for Molecular Sciences in Amsterdam, and the NIR sensor developed by the Swiss-based NIRlab, are two fine examples of the potential of NIR spectroscopy for on-site illicit drug detection[56, 58, 59].

1.2.2.2 Immunoassays for on-site illicit drug detection

Immunoassays are already mentioned for illicit drug testing as early as 1992, and are by no means a fresh newcomer in the illicit drug testing field[60]. Nevertheless, they are mentioned here since some end-users in my network performed exploratory performance tests with immunoassays during my PhD. Immunoassays employ antibodies or antigens to measure the presence or concentration of an analyte in a solution[61]. These analytes can be any kind of compound, as long as an antibody or antigen can be developed that specifically targets the analyte. Interestingly, the analyte can be measured in biofluids such as blood or urine, which can be of great use in roadside testing[61]. Immunoassays for illicit drug testing typically target drug classes, rather than individual drugs, and have a relatively high average price tag of 25 euros per test[62].

1.2.2.3 Electrochemical sensors for on-site illicit drug detection

There are three types of electrochemical sensors that have spawned useful applications over the past century: amperometric, potentiometric and conductometric sensors. The amperometric sensor has the best known application of electrochemistry in its repertoire: the glucose sensor[63]. The underlying principle is that a fixed potential is applied, after which the resulting (decaying) current is measured, which in turn can be related to the concentration of an analyte (e.g. glucose) in the solution[64]. An extension of amperometric sensors are voltammetric sensors, here the current is measured as a result of a varying potential. The resulting current-potential curves, called voltammograms, contain qualitative and quantitative information on the analytes present in the solution[64]. Voltammetric sensors are the central topic in this thesis, and will be discussed in more detail in Chapter 2. Potentiometric and conductometric sensors are of less importance for this work, however I want to mention some of their applications to highlight the impact of electrochemical sensors. Potentiometric sensors measure a difference in potential which can be linked to the analyte concentration in a gas or liquid solution[64]. Important examples are the pH meter which you will find in countless laboratories around the globe, and oxygen sensors that are used in e.g. planes[64, 65]. Conductometric sensors are based on measuring the specific conductivity of an analyte, a good application example is the sensor in a car that monitors the degradation of automotive engine oil[66]. Electrochemical sensors, especially voltammetric sensors, have emerged in a wide variety of fields, grace to their low price, miniaturization potential, short analysis time and high accuracy[67]. On-site illicit drug detection would greatly benefit from a technology with these features, and a vast amount of research has been conducted on electrochemical illicit drug sensors[6]. During the last 10 years, the amount of research articles published

on Raman and illicit drugs (app. 7200) is only slightly higher than the amount of electrochemistry and illicit drugs (app. 6600). Nevertheless, contrary to Raman spectroscopy, electrochemical sensors for illicit drug detection have not (yet) conquered the commercial on-site illicit drug detection world.

1.3 Aims of this thesis

My efforts to push the field of electrochemical sensors for on-site detection of illicit drugs to an application that is an added value for society, is the central topic of this thesis. Good order is the foundation of all good things. I will therefore start this thesis from the beginning, that is, by giving a detailed description on electrochemical sensors for on-site detection of illicit drugs (Chapter 2) (Figure 1.6). I will describe how these sensors generally work, what studies are important when developing them and specifically highlight the sensor aspects that are linked to end-user requirements. The latter is important to me, since I have strived to keep my research as application-oriented as possible. Via the A-Sense Lab network, the BorderSens project and a spin-off exploratory trajectory, I was able to work closely with end-users in both law enforcement and harm reduction, through demo's, workshops, on-site visits etc. This helped me to understand their needs and requirements, which I have endeavored to integrate in my research. A pivotal element is the educational background of most end-users, although highly skilled, they are typically not trained in exact sciences, let alone electrochemistry. Therefore, I made it a personal focus point during my trajectory to make sure that the link between end-user and electrochemistry was made, this is essential to bring the technology to the market.

To make this bridge between electrochemistry and end-users, we developed a peak recognition algorithm which performs the interpretation of the electrochemical signal for the end-user, requiring no prior knowledge of the technology or even science at all. The ins and outs of this peak recognition algorithm will be discussed in detail in Chapter 3, including several use cases to highlight its true power. Furthermore, I will make a comparison of our approach with the more traditionally employed pattern recognition algorithms.

After the general description on electrochemical illicit drug sensors and the data interpretation algorithm, I will describe the first sensor that I developed during my PhD - the MDMA sensor (Chapter 4). The purpose of this sensor is the qualitative, and uniquely the quantitative, detection of the psychoactive drug MDMA. I will give a detailed description of the studies that I performed to develop this sensor, including pH, buffer, concentration, temperature and binary mixture studies. Furthermore, I will describe how a Liquid Chromatography-Mass Spectrometry-Quadrupole Time Of Flight (LC-MS-QTOF) analysis provided insight in the oxidation pathway and the steps that I took to integrate the methodology in the peak recognition algorithm. I will describe how I validated the sensor with confiscated samples, which includes analysis of samples measured at the NICC in Brussels, the Laboratoire National de Santé in Luxembourg (LNS) and Sciensano facilities in Brussels, as well as a large validation study performed in collaboration with Police Amsterdam.

During the development of the MDMA sensor, it became apparent that the illicit drug 2C-B might cause false positives. Since 2C-B is an illicit substance, this is not a major drawback of the MDMA sensor, one could rationalize. Nevertheless, I developed an alternative strategy to diversify MDMA from 2C-B. It is important to provide end-users with this option, especially taking into account that 2C-B use is on the rise since the recent COVID-19 epidemic. This alternative strategy, which involves an *in-situ* derivatization with formalin, is the subject of Chapter 5. An additional sampling step is required, in return the strategy couples all the qualitative benefits of the MDMA sensor to the additional sensing of 2C-B. A second validation study was performed in collaboration with Police Amsterdam, comparing the MDMA sensor with the MDMA/2C-B sensor.

While I developed the MDMA sensor, several of my colleagues developed other so-called single drug sensors for detection of other commonly encountered drugs such as cocaine, heroin, amphetamine and ketamine. Rather than focusing on the development of a single drug sensor for a less commonly used drug, I decided to join forces with Jonas Schram and shift our focus to multidrug detection. Indeed, in certain contexts, it is more convenient for end-users to have a detection tool that can detect multiple drugs at once. Again, starting from the philosophy to work as close as possible to a real product, we decided to select a relevant context where our future multidrug sensor has the potential to make a difference, i.e. music festivals. The latter is a common setting where people tend to experiment with or use illicit drugs. We therefore decided to develop a multidrug electrochemical sensor for the four most commonly used drugs at Western European music festivals, i.e. cocaine, MDMA, amphetamine and ketamine. In the context of the BorderSens project, I also developed a flowchart based on visual appearance of drugs, that ties together the most important single drug electrochemical sensors developed at University of Antwerp: cocaine, MDMA, heroin, ketamine and methamphetmaine. The results of my work on multidrug sensors, are described in Chapter 6.

It may be clear that the link between technology and end-user markets is a central pillar of this thesis. The technology part is extensively covered in Chapters 1-6, whereas the end-user markets are only touched upon in those chapters. I will balance this with Chapters 7 and 8, which focus on the connection between technology and end-users. In Chapter 7, I will describe diverse topics that I have worked on in order to make an impact in real scenarios with the electrochemical sensing technology. This involves efforts in software development, but also in very practical things, often overlooked in academia. This chapter also includes a discussion on how I want to leave a legacy in the research field. This chapter lies close to my heart as it demonstrates how I went the extra mile to make an impact for the benefit of society, and not solely focused on academic outcomes.

At this point in the research, the technology is at a point that it is ready to be valorized. Since valorization is such a broad topic, I focused on several specific topics such as a market study, problem-solution fit and commercialization strategies. The findings are reported in Chapter 8. Since this is outside my field of expertise, I contacted dr. Iris Vanaelst (Valorisation Office UAntwerp) to provide guidance. Preferred witness interviews, a deep dive into business at Antwerp Management School and a literature study provided sufficient insights to report on the different end-user markets, and how the electrochemical technology can make an impact in these markets. It was enlightening for me to read up on literature from areas of research unknown to me, and to apply it to my own research.

Figure 1.6: Overview of the chapters in the thesis.

Overall, this thesis describes the development of electrochemical single- and multidrug sensors for on-site use, with a special focus on bridging the gap between academic lab and on-site use.

Chapter 2

Electrochemical sensors for on-site illicit drug detection

"Magicians and scientists are, on the face of it, poles apart. Certainly, a group of people who often dress strangely, live in a world of their own, speak a specialized language and frequently make statements that appear to be in flagrant breach of common sense have nothing in common with a group of people who often dress strangely, speak a specialized language, live in ... er."

Terry Pratchett, The Science of Discworld

Abstract

This chapter provides a detailed exploration of voltammetric illicit drug sensors, aiming to offer a comprehensive understanding of the topic. Initially, the broader research area of electrochemistry is introduced, setting the stage for the subsequent discussion. The focus then shifts to voltammetry, a crucial subfield of electrochemistry that assumes a central role in the following chapters. Following an outline of the underlying theory and equipment employed in this field, attention is directed towards voltammetric sensors, emphasizing their strengths and their relevance to illegal drug detection. The modus operandi of a typical voltammetric illicit drug sensor and the necessary steps involved in their development are outlined. Finally, the challenges faced by voltammetric illicit drug sensors are summarized, providing an overview of the current limitations in the field.

2.1 Electrochemistry

Electrochemistry is the study of electricity and how it relates to chemical reactions. In electrochemistry, electricity can be generated by movements of electrons from one element to another in a reaction known as an oxidation-reduction reaction, or shorter a redox reaction[68]. Even though electrochemistry can be described with this simple sentence, it encompasses many topics that have been discussed in countless books, courses and scientific publications. Electrochemistry is not a standalone subject of study in this thesis, it is the technology that facilitates the development of the sensors described in

the next chapters. Therefore, a basic understanding of electrochemistry is required to support the upcoming chapters. In this chapter, I aim to give the reader with sparse knowledge of chemistry and physics this background on electrochemistry to follow and understand the topics that will be discussed later on in this thesis.

Electrochemistry is the fusion of two major scientific fields: the science of electricity and chemistry[68]. These two fields, or at least the phenomena that they describe, have fascinated people since the dawn of modern humanity[69]. Unsurprisingly, both fields have attracted some of the greatest minds that have ever lived on this planet. Thales, Benjamin Franklin, Michael Faraday, Democritus, Jabir ibn Hayyan and Marie Curie, all were fascinated by electrical and/or chemical phenomena, and have spent their lives and great minds towards gaining more understanding of these phenomena. Since the 16^{th} century, electrochemistry, the overlap of electricity and chemistry, emerged as a field of study on its own. Initially, the objects of study were magnets and electrical generators. It can be observed that the chemical properties of certain specific compounds produced the latter's electrical phenomena (magnetism, electrical sparks). Intensive research followed during the 17^{th}, 18^{th} and 19^{th} century by pioneering researchers such as Sir William Watson, Luigi Galvani and Alessandro Volta. The latter is the inventor of the modern battery, a subfield of electrochemistry that is highly relevant to this day. In 1800, another major step was made in electrochemistry: William Nicholson and Johann Wilhelm Ritter developed a process to separate water into hydrogen and oxygen by using an electric current. This time, electricity is used to produce a chemical phenomenon, and not the other way around as was typical in the previous centuries. Many famous researchers, Michael Faraday, Svante August Arrhenius and Hermann Nernst to name a few, pushed the field of study forward in the 19^{th} and 20^{th} centuries. Nowadays, electrochemistry is omnipresent in our lives, with many applications that are indispensable in health care, industry and our daily lives in general.

2.2 Voltammetry

In the previous section, it was mentioned that electrochemistry relies on the interplay of electrical and chemical phenomena. Sometimes the chemical properties of a certain substance provoke electrical phenomena, and vice versa it also happens that with the help of electrical energy, a chemical reaction is set in motion that would otherwise not be possible. In this thesis, it is the latter that will be of interest. Specifically, it is the field of voltammetry that will be the central topic. Within electrochemistry there are many subfields, such as e.g. polarography, conductometry, voltammetry and coulometry. Interestingly, the aforementioned subfields can be linked to specific electrical quantities: polarography to electric current (I), conductometry to electrical resistance (R), voltammetry to electric potential (V) and coulometry to electric charge (q). Voltammetry thus has a special relation with electric potential, which is officially defined as the work energy needed to move a unit of electric charge from a reference point to a specific point in an electric field. Furthermore, in practice, the potential difference is used instead of potential, since it is only possible to measure a potential difference, and not the potential itself. The question arises: "How can we use potential (difference) to learn more about the chemical properties of an analyte?". In short, applying a potential difference to an electrode can result in the oxidation or reduction of the analyte. More specifically, the analyte is dissolved in a solution which is in contact with an electrode, and it is at the elec-

trode that oxidation or reduction takes place. The oxidation or reduction of a compound means that that compound loses, or receives, electron(s). And since the electric current is the flow of electrons (or ions), measuring such electric current can thus be used to learn more about the reduction and oxidation processes taking place. Therefore, (chemical) information about an analyte can be obtained by measuring the current as the potential is varied. The previous sentence is nothing but the general definition of voltammetry.

2.2.1 Theory

I scoped quickly towards voltammetry, since it is specifically this part of electrochemistry that is used in the rest of this thesis. In this section, the most important quantities and units are introduced, as well as the lingo associated with voltammetry. Reminiscing the definition of voltammetry, two major quantities come forward: the potential (difference) or voltage 'V' and the current 'I'. They are typically expressed, respectively, in the units of volts (V) and ampere (A). Voltammetric experiments are visualized with so-called voltammograms, i.e. 2D-plots of the current versus the potential (Figure 2.1F). Many voltammetric techniques exist, which differ from each other in the way the potential is varied as a function of time (Figure 2.1A-D). Once again, I will limit myself to voltammetric techniques that play a role in this thesis. The first, and most straight-forward, approach is *Linear sweep voltammetry (LSV)*, for which the potential is linearly swept over time. Secondly, in *Cyclic voltammetry (CV)*, the potential is swept between two potential values. First the potential is increased to a certain vertex, and subsequently decreased again to the starting vertex of the voltammogram. *Differential pulse voltammetry* (DPV) is an extension to LSV, where a series of regular voltage pulses is superimposed on the potential linear sweep. Finally, in *Squarewave voltammetry (SWV)*, a combined staircase and squarewave potential is applied. SWV is the star technique in this thesis and has therefore earned the right to have a section all to itself.

SWV is a more complex technique compared to LSV, with the benefit that increased sensitivity is obtained due to a minimum contribution of undesired nonfaradaic currents. The latter are currents that originate from a double-layer at the electrode surface (capacitive currents), and which cannot be attributed to any reduction or oxidation processes. Since it are specifically the redox processes that are of interest, it is beneficial to limit the interference of nonfaradaic currents. In practice, the potential is a superposition of a regular squarewave onto an underlying staircase. Instead of continuously measuring the current in function of the potential, the current is measured at two distinct points for each pulse: once at the end of the forward potential pulse and once again at the end of the reverse potential pulse (both times immediately before the potential direction is reversed) (Figure 2.1E). As such, the contribution of the nonfaradaic currents is minimized. Typically, the plotted current is obtained by subtracting the reverse current waveform from the forward current waveform.

A voltammetric experiment is defined by several parameters, which can be tuned toward the desires of the electrochemist. The first important parameter is the potential window, which is defined by the start and end potential. The step potential (ΔE_s), pulse amplitude (ΔE_p) and period (τ) are parameters that can be tuned to determine the shape of the square wave (Figure 2.1E). Finally, it is also possible to include a so-called pretreatment, in which a fixed potential is applied during a fixed amount of time prior to the measurement. Note that a classic electrochemical approach usually includes an optimization of these

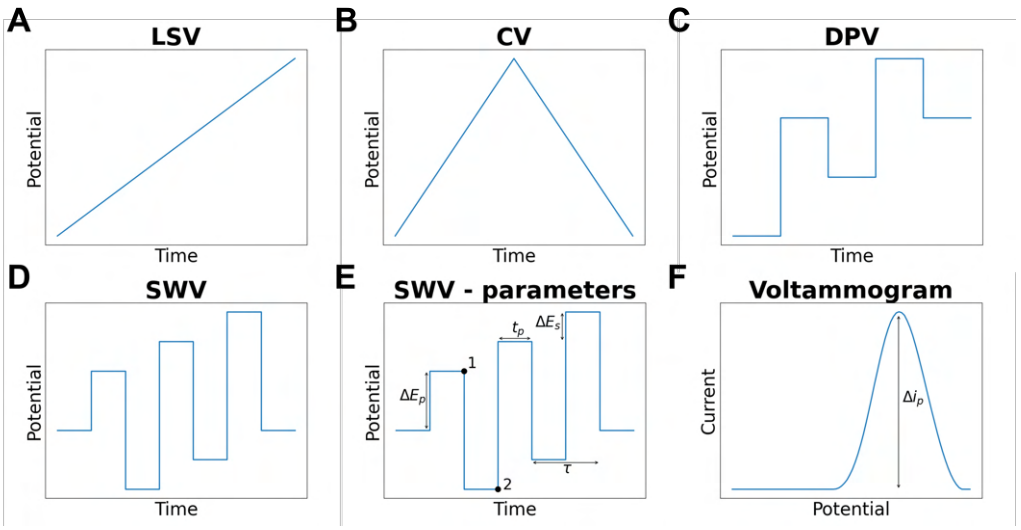


Figure 2.1: Subplots A to D show how the potential is varied in function of time for linear sweep voltammetry (LSV), cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV), respectively. Subplot E shows the parameters that determine the squarewave in SWV: the step potential (ΔE_s), pulse amplitude (ΔE_p), period (τ) and pulse width (t_p). Subplot F displays a voltammogram with peak current Δi_p.

parameters. The attentive reader will notice that this part is missing in this thesis because an optimal set of parameters was already determined in previous research at the A-Sense Lab. Finally, it is relevant to mention that temperature (°C), concentration of buffer and analyte (M), and pH influence the redox processes, and will thus play a role in this thesis.

Two important terms remain that still need some explanation: peak current and peak potential. In this thesis, a lot of attention is paid to the oxidation/reduction peaks present in the recorded voltammograms. The maximum current of the oxidation/reduction process is called the peak current (I_p). The potential at which this peak current is measured, is called the peak potential (E_p).

2.2.2 Electrochemical setup

Conducting voltammetric experiments requires a specific setup. It is important that the potential can be varied in an accurate manner, and that the resulting current is adequately measured. This is achieved by means of a so-called three-electrode setup, which consist of a working electrode, a reference electrode and a counter electrode. The working electrode is the one where all the chemistry action for the analyte of interest (oxidation and reduction) takes place. The role of the reference electrode is, as the name suggests, to act as a reference for the working electrode. It ensures that the potential of the working electrode can be controlled accurately. Finally, the auxiliary/counter electrode is there to close the electric circuit, passing all needed current to balance the current generated at the working electrode. Historically, these three electrodes were usually bulky rods,

made of e.g. inert metals, carbon or mercury, immersed in a solution that contains the analyte. Nowadays, this three-electrode setup is often screen-printed onto a plastic or ceramic substrate. Instead of immersing these so-called screen-printed electrodes (SPEs) into a solution, a droplet of a solution with the analyte is placed on the surface of the SPE (Figure 2.2).

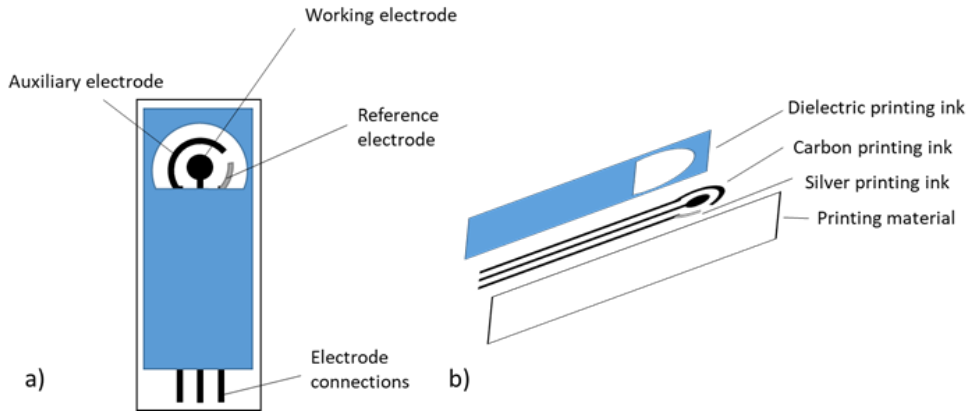


Figure 2.2: Overview of the three electrode system (a) and different layers (b) of a SPE.

Furthermore, there are two additional pieces of equipment of importance. First, there is the potentiostat, which is the electric hardware that controls the three-electrode setup (Figure 2.3). Potentiostats used to be rather bulky devices, however, the current state-of-the-art includes potentiostats the size of a human thumb. And secondly, an interface device such as a computer, tablet or smartphone is necessary to operate the potentiostat, i.e. select the correct parameters, start/stop measurements and display the recorded voltammograms (Figure 2.3). Potentiostat and interface devices can be connected via cable or Bluetooth.

2.2.3 Voltammetric sensors

The specific interest in voltammetry is for a very good reason, namely its grand potential for sensing applications. When the oxidation/reduction potential of an analyte is reached at specific interface conditions, i.e. the potential where the oxidation/reduction occurs, a current flows and an oxidation or reduction peak is observed in the voltammogram. As such, the voltammogram recorded for that analyte is a unique profile that can be used to identify said analyte. Thus, if an unknown sample is measured under the same conditions, and the recorded voltammogram corresponds to that of the previously mentioned analyte, it can be concluded with a high degree of certainty that that analyte is present in the unknown sample. This principle opens the door to voltammetric sensor applications, of which nowadays many examples exist.

Voltammetry is of course not the only scientific technique that opens up the option of sensing applications, so what is it that makes voltammetry of particular interest? First of all, there is the interesting 2D information that is enclosed in the voltammograms. The E_p provides information on the analytes present in solution, and the I_p facilitates the quantification of those analytes. As such, voltammetry has an edge over other

Figure 2.3: Overview of the SPEs (bottom left), potentiostat (top left) and tablet (right) mainly used during this thesis, with a 1 euro coin for reference.

electrochemical techniques such as chronoamperomtry or potentiometry. Furthermore, two other attractive characteristics come immediately to mind: portability and price. Since potentiostats, SPEs and measuring devices can be miniaturized, it is possible to get all required equipment into a lightweight box. This opens up the possibility for on-site point-of-care sensing. As such, voltammetry has a unique edge over a lot of established techniques that require more bulky equipment. Linked to the aforementioned is also the second advantage, namely the low cost. Since the SPEs are screen-printed in large amounts, and (dedicated) potentiostats are relatively simple electronic devices, costs can be kept low, in particular compared to typical scientific equipment prices. Couple this portability and low-cost with short analysis times and good accuracies, and it should come as no surprise that voltammetric sensors are being developed for all sorts of (point-of-care) applications.

It should be noted that there is a difference between developing voltammetric sensors, and having fully functional voltammetric sensors that create added value for the public. Further innovation is required, especially in more practical areas (e.g. sampling method, reproducibility, easy-to-use by non-experts,...) that are sometimes overlooked in a typical academic development process.

2.3 Voltammetric sensors for on-site illicit drug detection

Voltammetric sensors for on-site illicit drug detection have been explored extensively during the previous decade(s). The combination of assets of voltammetric sensors, discussed in the previous section, translates very well to the field of on-site illicit drug testing where accurate and fast testing at the point-of-care is a crucial part of the decision-making process. Currently, color tests and portable spectroscopic devices are however used for the on-site detection of illicit drugs, as discussed in Chapter 1. Although these techniques are effective, they are not the holy grail of on-site illicit drug testing. Both struggle with colored samples, which is a problem since drug cartels are aware of this and actively try to exploit this by mixing illicit drugs with dark agents such as charcoal or fishmeal. Furthermore, portable spectroscopic devices and color tests have some other disadvantages (high cost, subjective interpretation,...) that were already discussed in Chapter 1. Focusing on these shortcomings is not intending to discredit the current techniques. Rather it is to demonstrate that there is an opportunity for other point-of-care technologies to add value to on-site illicit drug testing. I strongly believe that voltammetric sensors are the ideal candidate to add this value, grace to their portability, low-cost, short analysis times, software integration, and indifference to colored samples.

2.3.1 Blueprint of a voltammetric illicit drug sensor

At this point, it is useful to have a look at the 'blueprint' of voltammetric on-site illicit drug sensors (Figure 2.4). This will help to better understand the upcoming sections and chapters.

Figure 2.4: Blueprint of voltammetric illicit drug sensor, displaying the different steps of a measurement. First, a few milligrams of a sample is dissolved in a buffer solution (1). Subsequently, a few droplets of the resulting solution are placed on the surface of a SPE (2). This SPE is inserted in a potentiostat, which is wirelessly connected to a measurement device such as a laptop or smartphone. Software is installed on this measuring device that allows to start a voltammetric measurement with a single click (3). After the measurement is done, data analysis software can be included which automatically converts the voltammetric output into a clear-cut interpretation thereof (4,5).

Typically, the operation of a voltammetric illicit drug sensor has several characteristic steps. First, a few milligrams of the suspicious samples are dissolved in a few milliliters

of a specific buffer solution. As discussed before, drugs appear in different forms (e.g. powder, liquid,. . .), and thus slightly different sampling procedures have to be developed to bring the illicit drug sample into the solution. Some unusual samples, such as in blotter or even impregnated in clothing, require additional sampling steps[14]. Additionally, if the illicit drug has low solubility in aqueous solutions (e.g. heroin), a small amount of organic solvent such as ethanol can be added to dissolve the sample. The buffer solution contains supporting electrolytes and ensures a constant pH. The type of buffer solution (e.g. phosphate buffer saline (PBS), acetate buffer (ACE),. . .) and pH of the buffer influence the voltammetric output, making it an important asset to allow diversification between target analytes. Choosing a favorable buffer is an important step in developing a successful voltammetric illicit drug sensor. Typically, a buffer solution has a stability of three to six months, which is something to consider when researching illicit drugs, as well as when bringing the technology to the market. In general, low detection limits (µM) can be reached with voltammetric sensors, requiring thus little sample amount for identification[6]. Remarkably, achieving low detection limits is less important in practice than it might seem if one were to focus exclusively on the literature. Especially in drug seizures, an end-user has access to milligrams, if not grams or even kilograms, of a sample. Furthermore, the buffer volume can be chosen by the electrochemist. Since a reasonable amount of sample can be dissolved in a volume of choice, it is rather straightforward to achieve a concentration that facilitates the detection of illicit drugs with voltammetric sensors (e.g. in the millimolar range), without the need to push the detection limits of the technology. Instead, the bottleneck for proper sampling in voltammetric illicit drug sensing is achieving a consistent concentration range in which the sensor can operate to its full capacity. The voltammetric illicit drug sensors operate at the point-of-care, which means that an analytical balance is not available to the end-user, and the weight of the sample has to be estimated. Furthermore, illicit drug samples come in a wide range of purities and appearances, which can make it cumbersome to achieve a consistent concentration, even if one could weigh a visually consistent amount of sample. Add to this the fact that the final sample method must work in different places for different people, and it should be clear that this is where the real challenge lies. This challenge, which is almost always overlooked in academia although crucial for the success of the final application, is discussed in detail in Chapter 7.

Secondly, a few droplets of the resulting solution are placed on the surface of a SPE. The low-cost and ease-of-use character of SPEs are some of the major contributors to the success of electrochemical sensors. A three-electrode configuration with a working, counter and reference electrode is generally employed. Figure 2.2 shows the different components of a SPE, on the left, the typical three-electrode system is shown, and on the right the different layers. The type of substrate (e.g. plastic, ceramic or paper) and conductive paste influence the electrochemical output of the measurement. The reference electrode is usually made with silver or silver chloride/silver ink, whereas the auxiliary/counter and working electrodes are made from carbon-based inks[70]. Note that in this work, the reference electrode is made of silver, which is a so-called pseudo-reference. It deviates from a conventional Ag/AgCl reference electrode, and could lead to potential shifts. This is accounted for by adding a fixed concentration of chloride ions (e.g. KCl) to the solution to approach the performance of a more classic setup. Since this setup is used for all measurements in this thesis, all potentials that are reported in this thesis are reported versus Ag/AgCl (which itself is 0.230 V ± 10 mV versus the standard hydrogen electrode). The electrodes are printed on a substrate and covered with dielectric ink to isolate the conductive tracks from the electrode's areas. Various types

of conductive materials can be employed at the working electrode[71]. Importantly, the nature of the screen-printed ink (which might vary depending on the supplier) can affect the voltammetric performance of the sensor. This favorably creates several additional variables that allow for optimization toward a specific target drug. At the same time, good control over the quality of the SPE production process, short and long term, is required to ensure good reproducibility and reliability of the voltammetric illicit drug sensor. This is an important aspect to consider when designing strategies for real applications in the field.

Interestingly, the surface of the working electrode of the SPE can be modified with a large variety of compounds, altering the voltammetric output, improving detection limits and in general facilitating an improved detection performance[72]. The improved detection performance is a major asset, however, the long incubation times and complex manufacturing process should not be neglected, especially when considering bringing the technology to the market (higher cost and cumbersome quality control). Depending on the type of modification, a distinction can be made between chemical sensors (e.g. modified with a biorecognition element such as polymers, ionic liquids or nanoparticles) and biosensors (modified with enzymes, antibodies or nucleic acids). Polymers, and molecularly imprinted polymers (MIPs) in particular, are artificial highly cross-linked polymeric receptors that are engineered towards the binding of specific analytes, in this case, illicit drugs[73]. Due to their high selectivity and excellent LOD in the nM-range, these sensors tend to be able to detect illicit drugs in biofluids as well[74]. Akhoundian *et al.* employed a combination of nano-sized MIPS with multiwalled carbon nanotubes (MWCNTs) to perform ultra-trace detection of methamphetamine in biological samples[75]. Nanoparticles are another type of modification that is commonly employed, Zhang *et al.*, for example, used Pt nanoparticles for the simultaneous detection of morphine and MDMA in biological samples[76]. Gold nanoparticles (modified exfoliated graphite electrode) are also employed, Masemola *et al.* successfully detected cocaine, heroin, amphetamine, 6-acetylmorphine and methylphenidate using this strategy[77]. Furthermore, several other types of modifications can be used, such as graphene and multi-walled carbon nanotubes[78], aptamers[79], and ionic liquids[80].

The SPE itself is inserted in a potentiostat that controls the voltammetric measurement. Typically, one of the following electrochemical techniques is used in electrochemical illicit drug sensors: LSV, CV, DPV or SWV. CV is usually employed to understand the electrochemical behavior of the analyte on the sensor (e.g. study the reversibility of the redox process). LSV, DPV and SWV are voltammetric techniques normally used for analytical purposes. Clearly, the voltammetric output will differ based on the employed technique and set of electrochemical parameters, again providing an opportunity to optimize the sensor toward a specific target drug. The choice of technique and parameters will depend on e.g. the need for reductive and oxidative scan, time of response, or complexity of the voltammetric scan leading to higher or lower sensitivities and enhanced LODs. SWV and DPV are usually employed for the electrochemical detection of illicit drugs due to their low background currents (low contribution of the nonfaradaic currents in the output signal) thus exhibiting higher sensitivities and better LODs. These features are mainly caused by the wave form of the applied voltammetric scan. The potentiostat is typically connected to an interface device (computer, tablet or smartphone) via cable or Bluetooth, which contains software that allows control over the measurement (parameters).

Identification software can be integrated with the measurement software to perform an analysis of the electrochemical output data, which is crucial to make the technology accessible to non-expert end-users. Typically, some preprocessing steps (e.g. baseline correction) are employed, followed by pattern recognition approaches, although electrochemical fingerprint-based peak recognition approaches are also used[81]. Note that a baseline correction is applied in some of the figures in this chapter to improve the interpretation of the data. Integrating the measurement and identification software into a user-friendly (mobile) application, makes the technology fully usable by the non-expert target audience and as such bridges the gap between lab and real use.

2.3.2 Current state-of-the-art

Voltammetric sensing literature has appeared on each of the illicit drugs introduced in Chapter 1. I have opted to discuss this literature more in-depth where it creates an added value and doesn't disturb the flow of the thesis. In practice, this means that I will omit a long list of scientific articles here, and will include e.g. a more detailed discussion of the literature on voltammetric MDMA detection in Chapter 4 prior to the introduction of the voltammetric MDMA sensor developed at the A-Sense Lab. I will list some good reviews here that can function as a stepping stone for further exploration of the research field to at least provide some point of reference for the interested reader. Florea *et al.* and Zanfrognini *et al.* give a nice, general overview of the state-of-the-art in voltammetric sensing for a wide range of illicit drugs[6, 82]. A more in-depth review of the possibilities of electrochemical sensors for illicit drug detection is given by Poltorak *et al.*[83]. Their review focuses specifically on cocaine detection, which is the most studied drug in voltammetry and as such a perfect model molecule to display the variety of electrochemical sensing strategies. Finally, I want to include the review of Teymourian *et al.* on wearable electrochemical sensors for (illicit) drugs, since I am convinced that the upcoming field of wearable sensors will play an important role in the future of voltammetric illicit drug sensors, and sensors in general[84].

2.4 Developing a voltammetric illicit drug sensor

In the previous section, I described the blueprint of a voltammetric illicit drug sensor, which gave insights into the different steps of a measurement with a voltammetric illicit drug sensor. In this blueprint, several factors were cited that can be chosen and tuned by the electrochemist to develop an optimal detection strategy, including e.g. buffer (type, volume, pH), type of electrode, type of voltammetric technique, voltammetric parameters,... The logical question that follows is: 'How does the electrochemist optimize these factors to develop an optimal detection strategy for a target illicit drug?'. During my time as a PhD researcher, I have had the opportunity to develop several detection strategies that were peer-reviewed and published in scientific journals. This has helped me to gain an understanding of the different steps and studies that are required to come to a detection strategy that is accepted by the scientific community, and probably more important, also works in a decentralized setting.

When I have to introduce myself during meetings with scientific peers or end-users, I tend to say that I work on electrochemical illicit drug sensors, both on methodology development and software development. Methodology and software are in my opinion two equally important parts of an electrochemical illicit drug sensor. From a broad perspective, the objective of the methodology (or in other words, detection strategy) is to obtain a unique and reliable voltammetric signal for the target analyte. Secondly, the software is then used to process this unique voltammetric signal, as a sensor befits, into an output that states the presence/absence of the target analyte in the measured sample. The methodology and software are thus strongly intertwined, and a sensor with good performance requires a strong symbiosis between both. At the A-Sense Lab, this symbiosis is one of our fortes, and I am proud that we can demonstrate this with the sensors reported in Chapters 4-6, especially considering that this symbiosis is not common in a research field that tends to strongly focus on solely methodology development. What follows in this section is a list of studies that are conducted by electrochemists to develop a detection strategy. Where possible, I will try to frame, on the one hand, the connection of each study to further software processing, and, on the other hand, the importance of the study to the final sensor.

2.4.1 Electrochemical behavior of a target analyte on SPE

The ultimate goal is to develop a voltammetric sensor for on-site detection of an illicit drug. The on-site part implies that the technology has to be portable, and therefore it is a straightforward choice to use SPEs. Additionally, keeping in mind cost, reproducibility and reliability, solely unmodified SPEs were initially considered during my PhD. This was a deliberate choice, following the design philosophy of Lockheed engineer Kelly Johnson: 'Keep It Simple Stupid!'. If necessary, there was always the possibility to make the sensors in this thesis more complex with electrode modifications, however time has shown that satisfactory results could be obtained with unmodified SPEs for the application of purpose in this thesis (i.e. detection of illicit drug samples).

The first studies that are conducted, have as the objective to understand the electrochemical behavior of the drug on the SPE. The first analysis is typically a CV study of the target analyte at a fixed concentration in various pH's (Figure 2.5A). This can be considered a reconnaissance mission to learn more about the oxidation and reduction processes taking place, which will vary according to the analyte. The first choice that is subsequently made based on the CVs, is the decision to conduct further measurements in oxidative or reductive mode. If there are oxidation signals, one will almost always decide to proceed with oxidative measurements. The reasoning is that in reductive mode, oxygen generates a broad signal which potentially overlaps with interesting signals from the analyte. In oxidative mode, the oxidation of water also leads to a signal, however, this background signal lies in a potential window that is less likely to overlap with interesting signals of the analyte. Furthermore, the CVs in various pH's are there to support this choice, not to determine the pH for the eventual detection strategy, since a more detailed pH study will be carried out later on. At this point, an initial, optional choice in pH can be made in order to subsequently get an initial idea about the influence of concentration (Figure 2.5B). The criterion for this preferential pH is typically the amount of voltammetric signals of the analyte. Generally speaking, the same rule applies here as with the number of meat balls in my grandmother's tomato soup: the more, the better. These signals will later on be

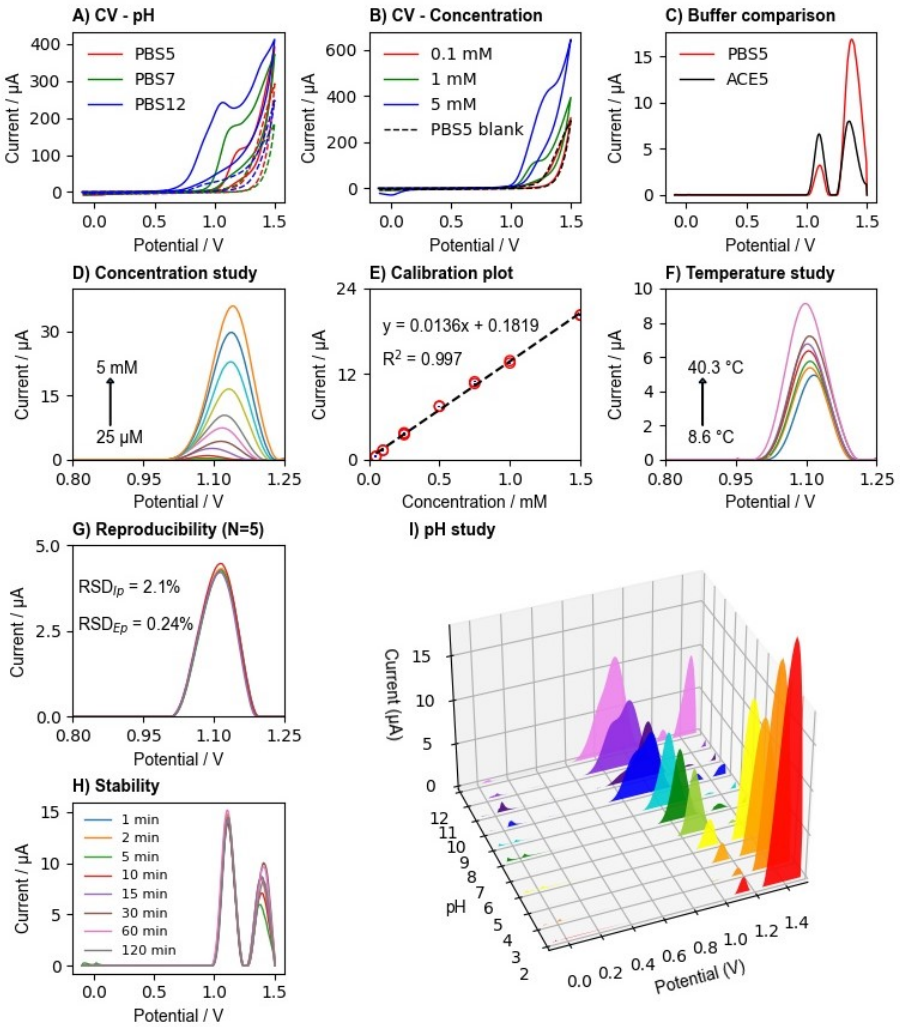


Figure 2.5: The electrochemical behavior of a target analyte, in this figure MDMA, on (unmodified) SPEs is mapped through a series of experiments. A) Cyclic voltammograms (CVs) of MDMA (1 mM) in various buffers (pH 5, pH 7 and pH 12). The dotted lines display the respective CVs of the blank buffers. B) CVs of MDMA in PBS5 buffer at various concentrations (0.1 mM, 1 mM and 5 mM). C) SWVs of MDMA (500 µM) in PBS pH 5 buffer and ACE pH 5 buffer. D) SWVs of MDMA in ACE pH 5 buffer at increasing concentrations (25 µM to 5 mM). E) Calibration plot of MDMA in ACE pH 5, based on the concentration study. F) SWVs of MDMA in ACE pH 5 buffer at increasing temperatures (8.6 °C to 40.3 °C). G) SWVs of MDMA (1 mM) in ACE pH 5 at new (unmodified) SPEs to evaluate the reproducibility. H) SWVs of MDMA (1 mM) in ACE pH 5 of the same solution, measured at various time points to evaluate the stability. I) SWVs of MDMA (500 µM) in various pH buffers to investigate the influence of pH on the electrochemical profile of MDMA.

used in the software for the unique identification of the target drug, so it is preferential to have as many signals as possible to work with. The CVs at various concentrations (e.g. 0.1 mM, 1 mM and 5 mM) at this preferred pH are there to gain an initial idea of potential, odd concentration behavior. Usually, the overall voltammetric profile remains present with varying concentrations, typical changes being an increasing peak current and potential of the voltammetric signals with increasing concentration. However, some illicit drugs, e.g. methamphetamine, have shown very different electrochemical profiles (EPs), depending on the concentration of the target analyte[85]. Finally, the CVs will also indicate if there is reversible or nonreversible behavior.

Subsequently, it is time to choose the voltammetric technique that will be used in the qualitative and/or quantitative identification itself, together with the parameters of said technique. Most of the time, one of the two high performing voltammetric techniques, i.e. DPV or SWV, is chosen. It seems that, to the best of my knowledge, there is no unambiguous approach to determine which one is the best, and that it is thus more a matter of personal preference. In the A-Sense Lab, SWV is preferred due to previous positive experiences and projects, and is therefore used for illicit drug detection as well. It was mentioned previously that a SWV measurement is defined by a set of parameters (e.g. step potential (ΔE_s), pulse amplitude (ΔE_p) and period), which need careful optimization. This optimization is laborious and time-consuming, and for time and resource management purposes not conducted again for each research study that is performed within a research line. Dr. Nick Sleegers and Dr. Mats de Jong conducted this optimization for cocaine detection at the A-Sense Lab, and it was decided to continue with their set of optimized parameters for all other illicit drug sensors at the A-Sense Lab[86]. Summarized, the following parameters are used for all SWV measurements, unless mentioned otherwise: potential range of -0.1 to 1.5 V, frequency 10 Hz, 25 mV amplitude, and 5 mV step potential.

What follows is the previously mentioned pH study, preferably performed in a Britton Robinson buffer (Figure 2.5I). The pH of a solution influences the peak current and peak potential in voltammetry due to the deprotonation of the analyte molecule. In an acidic environment, as the pH decreases, the analyte molecule tends to be protonated, which reduces its tendency to undergo oxidation. This results in a decrease in peak current and a shift towards more positive peak potential values. In general, a SWV of the target analyte is recorded for a pH range of 3 to 12, with increments of 1 pH value, at a fixed concentration (e.g. 0.5 mM or 1 mM). The first objective is informative, i.e. to learn more about the oxidation processes taking place. Plots can be made to show the change in peak potential and peak current with increasing pH. Secondly, based on the pH study, a certain pH will be selected for further development of the methodology. Typically, the guideline is that this is the pH with the maximal amount of oxidation peaks, although this is not necessarily always the case (see Chapter 4). Once a pH is selected, it is advised to evaluate if a PBS buffer is optimal, or if a buffer with better buffer capacities at the selected pH might be beneficial. As an example, if a pH of 5 is selected, an ACE buffer is preferred over a PBS buffer (Figure 2.5C). One should then perform an extra analysis to compare if a SWV of the analyte in both buffers shows the same electrochemical behavior.

The next study in line is a concentration study (Figure 2.5D). In this type of study, the pH is fixed, and a SWV of the target analyte is recorded over a wide range of concentrations, ranging from low concentrations (e.g. 10 µM) to high concentrations (e.g. 5 mM). A concentration study has several interesting outcomes. The most important one is

to learn more about the relationship between concentration and peak potential/peak current of the oxidation signals. Understanding this influence is crucial for the software development, and will eventually also determine the concentration range in which the sensor will operate. Furthermore, an effective calibration curve between concentration and peak current can be established, which can open up the integration of a quantitative module in the sensor later on (Figure 2.5E). Finally, the concentration study also provides the data to calculate the LOD and the limit of quantification (LOQ) of the methodology. The formulas used to determine these theoretical values are: 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 calibration curve. An additional analysis is thus needed here, i.e. a ten-fold measurement of blank buffer. The LOD is important to know and report, however as mentioned previously, not crucial for the success of the methodology.

Besides pH and concentration, a third parameter that is interesting to investigate is temperature. In general, increasing temperatures shift peak potentials to lower values, as described by the Nernst equation. The final application is intended for on-site use, which implicates that it should work in a wide range of temperature. Therefore, it is relevant to record SWVs of the target analyte over a range of temperatures (e.g. 5 °C - 40 °C, increments of 5 °C) at a fixed concentration at the selected pH (Figure 2.5F). The data obtained with this study will be used later on in the software development to make sure the eventual sensor holds up in various weather conditions.

Two more studies should be performed to fully map the electrochemical behavior of the target analyte at SPEs. First, the reproducibility should be checked with, what's in a name, a reproducibility study (Figure 2.5G). In this study, the target analyte is analyzed five times at new SPEs with fixed pH, concentration and temperature. The relative standard deviation (RSD) of the peak current and peak potential are calculated and reported. The purpose is to get an insight in the analytical performance of the methodology, although values below 5% and 1%, respectively, are preferred. Finally, a stability study over a prolonged time (e.g. 1 minute to 120 minutes) can be included to investigate, you will never guess, the stability of the analyte in buffer solution (Figure 2.5H). Again, the pH, concentration and temperature are fixed, however, this time the buffer solution with target analyte is prepared in advance, and a measurement is conducted at different time points after preparation. The purpose is to investigate the stability of the target analyte in the buffer, and more practical, if the methodology holds up if an end-user would leave some time between sample preparation and measurement. Again, the performance metric is the RSD of the peak current and the peak potential. This time, there are no preferred target values, since the purpose is more to understand the behavior. If it appears that the target analyte is only stable for five minutes, then it should come in the final protocol that there can only be five minutes between sampling and measurement.

2.4.2 Oxidation pathway of target analyte

Once the analytical performance of the methodology has been established, it is time to move on to the next step: unravelling the oxidation pathway of the target analyte. This study is not standard, nor crucial, for the methodology development of the voltammetric illicit drug sensor. It requires specific expertise and access to highly expensive scientific equipment (i.e. LC-MS), however, in return it provides great insights in the underlying mechanisms of the methodology.

The presence of an oxidation signal in a voltammogram indicates that the analyte gets oxidized, i.e. that the analyte loses one or multiple electrons. However, it is impossible to determine with voltammetry what the mechanism behind this oxidation is. A more powerful technique is required which can accurately identify the compounds that are present after oxidation, for which LC-MS is the ideal candidate. Before LC-MS can be applied, all oxidation products need to be present. This is achieved by applying a high voltage to a buffer solution with target analyte, i.e. conducting an electrolysis. The potentials at which the voltages are applied, are elegantly the peak potentials of the oxidation signals in the SWV of the analyte. After electrolysis, LC-MS (or even LC-MS/MS) is applied to the resulting solution to identify the oxidation products. Once these have been determined, all the puzzle pieces lie on the table, and the art is to piece those together into an oxidation pathway that connects the target analyte with its oxidation products. This is a difficult task that requires very specific expertise. Luckily, this expertise is present at UA, at the A-Sense Lab, and very importantly, at the Toxicological Centre of UA. In particular, prof. dr. Alexander L.N. van Nuijs and his research group at the Toxicological Centre, and dr. Nick Sleegers from the A-Sense Lab, are experts in this craft, and you can admire one of their art pieces in Chapter 4.

2.4.3 Electrochemical screening of a target analyte on SPE

At this point, the electrochemical behavior of the target analyte on SPE is well understood, and a preferential pH and buffer solution have been selected. The purpose of a sensor is to correctly identify a target drug's presence/absence in a sample. This seems evident and straightforward, however, one should be aware that there are millions and millions of different compounds on earth, and we ask from the sensor to correctly identify one specific compound out of all those compounds. It is clear that it is impossible to record the SWV for each compound to verify if they might be a treat for false positives (FPs) or false negatives (FNs). Therefore, smart choices should be made to evaluate the compounds that are a realistic threat for a false identification, and which compounds can be omitted. To do so, it is important to understand what causes FPs and FNs. Simply put, FPs are caused by compounds that have a similar EP as the target drug, and might be confused by the software as being the target drug. FNs are caused by compounds that are present in a sample together with the target drug, and alter the EP in such a way that the software does not identify the target drug anymore. Therefore, it is important to analyze compounds that: (i) might be analyzed with the sensor, as a FP check, and (ii) might be present in a sample together with the target analyte (cutting agents, diluents,...), as a FN check. Since the sensor will be used in a specific context, i.e. drug checking, it is possible to make a selection of compounds which covers the vast majority of potential FPs/FNs. Nevertheless, we have to be realistic that it is impossible to cover this risk for the full 100%.

The reports of the EMCDDA and UNODC are highly valuable to identify compounds that are interesting to investigate, however, the most important information is obtained by talking to the end-users. They can tell what samples they analyze with an on-site illicit drug sensor, and which are thus the potential FPs, and they know exactly what compounds are mixed with the target drug, and might thus cause FNs. This expertise is indispensable to develop a sensor that can make an impact, and to legitimize the sensor. At conferences and the like, the legitimate question often arises, triggered by the software

approach being followed (Chapter 3), whether there is not a very high chance of FPs. Technically this is indeed the case, but through a very thorough investigation of potential FPs and FNs we can largely mitigate this risk. Moreover, this is a risk that any kind of sensor has to live with, since it is impossible to analyze all compounds in the world. The specific challenge in drug testing, especially in a supply reduction context, is that criminal organizations actively try to circumvent the on-site detection technologies. Add to that the trend of NPS, and it is clear that this results in a continuous influx of new compounds that the on-site detection technologies have to withstand.

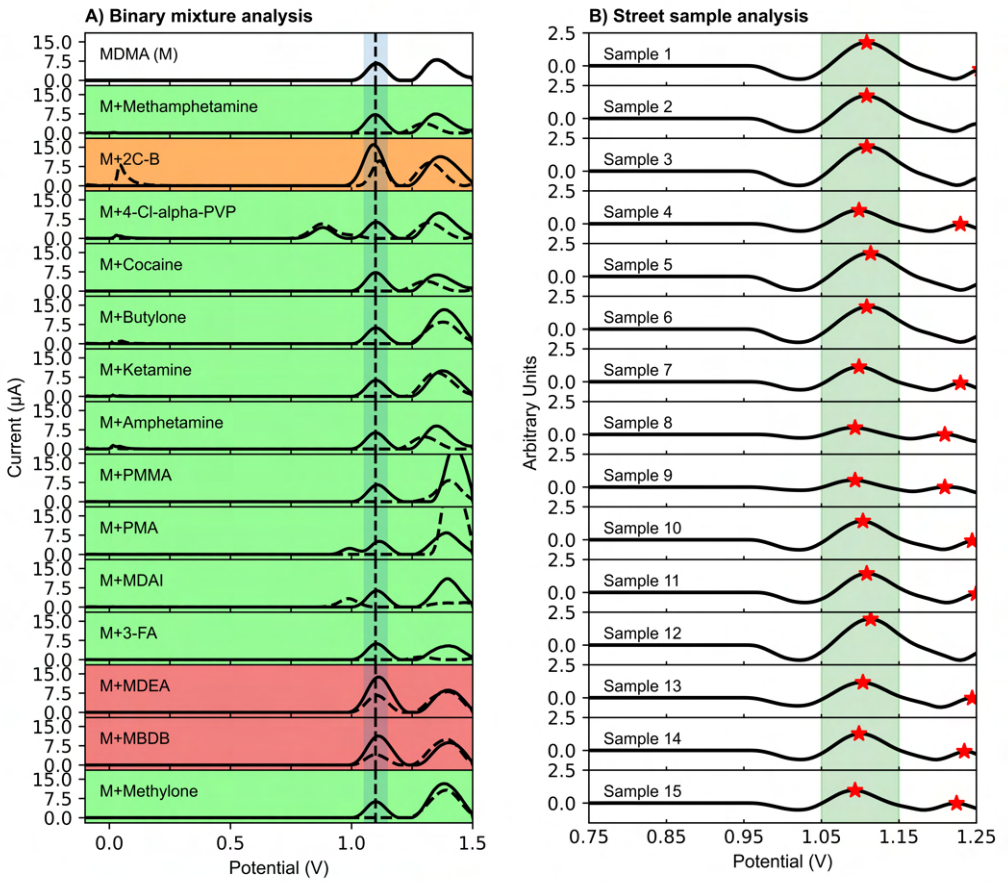


Figure 2.6: Part A displays a binary mixture analysis of MDMA in ACE pH 5 at SPE: the SWVs of 0.5 mM MDMA with 0.5 mM illicit drugs are displayed with full lines. The dashed line indicates where the signal of MDMA is located. The dashed SWVs indicate the EPs of the respective pure compounds. The detection window (1.05 V-1.15 V) is highlighted. Part B displays the processed SWVs (black) of 15 MDMA street sample mixtures in ACE pH 5 at SPE, in the light of a validation study. 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.

Once this set of relevant compounds has been determined, a screening study is conducted, which is coined a binary mixture analysis (Figure 2.6A). This study consists of two major parts: a first part to check for FPs and a second part to check for FNs. In the first part, a SWV of each compound of the set is recorded individually at room temperature in the selected buffer, with a concentration of e.g. 0.5 mM or 1.0 mM. Subsequently, a SWV of an equimolar mixture of the target drug and compound of the set is recorded at room temperature in the selected buffer, with a split concentration of e.g. 0.5 mM/0.5 mM or 1.0 mM/1.0 mM. Plotting the recorded curves and comparing them with the SWV of the target drug will provide visual clues on which compounds might raise potential FPs or FNs. However, the main purpose of this analysis is to provide data for software development. Indeed, one of the outcomes of the software should be that the target drug is identified by the software in all the binary mixtures and that all the individual SWVs of the selected set are returned negative by the software.

2.4.4 Software integration of methodology

The combined data of the electrochemical behavior studies and the binary mixture study, is sufficient to build the first version of the software. The ins- and outs of the software approach followed in this thesis are described in the next Chapter 3. Examples of the integration are described in e.g. Chapter 4. I will therefore not go into more detail here, and leave the more in-depth elaboration for the coming chapters.

2.4.5 Validation study of the sensor

A methodology and software have been developed at this point, what follows is a validation of the sensor. In voltammetric illicit drug sensing, a validation study usually consists of an analysis of confiscated samples (Figure 2.6B). The performance of the sensor is subsequently assessed with metrics such as accuracy, sensitivity (true positive rate) and specificity (true negative rate), providing an idea of how the sensor might work in real on-site scenarios. The validation study is the first time confiscated samples are analyzed with the methodology, and it is preferential to use the (first) validation studies as part of the development cycle. This means that the data collected during the validation study is used to further optimize the software. Evidently, a new validation study with new confiscated samples is then required to assess the performance of the improved sensor. Two important side comments need to be made: (i) the software developer needs to avoid overfitting the software on the validation set, and (ii) it is important to include negatives in the validation studies. Ideally, a validation set consists of a representative set of confiscated samples that an end-user would analyze with the sensor. Again, the input of the end-users is crucial in the development of the voltammetric illicit drug sensor.

The steps up until now describe the traditional steps for the development of a voltammetric illicit drug sensor. Most literature on the development of voltammetric illicit drug sensors will report (a selection of) these studies, and I have found that following these steps allows rapid development of a novel sensor for an (electroactive) target drug. However, additional studies (e.g. sampling methodology development, buffer stability studies, quality management system,...) are required to make the sensor truly usable by

end-users, and eventually make an impact with the sensor. These additional studies are described in Chapter 7.

I want to conclude this section with an important remark: validation of the sensor in the scientific world, as described above, is not the same as validation of the sensor in the real world. A more elaborate validation study is required to make the sensor, or even broader, the voltammetric sensing technology itself, eligible for e.g. forensic use. I am currently working on this more extensive validation study together with my talented colleague Florine Joosten, under the guidance of researchers from the National Institute of Standards and Technology (NIST). These additional studies investigate e.g. robustness, carryover, ruggedness,... of our sensors. All these studies will eventually culminate in a reference paper that should facilitate the acceptance of the technology in the forensic community.

2.5 Challenges

It should come as no surprise that voltammetric illicit drug sensors, just as every other sensing technology, have several challenges to overcome: (i) accuracy, (ii) validation with a large set of samples, (iii) bridging the gap from lab to end-user, and (iv) ability for multidrug detection. Some of these challenges are associated with the technology itself, whereas others are linked to the field and (complexity of the) market that is targeted.

Overall, the grand objective is to develop sensors that address the needs of society. Scientists, in general, will mainly use their time and energy to develop a sensor that ticks off scientific objectives such as high accuracy, excellent reproducibility, low LOD, short analysis time, etc. By trying to tick all these boxes, a disconnect might occur between the 'scientific' solution and a realistic application of the technology in the real world. Indeed, keeping the target market in mind, other aspects such as ease-of-use, reliability, validation, secure data handling and transmission, low cost and portability are important as well. There is often a trade-off between all these aspects, improving one might compromise another. Nevertheless, the objective should be to develop sensors that perform well in all areas that are important to the eventual end-users. In the coming section, I will discuss the challenges of voltammetric illicit drug sensors, with a special focus on bringing the advancements in the research field to fruition in a real world application.

The accuracy of an illicit drug sensor is in forensics typically expressed as the amount of true positive and negative analysis, divided by the total amount of analysis (including true positive and negative, and false positive and negative results). As such, good accuracy is obtained by developing a sensor that allows differentiation between target compounds and non-target compounds via a different voltammetric output, and by developing a data analysis approach that is capable of diversifying between these different electrochemical outputs. It should be noted that academic literature on voltammetric illicit drug sensors often fails to include such a data analysis approach, and simply employs a visual inspection of the voltammetric output. It may be clear that this approach might work if the expert is present, however, this is not a viable approach for a real-world application. Voltammetric output after a measurement is typically quite scarce in information, that is, a pure compound usually has a maximum of three oxidation/reduction

signals to work with. On the plus side, this typically provides the researcher with a good insight into the data since it is much more straightforward to interpret a voltammogram and understand the origin of each signal in comparison to e.g. a nuclear magnetic resonance (NMR) or Raman spectrum. However, the flip side is that there is often not as much information in the data to work with, which can make the unique identification of a target compound cumbersome. In addition, cutting agents and adulterants that are added to the drugs for profit, can suppress or shift the signals of an illicit drug in a voltammogram, and add their own signals to the voltammogram[87]. These different factors make it challenging, not impossible, to reach good accuracies with voltammetric illicit drug sensors. Fundamental research is of utmost importance here to understand the suppressing and shifting behavior of these cutting agents and adulterants. Besides the previously discussed electrode modifications, which allow a more target-specific EP, the electrochemist has another powerful tool to obtain good accuracies: chemometrics. This chemical discipline uses mathematical and statistical methods to maximally extract (electro)chemical information from (electro)chemical data. Linking chemometric tools to electrochemical illicit drug research has time and again proven successful to push accuracies to higher levels[88, 89]. Especially for applied illicit drug research, it seems unthinkable not to use chemometrics. Nevertheless, this is far from the standard, and it is a challenge for electrochemical illicit drug research to make more use of the tools provided by chemometrics. In the next Chapter 3, I will show how a chemometric algorithm can elevate voltammetric illicit drug sensors to a higher level.

Due to the constant emergence of NPS', there is an unrelenting supply of new drugs which can function as new targets for voltammetric detection strategies. Research papers describing such novel strategies for NPS targets appear relatively fast after a NPS rises to the forefront[90–92]. This highlights one of the qualities of voltammetric illicit drug research: its versatility and ability to quickly adapt to novel compounds. Typically, these research papers describe that the targeted drug has a voltammetric signal that can be used for detection. However, only a small amount of confiscated samples is included to prove that indeed, the drug can be detected with the new approach, followed by a statement in the conclusion that law enforcement agencies (LEAs) will greatly benefit from the new methodology. Importantly, LEAs are not too interested in these studies as long as no follow-up validation studies are performed with a larger number of confiscated samples, a substantial part of which should consist of negative samples. Only then will the new method be effectively validated, and LEAs will become familiar with voltammetric technology at the same time. From time to time, validation studies of this kind are carried out, but this still happens far too infrequently. In Chapters 4 and 5, I included validation studies on a large set of diverse samples that I gathered during the period 2019-2023, to legitimize that the voltammetric illicit drug sensors described in those chapters, and voltammetric illicit drug sensors in general, hold up when validated on a large set of diverse samples.

Similarly, it is a common sight in voltammetric illicit drug research, manuscripts that describe a certain type of modified electrode with great selectivity towards a target drug, obtaining great accuracy. Although very interesting and innovative from a research point-of-view, these modifications don't make the road to a real application shorter. Challenging reproducibility, long incubation times and an increased price are factors to be reckoned with. Little information can be found on modified electrode applications that made it out of academic research into a commercial application that is used in real life. This is not a statement to say it is impossible, but a critical look at the potential of a

modification to make it to such a stage would be beneficial for the research field. After all, the research field itself will blossom if at some point a commercial electrochemical illicit drug sensor is used by end-users. Further considerations about making voltammetric sensors really usable for end-users will be discussed in Chapter 7.

Additionally, as already mentioned previously, most electrochemical illicit drug research focuses on the detection of a single drug or single drug class. This is a drawback compared to other techniques, e.g. portable Raman or NIR[93–95], that can detect all illicit drug classes at once. Multiplexing, that is combining the electrochemical output of an array of SPE's, will open up electrochemical illicit drug detection to the simultaneous detection of multiple drug classes. It is expected that the field will evolve in this direction in the coming years as new fabrication technologies and nanomaterials use is converging. Similar to the single drug sensors, sufficient attention should be paid to the feasibility of these multidrug sensors for use in real scenarios. Aspects such as portability, easy sampling, short analysis times and low cost should be kept in mind during development besides the typical parameters such as reaching high accuracies. Chapter 6 is fully devoted to this voltammetric multidrug detection.

To summarize, electrochemistry and biosensors have the potential to deliver tools that meet end-user requirements: high accuracy, portable, low-cost, short analysis time, excellent reproducibility, safe data handling and transmission, and easy-to-use by non-experts. Currently, this potential is still largely contained within the research itself, and it is proving very challenging to bring the technology from the research world to the real world. In the following chapters I will describe how I have worked to change this over the past four years by eliminating the current disconnect between lab and real world.

Chapter 3

Data analysis

"I was always at my best when I was learning, when I was curious. When I had yet to see past the next horizon."

Reinhold Messner

This chapter is based on the manuscript "*Unlocking the full potential of voltammetric data analysis: a novel peak recognition approach for (bio)analytical applications*",
authored by *Robin Van Echelpoel, Mats de Jong, Devin Daems, Piet Van Espen & Karolien De Wael*,
which appeared in *Talanta, 223, 122605 (2021)*.

My contribution: Methodology, Visualization, Formal analysis, Software development, Writing - original draft.

Abstract

Bridging the gap between complex signal data output and clear interpretation by non-expert end-users is a major challenge many scientists face when converting their scientific technology into an on-site application. Currently, pattern recognition algorithms are the most frequently encountered signal data interpretation algorithms to close this gap, not in the least because of their straight-forward implementation via convenient software packages. Paradoxically, just because their implementation is so straight-forward, it becomes cumbersome to integrate the expert's domain-specific knowledge. In this chapter, a novel signal data interpretation approach is presented that uses this domain-specific knowledge as its fundament, thereby fully exploiting the unique expertise of the scientist. The new approach applies data preprocessing in an innovative way that transcends its usual purpose and is easy to translate into a software application. Multiple case studies illustrate the straight-forward application of the novel approach. Ultimately, the approach described in this chapter will play a crucial role in the coming chapters, since it allows the development of electrochemical sensors for illicit drug detection that require no prior knowledge of electrochemistry.

3.1 Introduction

Unraveling the valuable information hidden within complex data, and making that information understandable to non-experts, is a difficult task many scientists in (bio)analysis are confronted with when turning a scientific technology into a (bio)analytical application. Commonly, an expert in the research field is the only one capable of understanding and interpreting the complex data generated by a scientific device[96, 97]. However, if the complex output of the scientific device can be converted into a read-out comprehendible by non-experts, a major step is made towards the fulfillment of the point-of-need, paving the path for a successful application widely used. Software is the ideal solution to bridge this gap between complex data output and non-expert, grace to its cost-, time- and labor efficiency[98–101]. Furthermore, a software program can be integrated in the large majority of scientific devices. As such, a workflow can be created where an expert develops a software program and integrates it in the scientific device, after which any end-user can use the device without needing any scientific background. As a result, the group of potential users is drastically enlarged, and in extent the valuable time of the expert can be used elsewhere.

A suiting illustration of the aforementioned is given by the research field of electrochemical (bio)sensors, more specifically voltammetric sensors. It demands years of research to acquire the expertise to extract valuable information (qualitative and/or quantitative) from voltammograms. The development of an effective voltammetric detection method for the illegal drug cocaine is an excellent example of the aforementioned. It required many years of extensive research to come to a highly accurate (> 98%), portable voltammetric cocaine sensor that outperforms the existing on-site identification tools[102]. The suppressing and shifting nature of certain cutting agents (e.g. levamisole, benzocaine), in particular, made it challenging to develop an optimal strategy[103, 104]. The last major obstacle this voltammetric sensor has to overcome to become the ultimate tool law enforcement needs, is a translation of all the domain-specific knowledge that has been gathered over the years into a clear-cut interpretation thereof.

A commonly used approach to perform this translation, is the use of pattern recognition algorithms[105]. These algorithms have elevated research in many domains such as image processing and computer vision to new heights and are rightfully praised[106–108]. Voltammetry is no exception to this, and pattern recognition algorithms such as linear discriminant analysis (LDA), principal component analysis (PCA), soft independent modelling of class analogy (SIMCA) and more recently machine learning (ML), are frequently encountered[109–112]. One reason pattern recognition methods have become such a success is their easy implementation in software. Programming languages, such as Python or R, have highly convenient packages that allow a user to quickly run e.g. a PCA or construct and train a ML algorithm with limited prior knowledge. This is both a blessing and a curse, with the right expertise a user can quickly test different data analysis algorithms. However, the major risk occurs that the user no longer fully understands what happens between input and output, making the correct interpretation of the output cumbersome (so-called black box)[113–115]. The cohesion between the scientific technique that generates the data, and the data analysis method that interprets that data, is in danger of being lost. Especially, if it is also taken into account that the solutions these convenient software packages offer, are very general, precisely because they have to be so widely applicable. Incorporating domain-specific knowledge into

them can therefore be anything but straight-forward, while it is precisely this knowledge that makes a scientific technology so valuable and powerful. Furthermore, the performance of pattern recognition algorithms, ML in particular, is strongly intertwined with the amount of available data. A lack thereof can result in poorly trained algorithms that are prone to overfitting and have bad generalizability, which is especially dangerous in combination with a black-box approach[116].

In this chapter, a novel approach in which the domain-specific knowledge is the protagonist is proposed, rather than the algorithm itself. The expert's unique, subject-specific knowledge and insight in the data, is the starting point and the fundament on which the novel approach is built. The approach is developed for interpretation of voltammetric data, however it is envisioned that the scope of the approach can be extended to interpretation of signal data in other research fields. In voltammetry, the expert has generally excellent control over the different signals, i.e. the expert can commonly authenticate the origin and presence of each signal. Therefore, instead of trying to unravel patterns in the data, the individual peaks themselves will be used to extract information from the data. As such, it is assured that all the domain-specific knowledge of the expert is fully exploited, and in extent the risk of a black-box approach becomes non-existent. The bottleneck of this approach is thus the domain-specific knowledge, as opposed to e.g. the amount of available data to train an algorithm. This is far more desirable since a lack of domain-specific knowledge is a bottleneck for the scientific technology itself, and the data analysis as such does not create an additional bottleneck.

3.2 Experimental

3.2.1 Reagents and solutions

MDMA HCl standard was purchased from Lipomed (Arlesheim, Switzerland). A TNT sample was provided by the Dutch Forensic Institute (NFI, The Hague, The Netherlands). 2C-B and the cocaine and MDMA street samples were obtained from the National Institute for Criminalistics and Criminology (Brussels, Belgium). Analytical grade salts of potassium chloride and potassium phosphate, as well as potassium hydroxide, were purchased from Sigma-Aldrich (Overijse, Belgium). All solutions were prepared in 18.2 $M\Omega\, cm^{-1}$ doubly deionized water (Milli-Q water systems, Merck Millipore). The pH was measured using a CyberScan 510 pH-meter from Eutech Instruments (Landsmeer, The Netherlands) connected to a HI1131 glass bodied pH electrode from Hanna Instruments (Bedfordshire, United Kingdom). Adjustment of the pH was performed using a 100 mM KOH solution.

3.2.2 Voltammetric measurements

All samples in this chapter and throughout the rest of this thesis were weighed on a Sartorius Cubis MCE125P-2S00-U balance. This balance has a scale interval of 0.01 mg, has an automatic internal calibration, and is yearly externally calibrated with weights of 1 mg, 1 g and 100 g.

Electrochemical measurements, more specifically square wave voltammetric analyses, were carried out using a PalmSens4 potentiostat with PSTrace 5.7 software (Utrecht, The Netherlands). Disposable carbon ItalSens IS-C SPEs were purchased from PalmSens (Utrecht, The Netherlands) and were used during all electrochemical measurements. The SPE's contain an internal silver pseudo reference electrode and a carbon counter electrode. All experiments were performed by applying 50 µL of solution onto the SPE. All SWV measurements were carried out with a step potential of 5 mV, amplitude of 25 mV and frequency of 10 Hz. The following buffers were used during the electrochemical measurements: 0.1M phosphate buffer pH 5, 0.1M ACE buffer pH 5, 0.1M phosphate buffer pH 7 and 0.1M phosphate buffer pH 12. The measurement of the MDMA/2C-B mixture (Figure 3.3 – example 1) was preceded by a pretreatment (-0.8V/300s). The measurement of TNT (Figure 3.3 – example 2) was preceded by a 10 minute argon purge of the SPE cell to remove the influence of oxygen.

The bio-matrix samples using saliva, were prepared as follows: a stock solution of the drug in milliQ water was diluted in saliva to obtain a concentration of 500 µM (pure MDMA sample) or 0.3 mg/mL (street samples). Subsequently the stock solution containing the drug sample in saliva was diluted in the buffer to obtain the final concentrations (100 µM and 0.03 mg/mL respectively) for the pure sample and the street samples. A waiting time of five minutes was incorporated before starting the measurement.

3.2.3 Moving average baseline correction

The first step in the software framework is the removal of the baseline using a moving average baseline correction. The raw voltammogram is used as an input. For every two data points, the average current value is calculated, thereby reducing the amount of data points by a factor two. Every resulting data point A_i is subsequently compared to the average A'_i of the neighboring points A_{i-1} and A_{i+1}. Then it is checked if A_i is larger than A'_i (for oxidation peaks). If this is the case, A_i is replaced by A'_i. This is repeated until no more replacements take place or until the iteration threshold of 1000 is reached. Eventually, the number of data points is extrapolated to the original number. The resulting data points represent the increasing background and are thus subtracted from the raw voltammogram. The resulting voltammogram contains more visible oxidation peaks and is thus more easily interpretable.

3.2.4 Top hat filter

The top-hat filter is a so-called zero-area filter that has a central window with an odd number of channels w and two side windows each v channels wide. The value of the filter coefficients (k and h_k) follows from the zero-area constraint:

$$\mathrm{h}_k = \begin{bmatrix} -\frac{1}{2v}, -v - \frac{w}{2} \leq k < -\frac{w}{2} \\ \frac{1}{w}, -\frac{w}{2} \leq k \leq +\frac{w}{2} \\ -\frac{1}{2v}, +\frac{w}{2} < k \leq \frac{w}{2} + v \end{bmatrix}$$

The filtered (i.e. transformed) electrochemical response y_i^* is then obtained by the convolution of the electrochemical response with the filter:

$$y_i^* = \sum_{k=-v-w/2}^{v+w/2} h_k y_{i+k}$$

3.2.5 Peak identification

Two parameters are defined to identify the peaks: the minimum peak height and the minimum peak prominence. The first one speaks for itself, whereas the second one might require some more explanation. A marker is placed on the top of a potential peak. Subsequently, a horizontal line is drawn through this marker until (i) it crosses the signal because it encounters a higher peak or (ii) it reaches the left or right end of the signal. Then, the minimum of the signal in each of the two intervals defined in the previous step is searched. This point is either a valley or one of the signal endpoints. The higher of the two interval minima specifies the reference level. The height of the peak above this level is its prominence. Each peak that has a value higher than the defined minimum peak height and minimum peak prominence is identified as a peak that will be processed further throughout the approach.

3.3 Results

3.3.1 Approach

An innovative (voltammetric) signal data interpretation approach is developed which uses the locations of the individual peaks in a voltammogram to identify which compounds are present in a sample. Figure 3.1 illustrates the different steps of the developed approach: initially a raw voltammogram is preprocessed to enrich the electrochemical profile (i.e. the unique electrochemical signal or pattern specific for a certain analyte)[67]. After this preprocessing, the different peaks in the voltammogram are identified, followed by assignment of compounds to these peaks using an internal database. An exception module can be introduced in front of the peak assignment to include additional rules and requirements.

The novel approach utilizes individual peaks to assign the compounds present in the sample. It is therefore important to preprocess the raw voltammogram so that a voltammogram with distinct peaks is obtained. Preprocessing a raw signal to obtain a more easily interpretable signal is a common practice in signal processing[117]. The novel approach will however demand a more innovative use of the preprocessing that transcends its usual purpose. Solely obtaining a more easily interpretable signal is not enough, all meaningful peaks should be brought to light, even those that might initially be hidden by e.g. a shoulder. In the following steps, these meaningful peaks will be selected for further processing based on peak height and prominence. It is thus crucial that all meaningful

peaks are resolved and sufficiently separated, and a good preprocessing of the raw signal therefore plays a vital role in the success of the approach.

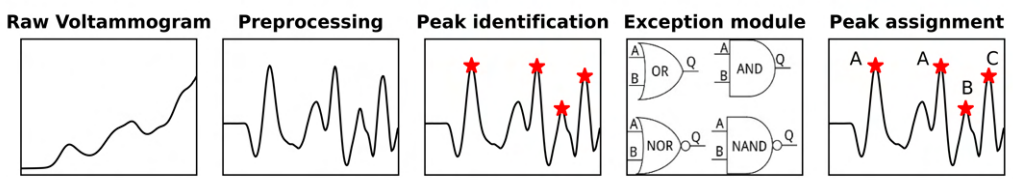


Figure 3.1: In the developed approach, a raw voltammogram is first modified with a baseline correction and a digital top hat filter to enrich the profile and as such improve sensitivity. Then the relevant peaks are identified, and assigned to a compound using an internal database. An exception module can be introduced to incorporate additional rules prior to the peak assignment step.

Initially, the baseline of the raw signal is corrected by using a moving average baseline correction, increasing peak distinction in the resulting voltammogram due to the removal of the background. However, overlapping peaks are not fully resolved by a baseline correction, limiting clear interpretation (Figure 3.2). A more sophisticated preprocessing tool, a digital filter, is thus subsequently applied to further improve peak demarcation. The general purpose of a digital filter is to smooth the data through a convolution, thereby improving the precision of the data without distorting the signal tendency[118]. Many different filters have been developed over time, often with great success, claiming a vital position in a wide variety of applications[119, 120]. For this approach, a zero-area filter, more specifically a top hat filter, was selected[121]. Besides their successful smoothing of the data, which is strived for here, zero-area filters have also proven to be beneficial for enhancing peak resolution[122]. This improved resolution is particularly useful here, as the approach is based on the identification of individual peaks. In this chapter, the zero-area filter of choice is the top hat filter, grace to its low computing time and promising performance in preliminary testing.

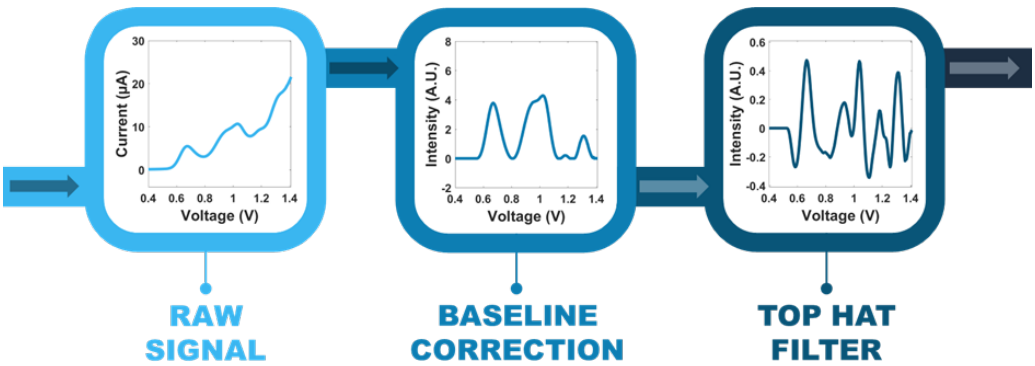


Figure 3.2: Illustration of the preprocessing steps.

After enrichment of the raw voltammogram with a baseline correction and digital filter, the resulting peaks are evaluated on their relevance. In a further stage, compounds will be assigned to these peaks, and it is thus important to solely consider peaks that hold valuable information. Two parameters are introduced to define which peaks are

interesting for further processing: (i) the minimum peak height and (ii) the minimum peak prominence. The prominence of a (signal) peak is a measure for how much the peak stands out relative to other peaks due to its intrinsic peak height and its peak location; it is akin to the concept of prominence in topography[123]. A low isolated peak can be more prominent than one that is higher but is an otherwise unremarkable member of a tall range. In the context of interpreting a voltammetric response, less prominent peaks are those which largely overlap with other peaks and/or which do not stick out considerably from the background signal.

Once the relevant peaks are identified, a compound is assigned to each of these peaks by exploiting the expert's domain-specific knowledge on voltammetric analysis or profile. The voltammetric profile of a compound represents the unique relationship between that compound and its specific voltammetric response, thus containing extremely valuable information[67]. Indeed, this relationship creates the perfect opportunity to link a (set of) voltammetric peak(s) to the presence of a specific compound. The approach takes full advantage of this opportunity by collecting all the voltammetric profiles into a database. The identified peaks are then one-by-one compared with the database, and a compound is assigned to a peak if a match is encountered.

The expert is the person par excellence to construct this database, since the latter is the sole person who has the required domain-specific knowledge. The database is one of the greatest assets of the novel approach, as it offers a general approach to incorporate subject-specific knowledge. Depending on the application, a further processing of the identified compounds can be included. In a detection sensor for example, an alarm or warning message could be associated with the detection of a specific compound to warn the end-user about its presence.

3.3.2 Illustration of the approach with three case studies

Figure 3.3 depicts three different applications of the developed software. In example 1, the analysis of an illicit drug mixture of 50.0% MDMA and 50.0% 2C-B (both commonly found ecstasy) in phosphate buffer pH 5 is shown. The baseline correction successfully removed the background, after which the top hat filter revealed two peaks that were not visible at first. This example truly demonstrates the power of the preprocessing steps, unravelling the presence of two peaks that were invisible in the raw voltammogram. The two revealed peaks are eventually correctly identified using the internal database, and assigned the appropriate tags, i.e. MDMA and 2C-B.

In the second example, a pure solution of the explosive 2,4,6-trinitrotoluene (TNT) in phosphate buffer pH 7 was analyzed. Baseline removal and filtering led to a modified voltammogram with distinct peaks. The three characteristic reduction peaks of TNT were subsequently correctly identified and assigned the right tag after comparison with the corresponding database[124]. Note that the intensities were inverted since the software searches for peak maxima instead of peak minima.

The third example, a street sample of cocaine, illustrates that more complex samples are still correctly handled by the software. After analysis in phosphate buffer pH 12, the baseline correction and top hat filter converted yet again with success the raw voltammogram into a processed voltammogram without background and clear peak distinction.

Cocaine was subsequently correctly assigned to the peak at 0.89 V, and in addition the cutting agents phenacetin, lidocaine and levamisole were correctly assigned as well[104].

The three case studies highlight the true power of the two data preprocessing steps. The preprocessing of voltammetry is a hot topic in voltammetry, involving the development and application of baseline removal algorithms and digital filters[125, 126]. The task of preprocessing the data is often unfairly limited to 'cleaning' the data (reducing noise and improving signal-to-noise ratio). Here, it is demonstrated that preprocessing the data can handle more ambitious tasks. The moving average baseline correction in combination with the top hat filter are the ideal tandem to reveal hidden features, improve peak demarcation and as such improve sensitivity. The resulting enriched voltammogram after preprocessing can thus be an objective in itself. An expert might come to new insights as new features that were masked in the initial voltammogram are revealed in the enriched voltammogram.

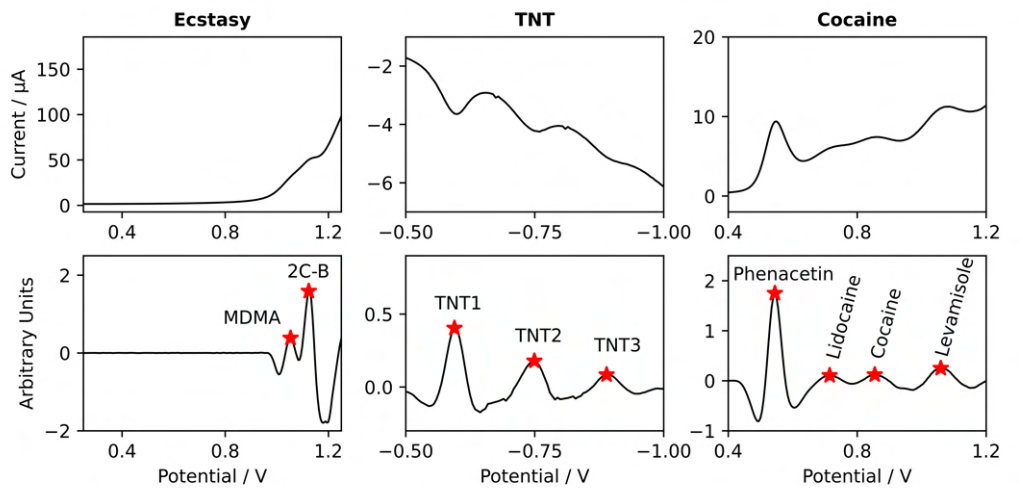


Figure 3.3: Three case studies of the approach are shown, from left to right: (i) an illicit drug mixture of 50.0% MDMA and 50.0% 2C-B in phosphate buffer pH 5, (ii) a 100.0% solution of the explosive 2,4,6-trinitrotoluene (TNT) in phosphate buffer pH 7 and (iii) a drug street sample containing 15.0 wt% cocaine, 23.2 wt% phenacetin, 16.0 wt% lidocaine, and 9.3 wt% levamisole analyzed in phosphate buffer pH 12 buffer. The top row displays the raw voltammogram, the bottom row the output after application of the peak identification approach.

3.3.3 Demonstration of the approach in bio-matrices

In the previous section, it was shown that the approach is highly suited to correctly identify a complex drug street sample. This result encouraged us to further explore the limits of the approach' applicability, this time by applying the latter to samples in bio-matrix. Since a database was already constructed for illicit drugs, it was decided to remain in this research area and investigate drugs samples in saliva. This bio-matrix is highly suited for illicit drug detection, especially when considering that many drugs can be found in saliva after administration[127]. The decision was made to focus on a highly

relevant target molecule, i.e. MDMA, as this illicit drug is almost exclusively administered orally, and research has proven its presence in saliva after a single dose[128]. In Figure 3.4, the approach is demonstrated on three MDMA samples in saliva. The first example depicts a pure MDMA sample (100 µM) spiked in saliva. Due to the higher complexity of the saliva, the electrochemical profile becomes more complex. However, the MDMA peak around 1.04 V is still visible and nicely highlighted by the preprocessing steps. The correct identification is subsequently executed by the approach. In the second example, the difficulty is raised by analyzing a MDMA street sample spiked in saliva (white crystal, 96.77%, 0.03mg/mL). The approach correctly identifies MDMA in this second example as well. The third example contains a MDMA street sample of low purity (orange tablet in form of Donald Trump, 39.46%, 0.03mg/mL). Once again, the approach correctly identifies MDMA. The bio-matrix, i.e. saliva, didn't cause any additional issues for the approach. It is interesting to note that some features at e.g. 0.36 V, 1.21 V and 1.39 V are recurring, probably originating from analytes in the saliva.

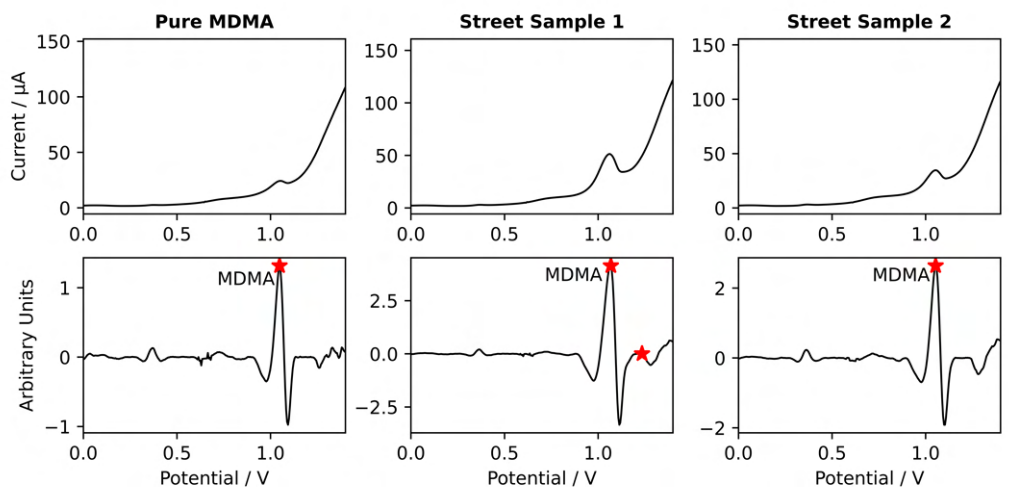


Figure 3.4: Three MDMA samples (one pure, two street samples) were spiked in saliva, after which the respective SWVs were measured in an ACE pH 5 buffer (top row). Subsequent application of the approach clearly indicates for all three samples that the assets of the approach translate to a bio-matrix (bottom row). The profile itself is, as expected, more complex due to higher complexity of the bio-matrix.

3.3.4 Special cases

In each of the examples presented in Figures 3.3 and 3.4, each peak could easily be assigned to a single compound. This 1-on-1 assignment will be sufficient in most of the cases where this software is applied. However, some applications require additional rules. The approach facilitates the incorporation of such additional rules through an exception module that is placed right in front of the compound assignment (Figure 3.5). This exception module is the perfect tool to bring domain-specific knowledge into the approach in a straight-forward manner. A parallel with the concept of logic gates is made to illustrate what an additional rule looks like[129–131]. However, the additional rules that can be incorporated are not limited to this concept. Figure 3.5A illustrates how

the presence of two (or more) characteristic peaks of a compound can be required when assigning a compound. An example is the voltammogram of the illicit drug ketamine in phosphate buffer pH 12[33]. This compound showcases two characteristic peaks, located at 0.90 V and 1.25 V respectively, and it can thus be required that both peaks need to be identified before ketamine is assigned to the voltammogram. The identification of only one of the peaks is thus not sufficient for assignment. Considering the TNT example in Figure 3.3, the aforementioned thus allows to incorporate an exception module that requires the presence of all three TNT peaks prior to assignment.

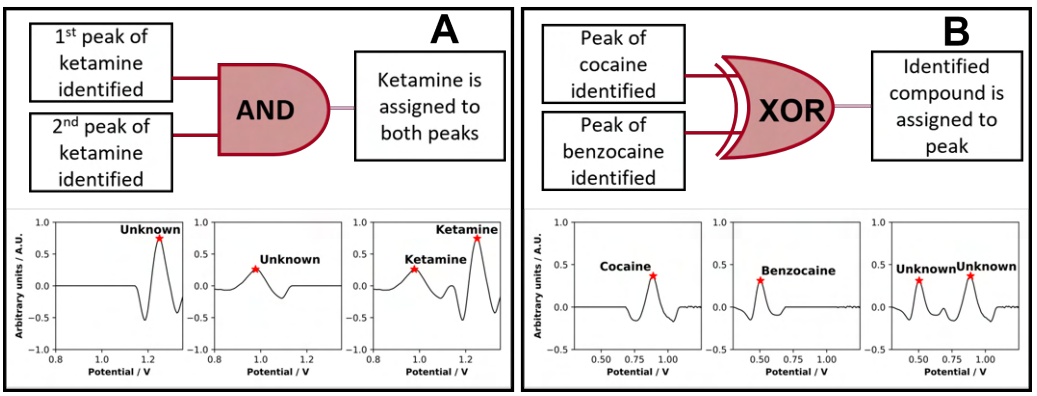


Figure 3.5: Additional rules, to extent the scope, can be integrated in the software by means of an exception module that is placed right before the peak assignment. The first example (**A**) shows how an additional rule can be incorporated that requires the presence of both peaks of ketamine ahead of assignment. Another possibility would be a rule where two compounds can never be assigned together (**B**).

Another possible exception is an additional rule that prevents two compounds from being assigned together (Figure 3.5B). Such an additional rule can be of great use when one of two compounds is known to cause a shift of the other one's signal. In that case it is a good safety measure to avoid their mutual assignment to prevent assignment errors. The implementation of this additional rule can be compared to an exclusive OR-gate, i.e. a true output is only given in case of an odd number of true inputs. In the example shown in Figure 3.5B, cocaine has one signal (0.82 V), as has the cutting agent benzocaine (0.41 V). Benzocaine is known to cause a shift of the signal of cocaine, and an additional rule is thus incorporated[104]. If both aforementioned signals (0.41 V and 0.82 V) are encountered together, cocaine and benzocaine will not be assigned to these peaks.

3.4 Discussion

In this chapter, a novel approach for the interpretation of voltammetric signal data was introduced. Instead of relying on the extraction and interpretation of complex patterns in the data, the interpretation is executed directly by comparing the individual peaks with an internal database. This database is constructed using the expert's domain-specific knowledge, thereby fully utilizing this unique expertise. Before comparison with the

database, two preprocessing steps, a baseline correction and a digital filter, are applied to the signal to remove the background and improve peak demarcation. This preprocessing tandem greatly enriches the voltammetric signal, revealing peaks that were visually hidden in the raw signal. These peaks are later on used for compound assignment and the preprocessing tandem consequently claims a key role in the approach. The increased sensitivity thus obtained is therefore of great importance in this approach, but can equally well be of exceptional value for any researcher seeking to extract hidden information from signal data.

Two parameters, minimum peak height and minimum peak prominence, are introduced to select the relevant peaks for further processing. A good parameter value choice is necessary to ensure that only those peaks are selected that hold valuable information. Values that are too lenient will result in a large pool of peaks that will further be processed, some of which might solely originate from noise.

Eventually, the selected peaks are compared with the internal database and assigned a compound if a match is encountered. The complexity of the database depends on the application and the amount of different compounds that need to be identified. The database of a detection sensor which targets a single compound could consist of that sole compound itself, whereas the database of an identification tool for e.g. waste water analysis might consist of a large list of compounds. Correct identification of samples in saliva demonstrates the approach' ability to transcend its assets into more complex bio-matrices. Additional rules, to e.g. build in restrictions or exceptions, can be included in the approach via an exception module. The database and exception module enable a very user-friendly integration of subject-specific knowledge into the approach, thereby providing quick access to tailor-made solutions. The approach allows integration of novel modules to facilitate further processing of the data. In voltammetry, amongst others research fields, the intensity of a signal can often be associated with the concentration of the corresponding analyte through means of a calibration curve. As such, a quantification module is an example of a potential module that could be introduced in the approach, in this case specifically dedicated to perform a quantitative analysis of the sample. Furthermore, the approach is designed in such a way that a single expert can optimize the approach (choose peak identification parameter values, build database, . . .), translate it into a suitable software program and integrate the latter in a scientific device. Ideally, a non-expert can then use the device without prior knowledge of voltammetry or even science. The expert is only involved in the setup and integration, which frees up time to perform more analyses.

3.5 Conclusions

A novel peak recognition approach is proposed, designed to interpret signal data. The approach combines a preprocessing tandem with a tailor-made database, thereby ensuring that the operating scientist can optimally integrate their expertise in the approach. In addition to the tailor-made database, an exception module grants the approach the versatility to readily be applied to different research problems. This adaptability makes the approach highly suited for integration in various scientific applications, e.g. (bio)sensors, that require interpretation of signal data. After integration by the expert, the approach

opens up the scientific application to non-expert end-users, allowing society to fully enjoy the benefits of the scientific technology.

Even though the approach was developed for interpretation of voltammetric signal data, it is envisioned that the scope can transcend the field of voltammetry and be applied in other fields of science that handle signal data such as optical (bio)sensors and applications in MedTech and life sciences.

Chapter 4

MDMA Sensor

"Anything simple always interests me."

David Hockney

This chapter is based on the manuscript "*Validated portable device for the qualitative and quantitative electrochemical detection of MDMA ready for on-site use*",
authored by *Robin Van Echelpoel, Marc Parrilla, Nick Sleegers, Saranya Thiruvottriyur Shanmugam, Alexander L.N. van Nuijs, Amorn Slosse, Filip Van Durme & Karolien De Wael*,
which appeared in *Microchemical Journal, 190, 108693 (2023)*.

My contribution: Methodology, Investigation, Visualization, Formal analysis with exception of LC-QTOFMS, Software development, Writing - original draft.

Abstract

Identifying and quantifying MDMA on-site in suspected illicit drug samples, both at recreational settings or manufacturing sites, is a major challenge for LEAs. Various analytical techniques exist to fulfil this goal, e.g. colorimetry 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 chapter, an electrochemical MDMA sensor is presented 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 the data analysis algorithm described in Chapter 3 and a 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 a large set of MDMA street samples provided by, and recorded at facilities of, NICC, LNS, Sciensano and Police Amsterdam. When possible, the performance of the electrochemical sensor was compared with the performance of a commercial portable Raman and ATR-FTIR device. Additionally, the electrochemical sensor was able to predict the purity of the tablets with a mean absolute error of 2.3%-2.6%. 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.

4.1 Introduction

MDMA was first synthesized in the 1910s by an employee of the pharmaceutical company Merck as a precursor to circumvent the patents of their rival Bayer[132]. Its structural similarities with hallucinogens, such as amphetamine and mescaline, attracted research interest over the next decades, but it was not until the 1970's that the compound really came to the forefront[133, 134]. 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[135–137]. 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[138]. Nevertheless, multiple side effects are associated with MDMA use, including brain damage, hypertension, depersonalization and nausea[139–142]. 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[1, 2].

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[1]. Some other established compounds found in ecstasy are 2C-B, 4-chloro-alpha-pyrrolidinovalerophenone (4-Cl-alpha-PVP), para-methoxyamphetamine (PMA), para-methoxy-*N*-methylamphetamine (PMMA), 5,6-methylene-dioxy-2-aminoindane
(MDAI), 3-fluoroamphetamine (3-FA) and methylone[12, 143–145]. 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[146].

MDMA usually comes in the form of (ecstasy) pills with a distinct color 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%[2]. 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[1]. It occurs that other psychostimulant agents are present besides MDMA, but this is rather rare. Specifically for ecstasy pills, some binders and coloring 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[2]. The MDMA content in ecstasy pills increases year after year, which in turn increases the risk of (unexpected) overdosing[2].

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)[1]. 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 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[147, 148]. The light, portable technologies on the other hand, with color tests (Marquis field test for MDMA) as a primary example, show the opposite pattern[149]. 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[51, 52, 93, 150–152]. Although showing promising results for on-site drug detection, these devices have a relatively high price tag, and the more portable Raman devices tend to struggle with colored samples (ecstasy pills are often colored) 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[6, 86, 153]. 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[81]. During previous years, electrochemical illicit drug sensors have emerged for the detection of various illicit drugs, including cocaine, heroin, ketamine and MDMA[16, 33, 86, 154]. 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[78, 155, 156]. 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 SPEs using DPV[145]. Their method demonstrated the opportunities of an electrochemical MDMA sensor, reaching low detection limits (0.17 μM), and showing an improved speed and cost over other analytical techniques such as Raman and High-Performance Liquid Chromatography (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 LSV[157]. 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, Shanmugam *et al.* reported an electrochemical approach for the detection of MDMA, using a combined PBS pH 7 and PBS pH 12 approach employing SWV at unmodified SPEs[41]. The two buffers were selected by the authors since MDMA has a rich 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. The novel detection strategy was subsequently validated on an initial set of 15 street samples, both qualitatively and quantitatively, and compared with the performance of a commercial portable Raman and ATR-FTIR on that same set of street samples. Later on, a more extensive validation was conducted on a set of 197 additional street samples, provided by end-users in Belgium, Luxembourg and The Netherlands. Overall, this new, electrochemical detection strategy provides LEAs with the rapid, low-cost, on-site detection of MDMA, without requiring specialized training.

4.2 Experimental

4.2.1 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 ecstasy street samples #1-#15 and #165-#212 were provided by the NICC in Belgium. Street samples #16-#20 were provided by LNS in Luxembourg, samples #21-#91 by police Amsterdam in The Netherlands, and samples #92-#164 by Sciensano in Belgium. The street samples were analyzed by GC–MS and

GC-FID to define their chemical composition (qualitatively). NICC employed GC-FID for quantification of MDMA, while Sciensano used UV-VIS spectrophotometry to quantify MDMA. The applied chromatographic methods are ISO 17025 accredited and are continuously evaluated through participation in international quality control programs (UNODC and European Network of Forensic Science Institutes—ENFSI). PBS solutions were prepared for the electrochemical measurements, containing 20 mM KH_2PO_4 and 100 mM KCl, purchased from Sigma-Aldrich (Belgium). ACE buffer 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.

4.2.2 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 (diameter = 3 mm), a carbon counter electrode, and a silver reference electrode, were used for all measurements (no preconditioning or pre-treatment required). The SWV parameters are the typical ones employed in this thesis: 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[86]. 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[16, 33, 41, 86, 154, 158] (i.e., PBS and ACE buffer) by applying 50 μL of the buffer onto the SPE. 100 mM KCl is sufficient for a fixed concentration of chloride ions and maintain 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 exci-

tation 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 min 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.

4.3 Results and discussion

In order to develop an electrochemical approach to detect MDMA (Figure 1.3) in one measurement within 30 s, 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 (Figure 4.1A and Figure 4.1F)[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 4.1A), since the oxidation peaks O2 and O3 are not visible in the considered potential range when employing a graphite SPE. The signal at 1.35 V 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 S4.1). 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 focusing on the corresponding signal will therefore result in a reduced amount of false positives, which in turn will improve the accuracy.

An ACE pH 5 buffer is selected over a PBS pH 5 buffer due the latter's lower buffer capacity at this pH, having a pKa of 4.76 and 7.21, respectively (Figure 4.1B). Increasing the concentration slightly shifts the diagnostic signal to more positive peak potentials (Figure 4.1C). 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 4.2 and S4.2).

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 (1.05 V-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[1, 2]. A detection window of 1.05 V to 1.15 V 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 ACE pH 5 buffer (Figure 4.1D). The linear relationship is described by the following equation: I_{O1} (μA) = 0.0136 (±0.0002) * [MDMA] (μM) + 0.1819 (±0.1412). A theoretical LOD and 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 μM and 52 μM), de Faria *et al.* (0.6 μM, no LOQ) and Alves *et al.*

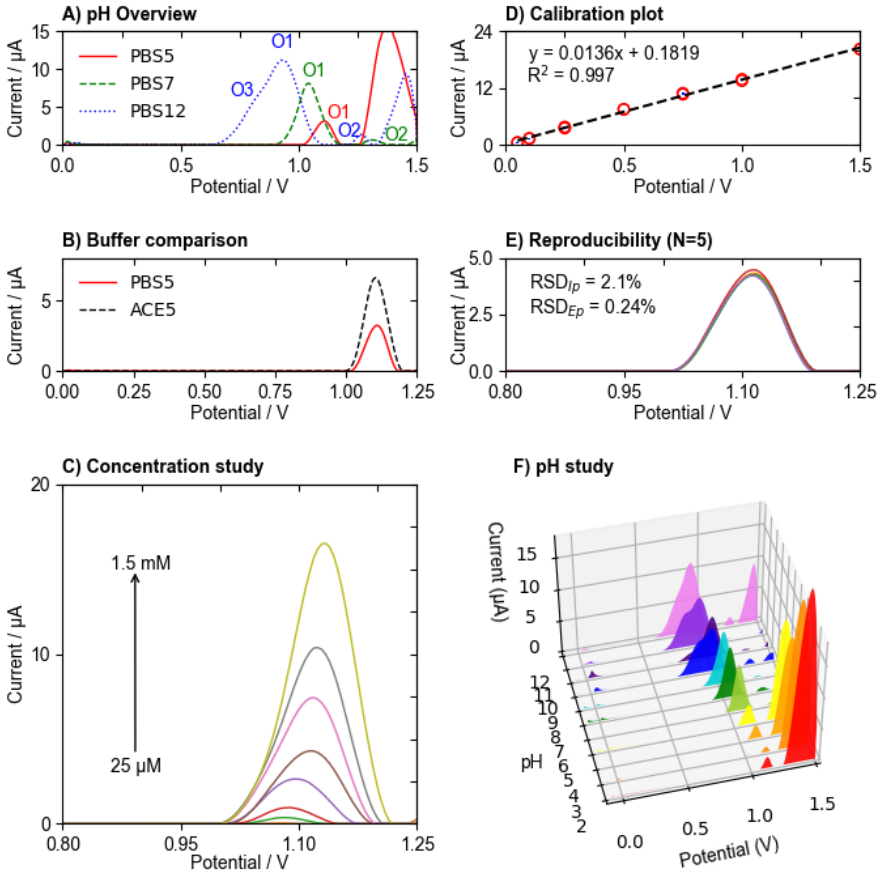


Figure 4.1: Electrochemical behavior of MDMA: A) SWVs of 0.5 mM MDMA solution in PBS buffer solutions at pH 5, 7 and 12 at SPE. Oxidation peak 1, 2, and 3 are abbreviated by O1, O2 and O3, respectively. B) SWVs of 0.5 mM MDMA solution in PBS pH 5 vs ACE pH 5. C) SWVs of increasing concentration of MDMA 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, E) Reproducibility study at 500 μM in ACE pH 5, and F) pH study of 0.5 mM MDMA in PBS buffer.

(1.83 μM and 6.11 μM)[41, 155, 157, 159]. Zhang *et al.* reach remarkably lower LODs (0.018 μM) by using Pt nanoparticles/carbon nanohorns[76]. 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

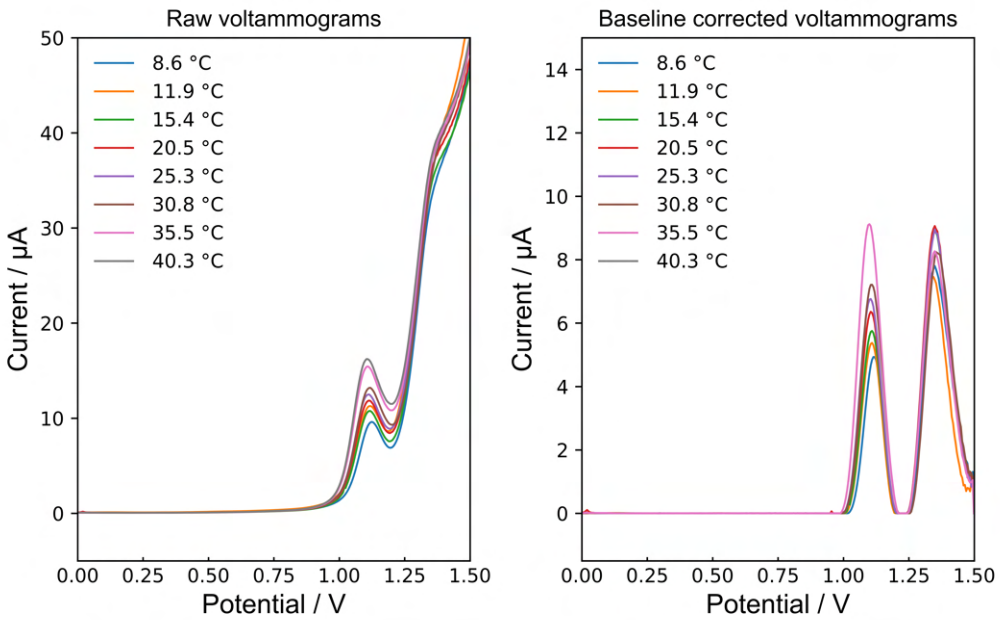


Figure 4.2: SWVs of MDMA (500 µM) in ACE5 at different temperatures. Both the raw voltammograms (left), and the baseline corrected voltammograms (right) are shown.

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 4.1E). Finally, a scan rate study confirmed that the mass transport mechanism is governed by diffusion of MDMA towards the SPE (Figure S4.3), which is in line with the findings of Shanmugam *et al.*[41].

4.3.1 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 EP. 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 4.1.

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 hypothesised that the product from pH 5 (1.11 V) is the same as found in the electrolysis at pH 7 (0.92 V). On

Table 4.1: Products found in electrolysis samples of MDMA. (DBE: Double Bond Equivalents, MFG: Molecular Formula Generator)

Compound	Retention Time (min)	Measured *m/z* $[M+H]^+$	Theoretical *m/z* $[M+H]^+$	Error (ppm)	DBE	Score (MFG)	Chemical formula
MDMA	4.23	194.1175	194.1176	-0.29	5	99.98	$C_{11}H_{15}NO_2$
P1	2.47	182.1171	182.1176	-2.51	4	98.93	$C_{10}H_{15}NO_2$
P2	4.16	178.0857	178.0863	-3.13	6	98.39	$C_{10}H_{11}NO_2$
P3	4.2	371.1984	371.1965	5.04	10	90.98	$C_{21}H_{26}N_2O_4$
P4	3.99	180.1016	180.1019	-1.7	5	99.51	$C_{10}H_{13}NO_2$

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 S4.4), it is concluded to be the product resulting from an oxidative demethylation reaction of the secondary amine (Figure 4.3). An identical reaction was reported for the oxidation of the secondary amine of ketamine[33]. In total, four products were found after electrolysis. P1 and P4 are products that are also formed during the metabolic oxidation of MDMA[160]. 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[161]. 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 electrolyzed 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 S4.4). 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 4.3), 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 S4.5)[160].

Moreover, this formed oxidation product P1 strongly resembles dopamine in its structure. The electrochemical mechanism and behavior of dopamine are described in depth in literature and are known to lead to follow-up reactions after its oxidation[162, 163]. Therefore, it is likely that product P1 exhibits similar behavior and consequently, a solution of dopamine was electrolyzed and analyzed by LC-QTOFMS (Figure S4.6). 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[163]. 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[162]. 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 S4.4). 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 4.3, which fits the EPs as well as the polymerization observed by Shanmugam *et al.*[41].

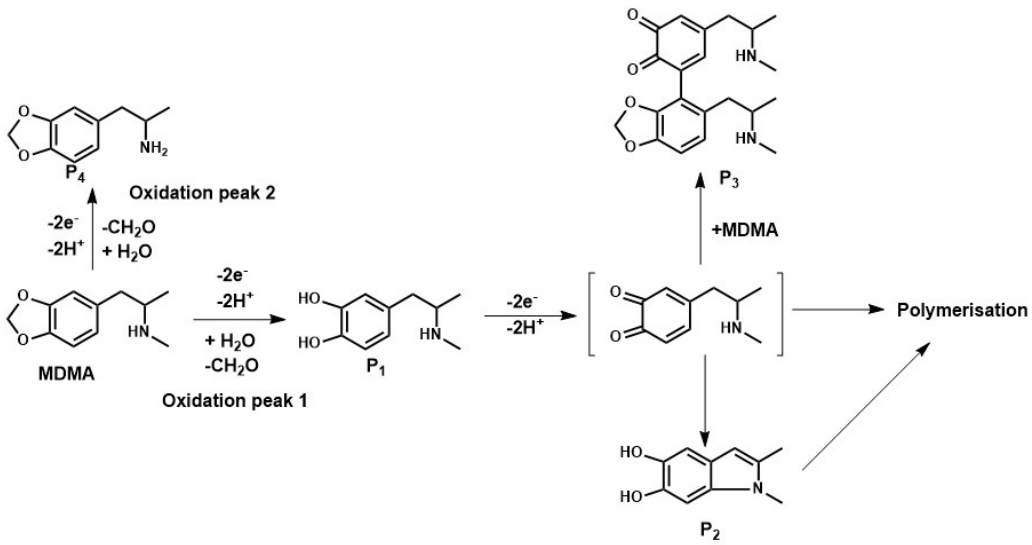


Figure 4.3: Proposed oxidation pathway of MDMA as elucidated by LC-MS.

4.3.2 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).

In Figure 4.4, 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 1.11 V. Listed below the EP of MDMA, with the dotted lines, are the EPs of pure cutting agents (Figure 4.4A) and pure illicit drugs (Figure 4.4B). 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 behavior,

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 (1.05 V – 1.15 V). Lidocaine and DXM have an oxidation signal at 0.99 V and 0.98 V, 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 Chapter 5. 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[92]. 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 EPs 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.

4.3.3 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[164]. 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 analyzed sample. Important for the accuracy of the predicted values is that the weight of the analyzed pill and the dissolved part, are carefully measured.

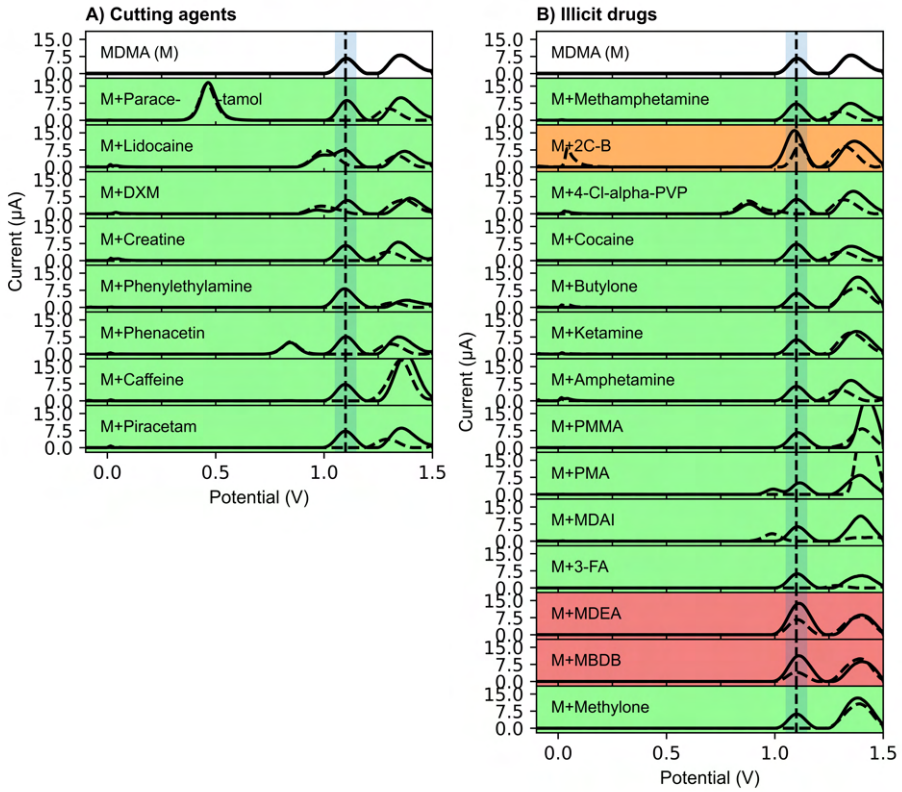


Figure 4.4: EP of MDMA binary mixtures in ACE pH 5 at SPE: SWV of 0.5 mM MDMA with 0.5 mM A) cutting agents and B) illicit drugs. The dashed line indicates where the signal of MDMA is located. The dashed SWVs indicate the EPs of the respective pure compounds. The detection window (1.05 V-1.15 V) is highlighted.

The calibration plot shown in Figure 4.1D, 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 analyzed sample.

$$c_{sample} = \frac{(I_{peak} - 0.1819)}{0.0136} \quad (1)$$

$$c_{100} = \frac{m_{sample}}{V * M(MDMA)} \quad (2)$$

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

The temperature has a (minor) influence on the observed peak currents, and thus on the quantification (Figure 4.2). The best results for quantification 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). The quantitative part of the sensor is therefore less straightforward to use on-site, since (i) there is a need for an analytical balance, and (ii) the temperature is best kept around room temperature. The qualitative sensor does not have these limitations, by working with an interval around the peak potential the sensor continues to perform optimally in both low and high temperatures. In addition, in Chapter 7 an extension of the software will be revealed that further addresses the temperature effect on the peak potential for qualitative identification.

4.3.4 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, the peak recognition approach described in Chapter 3 was integrated in the novel methodology, as such taking away the data analysis from the end-users[81]. 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. 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 at the given pH, 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 4.5 shows a screenshot of the final output screen, indicating that the analyzed sample contains MDMA. More impressions of the application are shown in Figure S4.7. Figure 4.5 also contains a QR code that, when scanned, connects to a video that 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.

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

The novel methodology (with the peak recognition approach and quantification module) was initially validated on 15 street samples containing MDMA (Figure 4.6). This set of

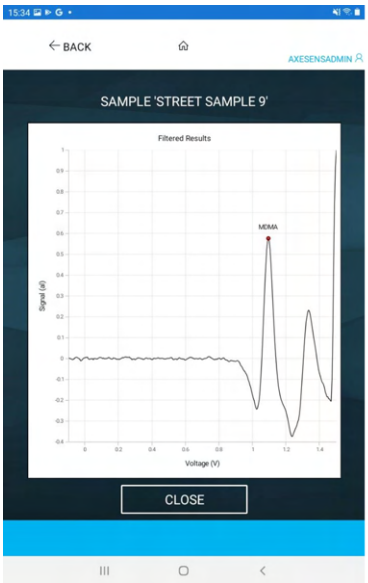


Figure 4.5: Screenshot of the processed voltammogram of street sample 9 measured in ACE pH 5 buffer, as shown in the mobile application (left). The diagnostic peak of MDMA around 1.11 V is clearly visible. Other screenshots of the mobile application are shown in Figure S4.7. Scanning the QR code will redirect you to a video that demonstrates the final sensor (right).

street samples represents the illicit ecstasy market, showing color variations (white, green, red,. . .), MDMA concentration (19.4%-96.6%) and appearance (powder, crystal and tablet). The overview of this set, together with the results of the electrochemical sensor on the set, are shown in Table 4.2.

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 color 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 4.2). 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 4.2 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 more lab-based, as it requires careful weighing with an analytical balance.

Table 4.2: 15 confiscated 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). The composition of the confiscated samples was determined with GC-FID.

Sample Name	Sample Content (w/w%) (GC-FID)	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

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 S4.8 and S4.9). Although the latter is indeed portable, it cannot be considered handheld, as it still requires a bench-top for its operation.

Contrary to the electrochemical methodology, not all samples are correctly identified as MDMA by the portable Raman device. In fact, 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, targeting 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.

4.3.6 Further validation of the technology

After the initial validation of the technology, I have striven to further validate the MDMA sensing technology at each opportunity that presented itself. Thanks to the strong net-

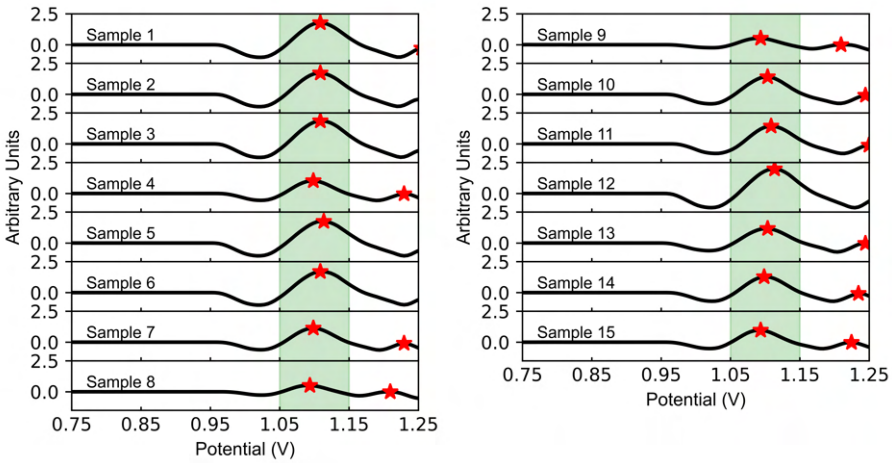


Figure 4.6: Processed SWVs (black) of MDMA on 15 street sample mixtures in ACE pH 5 at SPE. The red stars indicate the peaks detected by the software application, the green area represents the MDMA interval (1.05 V-1.15 V). If a peak is detected in the selected interval, the analyzed sample is set to contain MDMA. Figure S4.10 shows the baseline-corrected SWVs of the street samples.

work of the A-Sense Lab, I was able to perform measurements on relevant samples at various end-user sites, spread over three countries. This allowed me to expand the original validation set, consisting of 15 confiscated samples, to a validation set of 212 samples (Table S4.1). A number of 48 additional samples were measured at the NICC facilities in Brussels, five samples were measured at the LNS in Luxembourg, 73 samples were measured at Sciensano, and 71 samples were measured at the facilities of police Amsterdam. This last set will be discussed in more detail in the next Chapter 5. The samples measured at Sciensano consist of samples confiscated at the music festival Tomorrowland (2022), and were analyzed by a Master student working at Sciensano, after she received a training from yours truly. The gold standard techniques GC-FID and GC-MS were used to determine whether or not MDMA was present in the confiscated samples. If not, it was indicated if possible what other substance was present in the samples. NICC used GC-FID to accurately measure the m% of the first 15 confiscated samples, while Sciensano used a method based on the less accurate UV spectroscopy. The m% of samples from samples #92-#164 is therefore less reliable than samples #1-#15. It is noteworthy that 73 of the 212 samples (34.4%) did not contain MDMA. This is an important asset of this validation set, given that validation sets in literature tend to focus primarily on the target analyte. To conclude, it is also interesting to mention that every sample that was added to the validation set was measured on location, and therefore not in the safe environment of the A-Sense Lab.

Looking at the results, it can be observed that the voltammetric MDMA sensor achieves a nice accuracy of 91.5% (Table 4.3). A further analysis shows that there is a substantial difference between the sensitivity (99.3%) and the specificity (76.7%). From this it can be concluded that the method is much more sensitive towards false positives, compared to false negatives. Indeed, only one false negative sample was recorded on the entire set, i.e.

Table 4.3: Overall performance of the MDMA sensor described in this chapter. The sample set consists of 212 confiscated samples: 139 positives that contain MDMA, and 73 negatives that do not contain MDMA. The samples were measured at four different facilities: NICC, LNS, police Amsterdam and Sciensano, over a period of approximately two years. Three parameters, i.e. accuracy, sensitivity and specificity, are calculated to judge the overall performance.

Overall performance			
True positive (TP)	138	#samples	212
False positive (FP)	17	Accuracy (%)	91.5
True negative (TN)	56	Sensitivity (%)	99.3
False negative (FN)	1	Specificity (%)	76.7

a mixture of MDMA and ketamine. This is unusual to say the least, especially considering that almost two-thirds of the set carries a potential risk of false negatives. The risk of false positives from the method is therefore much higher. Digging deeper into the data, it becomes evident that false positives primarily arise from the presence of the illegal, psychoactive substance 2C-B, accounting for 10 out of 17 cases. Chapter 5 describes a solution that was sought for this issue. Additionally, false positives were also observed with the psychoactive drug mCPP (1x), as well as structurally similar compounds such as MDA (1x) and MMDPA (2x). Finally, three false positives were noted in the Sciensano festival set, the composition of which was not determined.

The ultimate goal of this validation study is to reveal the strengths, but certainly also the weaknesses, of the methodology. Here, the risk of false positives for 2C-B, and to a lesser extent MDA, MMDPA and mCPP, is clearly a disadvantage, and it is very important that this is communicated openly to the end-users. On a positive note, the technology demonstrates excellent accuracy and sensitivity, while also proving its effectiveness in on-site applications.

As mentioned previously, the samples from Sciensano provided quantitative information. The concentration was determined using UV-VIS spectrophotometry, relying on the Lambert-Beer law, which is considered less reliable compared to the GC-FID method employed by NICC. It's worth noting that the UV-VIS methodology is unable to quantify caffeine in the samples. However, the data from Sciensano can still be used to identify general trends. A MAE of 2.6% exists between the purities calculated at Sciensano via UV-VIS spectrophotometry, and the purities calculated with the electrochemical detection strategy described in this chapter. This result aligns closely with the MAE of 2.3% observed for the NICC set (samples #1-#15). This excellent result did not go unnoticed at Sciensano. Consequently, the organization reached out to employ the quantitative voltammetric MDMA sensor described in this chapter for the early warning system at the Pukkelpop music festival (2023). Due to external reasons, it was finally decided not to let this go ahead. Nevertheless, this recognition serves as a rewarding acknowledgment for the technology, which is now poised to make a positive impact on society.

4.4 Conclusions

The work in this chapter 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 that these devices do not provide any direct quantitative information.

Following the initial validation on 15 street samples, the methodology underwent further validation using novel street samples obtained from end-users in three different countries (NICC, LNS, Sciensano, and police Amsterdam). The validation set was expanded to include 212 street samples, of which 73 did not contain MDMA. Overall, the methodology exhibited an accuracy of 91.5%, a sensitivity of 99.3%, and a specificity of 76.7%.

It is important to note that the methodology carries a risk of false positives, particularly in relation to the psychoactive compound 2C-B. To provide additional quantitative validation, a separate analysis was conducted using 73 samples provided by Sciensano. The results revealed a MAE of 2.6%, which aligns with the MAE of 2.3% calculated on the initial set of 15 street samples provided by NICC.

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 decentralization of forensic analysis.

4.5 Supplemental figures and tables

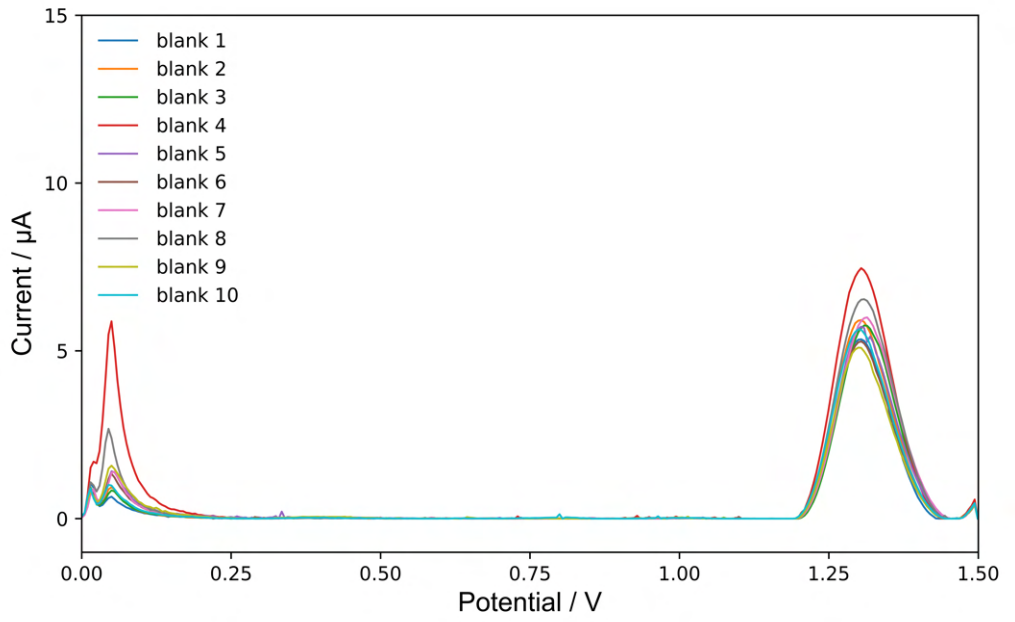


Figure S 4.1: SWVs of 10 blank measurements in acetate pH 5 (ACE5) buffer.

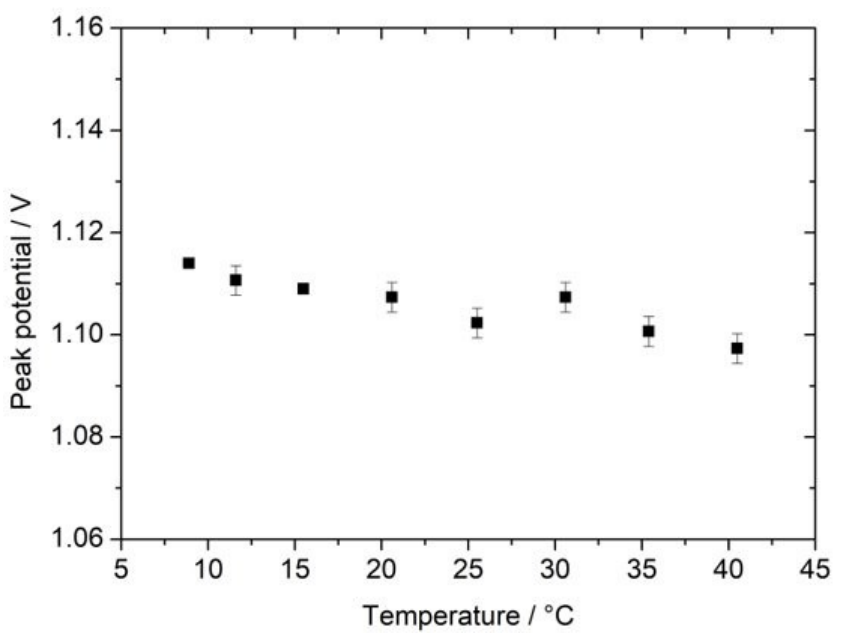


Figure S 4.2: Influence of the temperature on the peak potential of the diagnostic signal of MDMA (500 µM) in ACE5 buffer.

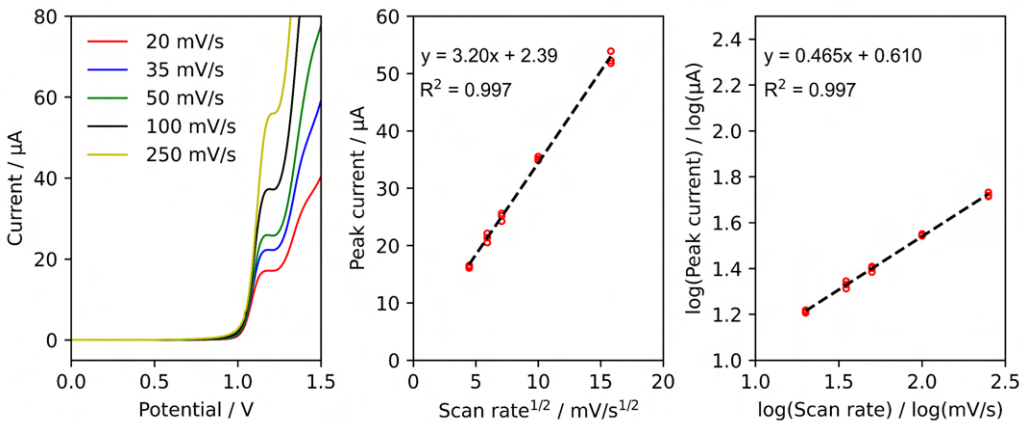


Figure S 4.3: Useful information about the electrochemistry of MDMA can be attained from the relationship between the peak current and the scan rate. Linear sweep voltammetry (LSV) measurements with various scan rates were carried out to assess whether the process at the working electrode of the SPE is adsorption or diffusion controlled. The figure left presents the LSVs at different scan rates (0.02 to 0.25 V/s). A plot describing the peak current in function of the square root of scan rate (middle) shows a linear trend, indicating the electrochemical process is diffusion controlled. A plot of log (peak current) vs log (scan rate) was found to be linear with a slope of 0.465 (right), which is close to the theoretical value of 0.5 for a purely diffusion controlled process, confirming that the process is diffusion controlled with the electroactive species of MDMA diffusing from bulk solution to the electrode surface as the rate determining step.

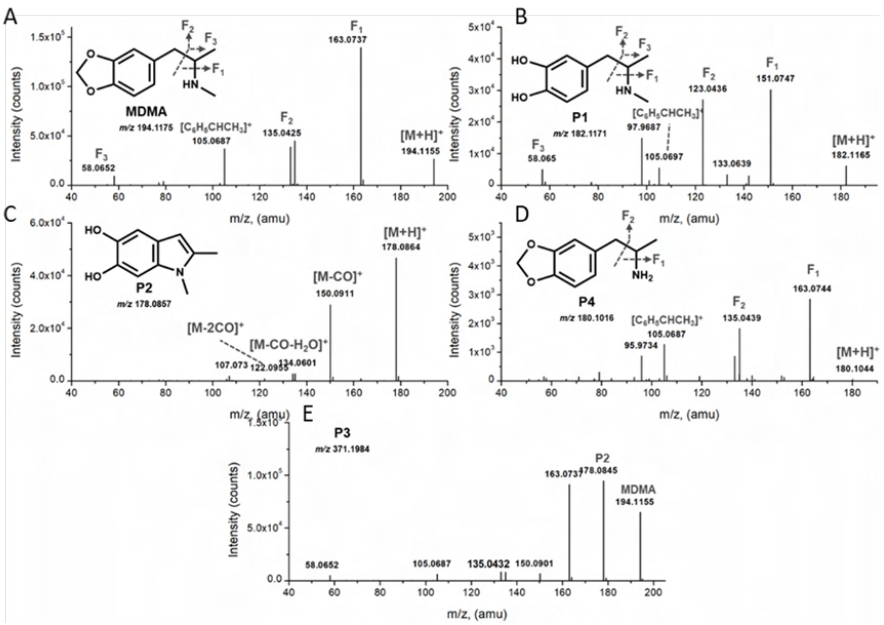


Figure S 4.4: MS/MS spectra of (A) MDMA (*m/z* 194.1175, $C_{11}H_{15}NO_2$), (B) oxidation product P1 (*m/z* 182.1171, $C_{10}H_{15}NO_2$), (C) oxidation product P2 (*m/z* 178.0857, $C_{10}H_{11}NO_2$), (D) oxidation product P4 (*m/z* 180.1016, $C_{10}H_{13}NO_2$) and (E) oxidation product P3 (*m/z* 371.1984, $C_{21}H_{26}N_2O_4$).

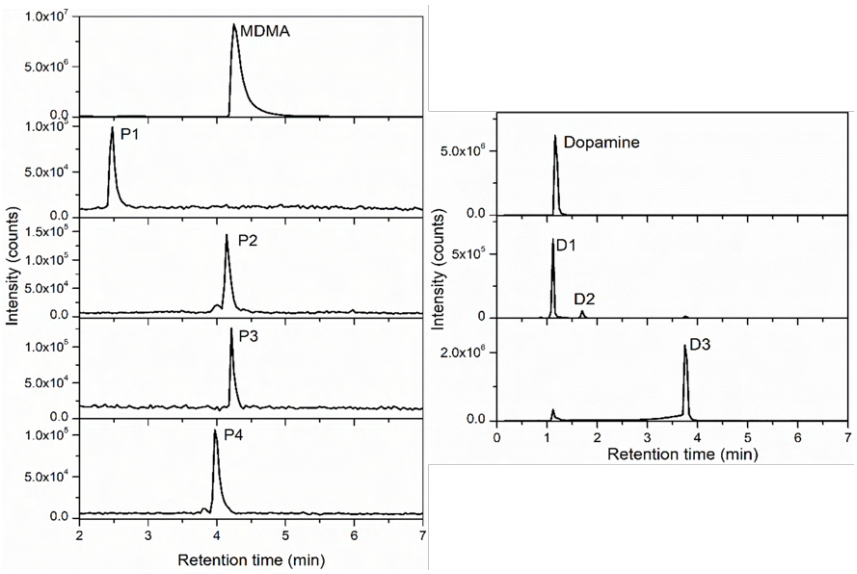


Figure S 4.5: Extracted ion chromatogram of MDMA standard (*m/z* 194.1175) and its oxidation products: P1 (*m/z* 182.1171), P2 (*m/z* 178.0857), P3 (*m/z* 371.1984) and P4 (*m/z* 180.1016) (left). Extracted ion chromatogram of dopamine standard (*m/z* 154.0860) and its oxidation products: D1 (*m/z* 152.0706), D2 (*m/z* 152.0706), D3 (*m/z* 150.0550).

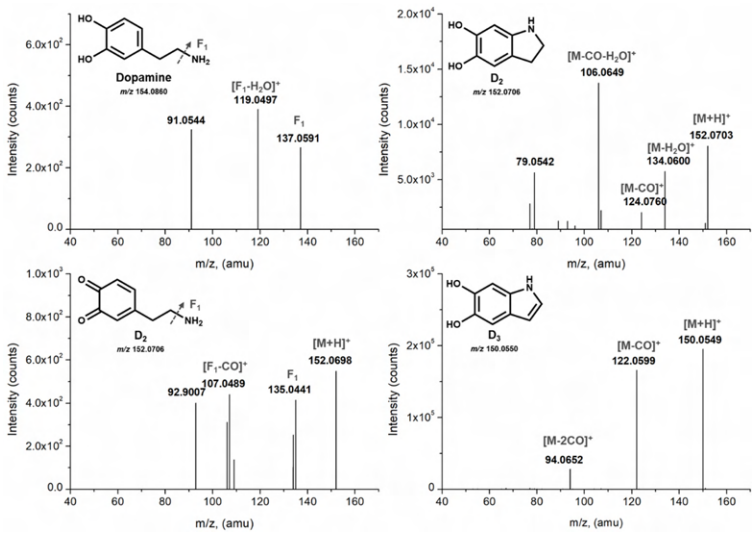


Figure S 4.6: MS/MS spectra of (A) Dopamine (*m/z* 154.0860, C8H11NO2), (B) oxidation product D1 (*m/z* 152.0706, C8H9NO2), (C) oxidation product D2 (*m/z* 152.0706, C8H9NO2) and (D) oxidation product D3 (*m/z* 150.0550, C8H7NO2).

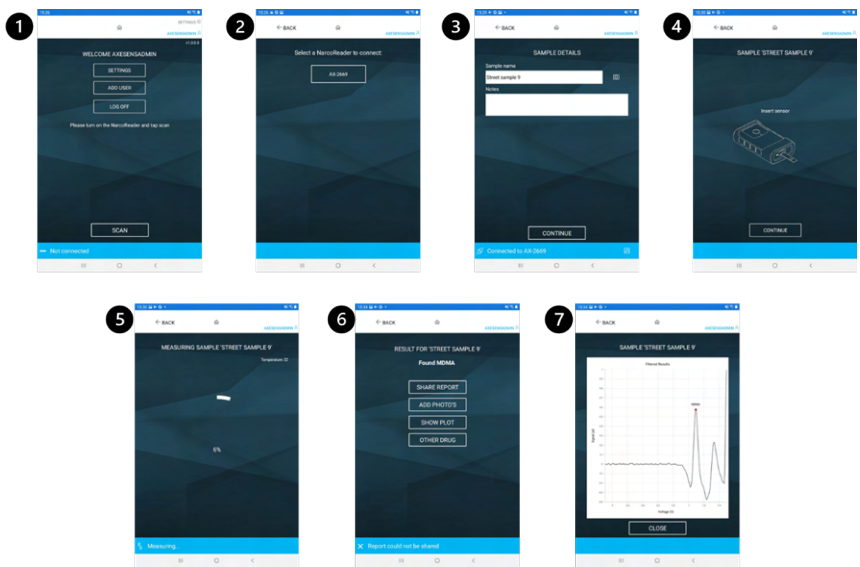


Figure S 4.7: Impressions of the mobile application, 1) Introduction screen, 'SCAN' will guide to screen 2; 2) Scanning for potentiostats via Bluetooth; 3) Once connected, the sample can be given a name, accompanied with some pictures and notes; 4) Animation shows end-user how to insert electrode in potentiostat; 5) Once the electrode is inserted, the measurement is started. Only a loading screen is shown, the user does not see the recording of the voltammogram; 6) Screen indicating presence or absence of MDMA. The user can also ask to share a report, add some photos or show the plot. An additional measurement can be started if desired.; 7) 'SHOW PLOT' in screen 6 redirects to this screen.

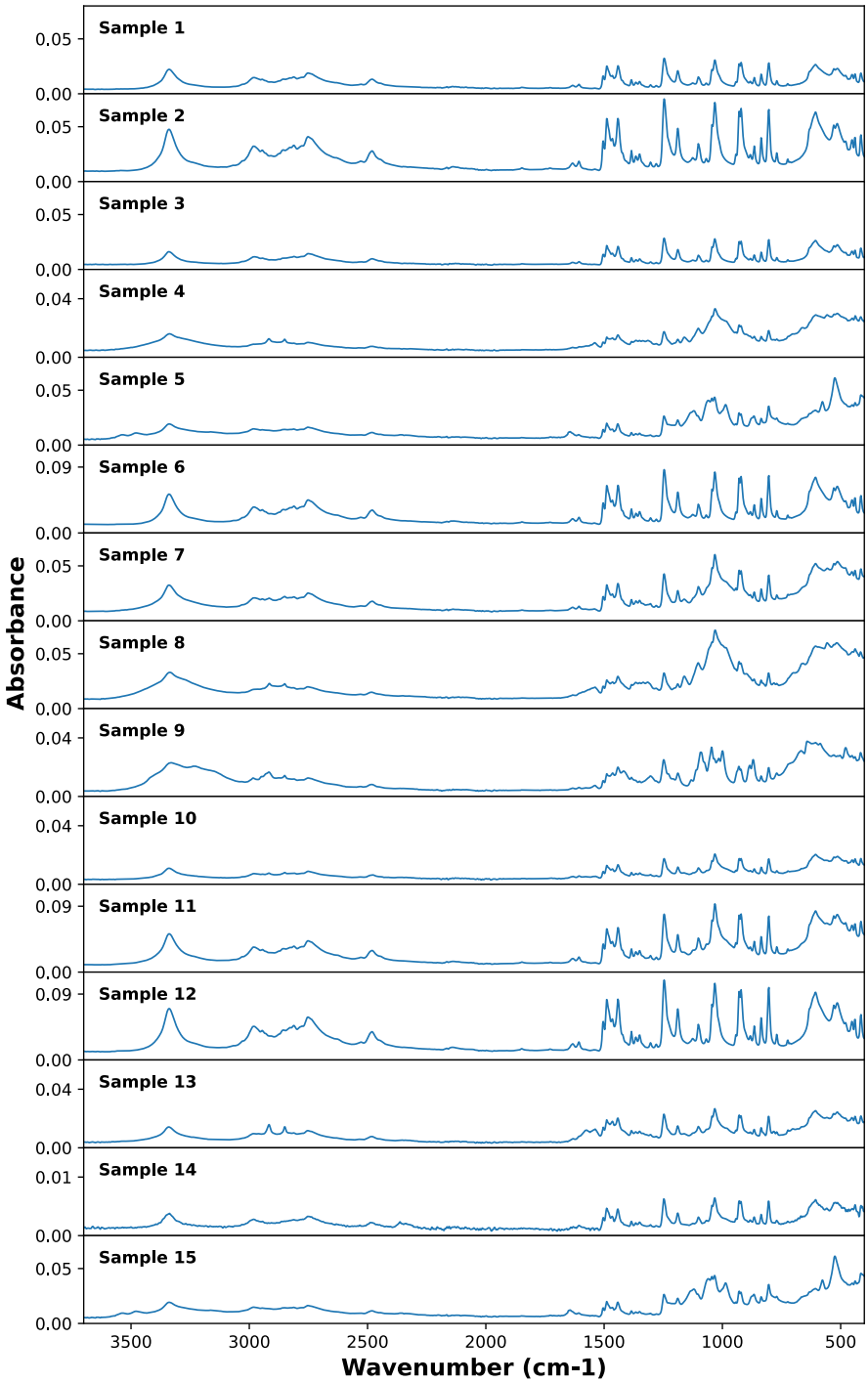


Figure S 4.8: FTIR-ATR spectra of the 15 street samples, composition can be found in Table 4.2.

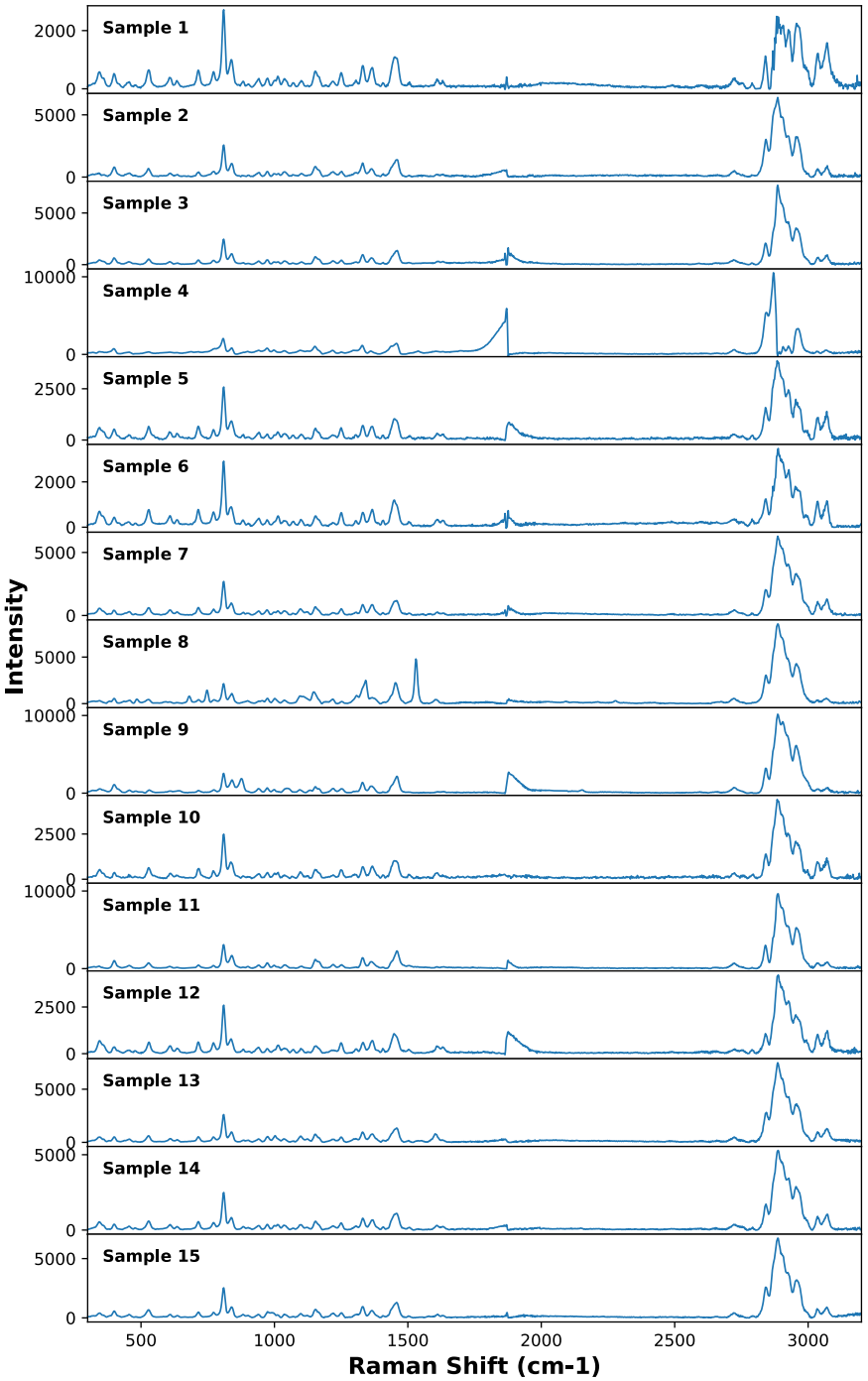


Figure S 4.9: Raman spectra of the 15 street samples, composition can be found in Table 4.2.

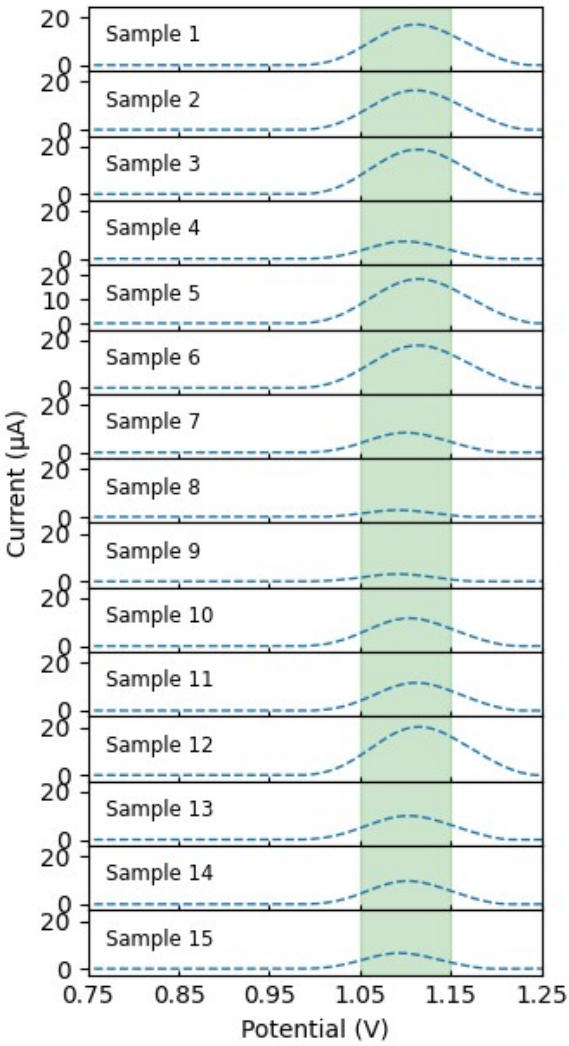


Figure S 4.10: Baseline-corrected SWV's of the 15 MDMA street samples, composition can be found in Table 4.2.

Table S 4.1: Overview of street samples analyzed with the ACE5 sensor described in Chapter 4. The samples are arranged by ascending date. A high performance technique (e.g. GC-FID or GC-MS) was used to determine the presence/absence of MDMA in each sample. NICC employed GC-FID to determine the exact m% of MDMA in the first 15 samples. Sciensano used a less accurate UV spectroscopy methodology to estimate the m% of MDMA in samples 94-164. All measurements were conducted by myself, except for those at Sciensano. The latter were conducted by a master student, who received a training by myself prior to the measurements.

Sample #	Institution	Code #	Content	m%	ACE5	ACE5 (m%)	Δm%	Result
1	NICC	1910693	MDMA	93.7	MDMA	89.2	4.5	TP
2	NICC	1910721.1	MDMA	92.6	MDMA	89.3	3.3	TP
3	NICC	2003105	MDMA	96.95	MDMA	96.4	0.55	TP
4	NICC	1912395.2	MDMA	40.4	MDMA	41.1	-0.7	TP
5	NICC	1912395.4	MDMA	92.7	MDMA	95.9	-3.2	TP
6	NICC	1912695	MDMA	88	MDMA	94.6	-6.6	TP
7	NICC	2001221.1.8	MDMA	40.4	MDMA	40.2	0.2	TP
8	NICC	2001221.2.1	MDMA	19.4	MDMA	18.6	0.8	TP
9	NICC	2001221.2.2	MDMA	24.7	MDMA	18.6	6.1	TP
10	NICC	2001221.2.3	MDMA	57.2	MDMA	57.4	-0.2	TP
11	NICC	2003420.3.2	MDMA	54.08	MDMA	52.8	1.28	TP
12	NICC	2003467.2	MDMA	96.77	MDMA	98.4	-1.63	TP
13	NICC	2003639.1	MDMA	39.46	MDMA	35.9	3.56	TP
14	NICC	2003639.2	MDMA	54.44	MDMA	50	4.44	TP
15	NICC	2003898.3	MDMA	35.33	MDMA	35.5	-0.17	TP
16	LNS	111A	MDMA		MDMA			TP
17	LNS	180B	MDMA		MDMA			TP
18	LNS	186	MDMA		MDMA			TP
19	LNS	229A	MDMA		MDMA			TP
20	LNS	342B	MDMA		MDMA			TP
21	Police AMS	1	MDMA		MDMA			TP
22	Police AMS	2	MDMA		MDMA			TP
23	Police AMS	3b	MDMA		MDMA			TP
24	Police AMS	4	MDMA		MDMA			TP
25	Police AMS	5	MDMA		MDMA			TP
26	Police AMS	6	MDMA		MDMA			TP
27	Police AMS	7	MDMA		MDMA			TP
28	Police AMS	8	MDMA		MDMA			TP
29	Police AMS	9	MDMA		MDMA			TP
30	Police AMS	10	MDMA		MDMA			TP
31	Police AMS	11	MDMA		MDMA			TP
32	Police AMS	12	MDMA		MDMA			TP
33	Police AMS	13	MDMA		MDMA			TP
34	Police AMS	14	MDMA		MDMA			TP
35	Police AMS	16	MDMA		MDMA			TP
36	Police AMS	17	MDMA		MDMA			TP
37	Police AMS	18	MDMA		MDMA			TP
38	Police AMS	19	MDMA		MDMA			TP
39	Police AMS	20	MDMA		MDMA			TP
40	Police AMS	21	MDMA		MDMA			TP
41	Police AMS	22	MDMA		MDMA			TP
42	Police AMS	23	MDMA		MDMA			TP
43	Police AMS	24	MDMA		MDMA			TP
44	Police AMS	25	MDMA		MDMA			TP
45	Police AMS	26	MDMA		MDMA			TP
46	Police AMS	27	MDMA		MDMA			TP

Sample #	Institution	Code #	Content	m%	ACE5	ACE5 (m%)	Δm%	Result
47	Police AMS	28	MDMA		MDMA			TP
48	Police AMS	29	MDMA		MDMA			TP
49	Police AMS	30	MDMA		MDMA			TP
50	Police AMS	31	MDMA		MDMA			TP
51	Police AMS	32	MDMA		MDMA			TP
52	Police AMS	33	MDMA		MDMA			TP
53	Police AMS	34	MDMA		MDMA			TP
54	Police AMS	35	MDMA		MDMA			TP
55	Police AMS	36	MDMA		MDMA			TP
56	Police AMS	37	MDMA		MDMA			TP
57	Police AMS	38	MDMA		MDMA			TP
58	Police AMS	39	MDMA		MDMA			TP
59	Police AMS	40	MDMA		MDMA			TP
60	Police AMS	101	2C-B		MDMA			FP
61	Police AMS	102	4-MMC		no MDMA			TN
62	Police AMS	104	2C-B		no MDMA			TN
63	Police AMS	105	2C-B		MDMA			FP
64	Police AMS	106	2-Br-4,5-DMPEA		no MDMA			TN
65	Police AMS	107	2-Br-4,5-DMPEA		no MDMA			TN
66	Police AMS	108	FA		no MDMA			TN
67	Police AMS	109	FA		no MDMA			TN
68	Police AMS	110	2C-B		MDMA			FP
69	Police AMS	111	2C-B		MDMA			FP
70	Police AMS	112	4-FMA		no MDMA			TN
71	Police AMS	113	2-Br-4,5-DMPEA		no MDMA			TN
72	Police AMS	114	2C-B		MDMA			FP
73	Police AMS	115	Pentylone		no MDMA			TN
74	Police AMS	116	2C-B		MDMA			FP
75	Police AMS	117	FMA		no MDMA			TN
76	Police AMS	118	2C-B		MDMA			FP
77	Police AMS	119	2C-B		MDMA			FP
78	Police AMS	120	2C-B		MDMA			FP
79	Police AMS	121	2C-B		MDMA			FP
80	Police AMS	122	2-Br-4,5-DMPEA		no MDMA			TN
81	Police AMS	123	FA		no MDMA			TN
82	Police AMS	124	FA		no MDMA			TN
83	Police AMS	125	FMA		no MDMA			TN
84	Police AMS	126	4-FA		no MDMA			TN
85	Police AMS	127	2C-B-fly		no MDMA			TN
86	Police AMS	128	FMA		no MDMA			TN
87	Police AMS	129	FA		no MDMA			TN
88	Police AMS	130	FMA		no MDMA			TN
89	Police AMS	131	mCPP		MDMA			FP
90	Police AMS	132	4-APB		no MDMA			TN
91	Police AMS	133	4-FA		no MDMA			TN
92	Sciensano	MP001	MDMA	19	MDMA	18	1	TP
93	Sciensano	MP002	MDMA	23.2	MDMA	25	-1.8	TP
94	Sciensano	MP003	MDMA	41.2	MDMA	48	-6.8	TP
95	Sciensano	MP004	Amphetamine		no MDMA			TN
96	Sciensano	MP005	Unknown		MDMA	7		FP
97	Sciensano	MP006	Amphetamine		no MDMA			TN
98	Sciensano	MP007	MDMA	79.4	MDMA	74	5.4	TP
99	Sciensano	MP008	MDMA	78.8	MDMA	87	-8.2	TP
100	Sciensano	MP009	MDMA	80.1	MDMA	82	-1.9	TP
101	Sciensano	MP010	MDMA	79.1	MDMA	76	3.1	TP

Sample #	Institution	Code #	Content	m%	ACE5	ACE5 (m%)	Δm%	Result
102	Sciensano	MP011	MDMA	30.1	MDMA	35	-4.9	TP
103	Sciensano	MP012	MDMA		MDMA	23		TP
104	Sciensano	MP013	MDMA	45.8	MDMA	44	1.8	TP
105	Sciensano	MP014	MDA		MDMA	83		FP
106	Sciensano	MP015	MMDPA		MDMA	20		FP
107	Sciensano	MP016	MDMA	31.6	MDMA	31	0.6	TP
108	Sciensano	MP017	MDMA	77.9	MDMA	81	-3.1	TP
109	Sciensano	MP018	no MDMA		no MDMA			TN
110	Sciensano	MP019	MDMA	27.1	MDMA	32	-4.9	TP
111	Sciensano	MP020	MDMA	26.1	MDMA	27	-0.9	TP
112	Sciensano	MP021	MDMA	27	MDMA	26	1	TP
113	Sciensano	MP022	no MDMA		no MDMA			TN
114	Sciensano	MP023	no MDMA		no MDMA			TN
115	Sciensano	MP024	MDMA	31.2	MDMA	30	1.2	TP
116	Sciensano	MP025	Ibuprofen		no MDMA	-1	1	TN
117	Sciensano	MP026	MMDPA		MDMA	17		FP
118	Sciensano	MP027	Trazodone		no MDMA			TN
119	Sciensano	MP028	Unknown		MDMA	26		FP
120	Sciensano	MP029	Amphetamine		no MDMA			TN
121	Sciensano	MP030	Amphetamine		no MDMA			TN
122	Sciensano	MP031	no MDMA		no MDMA			TN
123	Sciensano	MP032	Nordiazepam		no MDMA			TN
124	Sciensano	MP033	no MDMA		no MDMA			TN
125	Sciensano	MP034	Trazodone		no MDMA			TN
126	Sciensano	MP035	MDMA	26.9	MDMA	25	1.9	TP
127	Sciensano	MP036	MDMA	77.2	MDMA	85	-7.8	TP
128	Sciensano	MP037	MDMA	18.9	MDMA	20	-1.1	TP
129	Sciensano	MP038	Ketamine		no MDMA			TN
130	Sciensano	MP039	4-chloromethcathinone		no MDMA			TN
131	Sciensano	MP040	MDMA	33.9	MDMA	39	-5.1	TP
132	Sciensano	MP041	MDMA	27.6	MDMA	26	1.6	TP
133	Sciensano	MP042	MDMA	22	MDMA	24	-2	TP
134	Sciensano	MP043	MDMA	21	MDMA	23	-2	TP
135	Sciensano	MP044	no MDMA		no MDMA			TN
136	Sciensano	MP045	MDMA	28.5	MDMA	32	-3.5	TP
137	Sciensano	MP046	MDMA	16.2	MDMA	15	1.2	TP
138	Sciensano	MP047	MDMA		MDMA	34		TP
139	Sciensano	MP048	MDMA	76.6	MDMA	82	-5.4	TP
140	Sciensano	MP049	MDMA		MDMA	42	-42	TP
141	Sciensano	MP050	MDMA	25.9	MDMA	26	-0.1	TP
142	Sciensano	MP051	MDMA	30.9	MDMA	37	-6.1	TP
143	Sciensano	MP052	MDMA	28.2	MDMA	31	-2.8	TP
144	Sciensano	MP053	MDMA	80.3	MDMA	82	-1.7	TP
145	Sciensano	MP054	MDMA	15.5	MDMA	12	3.5	TP
146	Sciensano	MP055	no MDMA		no MDMA			TN
147	Sciensano	MP056	MDMA	74.5	MDMA	65	9.5	TP
148	Sciensano	MP057	MDMA	27.3	MDMA	33	-5.7	TP
149	Sciensano	MP058	MDMA	22.2	MDMA	25	-2.8	TP
150	Sciensano	MP059	no MDMA		MDMA	3	-3	FP
151	Sciensano	MP060	MDMA	27.2	MDMA	27	0.2	TP
152	Sciensano	MP061	MDMA	31.7	MDMA	35	-3.3	TP
153	Sciensano	MP062	MDMA	35.3	MDMA	42	-6.7	TP
154	Sciensano	MP063	MDMA	30.6	MDMA	31	-0.4	TP
155	Sciensano	MP064	MDMA	34.9	MDMA	36	-1.1	TP
156	Sciensano	MP065	MDMA	13.4	MDMA	15	-1.6	TP

Sample #	Institution	Code #	Content	m%	ACE5	ACE5 (m%)	Δm%	Result
157	Sciensano	MP066	MDMA	26.9	MDMA	27	-0.1	TP
158	Sciensano	MP067	MDMA		MDMA	24		TP
159	Sciensano	MP068	MDMA	20.7	MDMA	21	-0.3	TP
160	Sciensano	MP069	MDMA	24.5	MDMA	28	-3.5	TP
161	Sciensano	MP070	MDMA	29.3	MDMA	30	-0.7	TP
162	Sciensano	MP071	MDMA	79.4	MDMA	85	-5.6	TP
163	Sciensano	MP072	MDMA	29.1	MDMA	32	-2.9	TP
164	Sciensano	MP073	MDMA	28	MDMA	36	-8	TP
165	NICC	2209428_37_1	MDMA		MDMA			TP
166	NICC	2209428_66	4-CMC		no MDMA			TN
167	NICC	2209428_62_2	MDMA, ketamine		no MDMA			FN
168	NICC	2212260_5	no MDMA		no MDMA			TN
169	NICC	2212260_4	no MDMA		no MDMA			TN
170	NICC	2204748_3	no MDMA		no MDMA			TN
171	NICC	2210406_2	MDMA		MDMA			TP
172	NICC	2210411	MDMA		MDMA			TP
173	NICC	2210424	MDMA		MDMA			TP
174	NICC	2210416_1	MDMA		MDMA			TP
175	NICC	2210420_1	MDMA		MDMA			TP
176	NICC	2210427_1	MDMA		MDMA			TP
177	NICC	2210433_1	MDMA		MDMA			TP
178	NICC	2210413	MDMA		MDMA			TP
179	NICC	2209745	MDMA		MDMA			TP
180	NICC	2210434	MDMA		MDMA			TP
181	NICC	2209742	MDMA		MDMA			TP
182	NICC	2210431	MDMA		MDMA			TP
183	NICC	2209757_1	MDMA		MDMA			TP
184	NICC	2209757_2	MDMA		MDMA			TP
185	NICC	2210404	MDMA		MDMA			TP
186	NICC	2209428_69_1	MDMA		MDMA			TP
187	NICC	2209428_100_4	MDMA		MDMA			TP
188	NICC	2209428_103	MDMA		MDMA			TP
189	NICC	2209428_104	MDMA		MDMA			TP
190	NICC	2209428_105	MDMA		MDMA			TP
191	NICC	2209428_106	MDMA		MDMA			TP
192	NICC	2209428_107_1	MDMA		MDMA			TP
193	NICC	2209428_108	MDMA		MDMA			TP
194	NICC	2209428_109_1	MDMA		MDMA			TP
195	NICC	2209428_100_2	MDMA		MDMA			TP
196	NICC	2209428_110	MDMA		MDMA			TP
197	NICC	2209428_112	MDMA		MDMA			TP
198	NICC	2211779_1	Sucrose		no MDMA			TN
199	NICC	2211779_2	MDMA		MDMA			TP
200	NICC	2116232_7	Amphetamine		no MDMA			TN
201	NICC	2116232_3_1	Amphetamine		no MDMA			TN
202	NICC	2116232_3_2	Amphetamine		no MDMA			TN
203	NICC	2116232_9_1	Amphetamine		no MDMA			TN
204	NICC	2212289_3	Cocaine		no MDMA			TN
205	NICC	2116476_8	Methamphetamine		no MDMA			TN
206	NICC	2116476_18	Methamphetamine		no MDMA			TN
207	NICC	2212631_2	no MDMA		no MDMA			TN
208	NICC	2212260_1	no MDMA		no MDMA			TN
209	NICC	2210757_1	no MDMA		no MDMA			TN
210	NICC	2208363_1	no MDMA		no MDMA			TN
211	NICC	2207973	no MDMA		no MDMA			TN
212	NICC	2207965_1	no MDMA		no MDMA			TN

Chapter 5

MDMA/2C-B Sensor

"When the world is in trouble, chemistry comes to its rescue."

Carolyn R. Bertozzi

This chapter is based on the manuscript "*Electrochemical detection of MDMA and 2C-B in ecstasy tablets using a selectivity enhancement strategy by in-situ derivatization*",
authored by *Robin Van Echelpoel, Ruben F. Kranenburg, Arian C. van Asten & Karolien De Wael*,
which appeared in *Forensic chemistry, 27, 100383 (2022)*.

My contribution: Conceptualization (shared with Ruben), Methodology (shared with Ruben), Formal analysis (shared with Ruben), Data curation (shared with Ruben), Software development, Writing - original draft (shared with Ruben).

Abstract

As described in the previous chapters, 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 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 the previous chapter, an electrochemical MDMA sensor was described. The validation study of this sensor showed excellent results, although one clear downside could be identified: false positives on the illicit compound 2C-B. Since 2C-B is sometimes found in ecstasy pills, a solution was sought. In this chapter, a novel sensor is described that can distinguish MDMA and 2C-B, while keeping the same characteristics (fast, portable, easy-to-use) as the original MDMA sensor. An *in-situ* derivatization was introduced to achieve this goal. To this end, formaldehyde was used for *N*-methylation of 2C-B thereby enhancing its EP. The enriched EP 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.

5.1 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, 165]. In addition, the drug market also further diversified with over 1000 different NPS emerging in the last decade[165, 166]. 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[167]. 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 product that may lead to false positive results[40]. 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)[168]. Thirdly, the result of the color test may be obscured or misinterpreted by strongly colored samples as may be the case in smuggling scenarios[169] or aesthetically 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[170].

Spectroscopic techniques such as ATR-FTIR[52, 171], NIR[52, 56, 94] and Raman spectroscopy[93, 172, 173] 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[174]. However, limitations arise when samples have a dark color or are composed of multiple substances that show overlapping spectral signals[52, 93, 169]. In general, limits of detection between 10 – 40 wt% are reported for these direct spectroscopic techniques[56, 93, 94] where the higher limits of detection may be explained by strong signals from excipients partly obscuring the signal from the main active ingredient[93]. 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[173]. 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[43, 102], heroin[175] and ketamine[176]. Specific EPs can also be established for NPS, either as individual compounds[78, 177] or for specific classes such as cathinones[92]. Since electrochemical screenings are rapid and may be applied simultaneously using an array of electrodes, selectivity issues from identical EPs can be overcome by application of subsequent electrochemical strategies. De Jong *et al.*[103] 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.*[154] 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 EP of primary amines was introduced by Schram *et al.*[161]. 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 chapter 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 EP 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.

5.2 Experimental

5.2.1 Chemicals

Water (purified, Ph.Eur.), formaldehyde 35 wt% in H_2O (formalin) and sodium acetate were obtained from Sigma-Aldrich (Overijse, Belgium). MDMA, 2C-B and a wide range of 72 other drugs-of-abuse, pharmaceuticals, excipients and adulterants mentioned in Table S5.1 were provided by the Police Laboratory in Amsterdam 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 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 0.1M ACE pH 5 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 Figure 5.1. 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[178]. 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, 11x 2C-B, 4x 2-bromo-4,5-dimethoxyphenethylamine (2-Br-4,5-DMPEA), 7x fluoroamphetamine (FA), 5x fluoromethamphetamine (FMA), 1x mephedrone, 1x pentylone, 1x 2-(4-bromo-2,3,6,7-tetrahydrofuro[2,3-f][1]benzofuran-8-yl)ethanamine (2C-B-fly), 1x meta-chlorophenylpiperazine (mCPP), 1x 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[93]. The set of ecstasy tablets comprised of over 60 different designs (i.e. color, shape, imprint).

5.2.2 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.

5.2.3 Methods

5.2.3.1 Original MDMA Method (MDMA sensor)

The sample set was first tested using the electrochemical MDMA sensor described in Chapter 4. Figure 2.4 depicts the procedure that was followed for each measurement. First the sample was dissolved in a 0.1M ACE buffer pH 5 at a 0.3 - 0.4 mg/mL concentration (e.g. between 1.5 and 2 mg of sample in 5 mL buffer solution). Subsequently, a droplet (~0.05 mL) of the resulting solution is placed on the 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 data analysis script described in Chapter 3. 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 EP. Since the sensor in this application is tailored towards MDMA detection, only MDMA is included in this database. The EP of MDMA contains one single peak around 1.11V, thus if the EP 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 EP 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.

5.2.3.2 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 10s followed by a delay time of 30s. The latter is necessary to give the formalin time to react with the sample. 3. During EP 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.

5.3 Results and discussion

5.3.1 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 (Figure 5.1) 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,

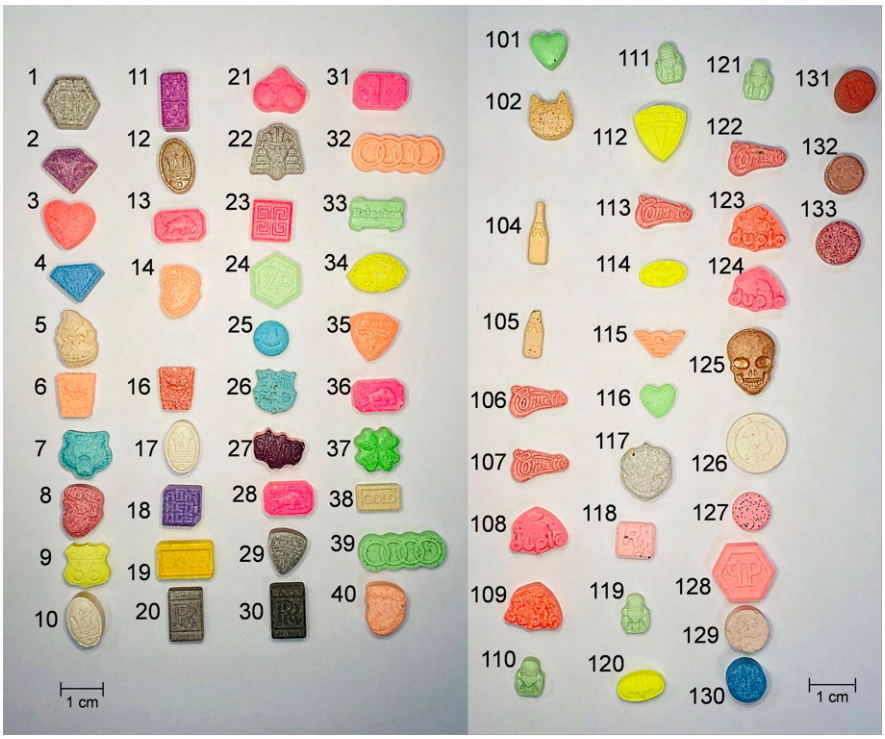


Figure 5.1: Ecstasy tablets selected for this study. Tablets 1 to 40 contained MDMA, tablets 101 – 133 contained other substances. Main active ingredients are given in Table 5.1. Note that samples #15 and #103 are omitted.

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 Figure 5.2A. 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 Figure 5.2B. 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[173]. 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 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 (Figure 5.2C) 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.

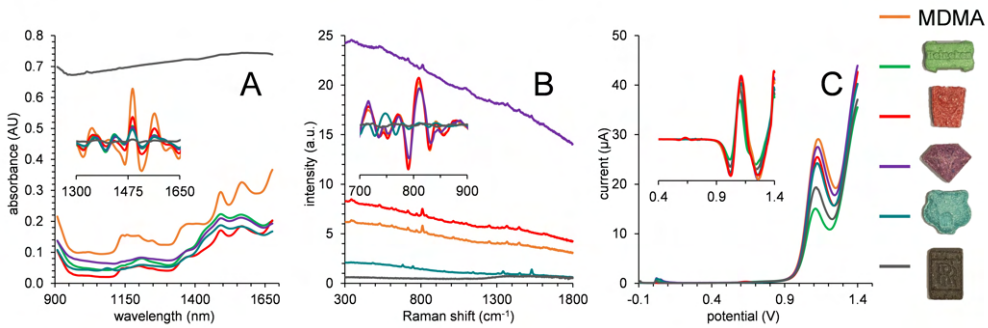


Figure 5.2: Influence of sample color on MDMA detection shown for pure MDMA (orange plots) and five MDMA-containing ecstasy tablets. NIR (A), Raman (B) and electrochemical analysis using the MDMA sensor (C). Insets depict preprocessed data of the most diagnostic part of the spectrum to emphasize limitations for certain colors. *Note that the Raman signal for the green tablet is missing due to saturation caused by fluorescence.*

5.3.2 Selectivity of the MDMA sensor

Table 5.1 shows the results of the electrochemical MDMA-sensor for the set of 71 tablets. 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 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 S5.1) were selected to assess the selectivity of the MDMA sensor. These substances comprised of common drugs-of-abuse, licit pharmaceuticals, NPS and adulterants. Figure 5.3 shows the EPs of the most frequently encountered substances compared to MDMA itself. The sensor results can be found in Table S5.1. 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 Figure 5.3B 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 EP, 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, promethazine and mCPP (Figure S5.1). Similar results were obtained for the seized casework tablets (Table 5.1), 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 5.1, 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.

5.3.3 EP enhancement for MDMA/2C-B differentiation

The MDMA sensor, described in Chapter 4, 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 EP of MDMA should be diversified from the EP of 2C-B. An electrochemical pretreatment has proven to be successful in the past for similar cases[154], 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[161]. A similar opportunity arises here, as 2C-B contains a primary amine while MDMA does not. Indeed, *in-situ* derivatization with formalin changes the EP of 2C-B, but leaves the EP of MDMA almost unaffected (Figure 5.4). Reproducibility studies show that the signals at 0.95 V and 1.14 V in the derivatized EP of 2C-B can reliably be used for identification of this compound. The EP of MDMA remains unaltered by the formalin, and the peak at 1.11V, 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 EP for MDMA and 2C-B, thereby creating the opportunity to develop a MDMA/2C-B sensor. Integration in the identification software of the EPs of MDMA and 2C-B after derivatization realizes the new MDMA/2C-B sensor. Note that the selectivity towards 2C-B is achieved by requiring the presence of both the peak at 0.95V and the

Table 5.1: 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.

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

peak at 1.14V after derivatization. In line with earlier work by Schram *et al.*[161] the observed reaction was proposed to be a Eschweiler-Clarke methylation whose reaction scheme is shown in Figure S5.2. 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 EP 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 figures S5.3, S5.4 and S5.5. 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

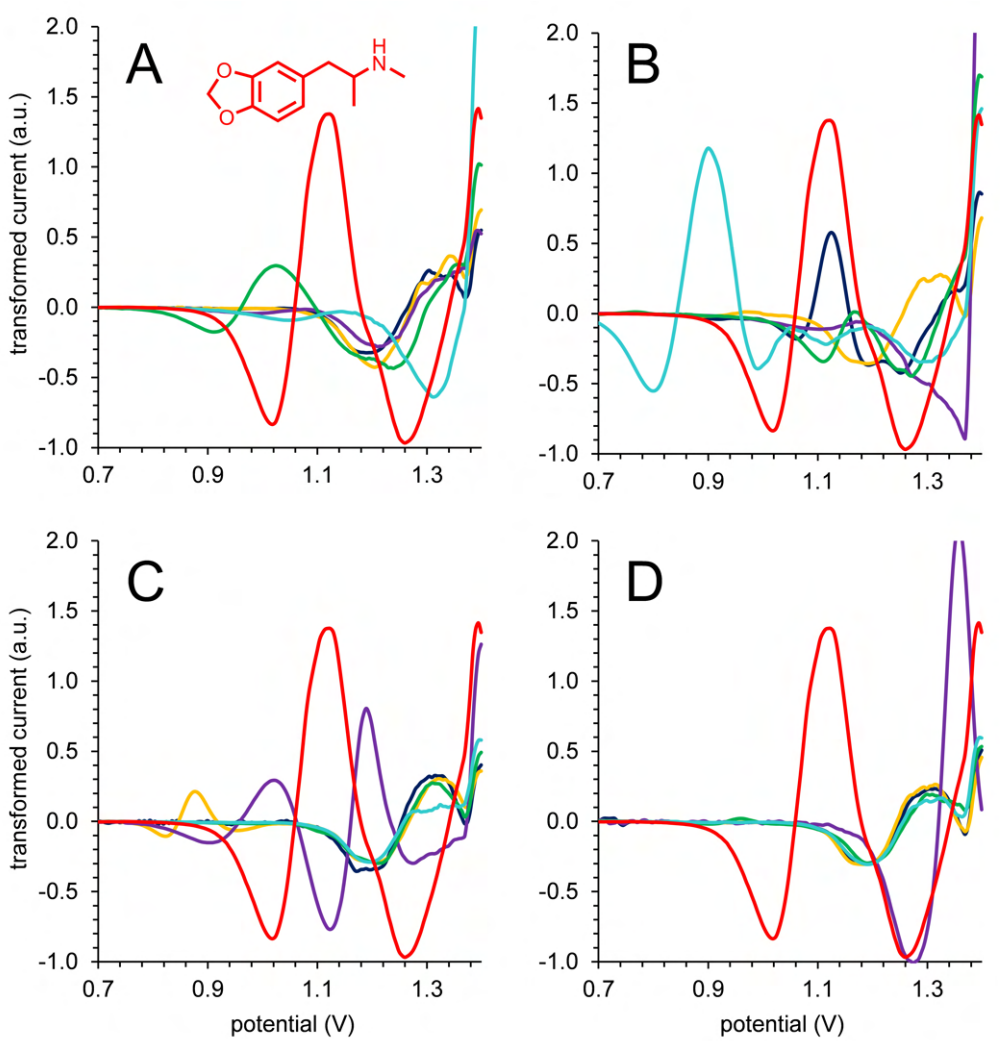


Figure 5.3: EPs following baseline correction and top hat filter transformation as preprocessing. MDMA (red trace) overlayed with common drugs (panel A): amphetamine (dark blue), methamphetamine (orange), cocaine (purple), heroin (green), ketamine (light blue); synthetic drugs (panel B): 2C-B (dark blue), 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).

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.

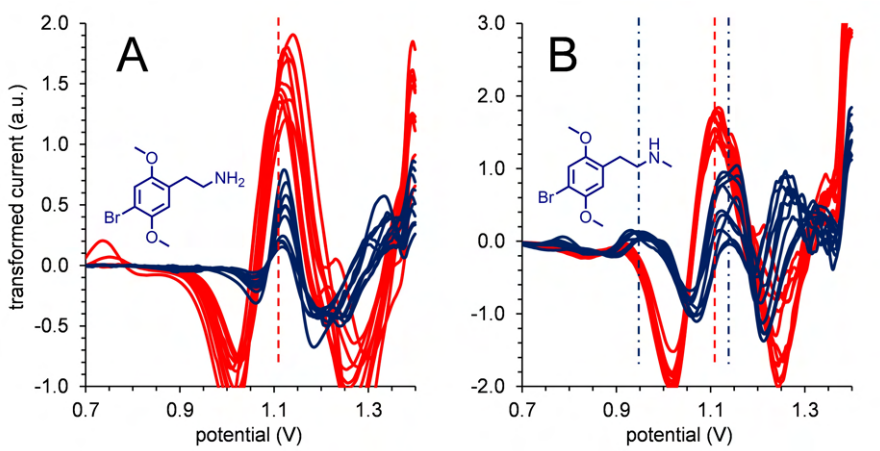


Figure 5.4: EPs following baseline correction and top hat filter transformation as preprocessing. Overlay of 10 MDMA-containing tablets (red) and 10 2C-B-containing tablets (blue) analyzed by the MDMA sensor (panel A) and the MDMA/2C-B sensor following *in-situ* derivatization with formaldehyde (panel 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.

5.3.4 Performance on case samples

All 39 samples containing MDMA are still correctly identified by the MDMA/2C-B sensor (Table 5.1), 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 (EPs are shown in Figure S5.6). 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 the illicit, psychoactive drug mCPP, caused a false positive for the MDMA-sensor, but not for the MDMA/2C-B sensor. When looking at the EPs of both reference mCPP (#46, Table S5.1) and the tablet sample (#131, Table 5.1) a major peak is observed ~ 0.88V, thus well outside the detection windows of both MDMA and 2C-B (Figure S5.1). It is likely that a very minor signal in the detection interval produced the erroneous results. These FPs caused by minor signals in the detection range show 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). Specifically for mCPP, no further solution is pursued since the compound is rather rare nowadays, presumably since its use results in a rather unpleasant experience [179].

Sample #115 containing pentylone exhibits the opposite behavior, creating a false positive

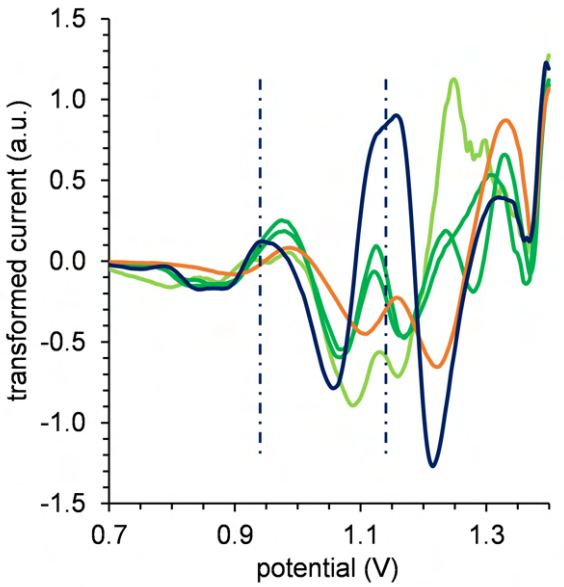


Figure 5.5: EPs following baseline correction and top hat filter transformation as preprocessing. False positive reactions on the MDMA/2C-B sensor: pentylone (orange) and 2-Br-4,5-DMPEA (dark green) with a true positive 2C-B sample (dark blue) and a true negative 2-Br-4,5-DMPEA (light green) plot for comparison. Blue dashed lines indicate the 0.95 V and 1.14 V peaks diagnostic for derivatized 2C-B.

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 EPs are shown in comparison with corresponding true positives in Figure 5.5. It can be seen that at both areas diagnostic for 2C-B (i.e. 0.95 V and 1.14 V) the false positive EPs also yield signal although their peak maxima and ratios are slightly different from the true positive EP. 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[81]. 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.

5.4 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 EP 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 EP. The secondary amine MDMA was not affected by formaldehyde, leaving its EP mainly unchanged. The applicability of the combined two-step sensor was demonstrated on a set of 71 ecstasy tablets seized in 2020. All 39 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 EP and an enhanced EP in combination with database-aided peak detection software therefore is a promising technique for on-site drug testing. As an outlook, the EPs 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 in 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.

5.5 Supplemental figures and tables

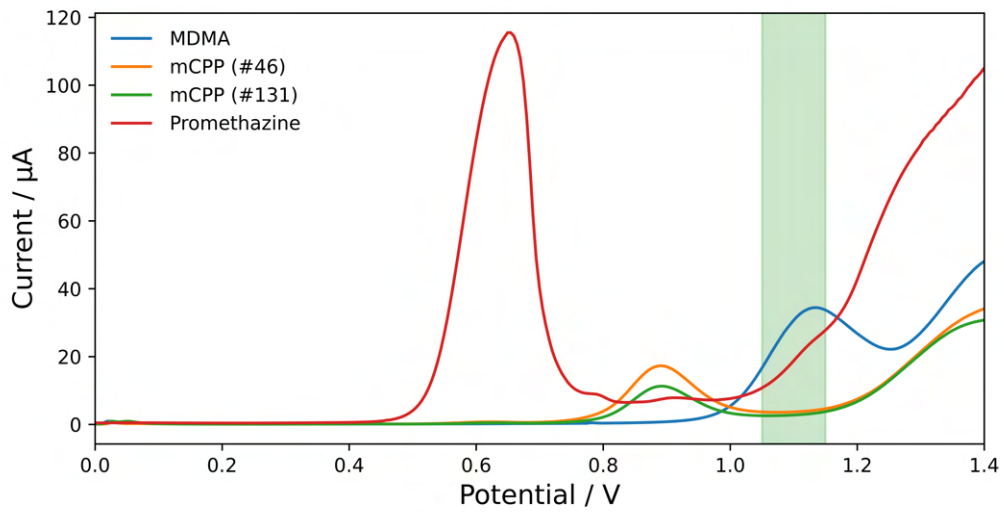


Figure S 5.1: EPs of MDMA, mCPP (#46), mCPP (#131) and promethazine. The latter three samples caused FPs for the MDMA sensor due to very minor signals in the detection interval.

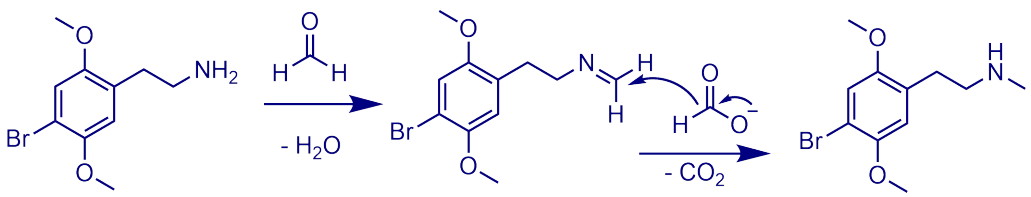


Figure S 5.2: Proposed reaction scheme of the *in-situ* derivatization of 2C-B with formaldehyde.

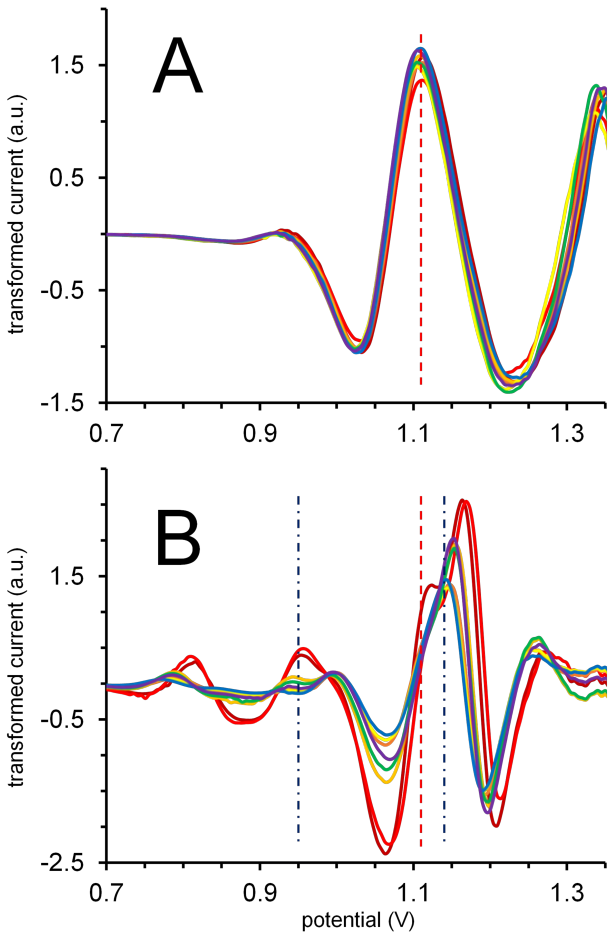


Figure S 5.3: Formaldehyde reaction rate plots for MDMA (A) and 2C-B (B). EFs analyzed without waiting time (purple plots), after 20s waiting (blue), 40s (green), 60s (yellow), 90s (orange), 150s (dark orange), 300s (red), 600s (dark red). Red dashed line indicates the 1.11 V peak diagnostic for MDMA, blue dashed lines indicate the 0.95 V and 1.14 V peaks diagnostic for derivatized 2C-B.

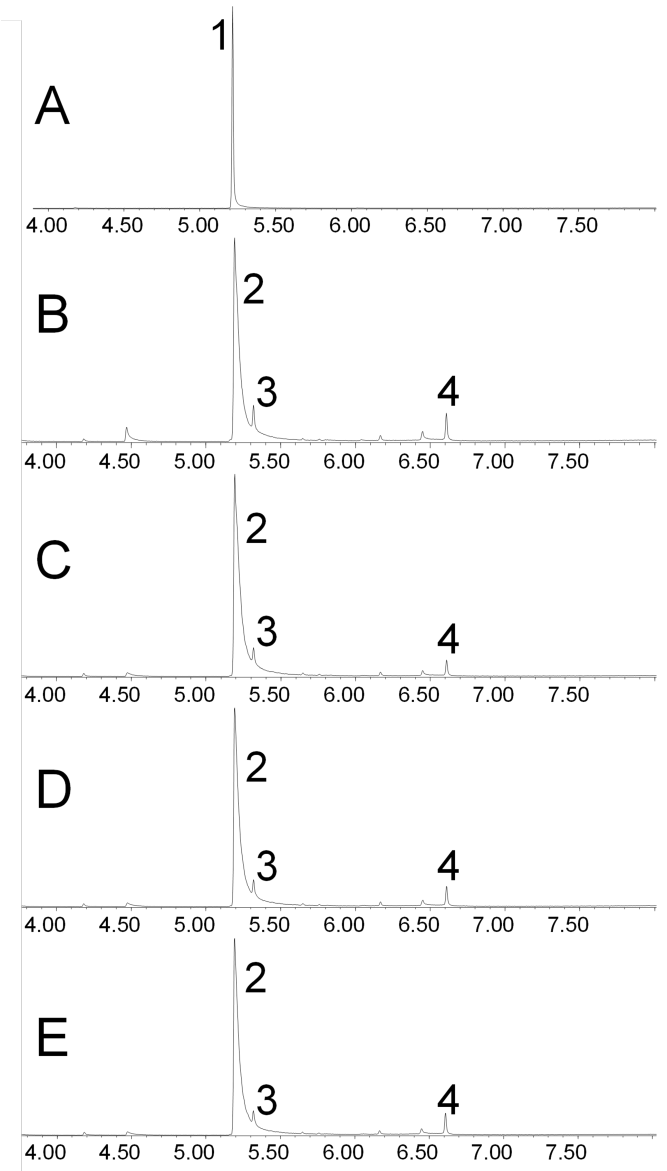


Figure S 5.4: Formaldehyde reaction analysis by GC-MS. A: 2C-B sample with no formaldehyde added; B: after 30 sec reaction with formaldehyde; C: 2 min reaction with formaldehyde; D: 10 min reaction with formaldehyde; E: 30 min reaction with formaldehyde. Mass spectra of peaks 1-4 are shown in Figure S5.5. GC-MS analysis of 1 μL dichloromethane from extracts obtained by an alkaline extraction of sample solution with 2 mL dichloromethane.

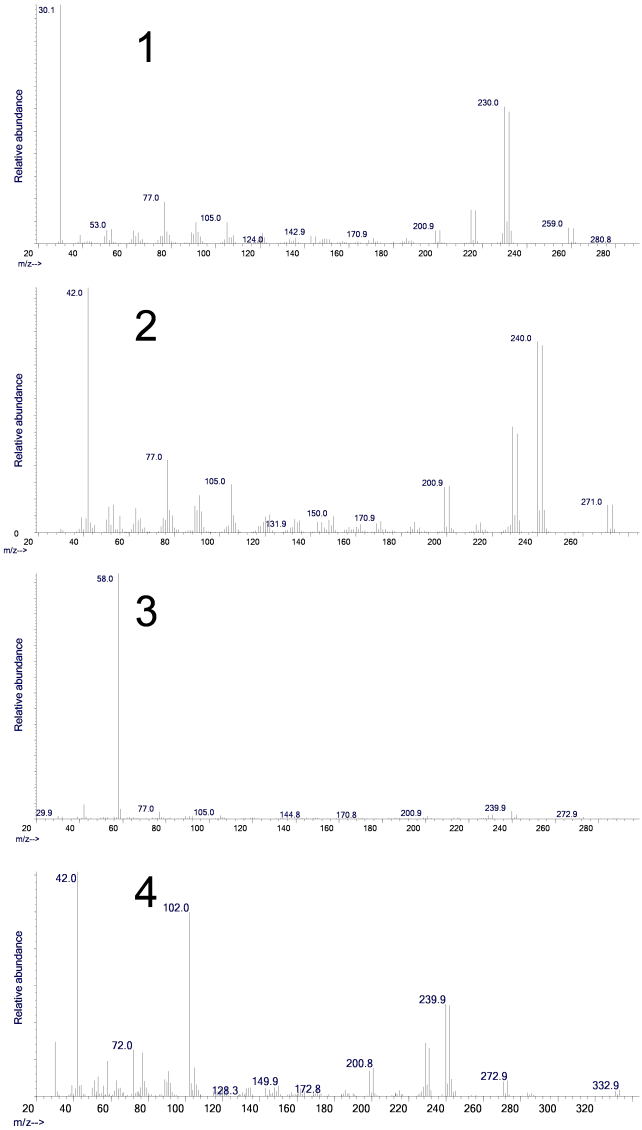


Figure S 5.5: Mass spectra of the GC-MS peaks in Figure S5.4. 1: 2C-B with m/z 30 base peak originating from the primary amine; 2: methylated 2C-B reaction product with m/z 42 base peak attributed to secondary amine; 3: dimethylated 2C-B with m/z 58 base peak attributed to tertiary amine; 4: secondary methylated 2C-B reaction product.

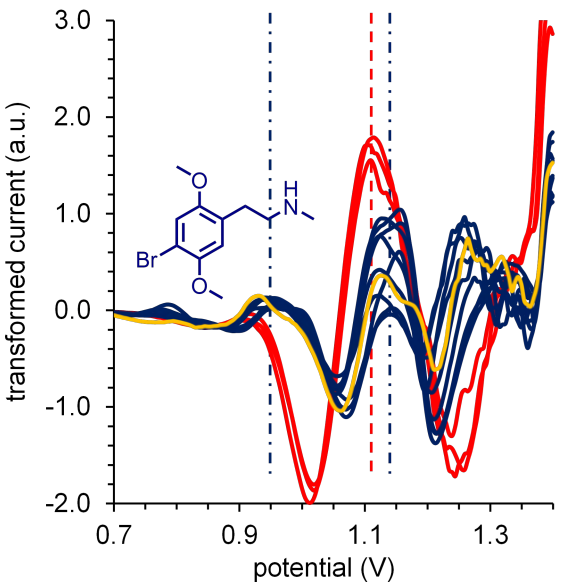


Figure S 5.6: Electrochemical profiles (EPs) following two preprocessing steps, i.e. a moving average baseline correction and a digital top hat filter transformation. Overlay of 5 MDMA-containing tablets (red) and 10 2C-B-containing tablets (blue) analyzed by the MDMA/2C-B sensor following *in-situ* derivatization with formaldehyde. All identified correctly. Orange plot depict the single 2C-B containing tablet #110 falsely identified as MDMA. 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.

Table S 5.1: Results of the MDMA electrochemical sensor including the obtained electrochemical fingerprint (EF) on drugs-of-abuse, common pharmaceuticals, NPS, excipients and adulterants. The red line indicates the 1.11 V peak diagnostic for MDMA.

#	Identity	MDMA sensor output	#	Identity	MDMA sensor output	#	Identity	MDMA sensor output
1	MDMA	MDMA	26	3-CMC	no MDMA	51	Phenacetin	no MDMA
2	Cocaine base	no MDMA	27	4-CMC	no MDMA	52	Procaine	no MDMA
3	Cocaine HCl	no MDMA	28	4-CEC	no MDMA	53	Benzocaine	no MDMA
4	Ketamine	no MDMA	29	Pentedrone	no MDMA	54	Manitol	no MDMA
5	Amphetamine	no MDMA	30	2,3-MDA	no MDMA	55	Lactose	no MDMA
6	Methamphetamine	no MDMA	31	3,4-MDA	MDMA	56	Inositol	no MDMA
7	Heroin	no MDMA	32	2,3-MDMA	no MDMA	57	Vitamin C	no MDMA
8	2C-B	MDMA	33	3,4-MDEA	no MDMA	58	Sugar	no MDMA
9	Sildenafil	no MDMA	34	Methylone	MDMA	59	Glucose	no MDMA
10	Oxazepam	no MDMA	35	Ethylone	MDMA	60	Boric acid	no MDMA
11	Flunitrazepam	no MDMA	36	2,3-Ehtylone	MDMA	61	Diltiazem	no MDMA
12	Diazepam	no MDMA	37	2,3-Methylone	no MDMA	62	Promethazine	MDMA
13	Methylphenidate	no MDMA	38	Dimethylone	no MDMA	63	Creamer	no MDMA
14	GHB	no MDMA	39	Pentylone	no MDMA	64	Starch	no MDMA
15	4-FA	no MDMA	40	5-APB	no MDMA	65	Aspirin	no MDMA
16	2-FMA	no MDMA	41	6-APB	no MDMA	66	Paracetamol/ Caffein	no MDMA
17	3-FMA	no MDMA	42	alpha-PVP	no MDMA	67	Levamisole/ Lidocaine	no MDMA
18	4-FMA	no MDMA	43	MDPV	no MDMA	68	Levamisole/ Paracetamol/ Lidocaine	no MDMA
19	2-MEC	no MDMA	44	AM-2201	no MDMA	69	Levamisole/ Phenacetin	no MDMA
20	3-MEC	no MDMA	45	5-F-APINACA	no MDMA	70	Phenacetin/ Lidocaine	no MDMA
21	4-MEC	no MDMA	46	mCPP	MDMA	71	Phenacetin/ Procaine	no MDMA
22	2-MMC	no MDMA	47	Paracetamol	no MDMA	72	Levamisole/ Phenacetin/ Procaine	no MDMA
23	3-MMC	no MDMA	48	Caffeine	no MDMA	73	Paracetamol/ Phenacetin	no MDMA
24	4-MMC	no MDMA	49	Levamisole	no MDMA	74	Cutting agent mix	no MDMA
25	3,4-DMMC	no MDMA	50	Lidocaine	no MDMA			

Chapter 6

Festival Sensor

"Make it look simple, the very complicated thing."

Massimo Bottura

This chapter is based on the manuscript *"Electrochemical methods for on-site multidrug detection at festivals"*,
authored by *Robin Van Echelpoel, Jonas Schram, Marc Parrilla, Devin Daems, Amorn Slosse, Filip Van Durme & Karolien De Wael*,
which appeared in *Sensors & Diagnostics , 1, 793-802 (2022)*.

My contribution: Conceptualization (shared with Jonas), Methodology (shared with Jonas), Visualization (shared with Jonas), Formal analysis (shared with Jonas), Software development, Writing - original draft (shared with Jonas).

Abstract

Music festivals have emerged as an important setting for the consumption of illicit drugs, harming both consumers and society. Therefore, law enforcement present at these events requires straightforward, robust and accurate screening tools to obtain a rapid indication of the presence of these drugs in suspicious samples encountered on-site. EP-based drug sensing has proven to offer the desired affordability, portability and high-performance for this purpose. However, previous studies have mainly focused on the detection of only one drug type, rather than the simultaneous detection of multiple drugs. In this chapter, two innovative electrochemical methods (i.e. the flowchart and dual sensor) towards the rapid and accurate detection of the four main illicit drugs encountered at festivals (cocaine, 3,4-methylenedioxymethamphetamine -MDMA-, amphetamine and ketamine) are developed and assessed based on their practicality, performance and limitations. The flowchart method employs sequential measurements in different measuring conditions, following a flowchart, combining good practicality, affordability and performance. The dual sensor method combines the EP recorded in parallel at two electrodes with different measuring conditions into a superprofile. As the combined electrochemical information of the recorded EPs provides an increased selectivity, this method obtains the highest accuracy (87.5% vs. 80.0% for the flowchart) when applied to a set of confiscated samples. Interestingly, both methods outperform a commerical portable Raman device (60%) that analyzed the same set of confiscated

samples. Overall, these electrochemical methods offer law enforcement a rapid, portable and accurate screening method for the analysis of the large variety of suspicious samples encountered at music festivals.

6.1 Introduction

Music festivals have become an important setting for the use of illicit drugs. Studies have shown the link between music, nightlife and substance abuse, as many people attending music festivals consider this setting as an ideal place to experiment with and/or consume illicit drugs[180–182]. However, the consumption of illicit drugs has an adverse health, social and economic impact on the user, while also negatively affecting society and the environment[183, 184]. Health-related risks associated with substance use at festivals include harmful side effects such as hyperthermia, seizures and multi-organ failure, which could even result in death[185]. Additionally, the presence of other substances (e.g. adulterants, diluents, other illicit drugs) in drug samples may cause harm, while strong variations in purity between different countries increase the risk of overdoses[185]. Moreover, concomitant consumption of alcohol or other drugs (polydrug abuse) occurs frequently in these settings and often exponentially increases the health risks[186, 187].

The monitoring of drug use patterns at festivals is performed in various ways, including through surveys, samples seized by law enforcement and the analysis of pooled urine or wastewater[188–191]. Depending on the type of festival and location, a wide variety of substances can be in circulation, going from traditionally popular drugs such as cannabis and cocaine to more exotic drug types such as synthetic cathinones and psychedelics[53, 192]. Furthermore, studies have shown the link between the music genre of festivals and drug use patterns[180, 193, 194]. Electronic music festivals in particular have been extensively linked with higher drug consumption and particularly increased use of amphetamine-type stimulants (ATS) such as MDMA[180, 193].

LEAs are tasked with preventing illicit drugs from reaching the festival site by performing searches at the entrance[195, 196]. When a suspicious sample is encountered, LEAs require a fast on-site indication of the presence of illicit substances to decide on further actions. Typically used laboratory techniques such as GC-MS, which are regarded as the gold standard in drug analysis, are not suitable for this purpose due to their low portability, time-consuming measurements and need for trained personnel[196, 197]. Therefore, LEAs present at music festivals need portable on-site screening methods for the rapid and accurate analysis of the expectedly large amount of samples encountered during these searches. Moreover, these methods need to be capable of analyzing different sample types such as tablets, capsules, powders, crystals and liquids which come in various shapes and colors[192, 196].

Electrochemical sensors have shown great potential for this purpose, due to their high portability, rapid measurements and affordability[6, 82, 84, 153]. Previous studies generally focused on the detection of one drug, while LEAs at music festivals and other nightlife settings require screening tools for a variety of drugs. The state-of-the-art approach used in electrochemical illicit drug sensors involves recording an EP at a single electrode and subsequently processing this EP with peak identification software (Figure 6.1 (green))[81]. This becomes cumbersome if multiple target compounds, some shar-

ing a similar structure, have to be detected by the same sensor. More electrochemical information of the sample is thus required, and it is hypothesized that this additional information can be obtained through extending the measurement from a single electrode towards multiple electrodes. By diversifying the measuring conditions at each electrode, different EPs can be obtained from the same sample, which will greatly enhance the electrochemical information available to decide on the sample's identity.

Herein we present innovative electrochemical methods for the simultaneous detection of the four illicit drugs most commonly encountered at festivals in Europe[53, 190, 191]: cocaine, MDMA, amphetamine and ketamine (Figure 1.3). After demonstrating the shortcomings of single sensors for the detection of multiple drugs, two novel multidrug methods (Figure 6.1 (black and yellow)) are assessed based on their practicality, performance and limitations: (i) a flowchart based on sequential measurements in different measuring conditions (pH, buffer composition, . . .) and (ii) a dual sensor which simultaneously measures an EP at two SPEs using different measuring conditions. A flowchart approach is familiar territory for end-users, since it is a strategy that is also followed in colorimetric testing of illicit drugs. In a dual sensor approach, the simultaneously recorded EPs are combined into a so-called superprofile. This superprofile links the information from both individual EPs, thereby creating a wealth of information about the measured sample. Both novel methods are developed using a training set of samples containing pure drugs, pure adulterants and relevant binary mixtures between drugs and adulterants. Thereafter, the methods are validated with a test set containing 10 confiscated street samples for each of the four target drugs (40 in total) and their performance compared to that of a portable Raman spectroscopic device which is commercially available for the analysis of confiscated samples. The test set was composed by randomly selecting confiscated street samples from the inventory of the A-Sense Lab. Overall, these methods aim to offer LEAs tools for the rapid, affordable and high-performance on-site screening for multiple drugs at music festivals, while also providing them with the possibility to use the device for other duties such as border control and cargo analysis.

6.2 Experimental

6.2.1 Reagents and confiscated samples

Standards of d,l-MDMA HCl, d,l-amphetamine sulphate, ketamine HCl, d-methamphetamine HCl, butylone HCl and codeine HCl were purchased from Lipomed (Arlesheim, Switzerland). A standard of cocaine HCl was purchased from Chiron AS (Trondheim, Norway). Standards of phenacetin, paracetamol, lidocaine, procaine and benzocaine were purchased from Sigma-Aldrich (Diegem, Belgium), a standard of levamisole was purchased from Acros Organics (Geel, Belgium), a standard of caffeine was purchased from VWR Chemicals (Leuven, Belgium) and a standard of creatine monohydrate was purchased from J&K Scientific (Lommel, Belgium). Confiscated samples containing cocaine, MDMA, amphetamine and ketamine were provided by the NICC in Belgium. Confiscated samples were provided in different physical forms (tablets, powders, crystals, pastes), colors (white, yellow, pink, orange), compositions (presence of adulterants and diluents) and purities (6.6 to 100%). Qualitative and quantitative analysis of the confiscated samples were performed by NICC using GC-MS and GC-FID respectively.

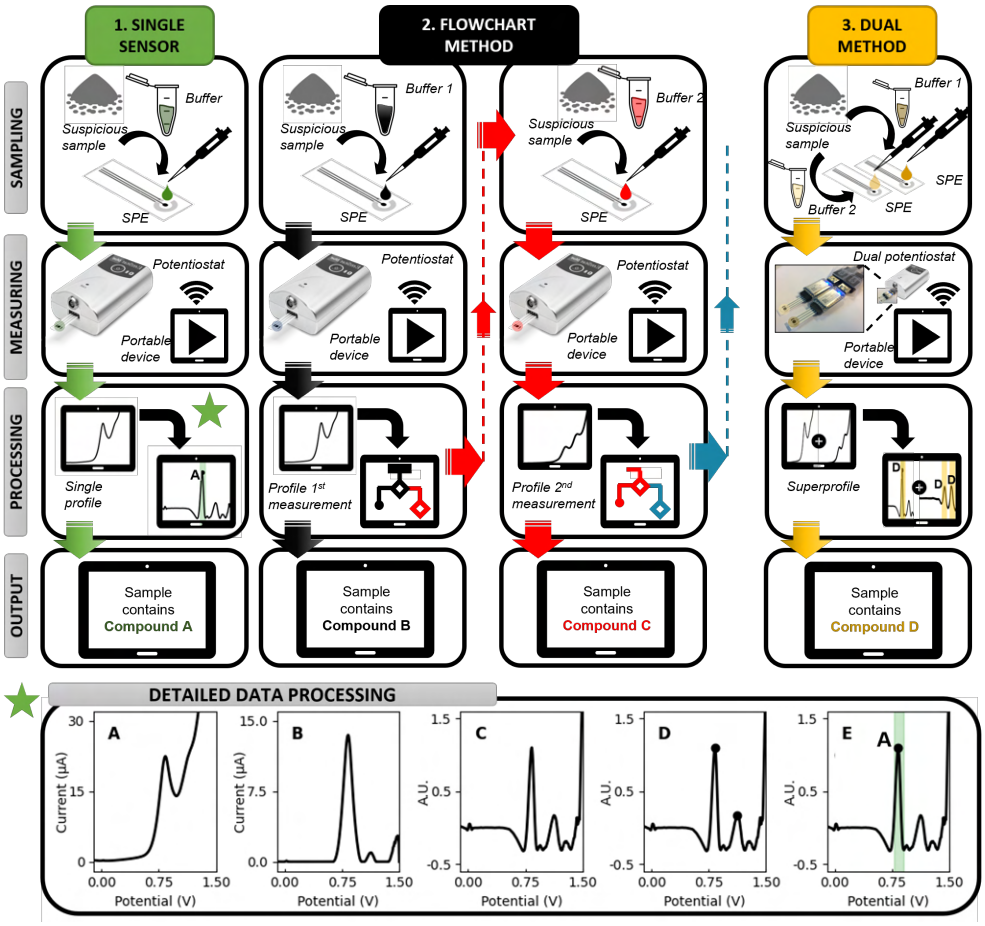


Figure 6.1: Schematic overview of the state-of-the-art approach used in electrochemical drug sensors (single sensor (green)). In this work, two novel methods are developed: a flowchart method (black) and a dual sensor method (yellow). A schematic display of the data processing of each method is displayed at the bottom of the figure. These data processing steps involve: preprocessing of the raw voltammogram (A) with a baseline correction (B) and digital filter (C), identification of the relevant peaks (D) and assignment of compounds to these peaks using an internal database (E).

Analytical grade salts of KH_2PO_4 and KCl, as well as KOH and HCl for pH-corrections and the formalin solution (aqueous formaldehyde solution, 37% w/w), were all purchased from Sigma-Aldrich (Overijse, Belgium). All solutions were prepared in 18.2 MΩ cm^{-1} doubly deionized water (Milli-Q water systems, Merck Millipore, Germany). Monitoring of the pH was performed using a 914 pH/conductometer from Metrohm (Herisau, Switzerland).

6.2.2 Instruments and methods

All SWV measurements were performed using a MultiPalmSens4 potentiostat (PalmSens, Houten, The Netherlands) with PSTrace/MultiTrace software. Disposable carbon ItalSens IS-C SPEs containing a graphite working electrode (Ø = 3 mm), a carbon counter electrode, and a silver (pseudo) reference electrode were used for all measurements (single use) and were also provided by PalmSens. All experiments were performed by applying 50 μL of the solution onto the SPE. The SWV parameters that were used: potential range of -0.1 to 1.5 V, frequency 10 Hz, amplitude 25 mV and step potential 5 mV.
PBS pH 12 used in the experiments contains 0.020 M KH_2PO_4 and 0.1 M KCl (further referred to as "pH 12"). Another buffer is also employed: PBS pH 7F contains 0.1 M KH_2PO_4 and 0.1 M KCl, as well as 30 %v/v formalin solution (11.1 %v/v formaldehyde) (further referred to as "pH 7F"). All measurements utilizing this buffer solution were started after a reaction time of 28 seconds (which adds to a total of 1 minute including the measurement time). Measurements of pure compounds and binary mixtures used a concentration of 0.5 mM per compound in the pH 12 buffer and 1 mM in the pH 7F buffer. Moreover, all street samples measured were diluted to a concentration of 0.3 mg mL^{-1} in the former buffer and to 2.0 mg mL^{-1} in the latter.
For the flowchart, a first sampling is performed in the proposed buffer solution for the first measurement. Depending on the result of the analysis, a second sampling could be proposed in a different buffer solution. In contrast, the dual sensor requires two samplings before each measurement, one in each of the proposed buffer solutions.
The spectroscopic measurements were performed using a Bruker Bravo Handheld Raman spectrometer (Bruker Optik GmbH, Ettlingen, Germany). Further information on the used parameters and library is included in the Supporting Information.

6.2.3 Data processing

All raw voltammograms (Figure 6.1A) were background corrected using the "moving average iterative background correction" (peak width = 1) tool in the PSTrace software and subsequently digitally filtered with a top hat filter (wt = 7) (Figure 6.1B and 6.1C, respectively). This digital filtering and all further preprocessing steps are executed utilizing an in-house developed MATLAB script. The resulting preprocessed voltammograms are displayed throughout the chapter. After the preprocessing of the voltammograms, the relevant peaks are selected based on a minimum peak prominence and minimum peak height threshold (Figure 6.1D). This is to make sure only peaks are used that hold information about the sample. Subsequently, compounds are assigned to the selected peaks using an internal database (Figure 6.1E). An exception module in the data processing software allows the incorporation of exceptions. A detailed description of this data processing approach can be found in Chapter 3.

6.3 Results and discussion

In the EP-based sensing of illicit drugs, identification is based on the peak potential associated with a particular redox signal of a target compound. First, the electrochemical behavior of the target is studied in specific measuring conditions to identify reliable signals. Second, a potential interval is defined around each peak to account for small variations in peak potential due to temperature, concentration or the effect of electroactive adulterants in drug samples. The collection of these intervals composes the internal database of the peak recognition approach in the data processing. The presence of a peak in a recorded voltammogram can then be linked to the presence of a specific compound in the sample if that peak lies within one of the predefined intervals of the internal database. Some compounds have multiple peaks, and thus an increased selectivity is obtained by requiring the presence of all those peaks. If these conditions are fulfilled for a specific drug, the analysis is positive for this compound.

6.3.1 Shortcomings of single sensors for multidrug detection

When developing a sensor for the detection of a single drug (Figure 6.1 (green)), the measuring conditions (buffer, pH, electrode material) are selected in such a way that the drug yields a clear and reproducible EP that can be successfully distinguished from other electroactive compounds present in confiscated samples. The optimization of measuring conditions has previously been reported for the detection of cocaine[198–200], MDMA[41, 145, 157] and ketamine[201, 202]. In particular, the use of pH 12 buffer on unmodified carbon SPEs is highly suitable for the detection of these three illicit drugs in the presence of their adulterants[33, 41, 103]. Meanwhile, the detection of amphetamine is complicated by the high oxidation potentials of primary amines[203], which fall outside the accessible potential window (>1.5 V) in an aqueous environment on unmodified graphite screen-printed electrodes.

To overcome this, derivatization approaches using various reagents have been reported in previous years[154, 158, 161, 204]. For example, the formaldehyde approach developed in our group successfully employs pH 7F buffer (containing 30 v% formalin solution) to detect amphetamine and enrich the EP of other drugs with additional characteristic peaks[161]. When multiple target compounds have to be detected by the same single sensor, this strategy becomes cumbersome due to potentially overlapping peaks. This is illustrated in Figure 6.2, which contains the EP of the four target drugs, measured in the previously mentioned pH 7F and pH 12 measuring conditions.

Table 6.1 summarizes the oxidation potentials of the characteristic peaks of these compounds. In both buffers, significant overlap between the signals of the target drugs is observed, especially considering that potential intervals need to be defined around each signal to account for variations caused by various factors (i.e. temperature, concentration and composition). Although some drugs yield multiple signals to facilitate detection, the strong similarities between the EPs of cocaine and ketamine in pH 7F and the non-detectable nature of amphetamine in pH 12 are only two examples to demonstrate the shortcomings of single sensors when selective multidrug detection is desired. As the use of multiple electrodes can provide the necessary additional electrochemical information, two multi-SPE methods are developed (Figure 6.1 (black and yellow)): (i) the flowchart

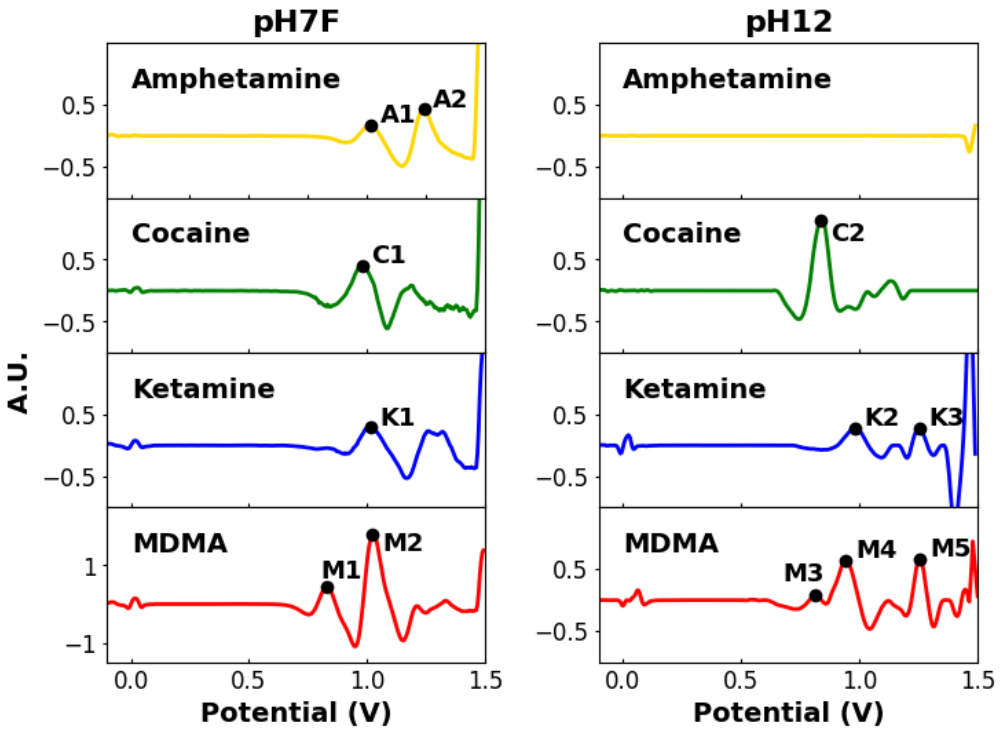


Figure 6.2: Overview of the preprocessed EPs of the four selected illicit drugs in pH 7F (left) and pH 12 (right). The relevant peaks are assigned a peak name. Concentrations in pH 7F: 1 mM. Concentrations in pH 12: 0.5 mM.

Table 6.1: Overview per compound of the peaks used for identification throughout this work. Each peak receives a unique code, also displayed in Figure 6.2.

	pH 7F		pH 12	
	Peak name	Peak potential (V)	Peak name	Peak potential (V)
Amphetamine	A1	1.01		
	A2	1.23		
Cocaine	C1	0.98	C2	0.83
Ketamine	K1	0.99	K2	0.98
			K3	1.26
MDMA	M1	0.81	M3	0.81
	M2	1.03	M4	0.95
			M5	1.24

method starts with a first measurement and depending on the resulting EP, a second measurement is executed in different measuring conditions. Exactly which conditions are used for this second measurement is linked to a specific output in the first measurement. Additional measurements can be performed if necessary (Figure 6.1 (black)). (ii) The dual sensor method measures an EP simultaneously at two SPEs using different

measuring conditions at each electrode. Both EPs are then combined into a superprofile that links the information of both individual EPs with each other (Figure 6.1 (yellow)). Both multi-SPE methods thus make use of the same EPs, but use them in a different way to come to a result. The flowchart can be seen as a stepping stone to the dual sensor, since the former tries to obtain a result with as few SPEs as possible, while the latter always uses the information from two SPEs to arrive at a result. The combination of these two recorded EPs into a superprofile, results in more than double the information since signals in the first EP can be linked to the presence/absence of signals in the second EP, and vice versa.

Table 6.2: Overview per compound of the potential intervals and required peaks used for identification throughout this work. (b): adjusted intervals in case of benzocaine detection (0.45 – 0.55 V in pH 12). * Detection of M3 is optional.

	pH 7F			pH 12		
	Peak name	Potential interval (V)	Required peaks	Peak name	Potential interval (V)	Required peaks
Amphetamine	A1 A2	0.96 – 1.04 1.15 – 1.35	2/2			0/0
Cocaine	C1	0.95 – 1.06	1/1	C2 C2(b)	0.78 – 0.90 0.80 – 0.97	1/1
Ketamine	K1	0.97 – 1.07	1/1	K2 K2(b) K3	0.92 – 1.02 1.02 – 1.15 1.23 – 1.40	2/2 1/1(b)
MDMA	M1 M2	0.78 – 0.86 0.98 – 1.08	2/2	M3* M4 M5	0.80 – 0.86 0.92 – 0.97 1.24 – 1.32	2/2*

6.3.2 Building the database through a selected training set

A set of samples, the training set, is selected to optimize the flowchart and dual sensor methods. It may be clear that both methods thus use the same set of EPs, i.e. the training set, for their optimization. An overview of the selected samples is given in Table 6.3. Apart from the four illicit drugs, this set contains eight adulterants and 21 relevant binary mixtures (drugs with adulterants). The adulterants are an important part of this training set as they are frequently added to drug samples and could influence the EP of the drug in the sample. These adulterants in particular are selected based on their common occurrence in formulations containing one of the drugs discussed in this work[18, 205, 206]. Furthermore, they could also be encountered as the main ingredient in legal formulations, in which case differentiation with the target drugs is necessary as an overlap could lead to false positive results. Therefore, it is important to carefully choose the potential intervals defined for each of the characteristic peaks of the targeted drugs, based on their electrochemical behavior in the used measuring conditions.

First, the adulterants in the training set were analyzed in pH 7F and pH 12 (Figure S6.1), with resulting peak potentials summarized in Table S6.1. It can be observed

Table 6.3: Training set containing four drugs, eight adulterants and 21 binary mixtures.

Training set			
Cocaine	+ Benzocaine + Caffeine + Levamisole + Lidocaine + Paracetamol + Procaine + Phenacetin	MDMA	+ Amphetamine + Caffeine + Creatine + Ketamine + Paracetamol + Phenacetin
Amphetamine	+ Caffeine + Creatine + Paracetamol	Ketamine	+ Amphetamine + Benzocaine + Caffeine + Creatine + Paracetamol
Codeine Lidocaine	Procaine Benzocaine	Caffeine Creatine	Paracetamol Phenacetin

that benzocaine, codeine, lidocaine and procaine produce signals located in a similar potential area to the target drugs (Table 6.1). This is mainly the case in pH 7F, as these measuring conditions have an enriching effect on the EPs of not only drugs but all compounds containing primary or secondary amines. The proximity of all these signals again highlights the necessity of combining multiple measuring conditions to obtain differentiation. The influence of these adulterants on the EPs of the drugs in the binary mixtures (Figure S6.2) is assessed based on the change in potential of the characteristic drug peaks (Table S6.2) they cause. Importantly, these findings lead to the definition of potential intervals for each peak. For example, if the presence of the adulterants of a drug tends to cause a positive shift in the peak potential of that drug, then the interval is made larger on that side of the peak. Several observations made from Table S6.2 are discussed below.

Firstly, the shifts of the C2 and K2 peaks (+0.09 V) in the binary mixtures of cocaine and ketamine with benzocaine in pH 12 stand out. Additionally, K3 is completely suppressed. In previous work, de Jong *et al.* found that, upon oxidation, benzocaine creates a local near-surface pH effect causing the redox peaks of other compounds (e.g. cocaine and ketamine) to shift or be suppressed[104]. To avoid false negative results, this effect needs to be taken into account when defining intervals. However, simply broadening it to compensate for this strong shift is not desirable, as several other compounds produce peaks in this potential zone (0.80 – 1.00 V) and narrow intervals are, therefore, preferred. As this effect is, to our knowledge, specific to benzocaine, a more selective solution is proposed. In the peak recognition software, it is programmed that, when a signal is detected in the interval 0.45 – 0.55 V (where the characteristic peak of benzocaine is located) in pH 12, the potential intervals of the C2 and K2 peaks are shifted to higher potentials and extended, while K3 is no longer a required peak (Figure 6.3). Secondly, large shifts are also observed for the A2 (+0.10 V) and M5 (+0.06 V) peaks in their binary

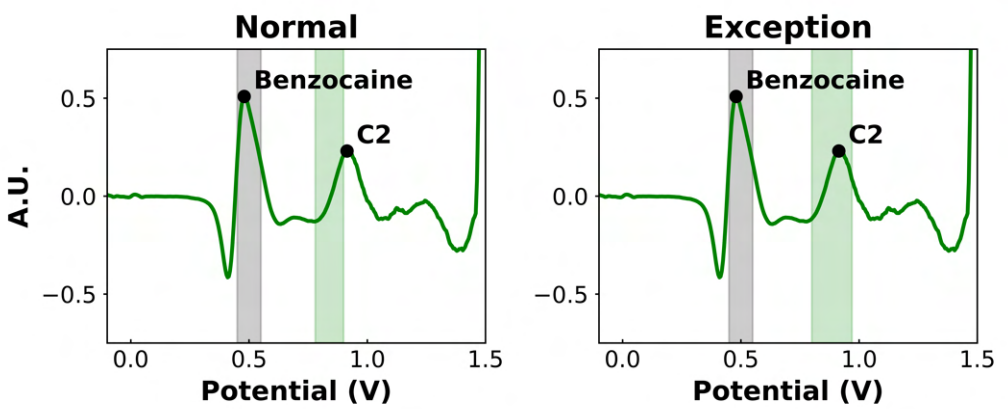


Figure 6.3: Left: a preprocessed voltammogram of an equimolar cocaine/benzocaine mixture (0.5 mM/0.5 mM) in pH 12 with the normal potential interval visually shown (in green). Right: same voltammogram but with a potential interval that is shifted/extended. If a peak is detected in the grey interval (0.45 – 0.55 V), it is positive for benzocaine and the exception is activated.

mixtures with caffeine (in pH 7F and pH 12 respectively). This is due to the overlapping of A2 and M5 with the caffeine signal, resulting in one broad peak. As fewer characteristic peaks of target drugs occur in this potential zone, a wide interval is proposed covering both the pure compounds and the binary mixtures (Table 6.2). Thirdly, the M3 peak (pH 12) is not detected in the binary mixtures of MDMA with amphetamine and ketamine due to a peak prominence below the threshold. This low peak prominence is a common issue for signals which are present as a shoulder on a different peak in the raw voltammogram and are separated by the preprocessing step, as is the case for M3 (Figure 6.2). To overcome this, the requirement of this peak for MDMA identification is set to optional as two characteristic signals in each pH 7F and pH 12 can provide the necessary selectivity (Table 6.2).

6.3.3 Flowchart method

A flowchart starts by performing a first test (on one SPE) to obtain an EP of the sample. Based on this, either a result is shown or a follow-up measurement, requiring a second sampling and measurement on a different SPE, is proposed by the software in different measuring conditions to improve differentiation. Thus, compounds that produce overlapping signals in the conditions used for the first test can be grouped in one joint interval. When a peak is detected in this interval, a follow-up measurement is proposed in conditions that allow differentiation between the drugs included. The overview of possible measurement sequences is subsequently summarized in a flowchart (Figure 6.4). Since LEAs at music festivals expectedly have a large number of suspicious samples to analyze, including numerous negative samples, it makes sense to minimize the measurement sequence in general and in particular for negative results. Therefore, the choice of measuring conditions for the first measurement needs to cover all the drugs targeted by the sensor, in this case by using the pH 7F conditions. As was demonstrated in Figure

6.2, the oxidation potentials of the C1, A1, M2 and K1 peaks in pH 7F are all located in a narrow potential zone (0.98 – 1.03 V). While MDMA can be selectively detected by utilizing the M1 peak, differentiation between the other three drugs is complicated.

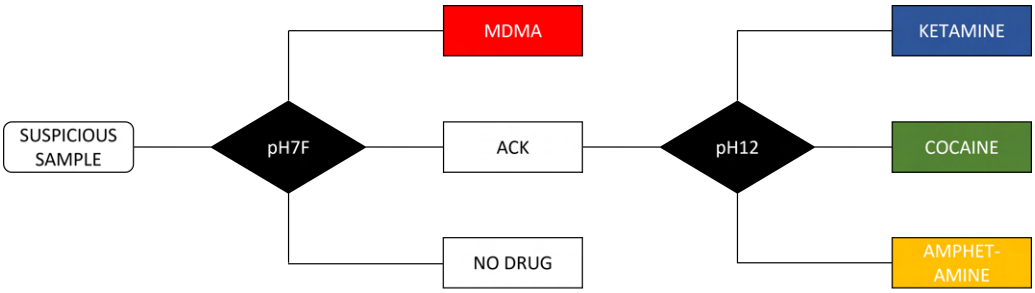


Figure 6.4: Representation of the flowchart method and its dedicated databases. First, a measurement is executed in pH 7F, and depending on the outcome, a second measurement might be executed in pH 12. ACK = joint interval for amphetamine, cocaine and ketamine in pH 7F (0.92 – 1.07 V).

Although the EP of amphetamine contains a second characteristic peak (A2), the presence of caffeine, considered to be one of the most prevalent adulterants in both cocaine and ketamine samples[18, 207], in the analyzed sample could also trigger this interval. Consequently, amphetamine, cocaine and ketamine are combined in one interval, named 'ACK' (0.92 – 1.07 V), to avoid wrongful identifications (Figure 6.4). For MDMA, both characteristic peaks are required for identification, and despite one of those occurring in the ACK interval, MDMA can be selectively identified based on the presence of the other. If neither the conditions for ACK detection are fulfilled nor those of MDMA, then the result of the measurement is negative. For this reason, a sample that does not contain any of the target drugs would only require one sampling and one measurement. If a peak is detected in the ACK interval, a follow-up measurement (in this case in the pH 12 condition) is proposed to differentiate between these three drugs. The EPs of cocaine and ketamine are sufficiently separated in these conditions, while amphetamine yields no peaks. It can be programmed in the peak recognition software that the latter is detected when neither the cocaine nor the ketamine intervals are activated in pH 12. However, Table S6.1 shows that three pure adulterants also activate the ACK interval in pH 7F: benzocaine, codeine and lidocaine. As none of them triggers the intervals of cocaine and ketamine in pH 12, the analysis of these compounds would give a false positive result for amphetamine. Since they are unlikely to be encountered in confiscated amphetamine samples (which mainly contain caffeine, creatine and non-electroactive diluents[17, 206]), an exception is built into the software that utilizes the difference in electrochemical behavior between the mentioned adulterants and amphetamine in pH 12. While the former all produce characteristic oxidation peaks between 0.47 and 0.80 V, the latter is non-electroactive. Thus, it is programmed that amphetamine is only identified by the sensor if: (a) the ACK interval is activated in pH 7F, AND (b) no peaks are identified in the potential range 0.47 – 0.80 V in pH 12. If a peak is detected in the defined range, the compound is deemed to be an adulterant and will give a negative result. The major advantage of the flowchart over the single sensor is that the results of a first measurement can be linked to an optional, second measurement. Next, a dual sensor method is investigated to further explore the possibilities of linking two EPs to each other.

6.3.4 Dual sensor method

A second method, coined the dual sensor method, involves the combined analysis of two EPs recorded simultaneously at two parallel SPEs. This method thus always requires only one sampling step (comprising sampling in one or multiple different buffer solutions), as opposed to the flowchart method which might require more than one sampling step (in one buffer solution each time). The fusion of the two simultaneously recorded EPs results in a so-called superprofile that holds much more information about the sample than either one of these two individual profiles separately. In fact, the information is more than doubled with the superprofile because the presence or absence of signals in both individual profiles can be linked to each other. The data interpretation approach of Chapter 3 has been extended for this dual sensor method to maximally exploit this information contained in the superprofile[81]. Both EPs are still preprocessed and subsequently assigned compounds using their own, unique database. However, only one, unique exception module which comprises both profiles is employed, rather than two exception modules for each profile separately. As a result, the presence of signals in both conditions can be required prior to the assignment of a specific compound.

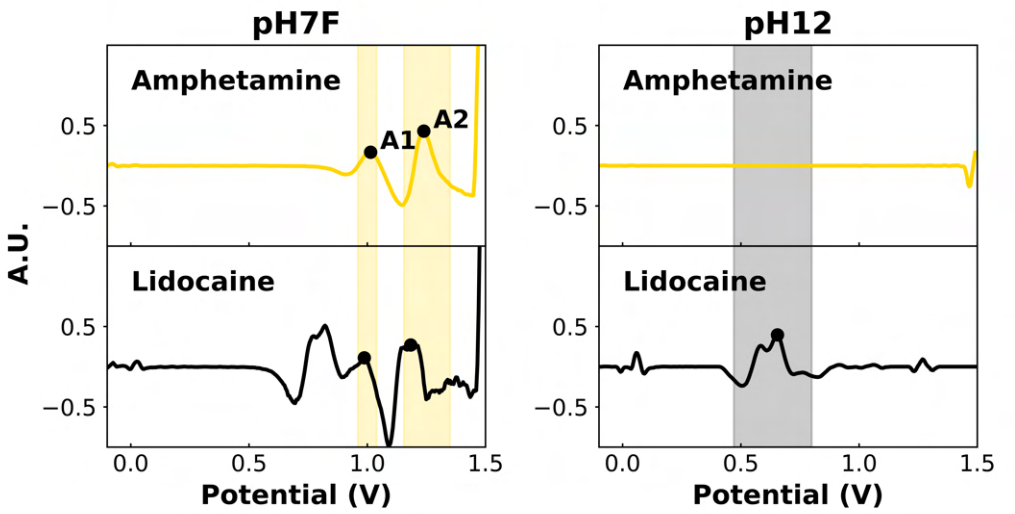


Figure 6.5: preprocessed voltammograms of lidocaine and amphetamine in pH 7F and in pH 12. Amphetamine is identified if both diagnostic peaks in pH 7F are present AND no peak is present in the interval (0.47 – 0.80 V). If a peak is present in the latter interval, as is the case for lidocaine, amphetamine is not identified, thereby avoiding potential false positives.

For these experiments, the pH 7F and pH 12 measuring conditions are used in parallel (Figure S6.3). The combination of a set of measuring conditions capable of detecting all drugs of interest (pH 7F) and another set of conditions to provide improved differentiation among those drugs (pH 12), is highly suitable for this purpose. As each analysis combines the EPs obtained in pH 7F and pH 12, a joint 'ACK' interval is no longer required and the intervals of the individual drugs can be used. The issue of false positives for amphetamine caused by adulterants is then reduced to those that activate both amphetamine intervals in pH 7F, which is the case for codeine and lidocaine (Figure 6.5). Again, it is programmed

that amphetamine can only be detected if: (i) both its intervals are triggered in pH 7F AND (ii) no peak is detected in the potential range 0.47 – 0.80 V in pH 12.

6.3.5 Validation using confiscated samples

Finally, the two multidrug sensing methods are validated by analyzing a set of confiscated samples (40 in total, Table S6.3). These confiscated samples, previously analyzed at NICC by the standard methods of a forensic laboratory (i.e. GC-MS and GC-FID), provide valuable information about what is encountered on-site and are difficult to mimic in the lab due to their varying composition and the limited information available in the literature on these compositions. The performance of both methods is subsequently assessed using several metrics related to the accuracy of detection and the practicality of the method, summarized in Table 6.4. In addition, their performance is compared to that of a commercial portable Raman spectroscopic device (Bruker Bravo) which is commercially available for the purpose of illicit drug detection.
The flowchart method uses the additional electrochemical information provided by follow-up measurements to improve performance. Application of this method to the voltammograms obtained for the 40 confiscated samples in pH 7F and pH 12 (Figure S6.4 and Table S6.4) leads to a correct identification in 80.0% of the cases (Table 6.4). The main source of wrongful classifications is the series of ketamine samples, with 6 out of 10 classed as MDMA (Table S6.4). It appears that a shoulder on the K1-peak of ketamine arises at high concentrations in pH 7F, which is separated into a peak by the preprocessing step and which activates the M1 interval of MDMA (Figure S6.4 and Table S6.4). This feature is not included in the EP of ketamine due to its presence depending on the concentration, but could be encountered in on-site measurements and, therefore, needs to be taken into account. In this flowchart, no follow-up measurement was proposed in the case of MDMA detection in pH 7F as it could be differentiated from the other drugs in these conditions. Moreover, this work aimed to make the flowchart as short as possible to showcase its balance between feasible practicality and performance. Indeed, the metrics displayed in Table 6.4 show that, of the two methods discussed, the proposed flowchart offers a good balance between being practical (limiting the number of samplings and SPEs used) and providing accurate results for this set of target drugs.

Table 6.4: Performance overview of the two electrochemical methods and portable Raman device employing various performance metrics.

	Flowchart method	Dual sensor method	Portable Raman
Accuracy confiscated samples (40)	80.0%	87.5%	60.0%
Time per analysis (seconds)	~90	~60	20-60
Average electrodes used	1.54	2	/
Different buffers required	1.54	2	/

The dual sensor method combines two parallel measurements (in two different measuring conditions) to achieve accurate detection. This method always requires the use of two SPEs and two samplings, but measurement time remains low due to the parallel character. Its key advantage is that, for each sample, the EP is recorded in two measuring conditions so each identification is well-founded. As expected, this method obtains the highest accuracy for the confiscated sample analysis in comparison to the standard methods

(87.5%, Table 6.4). Two of the wrongfully classed samples are false negatives (1C and 9C, Table S6.4), while the other three are classified as another illicit drugs. For all five, the expected characteristic peaks of the drug present in the sample were detected but fell outside the defined potential intervals (Figure S6.4). Optimizing the choice of interval limits is a gradual process and the more samples are analyzed, the more accurate these become. Therefore, this outcome is promising as further optimization on relevant drug samples will filter out these few false identifications and thereby improve the performance. Finally, the same set of confiscated samples was analyzed using the portable Raman device. The data evaluation was limited to finding the three main components as non-specialized personnel are unlikely to perform an extensive manual mixture analysis in the field. The results are summarized in Table S6.4 and the obtained accuracy is included in Table 6.4. The Raman device obtained a considerably lower accuracy (60.0%) than the electrochemical methods. Incorrect results include the identification of another (legal) substance present in the sample (e.g. caffeine in 3A and creatine in 8A) or of structurally related compounds (e.g. norephedrine in 1A and phenethylamine in 2A). In the field, LEAs often combine the use of a portable Raman device with other techniques to avoid false negatives. It is important to emphasize that these results reflect the capabilities of the tested device for this specific purpose, rather than those of Raman spectroscopy in general.

6.4 Conclusions

In this work, two innovative electrochemical methods for the simultaneous detection of the four most commonly encountered illicit drugs at festivals in Europe (cocaine, MDMA, amphetamine and ketamine) were developed and validated. LEAs at music festivals require portable screening tests for the rapid on-site analysis of suspicious samples encountered during searches. Therefore, both methods were compared based on their practicality regarding on-site use and performance on a series of confiscated samples. The flowchart method introduces the possibility of performing follow-up measurements based on the result of an initial test. These then provide the electrochemical information necessary to achieve the desired differentiation. Furthermore, this method has the practical advantage of eliminating the need for further measurements in some cases, such as for negative samples (when the recorded EP does not resemble that of any of the target drugs). Already reaching an accuracy of 80.0% in the proposed form, this can be further increased by introducing additional measurements. Expectedly, the dual sensor is the most reliable method (accuracy 87.5%) as it bases all identifications on a double EP recorded in different measuring conditions. For each of the wrongfully classed confiscated samples, further optimization of the potential intervals used in the peak identification software through testing more samples could offer the solution. When further expanding the scope of the sensor to detect other drugs, this principle of parallel measurements (two or more) collecting sufficient electrochemical information for selective detection will be essential, as the measurement sequences used in flowcharts will then become too laborious and time-consuming. Overall, these electrochemical multidrug methods proved to be viable options for the on-site screening of suspicious samples encountered during searches at music festivals, as evidenced by the performance comparison with the commercially available portable Raman device, which reached a considerably lower accuracy. They are portable, affordable, rapid, cover several different

drugs simultaneously and reach high accuracies. Moreover, these findings demonstrated the robustness of this electrochemical sensor for the detection of the target drugs in different physical forms (tablets, powders, crystals, pastes), colors (e.g. white, yellow, pink, orange), compositions (presence of adulterants and diluents) and purities (ranging from 6.6 to 100%). This work will serve as a foundation for the development of new electrochemical tools to detect illicit drugs in decentralized settings, thereby empowering the LEAs capabilities towards a safe society.

6.5 Supplemental figures and tables

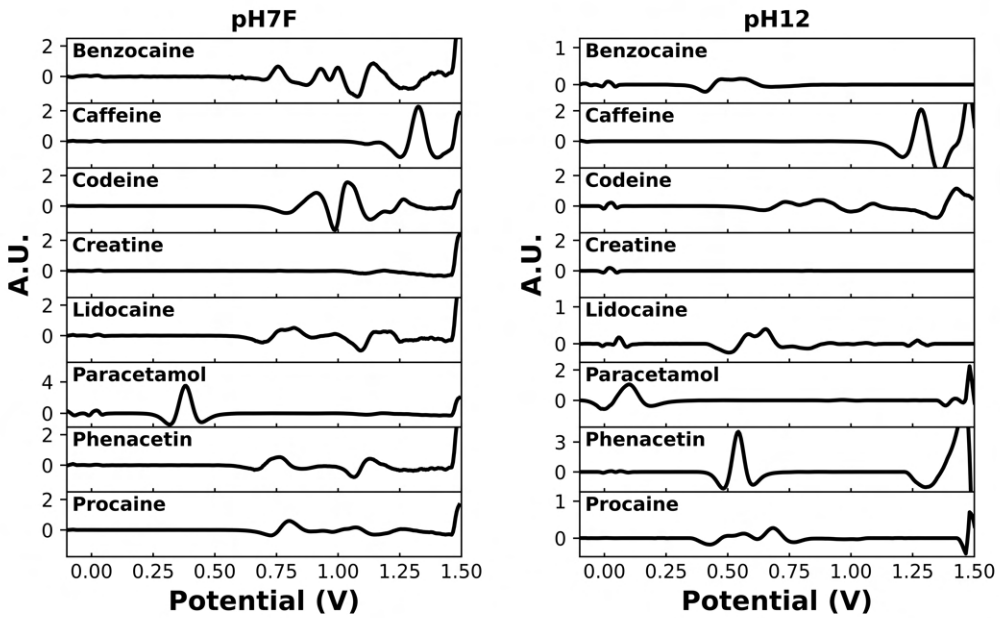


Figure S 6.1: Overview of the preprocessed EPs of the adulterants in the training set, recorded in pH 7F (left) and pH 12 (right). Concentrations in pH 7F: 1 mM. Concentrations in pH 12: 0.5 mM.

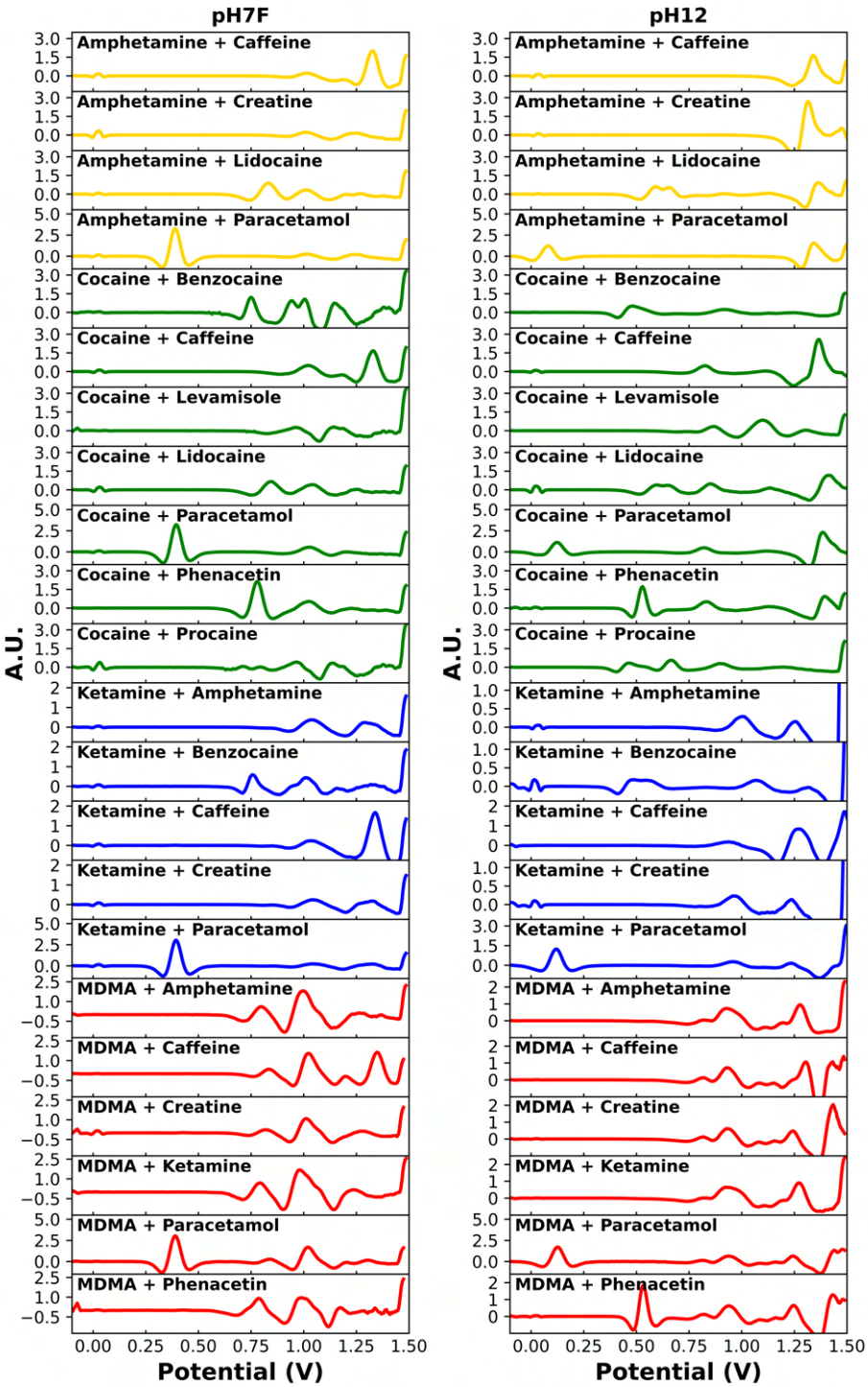


Figure S 6.2: Overview of the preprocessed EPs of the binary mixtures in the training set, recorded in pH 7F (left) and pH 12 (right). Yellow: amphetamine mixtures, green: cocaine mixtures, blue: ketamine mixtures, red: MDMA mixtures. Ratio in all mixtures: 1:1. Concentrations in pH 7F: 1 mM. Concentrations in pH 12: 0.5 mM.

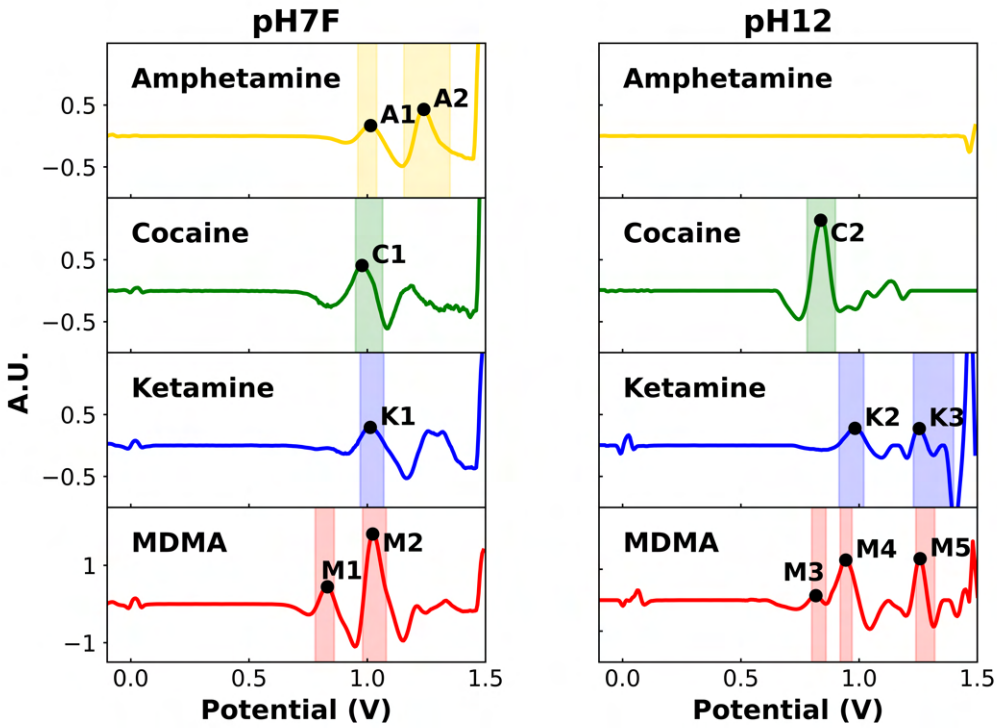
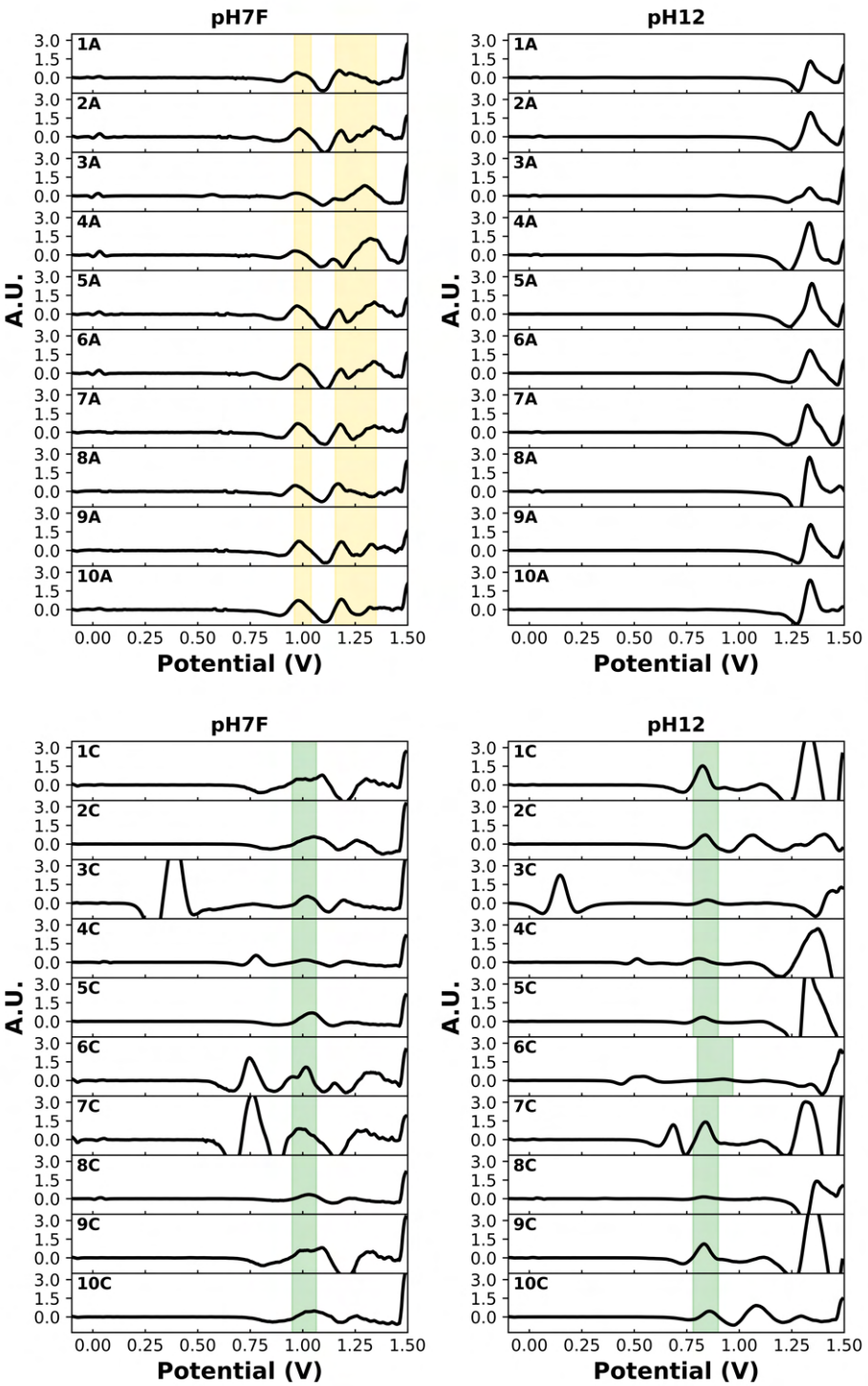


Figure S 6.3: Representation of the database used in the dual-sensor method (pH 7F + pH 12).


pH7F
pH12
1A
2A
3A
4A
5A
6A
7A
8A
9A
10A
1C
2C
3C
4C
5C
6C
7C
8C
9C
10C
A.U.
3.0
1.5
0.0
0.00 0.25 0.50 0.75 1.00 1.25 1.50
Potential (V)

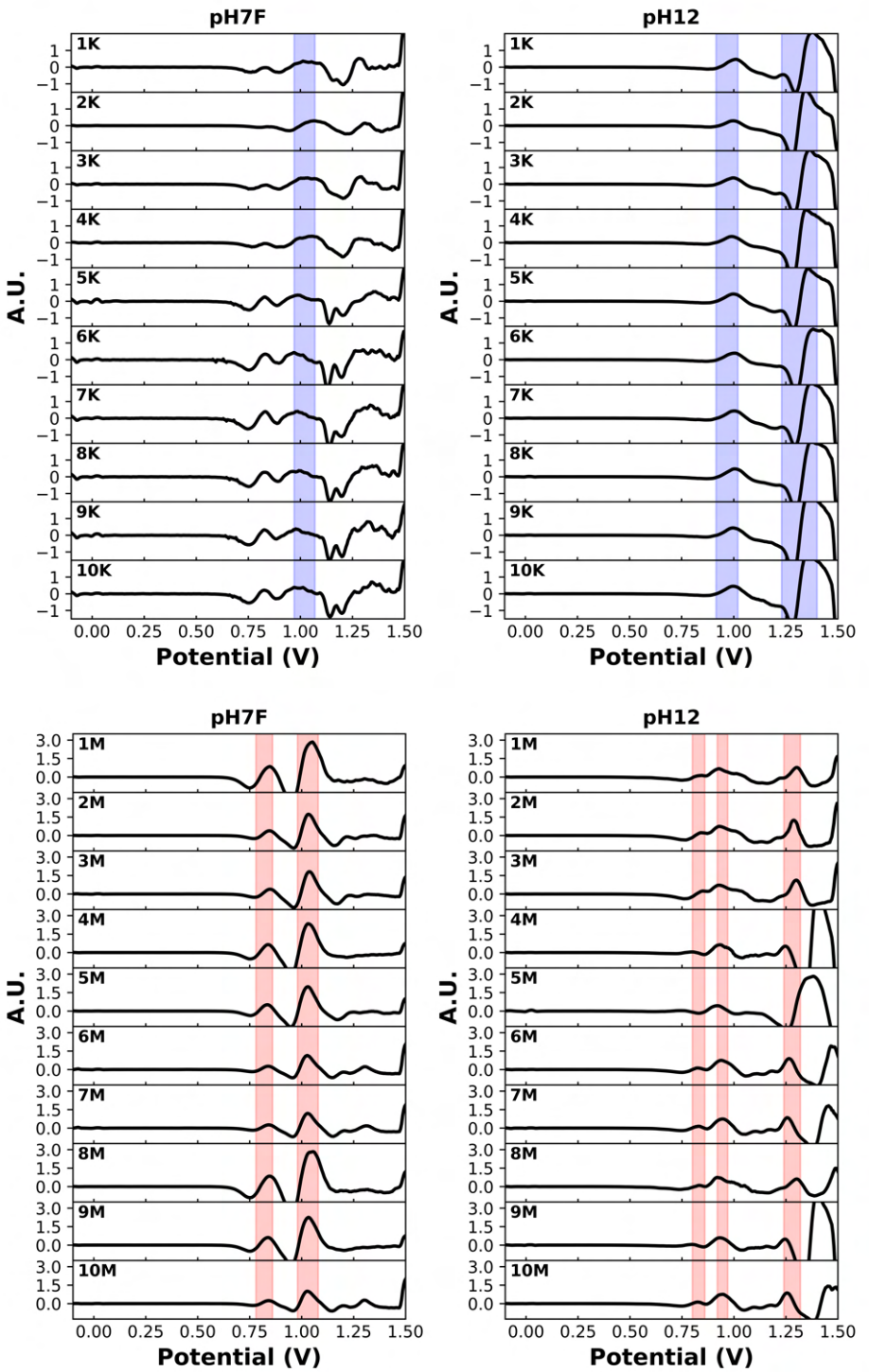


Figure S 6.4: Overview of the preprocessed EPs of the confiscated samples, recorded in pH 7F (left) and pH 12 (right). The potential intervals defined for each drug's peaks are visually shown in the color assigned to each drug. Yellow: amphetamine samples, green: cocaine samples, blue: ketamine samples, red: MDMA samples. Concentrations in pH 7F: 2.0 mg mL^{-1}. Concentrations in pH 12: 0.3 mg mL^{-1}.

Table S 6.1: Overview of the peaks produced by each adulterant and the corresponding peak potential in the pH 7F and pH 12 measuring conditions.

	pH 7F		pH 12	
	Peak number	Peak potential (V)	Peak number	Peak potential (V)
Benzocaine	1	0.76	1	0.53
	2	0.93		
	3	1.00		
	4	1.14		
Caffeine	1	1.33	1	1.28
Codeine	1	0.91	1	0.77
	2	1.04	2	0.91
	3	1.27	3	1.10
Creatine	1	/	1	/
Lidocaine	1	0.82	1	0.66
	2	0.99		
	3	1.18		
Paracetamol	1	0.38	1	0.10
Phenacetin	1	0.76	1	0.54
	2	1.13		
Procaine	1	0.80	1	0.69
	2	1.08		
	3	1.26		

Table S 6.2: Overview of the potentials of drug peaks in their binary mixtures in pH 7F and pH 12. Between brackets is the potential change of the characteristic drug peaks compared to those of the pure compound.

	pH 7F		pH 12	
	Peak name	Peak potential (V)	Peak name	Peak potential (V)
Amphetamine + caffeine	A1	1.01 (+0.00)		
	A2	1.33 (+0.10)		
Amphetamine + creatine	A1	1.01 (+0.00)		
	A2	1.25 (+0.02)		
Amphetamine + paracetamol	A1	1.02 (+0.01)		
	A2	1.26 (+0.03)		
Cocaine + benzocaine	C1	0.94 (-0.04)	C2	0.92 (+0.09)
Cocaine + caffeine	C1	1.02 (+0.04)	C2	0.83 (+0.00)
Cocaine + levamisole	C1	0.96 (-0.02)	C2	0.87 (+0.04)
Cocaine + lidocaine	C1	1.04 (+0.06)	C2	0.85 (+0.02)
Cocaine + paracetamol	C1	1.03 (+0.05)	C2	0.82 (-0.01)
Cocaine + procaine	C1	0.97 (-0.01)	C2	0.90 (+0.07)
Cocaine + phenacetin	C1	1.02 (+0.04)	C2	0.83 (+0.04)
Ketamine + amphetamine	K1	1.04 (+0.05)	K2	1.01 (+0.03)
			K3	1.26 (+0.00)
Ketamine + benzocaine	K1	1.01 (+0.02)	K2	1.07 (+0.09)
			K3	/
Ketamine + caffeine	K1	1.03 (+0.04)	K2	0.94 (-0.04)
			K3	1.27 (+0.01)
Ketamine + creatine	K1	1.03 (+0.04)	K2	0.96 (-0.02)
			K3	1.24 (-0.02)
Ketamine + paracetamol	K1	1.04 (+0.05)	K2	0.96 (-0.02)
			K3	1.23 (-0.03)
MDMA + amphetamine	M1	0.80 (-0.01)	M3	/
	M2	1.00 (-0.03)	M4	0.93 (-0.02)
			M5	1.28 (+0.04)
MDMA + caffeine	M1	0.83 (+0.02)	M3	0.82 (+0.01)
	M2	1.02 (-0.01)	M4	0.93 (-0.02)
			M5	1.30 (+0.06)
MDMA + creatine	M1	0.82 (+0.01)	M3	0.82 (+0.01)
	M2	1.01 (-0.02)	M4	0.93 (-0.02)
			M5	1.24 (+0.00)
MDMA + ketamine	M1	0.79 (-0.02)	M3	/
	M2	0.98 (-0.05)	M4	0.92 (-0.03)
			M5	1.27 (+0.03)
MDMA + paracetamol	M1	0.84 (+0.03)	M3	0.82 (+0.01)
	M2	1.02 (-0.01)	M4	0.93 (-0.02)
			M5	1.24 (+0.00)
MDMA + phenacetin	M1	0.79 (-0.02)	M3	0.82 (+0.01)
	M2	0.99 (-0.04)	M4	0.94 (-0.01)
			M5	1.25 (+0.01)

Table S 6.3: Overview of the composition and appearance of street samples. Identification and weight percentages were determined at NICC using GC-MS and GC-FID respectively.

Name	Sample nature (+ color)	Composition	wt.%
1A	Tablet (off-white)	Amphetamine	25.8
2A	Paste (orange)	Amphetamine	52.2
		Caffeine	29.2
3A	Tablet (red)	Amphetamine	6.6
		Caffeine	13.3
		Ketamine	/
		MDMA	1.36
		3-Fluoroamphetamine	1.84
4A	Powder (yellow)	Amphetamine	20.9
		Caffeine	48.4
5A	Powder (white)	Amphetamine	47.9
		Caffeine	41.8
6A	Powder (white)	Amphetamine	57.6
		Caffeine	31.2
7A	Paste (yellow)	Amphetamine	67.7
		Caffeine	20.7
8A	Paste (yellow)	Amphetamine	36.4
		Caffeine	1.3
9A	Powder (white)	Amphetamine	77.8
		Caffeine	13.4
10A	Powder (yellow)	Amphetamine	62.1

Name	Sample nature (+ color)	Composition	wt.%
1K	Powder (off-white)	Ketamine	83
2K	Powder (off-white)	Ketamine	5
3K	Powder (white)	Ketamine	82
4K	Crystals (white)	Ketamine	72
5K	Powder (white)	Ketamine	100
6K	Powder (white)	Ketamine	84
7K	Powder (white)	Ketamine	99
8K	Crystals (white)	Ketamine	99
9K	Powder (white)	Ketamine	100
10K	Powder (white)	Ketamine	100

Name	Sample nature (+ color)	Composition	wt.%
1C	Powder (white)	Cocaine	97.5
2C	Powder (white)	Cocaine	57
		Levamisole	41
3C	Powder (white)	Cocaine	19.2
		Paracetamol	73.2
		Levamisole	1.6
4C	Powder (white)	Cocaine	31
		Phenacetin	2.8
		Levamisole	5.7
5C	Powder (white)	Cocaine	29.6
		Boric acid	/
6C	Powder (white)	Cocaine	22
		Phenacetin	8.3
		Caffeine	15.5
		Lidocaine	12
		Levamisole	2.2
		Benzocaine	25.7
7C	Powder (white)	Cocaine	75.5
		Caffeine	3.1
		Hydroxyzine	9.6
		Lidocaine	<1
8C	Powder (brown)	Cocaine	20
9C	Powder (white)	Cocaine	>90.0
10C	Powder (white)	Cocaine	57
		Levamisole	41

Name	Sample nature (+ color)	Composition	wt.%
1M	Powder (white)	MDMA	93.7
2M	Powder (off-white)	MDMA	92.6
3M	Crystals (white)	MDMA	88
4M	Tablet (pink)	MDMA	40.4
5M	Tablet (grey)	MDMA	24.7
6M	Tablet (purple)	MDMA	57.2
7M	Tablet (grey)	MDMA	54.1
8M	Crystals (white)	MDMA	96.8
9M	Tablet (orange)	MDMA	39.5
10M	Tablet (yellow)	MDMA	54.4

Table S 6.4: Potentials of the peaks detected in the voltammograms of the confiscated samples in pH 7F and pH 12, as well as the results of the peak recognition software and the portable Raman analysis. For the Raman results, the three main components resulting from the library search were considered. The result displayed in the table is the most relevant one according to the analysis by the standard method (Table S6.3). ACK: joint interval amphetamine, cocaine and ketamine; AMP: amphetamine; COC: cocaine; COC(b): cocaine after activation of extended interval due to benzocaine detection (0.45 – 0.55 V in pH 12); KET: ketamine.

	pH 7F	pH 12	Flowchart result		Dual-sensor result	Raman result
Name	Peak potential (V)	Peak potential (V)	First analysis	Second analysis	Double analysis	
1A	0.97 1.17	1.34	ACK	AMP	AMP	Norephedrine
2A	0.99 1.18 1.34	1.34	ACK	AMP	AMP	Phenethylamine
3A	0.97 1.3	1.34	ACK	AMP	AMP	Caffeine
4A	0.96 1.33	1.34	ACK	AMP	AMP	AMP
5A	0.97 1.17 1.34	1.35	ACK	AMP	AMP	AMP
6A	0.99 1.18 1.34	1.34	ACK	AMP	AMP	AMP
7A	0.98 1.18 1.35	1.33	ACK	AMP	AMP	Norephedrine
8A	0.96 1.17	1.34	ACK	AMP	AMP	Creatine
9A	0.98 1.18 1.33	1.34	ACK	AMP	AMP	Norephedrine
10A	0.98 1.18 1.32	1.34	ACK	AMP	AMP	Norephedrine
1C	1.09 1.3	0.83 1.1 1.34	No drug	/	No drug	COC
2C	1.05 1.26	0.84 1.07 1.41	ACK	COC	COC	Paracetamol
3C	0.39 1.02 1.19	0.15 0.85	ACK	COC	COC	COC
4C	0.78 1.01 1.21	0.51 0.81 1.38	ACK	COC	COC(b)	Unknown
5C	1.04	0.83 1.33	ACK	COC	COC	COC
6C	0.75 1.02 1.33	0.54 0.92	ACK	COC	COC(b)	Unknown
7C	0.76 0.98 1.3	0.69 0.84 1.31	ACK	COC	COC	Unknown
8C	1.03 1.22	0.83 1.37	ACK	COC	COC	COC
9C	1.09 1.34	0.83 1.12 1.35	No drug	/	No drug	COC
10C	1.05	0.86	ACK	COC	COC	COC

	pH 7F	pH 12	Flowchart result		Dual-sensor result	
Name	Peak potential (V)	Peak potential (V)	First analysis	Second analysis	Double analysis	Raman result
1K	0.83 1.01 1.28	1.01 1.38	MDMA	/	KET	KET
2K	1.06 1.31	1 1.35	ACK	KET	KET	KET
3K	0.84 1.03 1.29	1 1.37	MDMA	/	KET	KET
4K	0.83 1.05 1.28	0.99 1.35	MDMA	/	KET	COC
5K	0.83 0.99 1.36	1 1.36	MDMA	/	KET	KET
6K	0.83 0.96 1.3	1.01 1.38	ACK	KET	AMP	KET
7K	0.83 0.99 1.34	1.01 1.37	MDMA	/	KET	KET
8K	0.83 0.99 1.37	1 1.38	MDMA	/	KET	COC
9K	0.83 0.98 1.32	1 1.36	ACK	KET	KET	KET
10K	0.83 0.97 1.35	1 1.36	ACK	KET	KET	KET
1M	0.85 1.05	0.93 1.3	MDMA	/	MDMA	MDMA
2M	0.85 1.03	0.93 1.29	MDMA	/	MDMA	2-aminopropane
3M	0.85 1.04	0.93 1.3	MDMA	/	MDMA	MDMA
4M	0.84 1.03	0.93 1.25 1.41	MDMA	/	MDMA	MDMA
5M	0.84 1.03	0.92 1.38	MDMA	/	KET	2-aminopropane
6M	0.84 1.03 1.3	0.94 1.26	MDMA	/	MDMA	MDMA
7M	0.84 1.03 1.31	0.83 0.95 1.25	MDMA	/	MDMA	2-aminopropane
8M	0.85 1.05	0.91 1.3	MDMA	/	KET	MDMA
9M	0.84 1.03	0.93 1.24 1.4	MDMA	/	MDMA	MDMA
10M	0.84	0.95	MDMA	/	MDMA	MDMA
Accuracy confiscated samples			32/40 (80.0%)		35/40 (87.5%)	24/40 (60.0%)

Chapter 7

Make an impact

"Without impact, innovation is just an idea with promise."

Judith Rodin

Abstract

This chapter has a pivotal role within this thesis, as evident from the similarity in the thesis and chapter title. The previous chapters frequently emphasized the aspiration to generate an impact with the voltammetric illicit drug sensing technology. The logical question that follows, is: "How was this goal exactly accomplished?". This chapter answers this question by delineating three fundamental pillars: impact through software, impact through practical considerations, and impact through the establishment of a lasting legacy. The first pillar extensively discusses software-related aspects such as single and multidrug sensing, temperature control, and app development. Subsequently, the focus shifts towards a range of diverse and highly relevant practical topics, often overlooked within the research field. Notably, specific areas such as sampling methods and buffer stability are brought to the forefront. Concluding the chapter, attention is paid to two topics that endeavor to leave a legacy beyond the thesis itself. These comprise a comprehensive guide for organizing successful demonstrations targeting end-users, and my perspective on the future trajectory of the research field. In summary, this chapter offers a comprehensive overview of the multifaceted efforts undertaken to position voltammetric drug detection technology as a catalyst for societal impact.

7.1 Introduction

It is a recurring theme in this thesis: the desire to make an impact with my research. This desire originates from my personality, I find it important to use my time and energy for a task with purpose. Furthermore, I feel a responsibility towards the public, this project is funded with tax-payers money, and I always felt a strong desire and drive to show them a tangible product by the end of the project. The technology described in this thesis has a grand potential to make an impact. However, it is commonly underestimated what gap exists between the finished product in an academic lab, and the finished product

for an end-user in the field. In an academic context, the end-point is often an academic publication, whereas for the end-user product a lot of (practical) aspects need to be covered that are not necessarily covered in the academic (analytical) research. I was lucky that I could work on these (more practical) aspects that facilitated the transition of the electrochemical technology from lab to true on-site application. The next pages contain a summary of the work I did to make sure the electrochemical technology can make an impact, described in three pillars: making an impact (1) with software, (2) by caring about the practical aspects, and (3) by leaving a legacy. Being able to work on these aspects had a very positive effect on my general feeling of purpose and daily motivation, and I am very happy to be in the position to present this chapter.

7.2 Make an impact with software development

The importance of software to bridge the gap between electrochemical output and non-expert end-user has extensively been covered in Chapters 3, 4, 5 and 6. These chapters are based on published research articles, and additional research has been conducted since the publication of these articles. I want to avoid repetition, so I will mainly focus on this additional research, and other software aspects that are not covered in the previous chapters.

7.2.1 Single drug sensor

Chapter 3 describes in detail the identification software that was developed to convert the complex electrochemical output into a clear yes/no result that is understandable for non-expert end-users. This software is crucial to make an impact with the electrochemical sensing technology, since it drastically increases the group of potential users.

In Chapter 3, an exclusion case is discussed where benzocaine and cocaine can never be assigned together, because benzocaine is known to shift the diagnostic signal of cocaine. The presented exclusion rule provides some relief, however, it accepts that cocaine will never be identified if benzocaine is present as a cutting agent. Although benzocaine is not a common cutting agent of cocaine, including this exception will inevitably lead to a loss in accuracy. This is why I included a more elegant solution in the current version of the cocaine sensor. The diagnostic cocaine peak shifts to higher potentials when in the presence of benzocaine. This shift is concentration dependent, so a larger relative presence of benzocaine shifts the cocaine signal to higher potentials, although this shift remains limited. Therefore, as a solution, an exception rule is included that if the diagnostic signal of benzocaine is detected, an alternative, more broad, detection window is allowed for detection of the diagnostic cocaine signal (Figure 7.1 - top row). This extended detection window anticipates the shift of the diagnostic cocaine signal, so that cocaine is still detected in cocaine/benzocaine mixtures. This same solution was also incorporated in the festival kit sensor described in Chapter 6.

The cocaine sensor contains another exception as well. During development of the sensor, it became apparent that the EP of heroin resembled the EP of a cocaine/paracetamol mixture (Figure 7.1 - middle row). Heroin has two diagnostic signals in a pH 12 buffer,

at 0.15 V and 0.88 V, which resemble the diagnostic signals of paracetamol and cocaine, respectively[16]. Since paracetamol is a common cutting agent of cocaine, it is not an option to use the heroin signal at 0.15 V as a diagnostic signal for identification of heroin[14]. Interestingly, heroin has a diagnostic signal in a pH 5 buffer at 1.02V, whereas cocaine has no signal at all in this buffer. This is why an exception was included in the cocaine sensor that if a signal is detected at 0.15 V and at 0.88 V in pH 12 buffer, a second measurement is requested in a pH 5 buffer to get a definite result. If a heroin signal is present in the EP generated with the second measurement in a pH 5 buffer, then the sample contains heroin. If no heroin signal is observed in the second measurement, the sample contains cocaine and paracetamol.

A third, and final, example is the exclusion of specific interfering agents (Figure 7.1 - bottom row). During validation of the heroin sensor with street samples, it became apparent that the illicit drug *N,N*-dimethyltryptamine (DMT) gives false positives. Interestingly, DMT has three signals in its EP. Therefore, if all of these three signals are identified, an exclusion is included that says the sample contains DMT, and not heroin. I feel comfortable to include this exception since an EP with three diagnostic signals is highly unique. If it would have been two diagnostic signals instead of three diagnostic signals, as is the case for heroin in the second example, I would avoid this type of exception and opt for: a) a second, confirmatory measurement in different conditions, or b) show a dialog box that the result is uncertain.

The possibility to include exceptions makes the identification software very versatile, allowing tailor-made solutions that maximize electrochemical insights. However, one should be aware that the inclusion of every exception module has a trade-off between a benefit, and a consequential downside. The benefit will be clear since it usually corresponds to the problem you are trying to solve with the exception. The downside, however, might not be clear at first sight, and a thinking exercise is required to map the downside(s). The first example raises a (small) risk for false positives, the second example requires an additional measurement that increases time and costs and the third example has a (small) risk to cause false negatives for heroin. To conclude, it is highly recommended to take this trade-off into consideration when implementing a novel exception in the software.

7.2.2 Multidrug sensor

Single drug sensors are the natural stepping stone to multidrug sensors. It makes sense that the software used in the single sensors is also the stepping stone for the software of the multidrug sensors. However, as discussed in Chapter 6, it is not possible in most cases to simply use the single drug sensing software for multidrug detection. An extension is required, and two different approaches were developed for this purpose: 1) a flowchart which ties the single sensors together, or 2) an array with superprofile software. Major progress was achieved for the flowchart approach, which will be discussed in the following section.

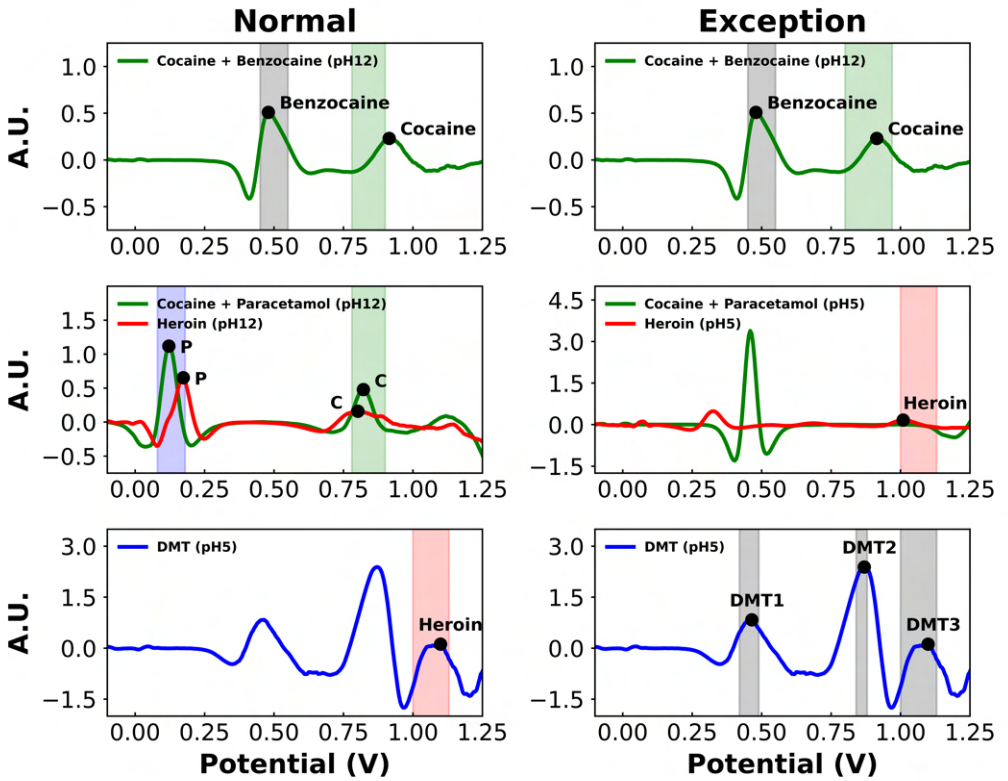


Figure 7.1: Visualization of three exceptions included in the single sensor software. The top row depicts the cocaine - benzocaine exception. If the diagnostic signal of benzocaine (0.45 V - 0.55 V) is detected, an alternative detection interval is used for cocaine (0.80 V - 0.97 V). The middle row shows the exception that handles a potential false positive for heroin in the cocaine sensor. Heroin and a cocaine/paracetamol mixture have a similar EP in pH 12 buffer. Therefore, if a cocaine/paracetamol mixture is detected, a second measurement in a pH 5 buffer is requested. In this second measurement, the diagnostic signal (1.00 V - 1.13 V) of heroin can be used to make a correct identification. Finally, the bottom row shows an exception that prevents potential FPs caused by DMT for the heroin sensor. If all three diagnostic peaks (0.42 V - 0.49 V, 0.84 V - 0.88 V, 1.00 V - 1.13 V) of DMT are detected, the analyte is said to be DMT, and not heroin.

7.2.2.1 Flowchart

The flowchart described in Chapter 6, for simultaneous detection of cocaine, MDMA, ketamine and amphetamine, works adequately. However, that flowchart neglects one major asset that is inherent to a flowchart: the incorporation of the users interpretation. In the context of illicit drug detection, the user can interpret the visual appearance of the sample, which can be integrated in the flowchart. The visual appearance of illicit drugs contains major clues on its content, e.g. a colored pill has a bigger chance to contain MDMA than heroin, where for a brownish powder it is the other way around. Therefore, if this visual appearance can be included in the flowchart, the accuracy should benefit.

The research conducted in this thesis falls within the BorderSens project, a European project that focuses on 'border detection of illicit drugs and precursors by highly accurate electrosensors'. In this project, there are five major drugs of interest that cover a major part of the illicit drug landscape in Europe: cocaine, heroin, MDMA, methamphetamine and ketamine. For all of these drugs, single drug sensors were developed in a similar fashion as the MDMA sensor described in Chapter 4, using the single drug sensor software[14, 16, 33, 85]. This selection of drugs was subsequently used as targets for a multidrug sensor that is based on a flowchart with visual appearances incorporated (Figure 7.2). In the following sections, I will dive into the specifics of the flowchart, followed by a validation study performed at NICC facilities.

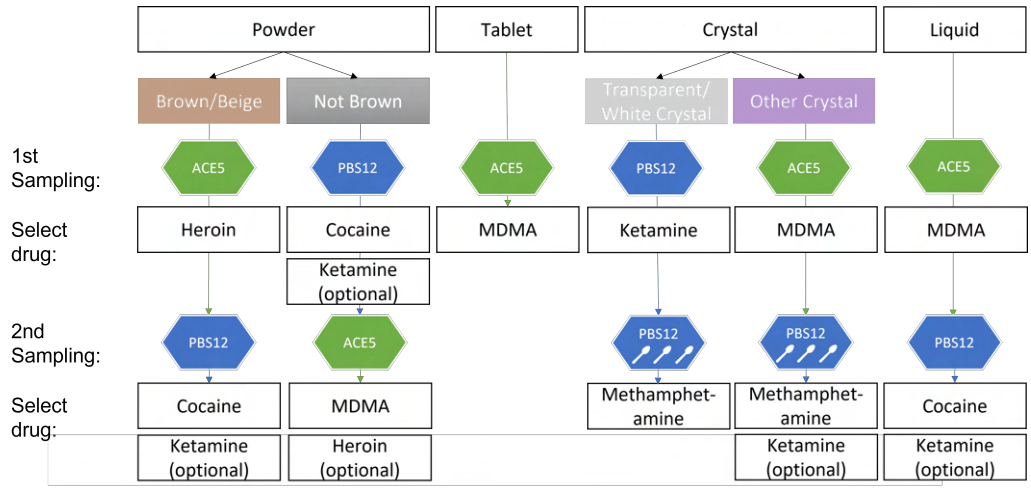


Figure 7.2: A flowchart based on visual appearance, guides a user through the different single drug sensors to efficiently come to an identification.

Development

The goal of the flowchart is to get a correct drug identification if one of the five target drugs is present in the analyzed sample, in as little measurements as possible. Evidently, if none of the five drugs is present, the result should come back as negative. Table 7.1 contains the information I used to construct the flowchart. The first line of the flowchart is based on the visual appearance of the sample. The appearances for each drug were determined based on talks with end-users, divided into a primary appearance and secondary appearances. It makes great sense to follow this approach, since by testing the drug that is most strongly associated with a specific visual appearance, the probability of finding the correct drug with the first test is greatly enlarged.

Interestingly, cocaine & ketamine, and MDMA & heroin, use the same buffers and sampling amount, respectively. This means that e.g. if a sample is tested with the cocaine sensor, the recorded EP might contain peaks that hint to the presence of ketamine in the sample. This is why, if the visual appearance could correspond to both e.g. cocaine and ketamine, an 'optional' ketamine analysis can be performed if the first cocaine measurement reveals the potential presence of ketamine. The cocaine and ketamine sensor use different minimum peak height and minimum peak prominence parameters, which makes it cumbersome to fuse them into one sensor that tests for both drugs. Therefore,

the best solution, which is the one integrated now, is that a pop-up screen is generated in the cocaine sensor if the two diagnostic signals of ketamine are detected. The sample can subsequently be analyzed with the ketamine sensor, using the same sample that was prepared for the cocaine measurement. Only a new SPE is thus required, keeping the workflow low-cost and time-efficient.

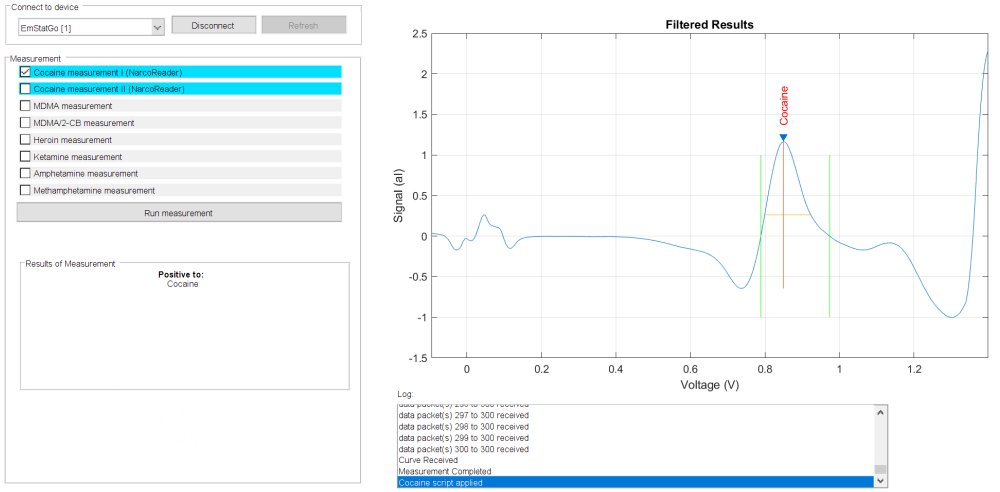


Figure 7.3: A graphical user interface (GUI) developed in Matlab, allows a user to select the single drug sensor that is suggested by the flowchart.

Practically, the single sensors are all integrated in a graphical user interface (GUI). Following the flowchart, the user is instructed which of the single sensors needs to be selected. There are two different setups, one with a GUI in Matlab (Figure 7.3), and one with a GUI integrated in a mobile application, which will be described in a section later on.

Table 7.1: Overview of the information used to construct the flowchart.

	Cocaine	Heroin	MDMA	Methamphetamine	Ketamine
Primary appearance	White Powder	Brown/Beige powder	Tablet	Crystal	White crystal
Secondary appearances	Colored powder, liquid		Crystal, liquid, colored powder		Powder, liquid
Buffer	PBS12	ACE5	ACE5	PBS12	PBS12
Sampling amount	5 mg/mL	5 mg/mL	5 mg/mL	7.5 mg/mL	5 mg/mL
Identification intervals	1	1	1	1	2

If the first round of measurements turns out negative, the flowchart might suggest a second sampling (different buffer or sampling amount) to test the sample on the presence of another drug that might correspond to the visual appearance of the sample. Again, some optional measurements are included, following the principle described in the previous paragraph. If the measurements in this second round are negative as well, the sample does not contain any of the five targeted drugs. Therefore, a maximum of two samplings is required to come to a result.

I would like to follow this up by briefly explaining the rationale behind the link between appearance and selected drug test. First of all, it is important to mention that the drug testing game is a numbers game. Cocaine is the second most encountered drug, after cannabis, in Western Europe, and this is reflected in the flowchart. The probability that a sample contains cocaine, is larger than the probability it contains e.g. ketamine,

which is why the flowchart will suggest to test cocaine first. Cocaine itself is typically encountered as a white powder, although drug cartels mix it with other agents to increase profits and/or mask its presence. Taking this into account, combined with the fact that cocaine is such a prevalent drug, all powders are first tested for cocaine. Heroin, which typically appears as a brownish/beige powder, is the sole exception. Nevertheless, if a brown powder is tested for heroin and returns a negative result, the sample must still be tested with the cocaine sensor. If a non-brown powder returns a negative result for cocaine, a second test for MDMA is performed, with an optional second heroin analysis in the same pH 5 buffer.

MDMA is the only drug of the five included in the flowchart that sometimes appears in (ecstasy) tablets. It is thus straightforward that a tablet is solely tested on the presence of MDMA. As a reminder, if the result is negative, this will only mean that the tablet does not contain any of the five target drugs of the flowchart, not that it does not contain any illicit substance.

Interactions with end-users provided much needed insights in the potential drugs present in crystals. Three of our target drugs qualify when a crystal is encountered: methamphetamine, ketamine and MDMA. Since methamphetamine is the least prevalent of the three in Western Europe, it is always tested second. A division is made based on color for the first test: transparent or white crystals are tested for ketamine, while all other crystals are tested for MDMA.

Finally, for liquids, MDMA is most likely to be present out of the five target drugs. Therefore, first a MDMA test is performed, followed by a cocaine test if the first result is negative. Cocaine is tested second because customs has encountered cases in the past where cocaine was dissolved in liquids such as wine or orange juice to mask its identity.

Validation

The flowchart described in the previous sections is an updated version of an initial flowchart that was developed in October 2022 (Figure 7.4). This flowchart will not be discussed in-depth, since it doesn't differ that much from the final flowchart and the emphasis should remain on the latter. However, the old flowchart has relevance to it because a two-day validation study was performed at NICC facilities to assess its performance. The results of this validation study will be discussed in short here, as they demonstrate the value of a flowchart based on visual appearance.

In total, 118 samples were analyzed with the flowchart shown in Figure 7.4: 12 cocaine, 30 MDMA, 16 ketamine, 18 heroin, 4 methamphetamine and 38 negative samples. These 38 negative samples contained both licit and illicit compounds. A sample is a true negative (TN) if the final outcome is that the sample does not contain any of the five target drugs. The sample might thus contain a drug (e.g. 2C-B) outside of the target group, and correctly be identified as a TN even though it does contain an illicit substance. A sample is a true positive (TP) if the correct target drug is identified in the sample. FNs and FPs are the opposites of TNs and TPs, respectively. Three different metrics (accuracy, sensitivity and selectivity) are employed to assess the performance of the flowchart.

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$

$$Sensitivity\ (True\ positive\ rate - TPR) = \frac{TP}{TP + FN}$$

$$Selectivity\ (True\ negative\ rate - TNR) = \frac{TN}{TN + FP}$$

Overall, the flowchart reaches an accuracy of 91%, which is proof that a flowchart approach based on visual appearance is a valid approach for multidrug sensing. Remarkably, there is some difference between sensitivity (TPR) and selectivity (TNR), 94% compared to 84%, respectively. The TNR of 84% takes into account FPs, which are not only caused by negative samples, but also by falsely identified drugs of the target set (e.g. cocaine identified as ketamine). Furthermore, the TNR is determined by the TNs and FPs, of which there are less representatives in the set (38), compared to the TPs and FNs that are used for the TPR (80). Therefore, the number of FPs has a higher impact on the TNR, than the number of FNs has on the TPR. Overall, more samples should be added to the validation study to determine if this difference in sensitivity and specificity is a recurring characteristic of the method. Finally, a total of 156 tests was required to come to a conclusive result for the 118 samples, which means that on average 1.32 tests are necessary.

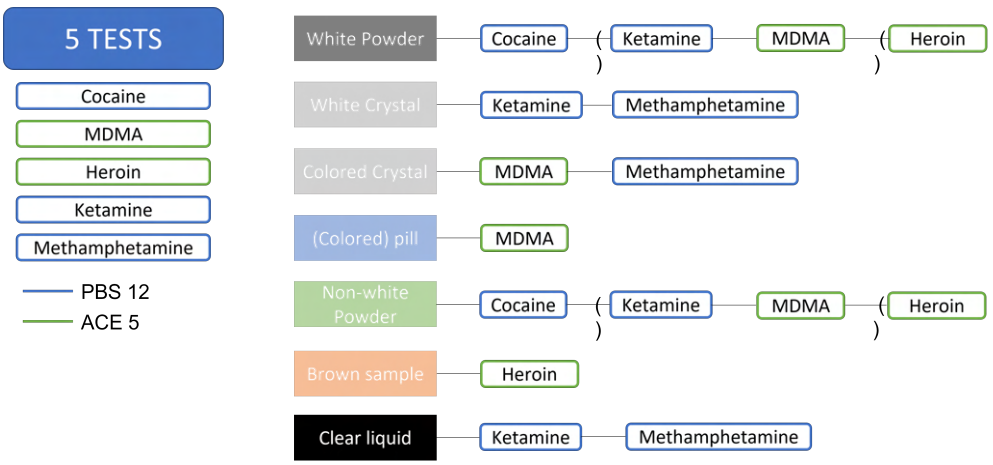


Figure 7.4: A first attempt for a visual appearance flowchart was developed in October 2022.

The same metrics could also be calculated for the individual single drug sensors. The total amount of tests is the highest for cocaine and MDMA, which is no surprise since they are the common 'first' tests in the flowchart. The cocaine and heroin sensor perform slightly worse than the other ones, however still very acceptable. The MDMA and ketamine sensors deliver excellent results. The methamphetamine sensor does too, although five analyses is too little to make meaningful conclusions. Overall, 11 false identifications were observed, divided in six false positives and five false negatives. Two false negatives were caused by a very high levamisole content, and low cocaine content, which troubled identification. Four times, an electroactive agent caused a false positive. One of these four cases is the DMT case, described in single drug sensor section, for which a solution was found. In one instance, the cocaine + paracetamol exception, described in the same

section, was not triggered. This issue will be solved by slightly extending the paracetamol interval. The other five false identifications are odd one out events for which no clear solutions can be provided. To conclude, this validation study demonstrates the added value of a visual appearance flowchart approach. Some tweaks to this validated flowchart were made based on input from end-users to come to the final flowchart described in the previous section. Finally, the performance of the individual single sensors shows that the software parameters and detection intervals are well optimized.

Table 7.2: Overview of the validation study performed for the flowchart. The table summarizes the amount of tests performed by each single sensor, together with several performance metrics such as accuracy, sensitivity, specificity and number of tests performed.

	Flowchart	Cocaine	Ketamine	MDMA	Heroin	Methamphetamine
True positives	75	9	16	29	17	4
True negatives	32	37	12	11	9	1
False positives	6	3	1	0	2	0
False negatives	5	3	0	1	1	0
Total	118	52	29	41	29	5
Accuracy (%)	91	88	97	98	90	100
Sensitivity (%)	94	75	100	97	94	100
Specificity (%)	84	93	92	100	82	100
Amount of tests	156					
Required tests	1.32					

7.2.3 Temperature sensor

As mentioned many times in previous chapters, one of the major assets of the electrochemical illicit drug sensing technology is its portability. This allows the technology to make an impact on-site, something which is not possible for other technologies such as LC-MS or GC-FID. This opportunity has several implications, mainly related to the conditions on-site, which are subject to environmental changes (temperature, humidity,...). A lab-based technology will typically work in room temperature and humidity conditions, while an on-site technology needs to function at low temperatures in winter, and high temperatures in summer. It is well-known in voltammetry that temperature has an influence on the EP of an analyte. Since the technology uses these profiles for its identifications, the effect of temperature has to be investigated, and accounted for, to make sure that the technology has the same performance inside and outside of the lab.

Extensive work was conducted in the A-Sense Lab to investigate the influence of temperature on the EPs of illicit drugs and their cutting agents [208, 209]. More specifically, the influence of temperature on the peak potential of the analyte was of interest, since these peak potentials are used for identification by the method described in Chapter 3. Even though for most compounds peak potentials shift to lower values with increasing temperatures, no common trend can be observed. Therefore, a methodology was developed by colleagues of mine, dr. Mats de Jong in particular, which facilitates a consistent approach to deal with the temperature effect. This approach is described in patent

EP4063867A1. Going into detail on this approach would lead us too far away from the topic of this chapter. However, I will give a description on the practical implementation of this approach in the detection software, since that was the part I was involved with.

The identification software described in Chapter 3 uses detection intervals to identify the target analytes. It was mentioned many times that these intervals are necessary to deal with variations in concentration and temperature. The size of these detection intervals has a major influence on the accuracy of the sensor: a very small detection interval will result in false negatives, while a very large interval will result in false positives. The size of the detection intervals is usually chosen in such a way that it is exactly the right size to deal with the variations in temperature and concentration. As a result, if changes in temperature (or concentration) have a large influence on the peak potential of the target analyte, the detection window needs to have a substantial size, which in turn is reflected in the accuracy of the method (higher risk of false positives). The methodology developed by Mats deals with this problem by splitting the initial unique detection window, into several detection windows that are linked to specific temperatures. I will explain this with an example, in honor of Mats, I will use the cocaine sensor as example. Initially, the detection window of cocaine in PBS12 buffer ranged from 0.788 V to 0.938 V, which allowed the detection of cocaine in temperatures ranging from 7°C to 40 °C. After applying the methodology of Mats, this one detection interval (0.788 V - 0.938 V) was split into three separate intervals: 0.788 V - 0.898 V for temperatures above 25 °C, 0.808 V - 0.928 V for temperatures between 12.5 °C and 25 °C, and 0.848 V - 0.938 V for temperatures below 12.5 °C. How these specific intervals are determined, is described in patent EP4063867A1. The final result are smaller detection windows which will decrease the risk of false positives, without increasing the risk of false negatives, thus improving the accuracy of the methodology.

Now how does this work in practice? The latest generation of portable potentiostats have a built-in temperature sensor. The current temperature can thus be extracted from the hardware, and be used as a variable in the detection software. After this, only an extra layer of 'if', 'else if' and 'else' operators needs to be defined in the software which, depending on the measured temperature, selects the desired detection interval. Again, using the cocaine sensor as an example, if e.g. on a hot day the temperature measured by the potentiostat is 26 °C, then 0.788 V - 0.898 V is selected as the detection window for cocaine. This section shows very well that the methodology can be improved by understanding the environment in which it has to operate.

7.2.4 App development

The detection software described in Chapter 3 and the previous sections is the engine of the electrochemical illicit drug sensing technology. And just as an engine is packaged in a beautiful car, the detection software deserves to be packaged in a beautiful solution: a mobile application. During 2021, I was involved in a project that oversaw the development of a tailored mobile application for the electrochemical single sensors (cocaine, MDMA, ketamine). Myself, dr. Mats de Jong and dr. Devin Daems conceptualized the mobile application together with PalmSens BV, an electrochemical software and hardware provider. The application itself was built by PalmSens BV in C#/.NET, after the project was done, I received the code of the application. During the last few years, I have

made several changes to the application, most importantly, the integration of the heroin and methamphetamine single sensors.

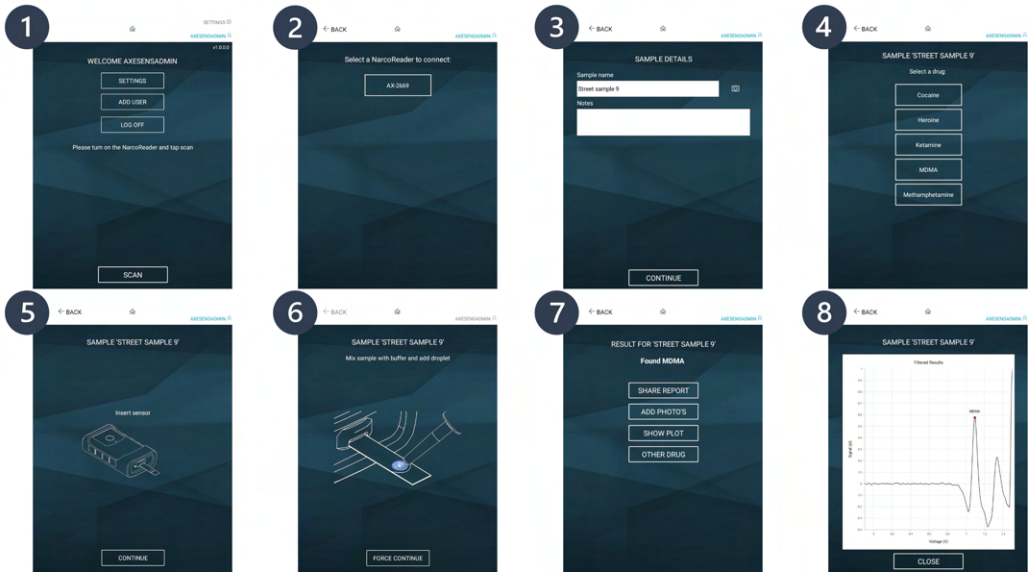


Figure 7.5: Impressions of the mobile application, 1) Introduction screen, 'SCAN' will guide to screen 2; 2) Scanning for potentiostats via Bluetooth; 3) Once connected, the sample can be given a name, accompanied with some pictures and notes; 4) Select the drug that needs to be tested; 5) Animation shows end-user how to insert electrode in potentiostat; 6) Once the electrode is inserted, an animation shows how to apply a droplet to the SPE. An automatic droplet detection will subsequently initiate the measurement once a droplet has been applied; 7) Screen indicating presence or absence of tested drug. The user can also ask to share a report, add some photos or show the plot. An additional measurement can be started if desired.; 8) 'SHOW PLOT' in screen 7 redirects to this screen.

I want to highlight several aspects of the app here, since they will play an important role in making the product attractive for end-users. The fundamental goal of the application is to make it as easy as possible for an end-user to perform a measurement. The application is not unnecessarily complex, and has an intuitive flow. Figure 7.5 represents a walk-through of a measurement, and shows the different screens that are encountered upon performing a measurement. After logging in with a personal login, a home screen (Screen 1) is encountered with three (or four for the admin) options: 1) a settings button that will lead to a screen to select e.g. language, 2) a button to add a user, only visible for the admin, 3) the option to log off, and 4) a scan button. The latter will start the flow for a measurement, upon pressing the scan button, the application will start looking for a potentionstat via bluetooth. A list will subsequently be shown with potentionstats that are ready for connecting (Screen 2). After selecting the desired potentionstat, the application will proceed to a screen that allows a user to enter sample details such as the sample name, some notes and the option to take a picture of the sample (Screen 3). Later on, this information will be used to generate a secure report of the sample together with the result of the test. This report is an important asset for end-users, since it allows them to efficiently perform measurements on-site, and come back to the results at a later

point in time. Once the sample details are entered, the app will ask to select the drug sensor that is required for the test (Screen 4). Five tests are available: cocaine, MDMA, heroin, ketamine and methamphetamine. Once the correct test is entered, an animation screen is shown that demonstrates how to correctly enter the SPE in the potentiostat (Screen 5). This facilitates the use of the technology by non-expert end-users. After pressing continue, a new animation screen is shown that demonstrates how to apply the sample droplet to the SPE (Screen 6). An automatic droplet detection is included that, once the droplet is detected, automatically proceeds to the measurement screen. Again, the animation and the droplet detection make sure that the technology is attractive for non-trained end-users. The measurement screen shows a circle that fills to 100% to show the progress of the measurement, and indicates the temperature measured by the potentiostat. Once the measurement is done, the app automatically proceeds to the final screen (Screen 7). The result screen simply shows if the tested drug was or was not present in the analyzed sample. Furthermore, the user has the option to share the report, add some photos, have a look at the electrochemical curve (Screen 8) or proceed to a new measurement.

7.3 Making an impact with practicalities

Bringing a technology to the market means, among other things, that there comes a time when the technology leaves the safe space, i.e. the lab, where it was originally developed. In the safe space, there is a very good control over all aspects (operation, storage,...) of the technology, which makes that the technology works and behaves as intended. However, the objective is that the technology can make an impact outside of this safe space, which comes with certain challenges. Previous chapters already described how the electrochemical technology was made accessible for non-expert end-users by e.g. implementing data analysis software. However, during my PhD, I have experienced and learned that several other, often more practical things, need to be considered as well to ensure that the technology is viable outside its safe space.

7.3.1 Sampling method

Before diving into this section, I would like to inform the reader that accompanying video material for the upcoming section can be found via the QR code in Figure 4.5. Bearing in mind the risk of repeating myself: the identification software uses detection intervals to accommodate variations in peak potential due to concentration and/or temperature. In one of the previous sections, I described how a temperature sensor coupled to an extension of the identification software mitigates variations in peak potential of the target analyte due to temperature variations. The question arises, is there a similar solution to deal with variations in peak potential due to concentration variations? The answer is yes, although only to a certain extent, since certain boundary conditions need to be respected. Most importantly, since the sensor needs to be used on-site, it is not possible to accurately weigh the amount of sample that is dissolved in the buffer solution. Furthermore, it may be clear that purely by visual interpretation, it is not possible to determine which compound is present in the sample (this is why we need the sensor), nor is it possible to determine the purity. This implies that even if one could measure the exact amount

of sample that is dissolved in the buffer solution, the concentration of the target analyte cannot be guaranteed due to variations in purity. Luckily, it is possible to find values on purity ranges for each drug in reports from the UNODC and EMCDDA, e.g. in 2021, the purity of cocaine ranged from 31% to 80%[2].

Keeping these aforementioned boundary conditions in mind, it is possible to develop a sampling method that ensures a concentration that can be handled by the sensor, i.e. avoid under- and oversampling. The concentration is determined by dividing the weight of the sample by the volume of buffer solution in which it is dissolved. As mentioned previously, the amount of dissolved sample is variable, however, the amount of buffer can be chosen. Two challenges arise: first of all, how can we make sure that the amount of dissolved sample is as constant as possible? And secondly, what is the desired concentration range, and subsequently, what is the sample weight that we aim for, keeping in mind that we can choose the buffer volume?

A solution for the first problem was found in the disposable 140 mm smartspatula's, provided by companies such as Merck and Novolab. These spatula's are designed in such a way that filling the tip results in a consistent sampling amount. Furthermore, it is easy to explain to end-users what the required amount of sample is: 'Fill the tip of the spatula once.'. During demonstrations and trainings, I have noticed that end-users tend to take too much sample to be sure that 'enough' sample is dissolved. This room for interpretation is taken away with the smartspatula's, as it provides a very clear instruction on the amount of sample that needs to be dissolved. Furthermore, a study was conducted at A-Sense Lab to assess the average and standard deviation of the weight of the sampled amount when using these smartspatula's. Two researchers sampled a sugar sample ten times in a row, each time taking a new spatula (Table 7.3). With averages of 4.59 mg and 5.36 mg, and standard deviations of 1.80 mg and 1.49 mg, respectively, it can be concluded that the consistency in sampling weight is adequate, however not perfect. Furthermore, samples can have different mass densities depending on their appearance (e.g. powder versus crystal), which will inevitably lead to variations in dissolved weight. Nevertheless, this solution is the best compromise between consistent sampling and ease-of-use for end-users.

Note that currently a collaboration project between the A-Sense Lab and the NIST institute is running to further validate the voltammetric illicit drug sensing technology. The sampling methodology is one aspect that is further validated, this time by involving people with various levels of expertise in sampling handling.

Table 7.3: Two researchers sampled a sugar powder ten times to assess the average and standard deviation of the sampling method with the smartspatula's.

	S1 (mg)	S2 (mg)	S3 (mg)	S4 (mg)	S5 (mg)	S6 (mg)	S7 (mg)	S8 (mg)	S9 (mg)	S10 (mg)	**Average (mg)**	**StDev (mg)**
Person 1	5.40	3.23	3.31	9.07	3.95	5.30	4.14	4.37	4.50	2.66	4.59	1.80
Person 2	3.54	8.4	4.45	4.26	4.86	7.36	5.39	5.11	4.41	5.79	5.36	1.49

Now that a target value for the sample weight is established, the required buffer volume can be determined. Two things are of importance: the range of the sensor, and the purity statistics of the target analyte. The working range has as a lower limit, in theory, the LOD of the sensor. The upper limit has no distinct value, however, a good target value is a concentration where oversampling starts to occur. There is no exact definition for oversampling in this context, however, it can be considered oversampling when the

diagnostic peak becomes very broad and the peak potential rapidly drifts away from the typical peak potential. I will use the cocaine single sensor as an example, which has a LOD of 2 μM, and an oversampling limit of approximately 5000 μM[102]. The purity of cocaine in 2021 ranged from 31% to 80%[2]. Therefore, taking these purity values in account, and using the lowest and highest sampling amounts of the sampling experiment, i.e. 2.66 mg and 9.07 mg, the theoretical minimum and maximum concentration amount to 404 μM and 3559 μM, respectively, if a buffer volume of 6 mL is used (Table 7.4). These theoretical lower and higher concentration limits fall well within the sensor range (2 μM - 5000 μM). Similar calculations can be performed for each of the single sensors.

Table 7.4: A lower and higher concentration are calculated using a buffer volume of 6 mL and sampling amounts based on the sampling experiment and purity.

	sampling amount (mg)	M(Cocaine HCl) (g/mol)	n (mol)	Purity	n (mol) after purity correction	sampling volume (L)	final concentration (µM)	Range (µM)
Lower limit	2.66	339.8	7.82E-06	31%	2.43E-06	0.006	404	2
Upper limit	9.07	339.8	2.67E-05	80%	2.14E-05	0.006	3558	5000

Summarized, a good concentration window can be provided by using disposable smartspatula's and a fixed buffer volume (of e.g. 6 mL for the cocaine sensor). The buffer solution is stored in a closed vial. Upon use, the cap of the vial is broken, after which the sample, sampled with the smartspatula, is mixed with the buffer in the opened vial. After mixing, a transparent cap is placed on top of the vial, which facilitates the application of a droplet of the resulting solution to a SPE.

7.3.2 Buffer stability

I was involved in several collaborations where the electrochemical illicit drug testing technology was used by end-users (Sciensano, Customs at Port of Antwerp, Dutch customs, Police Amsterdam,...) without the presence of an expert. Typically, I would deliver the technology in a package to the end-users, accompanied by a training session of the end-user(s) that would be responsible for operating the technology. This technology package consists of the potentiostat, vials with the buffer solution, SPEs, a tablet and spatula's. It goes without saying that the technology should perform the same at the facilities of the end-users, as it did in the facilities of the A-Sense Lab where it was developed. An important aspect here is the stability of the buffer solution and the SPEs. At the A-Sense Lab, buffer solutions are regularly made, and the pH is checked and adjusted if it has slightly drifted away from the target value. This option, however, is not available at the facilities of the end-user. Therefore, I conducted a buffer stability test to investigate the behavior of the ACE5 buffer over a prolonged time.

I prepared a fresh ACE5 buffer solution (20 mM ionic strength) with 100 mM KCl on the 17th of January 2021 with the goal to assess the stability of the buffer weekly over a period of at least three months (Table 7.5). Two separate situations were compared, one where the buffer is stored in a fridge, and one where the buffer is stored at room temperature. Four metrics were used to assess the stability of the buffer, of which the most important one is the pH. Furthermore, the visual appearance was monitored as well, since ACE buffers are known to form mold. Finally, a stable analyte was selected, lidocaine (500 µM), to assess variations in its peak potential or peak current. The average and standard deviation of three measurements were reported.

Table 7.5: A shelf life study was performed for the ACE5 buffer. Two conditions, storage in fridge and storage at room temperature, were compared using pH, visual appearance, peak potential and peak current as metrics.

	Fridge						Room Temperature				
Date	pH	Mold	$\overline{x}$ (Ep)	σ (Ep)	$\overline{x}$ (Ip)	σ (Ip)	pH	Mold	$\overline{x}$ (Ep)	σ (Ep)	$\overline{x}$ (Ip)	σ (Ip)
17/01/2021	4.99	clear	0.994	0.004	7.57	0.14	4.99	clear	0.987	0.002	7.37	0.16
24/01/2021	4.92	clear	1.006	0.002	7.87	0.42	4.95	clear	1.009	0.000	7.36	0.06
31/01/2021	4.98	clear	1.007	0.005	7.64	0.36	4.95	clear	1.009	0.004	7.54	0.20
7/02/2021												
14/02/2021												
21/02/2021	5.14	clear, 1 vial with minor mold	1.009	0.004	8.30	0.39	5.02	clear	1.009	0.004	6.49	1.24
28/02/2021	5.15	clear, 1 vial with minor mold	1.009	0.004	7.32	0.27	4.98	clear	1.002	0.002	6.45	0.31
7/03/2021	4.96	clear, 2 vials with minor mold	1.016	0.006	7.37	0.31	4.96	clear	1.007	0.006	8.06	0.52
14/03/2021	5.08	clear, 2 vials with minor mold	1.007	0.002	7.94	0.09	4.94	clear	1.014	0.000	8.11	0.03
21/03/2021	5.10	1 clear, 1 major mold, 2 minor mold					4.98	clear	1.001	0.002	7.93	0.28
28/03/2021	5.09	1 clear, 1 major mold, 2 minor mold	1.001	0.002	8.53	0.38	5.02	clear	0.994	0.004	9.36	0.26
4/04/2021												
11/04/2021	5.12	2/4 minor, 2/4 major mold	1.001	0.002	8.45	0.10	5.04	clear	1.002	0.002	8.47	0.15
18/04/2021												
25/04/2021												
2/05/2021	5.15	2/4 minor, 2/4 major mold	0.997	0.002	8.61	0.05	5.02	2 minor mold, 2 clear	1.001	0.005	8.63	0.17
9/05/2021	5.12	2/4 minor, 2/4 major mold	0.999	0.000	7.98	0.16	5.03	2 minor mold, 2 clear	0.992	0.005	8.51	0.24
16/05/2021	5.19	2/4 minor, 2/4 major mold	0.989	0.004	8.83	0.29	5.04	2 minor mold, 2 clear	0.989	0.004	8.78	0.14
23/05/2021												
30/05/2021	5.19	2/4 minor, 2/4 major mold	1.006	0.002	7.72	0.08	5.04	3 minor mold, 1 clear	1.002	0.002	8.00	0.17
6/06/2021												
13/06/2021	5.19	2/4 minor, 2/4 major mold	0.989	0.000	8.00	0.06	4.99	3 minor mold, 1 clear	0.979	0.000	8.49	0.12
20/06/2021	5.23	2/4 minor, 2/4 major mold	0.990	0.006	7.87	0.58	5.02	3 minor mold, 1 clear	0.980	0.002	8.61	0.04
27/06/2021	5.04	2/4 minor, 2/4 major mold	0.986	0.002	8.46	0.02	4.98	3 minor mold, 1 clear	0.987	0.002	8.88	0.22

Interestingly, the pH does not drift to higher or lower values over time, but rather swings around the starting 4.99 value. Furthermore, it appears that this swinging is more stable for the buffer solution stored outside of the fridge at room temperature. General consensus is that storing a buffer solution at lower temperature should stabilize the buffer solution, so this result is somewhat counter-intuitive. A possible explanation is that the buffer solution stored in the fridge undergoes more temperature fluctuations, which in turn might be reflected in the larger pH fluctuations. Nevertheless, both buffer solutions are stable over the three months, and remain stable in the weeks and months thereafter. Remarkably, after five months, the buffer stored at room temperature has a pH equal to the starting value.

The buffer solution was divided over four vials to monitor the formation of mold. As expected, some mold formed over time, starting of in minor amounts and increasing over time. The behavior is not the same for each vial, but eventually, after five months, mold was formed in all but one vial that was stored at room temperature. Again, the buffer solution stored at room temperature appears to be more stable, with delayed mold formation compared to the buffer stored in the fridge.

The EPs of lidocaine recorded over time show no signs of the mold, meaning that the latter is not composed by electroactive parts. Mold formation as such is therefore not a limitation for the electrochemical illicit drug testing methodologies that employ the ACE5 buffer, however, one should be cautious when using this buffer for commercial purposes. Mold is usually associated with something that has gone bad, even though this is not the case for this specific application. Non-transparent buffer storage vials might be a good solution. Furthermore, the EPs of lidocaine remain consistent over time, again proving that the buffer is stable over a prolonged period. The peak potential is the most important factor for qualitative identification by the electrochemical technology, and all peak potentials fall within a 30 mV and 35 mV range for the fridge and room temperature buffer solutions, respectively. The shifts in peak potential and peak currents are not correlated with shifts in pH, and are most likely related to variations in the electrodes.

It can be concluded that the ACE5 buffer solution can be stored over three months, and even up to five months, without posing any burdens on the novel electrochemical technology. It appears that it is beneficial to store the buffer solution at room temperature. Some mold will inevitably be formed over time, which is an aesthetic concern, but not a scientific one, since it does not influence the EPs recorded with the buffer solution.

7.3.3 Intelligent product design

The electrochemical illicit drug sensing technology is intended to be used by end-users that are not familiar with electrochemistry, or even science. This means that they are not accustomed with the actions required to successfully perform a measurement. However, by integrating some smart design practices in the illicit drug sensor, several potential errors can be avoided.

First of all, the screen printed electrodes use a three-electrode setup. After sampling the product, it is important that a droplet of the resulting solution is applied to the SPE and covers all three electrodes. To mitigate the risk that not all three electrodes are covered, a circle in a different color is drawn around the three electrodes. A user intuitively wants to fill the circle, which in turn will ensure that all three electrodes are covered. A second potential risk is a bad connection between the SPE and the potentiostat. Therefore, a small light is integrated in the potentiostat, which turns to green when a proper connection is established between the SPE and the potentiostat. Finally, an automatic droplet detection is integrated in the application as well.

7.3.4 Quality management systems

Previously, it was described how the technology was delivered as a package to end-users in several collaborations. This package consists of a potentiostat, a tablet, spatula's, SPEs and the buffer solution. Over time, new potentiostats and tablets are ordered, novel batches of spatula's and SPEs are required if the previous ones are finished, and fresh buffer is prepared. This means that every time the risk exists that the newly added part is faulty and might compromise the proper functioning of the technology. Therefore, a quality management system (QMS) needs to be put in place to ensure the quality of the technology in every delivery.

In consultation with Prof. Dr. Karolien De Wael and Dr. Devin Daems, it was decided that measuring a small set of standard samples is the best way to guarantee the quality for a delivery. Taking the cocaine sensor as an example, it was decided to conduct the following measurements: 3x blank PBS12 buffer, 1x blank ACE5 buffer, 3x Cocaine (500 µM) in PBS12, 1x Cocaine + Caffeine (500 µM + 500 µM) in PBS12, 1x Cocaine + Lidocaine (500 µM + 500 µM) in PBS12, 1x Cocaine + Paracetamol (500 µM + 500 µM) in PBS12 and 1x Cocaine + Paracetamol (500 µM + 500 µM) in ACE5. The blank measurements should return a negative result, whereas the cocaine measurements should return a positive result, even in the presence of cutting agents. The cocaine/paracetamol mixture should trigger the exception described in the single drug sensor section, requiring a second measurement in ACE5 buffer. Important is that the exact potentiostat, tablet, SPEs, spatula's and buffer are used that are intended for the technology delivery.

Performing more measurements, e.g. assessing variations in concentration, would be beneficial. However, one should be aware that a QMS costs money. A trade-off exists between the potential financial costs if the quality was not ensured, and the financial cost of the QMS itself. In my opinion, this trade-off is scalable, meaning that the current QMS is sufficient for the current size of deliveries (50-200 consumables), but a more extensive QMS should be put in place if the size of the deliveries increases. As a side note, one could argue that the starting phase of a novel technology is crucial for building a reputation, and thus justifies the increased cost of an extensive QMS.

7.4 Make an impact by leaving a legacy

7.4.1 Guideline to perform a technology demonstration

Finally, a crucial aspect of making an impact with a novel technology, is making sure that people learn about the existence of said technology. There are several ways of doing this, with probably the most effective one being demonstrations. During my PhD, I have performed many demonstrations for various audiences, ranging from high school students, over customs officers, to politicians. Each type of audience has a different background, and requires a different approach. Furthermore, the incentive of the demonstration can differ as well, sometimes it is just providing information, while in a different case the objective might be to convince the audience of the added value of the technology to obtain a grant or investment.

In my opinion, the most optimal structure for a demonstration, consists of three parts: i) a short powerpoint presentation or verbal introduction of the technology, ii) followed by a prerecorded video of a measurement and iii) finished off with a live demonstration of the technology.

The introduction is crucial to situate the technology. The way to approach this is highly dependent on a) the knowledge level of the audience, and b) the purpose of the demonstration. In August 2022, I gave a demonstration of the technology for a venture builder from the Netherlands. The audience in this case was highly educated, and interested in potentially commercializing the technology. Therefore, the introduction part went into great scientific detail since the education level of the audience allowed them to understand the technology behind the electrochemical illicit drug sensors. Furthermore, this allowed us to prove that our technology is scientifically sound. Additionally, extra attention was placed on the added value of our technology over the current existing technologies. The main goal of the demonstration was to make the technology attractive for commercialization purposes, which is achieved by highlighting the strong points of the technology in great detail.

Six months later, in February 2023, I demonstrated the technology to a European delegation that included, among others, Ms Annelies Verlinden, Belgian Minister for Interior, Institutional Reforms and Democratic Renewal; Ms Ylva Johansson, European Commissioner for Home Affairs, and Mr Alexis Goosdeel, Director of the EMCDDA — European Monitoring Center for Drugs and Drug Addiction. The demonstration of the technology was part of a session organized by Belgian customs in the port of Antwerp. They described how drug cartels employ advanced smuggling tactics to import cocaine, and how our technology helped them in detecting cocaine in masked samples. Therefore, I placed more emphasis on the added value of our technology for the fight against the drug cartels. The delegation members had a very busy schedule that (and probably every) day, so it was key to grasp their attention and efficiently pass on the key message. Therefore, I did not discuss the scientific part of the technology. Finally, it was also a nice opportunity to show to some of the highest European authorities that a European-funded project makes a true impact.

After the verbal introduction, I like to show, if possible, a prerecorded video of a measurement. There are two main reasons for this: 1) it gives me more time to narrate the different steps, and 2) it ensures that the first encounter with the technology is a positive one. Even though the technology has rarely failed me, there is always a risk that something doesn't go as planned. Some audiences, especially law enforcement, tend to confuse a demonstration with a (blind) test of the technology. The technology is perfectly capable of withstanding such blind tests, however it obviously entails additional risk, and it is thus nice to have already shown a first positive measurement.

The live demonstration is the final, and most crucial part, of a demonstration. It shows the technology in full action. Nothing is more efficient in capturing an audience for the technology than a successful live demonstration. The choice of analyte is the most important one to make. A blind drug testing makes the most impact, because the stakes are high. As said previously, the technology is able to handle this heat. A blind testing is not always possible, in that case, a licit compound makes the least impact, but sometimes there is no other option if the location is not licensed to have illicit drugs at the premises. If it is possible to test an illicit compound, it needs some thinking on what

compound can make the most impact. As an example, Belgian customs in the port of Antwerp deal almost solely with import of cocaine. Therefore, it makes the most sense to demonstrate the cocaine sensor to them with a cocaine sample, preferably a sample that causes trouble for their current technologies, such as a charcoal-cocaine mixture. Harm reduction organizations are more interested in ecstasy, so for them it makes more sense to demonstrate the MDMA sensor with an ecstasy sample. Again, preferably with a targeted, difficult sample, such as a dark-colored pill.

Finally, the live demonstration itself is quite straight-forward. I found it is best to start by shortly explaining the key parts of the technology (e.g. potentiostat, SPE,...), making the audience familiar with the technology. This is followed by going through the motions of a full measurement, taking the time to explain the different steps. It ends when the correct identification is shown on the display of the measuring device.

7.4.2 Guideline for future work

My main legacy in the field of electrochemical illicit drug sensors is that I have made the technology more accessible to end-users. The future will tell if this legacy will be a lasting one. I will use the next section and chapter to have a look at this future of the technology. In my opinion, this can be divided into two parts: the scientific future, and the commercial future. The first part will be described in this section, whereas the second part will be discussed in the next chapter. It is no coincidence that more pages are dedicated to the commercial future compared to the scientific future. In my opinion, the technology is ready to be commercialized, and society will benefit more from efforts put towards commercialization of the technology, compared to efforts put towards further scientific improvement of the technology. This does not mean that further scientific research has no point, in the contrary, I think many interesting developments can still be made to improve the technology. However, it seems opportune that this additional research supports the valorization of the technology. A great deal of time, effort and taxpayer money has already been invested in the technology, and at some point the fruits of these efforts must be reaped in order to pay back these investments to society.

7.4.2.1 Single sensor - future developments

Currently, a repertoire of several single sensors have been developed in the research group, covering the most important drugs found in Western-Europe: cocaine, MDMA, heroin, ketamine, methamphetamine and amphetamine. The technology readiness level (TRL) is already very high for the single sensors, i.e. it is estimated that the TRL is in between seven and eight. A prototype can successfully be demonstrated in an operational environment, and several smaller projects have shown that the technology works in an operational environment. Gains are still possible, but will probably be marginal compared to the effort that is required to achieve those gains.

A good approach to achieve these gains, and thus improve the sensors, is to keep measuring new samples with the voltammetric sensors. Each set of samples that is measured, can first serve as a validation set to assess the performance of the sensors, and later on be used to improve the sensors with the insights from the validation study (e.g. opti-

mize sensor parameters, include novel exceptions,...). Repeating this process with new sample sets, results in a continuous improvement cycle that will eventually benefit the performance of the sensors.

This approach sounds very good in theory, however it is necessary to consider the selection of the sample sets that are used (for validation and optimization) to truly make this approach work. The identification software, based on the peak recognition approach of Chapter 3, strongly relies on domain-specific knowledge, so overfitting on the sample set might seem like less of a threat than for e.g. an artificial neural network approach that is more prone to a black box approach. Nevertheless, a similar risk exists in which a bias is created towards the data that is used to further optimize the identification software. The threat here is that this data, i.e. the sample sets used for further optimization, is not representative for the samples for which the sensor will be employed. Therefore, it is of utmost importance to involve end-users in the choice of these sample sets.

Again, this sounds very good in theory. However, in practice, each end-user encounters different samples, has different procedures, and envisions a different use or purpose for the voltammetric sensors. Therefore, many different interpretations exist for the samples that should be used to validate and further optimize the sensors. It is wise to consult with different end-users, and find a good compromise in the samples used to further optimize the sensors. Here, the aforementioned observation is important that implementing exceptions in the software also carries risks, and it is therefore very important to consider whether it is relevant to take this risk. That being said, there are certainly some areas where progress is up for grabs. The samples that were currently used for validation and optimization, have always had an over-representation of 'positive' samples, i.e. samples that contain the target drug. Measuring more 'negative' samples would learn more about the selectivity of the sensors, and facilitate their further optimization.

Another important observation can be extracted from the previous paragraph: there is no such thing as a universal validation set that is meaningful for everyone. We tend to place very high value on performance metrics, such as accuracy or sensitivity, of a sensor. These metrics are calculated using an independent validation set. What that validation set should look like is however nowhere firmly noted. The main guideline is that it should consist of confiscated samples, which are supposed to reflect the samples for which the sensor will ultimately be used. However, experience has shown that this selection varies greatly depending on the end-user to whom you ask. It is normal that this selection varies since they all have their own procedures, and the drug market can vary greatly depending on location. As a result, there is a very real chance that the accuracy of the sensor will vary greatly, depending on which validation set is used. For example, the amount of 2C-B samples present in a validation set will have a substantial impact on the calculated accuracy of the MDMA sensor proposed in Chapter 4. Strangely enough, the performance of different sensing techniques is sometimes compared by metrics such as accuracy, while the validation sets that were used to calculate these metrics are not comparable at all. Therefore, it is opportune to be cautious with performance metrics such as accuracy or selectivity, and as a minimum use the same validation set to calculate these metrics.

Going back now to the optimization of the single sensors, it is important to acknowledge that a good sensor will have a different meaning for each end-user. It is not necessary to overly focus on performance metrics, but preferable to investigate how the sensor can

create value for the end-user, and which samples are relevant to include in the sample sets for validation and optimization to fulfill this purpose.

Besides optimizing the current single sensors, further development can go towards developing new electrochemical illicit drug sensors. The A-Sense Lab has two major assets in this regard: the expertise to efficiently develop new electrochemical illicit drug sensor (Chapter 2) and an extensive database of voltammograms of licit and illicit compounds in various buffers. This database will facilitate a rapid development of novel electrochemical sensors, providing the developer with the opportunity to rapidly explore the feasibility of different sensing approaches. This could be of particular interest for the detection of NPS. They emerge (and disappear) rapidly, which makes it difficult for the current detection technologies to keep up. In this regard, the short development time of the electrochemical sensing technology described in this thesis could be an asset. A use case could be NPS that are known to be extremely dangerous for the health of PWUD. Over the last few years several examples come to mind such as PMA or 3-MMC. These NPS are sometimes sold as ecstasy, tricking the user into believing that the active psychoactive ingredient is MDMA. As such, a sensor that is developed in a short period of time will make a positive impact on the health of society.

7.4.2.2 Multidrug detection

The greatest advances in electrochemical drug testing technology, in my opinion, can be made in multidrug detection. Two strategies have been proposed in this thesis: a flowchart approach and an array approach. The flowchart approach was further elaborated upon in this chapter, and it was shown that incorporating the visual appearance of the analyte had a very beneficial impact on the overall performance. It is possible to incorporate further drugs into a flowchart approach, however, this does not seem opportune as it will have an unfavorable effect on the ease-of-use of the end-user. I will focus more on the array approach since I believe the biggest gains can be made by further improving and extending array approaches.

Before diving into the technical challenges, it is important to have a look first at the bigger picture. It seems straight-forward that it is better to have a test that can detect multiple drugs at once, compared to a test that can only detect one specific drug. However, since the challenge is more complex, the solution tends to be more complex and thus, also more expensive. Talking to end-users, it is clear that they are interested in electrochemical multidrug detection, if the price, analysis time and ease-of-use are not compromised. This is very important to keep in mind. Furthermore, it should be noted that electrochemical data comprises of a little amount of signals compared to e.g. Raman or infrared spectroscopy. The complexity of e.g. Raman spectra, allows a library approach where a spectrum is compared to a very large database of compounds. This is not the case for electrochemistry, even if the amount of electrochemical data retrieved from one measurement is extended via an array approach, I believe that the library of compounds that can be detected, will remain rather limited. Since this set of target compounds for electrochemical multidrug sensors will be limited compared to competing techniques, it becomes even more important to talk to end-users and listen to their problems and challenges. A good example is the festival sensor described in Chapter 6 or an ecstasy pill multidrug sensor that is currently in development at the A-Sense Lab. For the latter, we sought contact with end-users within supply reduction and harm reduction

organizations to learn more about their challenges when it comes to analyzing (ecstasy) pills. These talks helped to define the specifications for an impactful multidrug sensor targeted towards detection of illicit drugs commonly found in (ecstasy) pills.

Moving on to the technical challenges and future perspectives, I see two major areas: data processing and sampling. The idea of an array approach is to measure the same sample at multiple SPEs under different conditions, created by various electrode modifications and/or buffer solutions. As a result, multiple diversified EPs are recorded. This combination of multiple single EPs into an enriched 'superprofile' holds more electrochemical information about the analytes present in the sample, allowing improved (multidrug) identification. Advanced data processing is required to extract this electrochemical information from the superprofile and convert it into an clear-cut interpretation. An extension of the peak recognition algorithm described in Chapter 3, as illustrated in Chapter 6, will bring this to a good end. However, every time an additional electrode is added to the array, the practical implementation of the peak recognition approach will become more complex. The peak recognition approach for multidrug detection relies heavily on the exception module. Even for the festival sensor, which employs a fairly simple two SPE setup that targets four drugs, the exception module already becomes quite complex. Therefore, especially from three SPEs onward, I would suggest to seriously consider pattern recognition approaches. At that point, the electrochemical superprofile of a compound will be considerably more 'unique' compared to the EP recorded at a single SPE, which makes pattern recognition approaches attractive. Importantly, the (size of the) database of EPs that was built over the previous years works in favour of pattern recognition approaches as well.

The second major hurdle for electrochemical multidrug detection, is an efficient sampling method. As always, making an impact means, amongst other challenges, making it as convenient as possible for the end-user to use the technology. Therefore, since array approaches will most likely require multiple buffers, it will be necessary to rethink the sampling procedure. Simply extending the current sampling procedure would require a separate sampling per buffer solution, which is too impractical for use in on-site scenarios. The main challenge is thus reducing the amount of sampling steps, which ideally, could be achieved with only small tweaks to the single sensor sampling procedure. Paper-based microfluidics coupled to a single sampling step in water instead of buffer solution might be the key to success here. As such, the sample is dissolved only once in solution. My talented colleague Dr. Annemarijn Steijlen is currently working on such a paper-based microfluidics system, with promising results.

Summarized, an array approach holds great promise for electrochemical multidrug detection. First, it should be determined which combination of buffers and/or array modifications results in an electrochemical superprofile that allows discrimination of the target drugs. This is closely intertwined with the development of a data analysis algorithm, based on peak or pattern recognition, that facilitates this discrimination. Furthermore, the development of a suitable sampling method should not be lost sight of. The development of electrochemical multidrug array sensors is a very exciting challenge from a scientific point of view. Interestingly, the knowledge gathered with the development of these sensors will be of great value for electrochemical applications in other fields.

7.4.2.3 Decentralization of the forensic laboratory

The traditional two-step process in forensic process, with a first presumptive test on-site and a second-confirmatory test at a centralized forensic laboratory, creates logistical challenges, long analysis times and a backlog at the forensic laboratories[170]. This has prompted forensic laboratories to evaluate and innovate this process. A solution is found in decentralizing the forensic laboratory for illicit drug analysis, i.e. measuring a suspicious sample with multiple (orthogonal) techniques on-site. Decentralizing the forensic laboratory as such omits the transport of the samples to a central place, which greatly reduces the overall time to come to a conclusive result. Furthermore, it resolves the backlog created at the forensic laboratory, and frees up time for the forensic expert.

The idea is to integrate several on-site technologies into a single data platform that exploits the combined (orthogonal) data of several on-site detection technologies into a single detection strategy. Large, reliable databases, powerful (machine-learning) algorithms and orthogonal on-site detection technologies are crucial for the success of this approach. Notably, portable spectroscopic techniques (NIR, MIR and Raman spectroscopy), are currently the leading contenders for this purpose. These techniques are well-suited due to the richness of chemical information contained within their spectra, enabling the distinct identification of compounds. Equally significant is their ease of integration into a centralized data platform.

It is worth noting that each of these techniques is grounded in the interaction of the analyte with electromagnetic radiation. In light of this, the incorporation of electrochemical sensors offers a valuable dimension of orthogonality, as they focus on the redox properties of the analyte. Furthermore, electrochemical sensors can be seamlessly integrated into a centralized data platform. These features make electrochemical sensors highly suited to play a role in the decentralization of the forensic laboratory.

7.5 Conclusions

My efforts to make an impact with the technology run like a thread through this chapter. Since this is a broad and slightly unconventional topic for a thesis chapter, it might feel like a patchwork of unrelated sections. Nevertheless, I hope it was an interesting read, and that you agree with me that it is worthwhile to invest time and energy into these often more practical aspects. At the end of this chapter, I can conclude with confidence that the electrochemical drug sensing technology is ready for use in the real world. In the final section, I already had a look at the scientific future of the technology. In the next chapter I will also have a look at the economic future of the technology, which at this point in the development of the technology is of equal, if not of more, importance.

Chapter 8

Valorization

"You have to live spherically - in many directions. Never lose your childish enthusiasm - and things will come your way."

Federico Fellini

Abstract

The previous chapters describe the work that I have done to bring electrochemical sensors to a point that they are ready to leave the lab and make an impact in the real world. This final product has been demonstrated for many end-users, and time after time, these demonstrations were met with interest and enthusiasm. Additionally, several trial projects (with e.g. Sciensano and Port of Antwerp) have shown that the device can be used by end-users, without the presence of myself or any colleagues, with good results. Therefore, it becomes time to think about the valorization of the product. Valorization encompasses many aspects, describing and applying all of them to this product would require a thesis on its own. This is why I selected several topics that I think are most relevant (market segmentation, problem-solution fit, value proposition and commercialization strategies). In this chapter, I will introduce these topics and apply them to our product.

8.1 Why is valorization of research important?

The ultimate goal of the research described in this thesis is to bring a high-performance scientific technology to a level so that it is ripe to be commercialized. Crucial in this are both the scientific technology, which is typically thoroughly discussed in scientific research, and the end-users, who are often overlooked or only considered in a late stage of the research. This is understandable, since a lot of time and energy in scientific research is centered around fundamental research, i.e. building understanding and insight in scientific topics. Fundamental research is the foundation on which application-oriented research can flourish. However, in my experience, application-oriented research has a tendency to stay too much in the realm of research, failing to connect with end-users. Focusing now on electrochemical illicit drug research, it is remarkable how often the

probable end-user, who is usually not trained in science (let alone electrochemistry), is overlooked in scientific publications on the topic. Understandably, data interpretation software is not included in every manuscript, but not even a single mention of the path of technology towards non-expert end-user seems like too little effort for application-oriented research. Typically, a researcher works in academics due to their passion for research, and might therefore be more likely to discuss the research itself in depth, rather than any valorization aspects of the research.

Luckily, there is the space and opportunity in this thesis to discuss the valorization aspects of the technology in a separate chapter. One of the personal goals during my PhD is to make an impact with my work, and linking research to valorization plays a crucial part in this. This chapter is written based on the experience build during the PhD trajectory, including many interactions with end-users from different market segments. Furthermore, I voluntarily followed dr. Devin Daems during 2019-2021 when he attempted to launch a spin-off company with the electrochemical illicit drug sensing technology. This includes intellectual property (IP) meetings, demo's for end-users (Port of Antwerp, MARINFO), measurements on-site with customs in Antwerp, Luxembourg, Brussels and Amsterdam, meetings with a product development company and a packaging company,... I was also coached for this chapter by: 1) dr. Sara Melis, valorization manager for the Enviromics consortium, and 2) dr. Iris Vanaelst, the Venturing & Licensing Manager at the UAntwerp. And last, but not least, I was allowed to follow the Deep Dive into Business course, organized by the UAntwerp Valorization Office and offered in collaboration with the Antwerp Management School. During this course, me and a few colleagues investigated the valorization potential of the electrochemical illicit drug detection technology via the creation of a spin-off.

Valorization of research can encompass many different topics, and it is impossible to discuss them all in depth in a single thesis chapter. Therefore, I made a selection of interesting topics that should give you, the reader, a comprehensive overview on several important valorization aspects, with an emphasis on creating the right solution for the right problem. I will start with a strengths, weaknesses, opportunities and threats (SWOT) analysis of the technology. Understanding these aspects is crucial to define the markets segments that could benefit from the technology. Research by Gruber *et al.* shows that entrepreneurs who identify a "choice set" of market opportunities prior to first entry derive performance benefits by doing so[210]. Even more so, Gruber *et al.* showed that technology start-ups that considered more than one market opportunity prior to first market entry are more successful than technology start-ups who fail to do so. I will therefore take a look at different market segments that could benefit from the novel technology, looking beyond the typical law enforcement market, and zoom in on the characteristics of those market segment(s). Understanding the strengths and shortcomings of those market segments is necessary to create a good problem-solution fit with the technology. This is crucial for the commercialization of the technology, no one benefits from a solution for a non-existent problem. Once a good problem-solution fit is established, I will focus on the value proposition, i.e. how does the technology create value for the market segment (product-market fit). I will employ the value proposition canvas as designed by Osterwalder *et al.* to visualize the value proposition[211]. Finally, I will look into several potential commercialization strategies, using the model of Gans *et al.*[212], to define the most effective commercialization strategy to bring the technology to the market and make an impact.

8.2 Background, technology scope & SWOT analysis

I will first briefly discuss the background of the project and the technology scope to ensure that the chapter can stand on its own. Subsequently, I will perform a SWOT analysis to give more insight in the technology and its place in the current on-site illicit drug testing landscape. It is not my intention to perform a competitive analysis, for this I refer the reader to the thesis of my colleague Noelia Felipe Montiel. However, the SWOT analysis should facilitate the next sections that handle market segmentation, problem-solution fit, value proposition and commercialization strategy.

8.2.1 Background

The research conducted in this thesis falls within the BorderSens project, a European project that focuses on 'border detection of illicit drugs and precursors by highly accurate electrosensors'. Many partners from different backgrounds (academics, commercial hardware providers, forensic institutes, customs) are involved in the project, which results in an interesting network that I could benefit from for the research in this Chapter.

8.2.2 Technology scope

The novel technology involves electrochemical sensors for on-site illicit drug detection (Figure 2.4). In this chapter, the focus is on five so-called single drug sensors that were developed at A-Sense Lab[16, 33, 85, 102, 158]. These five sensors each target one specific drug (cocaine, MDMA, ketamine, heroin and methamphetamine). The sensors are all ready for use, no additional development is required anymore to bring them to the market. Summarized, a few milligrams of sample are dissolved in a buffer solution, after which a droplet of the resulting solution is placed on a SPE. This SPE is inserted in a light, portable potentionstat that is connected wirelessly to a smartphone or tablet. A customized app is installed on this monitoring device to guide the non-expert end-user through the different steps, and additionally, also performs the analysis of the electrochemical signal. Approximately 30 seconds after starting the measurement, the end-user will see a message on the screen that indicates if the targeted drug is present in the analyzed sample.

8.2.3 SWOT analysis of the technology

Strengths

The core strengths of the technology (random order) are:

- low cost per analysis (<5 euros),
- short analysis time (<1 min),
- portability (<1 kg),

- low detection limits (50 µM),
- suitability for non-expert end-users,
- digital read-out,
- high accuracy (>90%),
- low sample amount required (5 milligrams),
- quantification of MDMA in ecstasy samples,
- possibility to handle complex samples (colored, smuggled,...).

Other strengths, although less important, include:

- several companies supply hardware,
- potential to extend technology to other targets,
- versatility towards targeting new illicit drugs in ever changing drug market.

Strengths specifically associated with research group:

- IP-protected (4 patents).

Weaknesses

- Dependence on electrode supplier,
- new technology without established success stories on other targets,
- invasive sample handling,
- targets only a single drug (no library of multiple target compounds),
- novel cutting agents might negatively influence accuracy,

Weaknesses specifically associated with research group:

- Little to no in-house commercial experience (no sales channels, marketing,...).

Opportunities

- Take SPE production in own hands,
- illicit drug market is ever increasing and diversifying,
- exploit network built during development of technology,
- backbone of excellent research (including many scientific publications),

- create encrypted, cloud-based database approach,
- extend technology towards human sample analysis.

Threats

- Suppliers of hardware running out of business or halting production,
- competitor emerges within field of electrochemical illicit drug sensors,
- competitor emerges outside field of electrochemical illicit drug sensors,
- existing competitors improve their technology.

Overall, the technology has plenty of strengths that make it attractive for end-users, especially for on-site use. On the other hand, it may be clear that other technologies have an edge over the presented technology in some aspects, such as invasiveness or multidrug detection. Some weaknesses are inherent to the technology (e.g. invasiveness), however, there are opportunities to turn some weaknesses into strengths (e.g. by taking SPE production in own hands). There are threats present as well, which are mainly related to supply chain and competitors in the field.

8.3 Market Study

8.3.1 Market segmentation

A novel technology often creates benefits in multiple market domains, which gives an entrepreneur the option to enter one, two or more market domains at once or sequentially[213]. In this subsection, I will define the different market segments that can be targeted by the novel technology. I will do so by using the criteria defined by Osterwalder *et al.* that describe when different customer groups represent separate segments[211]. These criteria go as follows:

- their needs require and justify a distinct offer,
- they are reached through different distribution channels,
- they require different types of relationships,
- they have substantially different profitabilities,
- they are willing to pay for different aspects of the offer.

Keeping these criteria in mind, two different market segments can be distinguished: organizations related to supply reduction of illicit drugs, and organizations related to harm reduction of illicit drugs. The first ones typically fall within the realm of government, and include law enforcement, customs and forensics. Although they are all charged with different tasks, the main purpose of the technology for all of them is to determine

whether or not a drug is present in a suspicious sample. This is why, especially with the Osterwalder criteria in mind, they are considered to be in the same market segments. On the other hand, harm reduction-related organizations do form a separate customer group. Their use of the technology is different, e.g. UAntwerp collaborated on a harm reduction project with Sciensano where the single sensor technology was employed to monitor how heavily heroin street samples are cut. Furthermore, the other Osterwalder criteria are met as well. Harm reduction organizations are not necessarily related to government which requires a different distribution channel. Additionally, their vision on illicit drug policy is intrinsically different from supply reduction, which clearly requires a different type of relationship. All this combined justifies their definition as a separate market segment.

8.3.1.1 Supply reduction organizations

The actions of organizations that enforce supply reduction policy are targeted towards identification and confiscation of illicit drugs. This can be situated at the production site, during transport, or when in possession by dealers or consumers. Customs in this context are specifically tasked with identifying and confiscating illegal drugs during transport when a country border is crossed (port, airport or access roads). The other situations are dealt with by law enforcement agencies, mainly police forces but also military forces can be included here. Interestingly, if customs or police identify illicit drugs on-site with portable detection techniques, the suspected samples are sent to forensic institutes for confirmatory testing. Although all three types of organizations come into action in different situations and stages of the supply reduction process, all aforementioned organizations have the same goal: identifying illicit drugs in suspicious samples.

8.3.1.2 Harm reduction organizations

Harm reduction organizations have a different approach compared to supply reduction organizations, they are interested in information rather than confiscation. This means that the use cases of the technology for harm reduction organizations are more diverse. Sometimes identification of a drug in a sample is still the main goal, e.g. UAntwerp participated in a harm reduction project that analyzed 73 ecstasy samples confiscated at the Tomorrowland 2022 festival to gain insight in the MDMA content in those samples. Another use case with identification as a central goal, is the TRIP project of Modus Vivendi, a harm reduction organization located in Brussels. This project allows PWUD to test their samples to get more information on the content of their samples. Besides these identification-oriented projects, UAntwerp participated in the RADAR project, coordinated by Sciensano. In this project, the technology is used to analyze heroin samples in drug consumption rooms. Not solely to identify heroin in the samples, but also to estimate how heavily cut the samples are. Since harm reduction organizations place the health of PWUD central, their user requirements differ from those of supply reduction organizations, i.e. gaining information to protect PWUDs rather than simple identification prior to confiscation, creating more diverse use cases.

8.3.2 Market potential

Market potential is difficult to estimate accurately. According to Clarysse *et al.* the best approach is to combine the insights of a so-called top down and bottom up approach[214]. A top down approach involves studying market reports, literature, etc. to gain insights in the market potential. The quality of these insights is highly dependent on the quality of the market report. Some market reports can be freely accessed online, however the high quality ones are typically behind a (hefty) paywall. I reached out to the valorization office, and received a temporary access to the well-renowned Frost & Sullivan market reports database. The insights found in those reports, can be found below.

Contrary to the top down approach, the bottom up approach uses insights from practitioners in the field. Informal discussions, preferred witness interviews and questionnaires are the main tools to obtain these insights. Since many practitioners in the first segment are involved in the project, a questionnaire has been sent out to provide information for the bottom up approach. Additionally, I performed a preferred witness interview with practitioners at Modus Vivendi to gain more insights in the second market segment.

8.3.2.1 Top down approach

Supply reduction organizations

Several metrics can be considered to assess the market potential of the technology for supply reduction organizations, but the one that makes most sense is the total amount of tests used by the market. Although a thorough investigation was performed, it seems impossible to find reliable figures that describe this value. Another metric that can lead to this value, is the amount of confiscations. It can be generalized that each confiscation requires at least one test. Moreover, not every test has a positive result, quite often multiple negative tests are performed prior to a test of a sample that does give a positive result. It is estimated that an average of three to five tests is required per confiscation. The EMCDDA reports in its statistical bulletin a total of 531 912 confiscations in Europe (even without the Netherlands, France and Germany) in 2020[2]. This translates to roughly 1.5 to 2.5 million tests. This figure is based on some big assumptions, which is inherent to the top down approach. Even more assumptions are necessary to come to the market potential. Multiple technologies are currently used in the market, mainly color tests and portable spectroscopic technique. A new technology entering the market, like the electrochemical technology, will initially be used to complement the existing technologies, i.e. to solve cases that are difficult with the current technologies. It is only in a later stage, after proving its worth, that a new technology might replace the current technology for specific cases. Since it is very hard, if not impossible, to predict this process, no estimation on the market potential is given here. The summary of this part is that the market for on-site illicit drug testing uses roughly 1.5 to 2.5 million tests per year in Europe (minus the Netherlands, France and Germany).

Even though no specific numbers are given, the Frost & Sullivan market reports do give some insights in the supply reduction (here called the security screening and detection) market. It appears there are three strategic imperatives on the security screening and detection market: competitive intensity, disruptive technologies and geopolitical chaos. It is projected that innovative technologies will considerably impact the first two imperatives,

i.e. creating more competitive intensity and disrupt the existing technologies. The latter is interesting, digital enhanced systems for quick and accurate security processing are projected to play an important role in disrupting the existing technologies. Indeed, the novel electrochemical technology can easily be integrated in a digital enhanced system, which is much more difficult for a current technology such as color tests. As such it has an edge over the existing technology, and might thus function as a disruptor. Interestingly, the market report also provides information on the targets to influence to sell a novel technology in this market, i.e. who makes the purchasing decision relating the on-site detection technology. In Europe, this is customs and the ministry of finance/interiors, whereas in North-America, South-America, Asia and the Pacific it is the customs admin. Since the project is located in Europe, and the European market will be considered first, it is thus very important to involve the ministry through demo's and projects. BorderSens is actively doing this, e.g. the 7th of February 2023, I demonstrated the electrochemical technology with success to Ms Annelies Verlinden, Belgian Minister for Interior, Institutional Reforms and Democratic Renewal; Ms Ylva Johansson, European Commissioner for Home Affairs, and Mr Alexis Goosdeel, Director of the EMCDDA — European Monitoring Centre for Drugs and Drug Addiction (Figure 8.1).

Figure 8.1: I demonstrated the novel electrochemical technology to Ms Annelies Verlinden, Belgian Minister for Interior, Institutional Reforms and Democratic Renewal; Ms Ylva Johansson, European Commissioner for Home Affairs, and Mr Alexis Goosdeel, Director of the EMCDDA — European Monitoring Centre for Drugs and Drug Addiction. In Europe, it is crucial to get the Ministery of Interior on board, since they are the major decision maker when it concerns the purchase of technologies for on-site drug testing.

Harm reduction organizations

A top down approach for harm reduction organizations is very difficult, since no reports exist that bundle information of the different harm reduction organizations in Europe. A European organization that encompasses the different harm reduction organizations does exist, i.e. the Trans-European Drug Information (TEDI) network, but they don't have official statistical reports. Some individual harm reduction organizations, however, have their own reports. One of, if not the, most prominent harm reduction organization in Europe, is the Dutch 'Drugs Informatie en Monitoring Systeem' (DIMS)[215]. In 2021, they analyzed 10 302 samples, of which more than half were ecstasy pills. Similar information cannot be accessed for other prominent organizations such as Energycontrol (Spain) or The Loop (United Kingdom). The two Sciensano projects I was involved with, used 70 and 345 tests, respectively. What these numbers prove, is that there is a market for on-site illicit drug detection tools in harm reduction organizations. Additionally, it can be concluded that ecstasy, and thus MDMA, is the main drug of interest for harm reduction organizations.

8.3.2.2 Bottom up research

The valuable information typically lies within bottom up research, rather than top down information. Indeed, it makes sense that the numbers and information provided by practitioners in the field give a more accurate depiction of the situation than a very high-level market report.

Supply reduction organizations

Multiple supply reduction organizations are involved in the BorderSens consortium, which allows for a direct line to preferred witness information. In November 2022, a questionnaire was send out to these organizations to gain insights in several areas. This includes their preference for a single versus multidrug sensor, predicted amount of tests that would be ordered and the decision-maker within the organization for purchasing the novel technology (Table 8.1). No specific countries are mentioned to anonymize the organizations that participated in the questionnaire.

Even though the sample set is limited, some very valuable information can be extracted from the questionnaire. It is clear that the preference for type of sensor (single or multidrug detection) differs amongst the various agencies. Each organization has specific challenges, which makes that they have different use cases for the electrochemical technology. The number of tests needs some nuance to interpret correctly. The question in the survey asked how many tests the organization would purchase from the BorderSens product. This means that these figures are based on the current position of electrochemistry in the testing landscape, and might change if end-users get familiar with the added value of the technology. Interesting are the figures of Country E Customs and Country F Police since they report on the currently used amount of quick tests. As indicated in the beginning, supply reduction organizations involve customs, forensic institutes and police forces. This means that the numbers given by Country E Customs and Country F Police are not necessarily representative for the complete countries. Nevertheless, it is interesting to check how those numbers of tests compare to the amount of inhabitants. Country E and Country F have an approximate population of 10 - 12 million and 4 - 6

Table 8.1: A questionnaire was distributed amongst the supply reduction organization partners within the BorderSens consortium to gain more insight in: the preferred type of solution (single drug vs multidrug), their interest in purchasing the electrochemical technology, and the decision-maker for purchasing the technology. No specific countries are mentioned to anonymize the organizations that participated in the questionnaire. Approximate population per country: A - 10-12M, B - 17-20M, C - 17-20M, D - 0.5-1M, E - 10-12M, F - 4-6M.

	Preferred technology	Predicted #tests	Decision-maker
Country A Customs	Single sensor for port (+ multidrug for airport)	Single sensor: 300 - 3 000/year Multidrug: 5 devices	Public procurement procedure
Country B Customs Lab	Single sensor	Tender of 33 000 euro/year	Tendering procedure
Border Police Country C	Multidrug sensor		General inspector of Country C Border Police
Country D Police	Multidrug sensor	100 readers with 1500 test/year	General director
Country E Customs		Not interested, but use 10 000 quick tests per year	Country E Customs
Country F Police	Multidrug sensor	4 000 tests per year	Crown office (Country F)

million, respectively. This means an approximate average of one test per 1 000 - 1 200 and 1 000 - 1 500 inhabitants, respectively. Since these numbers are close to each other, it makes sense to make a calculation for all of Europe, with e.g. a value of one test per 1 200 inhabitants in Europe. 746.6 million people live in Europe, which then corresponds to 622 000 tests. Again, it should be emphasized that some heavy assumptions are made to come to these figures. Europe without France, Germany and the Netherlands, has a population of 577 920 000 inhabitants, which corresponds to 481 600 tests.

Finally, it is interesting to see that the decision-making process is different per organization. In Countries A and B, a tendering procedure has to be followed, whereas in the other regions the decision can be made by the organization itself or a more centralized governmental organization.

Harm reduction organizations

During my research I came into contact with harm reduction in three ways. In March 2021, I followed a two week winter school organized by the EMCDDA in collaboration with the University institute of Lisbon, centered around the topic of 'Illicit drugs in Europe: epidemiology, responses and policies'. The winter school taught me about harm reduction for the first time, and gave me good insights in its goals and methods. Secondly, I participated in two projects (Tomorrowland, Radar) organized by Sciensano, which fall within a harm reduction context. I was responsible for the deliveries, demo's and trainings within these projects. Discussions with dr. Eric Deconinck, dr. Maarten Degreef and Margot Balcaen gave me additional valuable insights in harm reduction policy, in particular with harm reduction initiatives performed in Belgium. Although these two sources provided valuable insights concerning user requirements and use cases of the technology, information on e.g. their experience with current technologies,

amount of tests used per month,... was still limited at this point. This is why I also performed a so-called preferred witness interview with a representative (Nicolas Van Der Linden) of Modus Vivendi. The goal of this interview was to get a more complete picture of this market segment, especially concerning the challenges they are experiencing with current technologies and how a new, emerging technology could solve some of these challenges (problem-solution fit & value proposition). Additionally, this preferred witness interview was also useful to learn more about figures such as the amount of monthly tests, associated with harm reduction.

This is not the place to report all (interesting) information that was gathered during that meeting, so what follows is a condensed summary. Modus Vivendi operates one stationary, and several mobile drug checking services. They employ a combination of color tests, thin-layer chromatography and portable FTIR to analyze samples at those locations. If quantification or more detailed results are required, they send samples to Sciensano for GC-MS analysis, and for which they have to pay. The budget of Modus Vivendi is limited, so an on-site quantification technique would be very welcome. The purpose of the drug checking is two-fold: 1) monitoring, which requires strict tests, and 2) harm reduction, which requires more loose, indicative tests. Per year, they perform approximately 300-400 tests. The number of harm reduction tests per year is strongly intertwined by the vision and policy of the government. In the Netherlands, the government is supportive of drug checking, and this has as a result that the corresponding organization DIMS performs many times the amount of tests that Modus Vivendi performs. The limiting factor for drug checking in harm reduction is currently the budget, not the demand. A positive evolution is on-going for harm reduction, which means that an increasing amount of governments is paying attention to, and subsidizing harm reduction initiatives. Overall, the volume for harm reduction drug testing as a market is currently not comparable to supply reduction, however this market should not be ignored either.

8.3.2.3 Combining bottom up and top down research

Combining the results of the bottom up and top down research should give a good insight in the market segments. Both approaches rely on assumptions, however, if the conclusions of both approaches can be reconciled, it can be concluded with acceptable certainty that those assumptions were realistic.

Supply reduction organizations

The number of illicit drug tests performed each year in Europe (without the Netherlands, France and Germany) by supply reduction organizations amounts to 1.5-2.5 million according to top down research, and roughly 0.5 million according to bottom up research. Since these are large volumes, the difference between both approaches is significant. However, both figures are in the same order of magnitude, despite relying on some heavy assumptions. Speaking with some precaution, it can be concluded that the true number of tests will be in the same ballpark. If one had to choose, the true figure will probably be closer to the number estimated by the bottom up approach, i.e. approximately half a million tests per year. Subsequently estimating the market share the novel technology could potentially conquer, is a very difficult task. This would require more extensive and specialized market research, which is out of scope for this chapter.

Interestingly, both bottom up and top down research agree that, in Europe, the customs and ministry of interiors/finance are the decision makers when it concerns purchase of on-site illicit drug tests. When the technology gets commercialized, these instances should be targeted.

Harm reduction organizations

Due to the absence of global reports for harm reduction organizations, together with the scattered nature of these organizations, it is hard to combine bottom up and top down research. Nevertheless, the limited information that can be found online, is in line with the figures obtained during the preferred witness interview. Overall, the amount of on-site illicit drug tests performed by harm reduction organization in Europe, will be in the range of 10 000 – 100 000 tests. DIMS in the Netherlands performed 10 302 tests in 2021, whereas this number in Belgium is only in between 300 and 400 tests. The spread in these figures show that it is very difficult to generalize for all of Europe, since countries with more established harm reduction practices will have a test per capita ratio that resembles that of the Netherlands, whereas other countries might resemble the test per capita ratio of Belgium. The final figure for tests performed by harm reduction organizations in Europe will definitely be smaller than that of the supply reduction organizations, but by no means negligible. Furthermore, harm reduction organizations are highly dependent on external funding, often by the local government. It is not entirely clear who is the final purchase decision maker, but it will probably be the harm reduction organization itself, potentially with input from the funding provider.

8.4 Problem-solution fit

8.4.1 General

It seems very straightforward that a solution developed by an innovator solves a problem, i.e. that there is a match between problem and solution. However, history teaches that a good problem-solution fit is far from straightforward, in fact many examples exist of multinational firms with large marketing divisions that got the problem-solution fit wrong. Probably one of the most well-known examples is the New Coke story, which took place in the eighties of the previous century (Figure 8.2). In 1985, Coca-Cola had been losing market share for several years to non-cola beverages and diet soft drinks. The company performed blind taste tests, which showed that the public, or at least the test public, preferred the sweeter taste of their main competitor Pepsi. Coca-Cola deemed that their problem was a declining interest in the taste of their product, and that the right solution was a reformulation of the drink. The reformulated drink was launched as New Coke in 1985 and, after an initial success, quickly became a massive flop. The drink was rebranded as Coke II in 1990, and discontinued in 2002. The origins for the failure can be traced back to the problem-solution fit. First of all, the problem the company was trying to solve was solely determined by the results of the taste tests. The company performed so-called sip tests, which, as research later showed, are not necessarily representative for the buying behavior of the people that performed the test[216]. It appears that a phenomenon, called sensation transference, occurs where people unconsciously link their taste satisfaction to the packaging[217]. The company

overestimated the importance of the taste, and thus the role of the taste of their drink in the declining market share. Secondly, the company made a big error when it didn't fully consider the difference in target audience. Coca-Cola had a strong user base in the southern states of the United States, who considered the drink part of their regional identity. The public in this region didn't feel they were considered in the decision-making of the reformulation, for them messing with the drink was messing with their identity. The aforementioned two reasons show that Coca-Cola got the problem wrong, and as a result, developed the wrong solution.

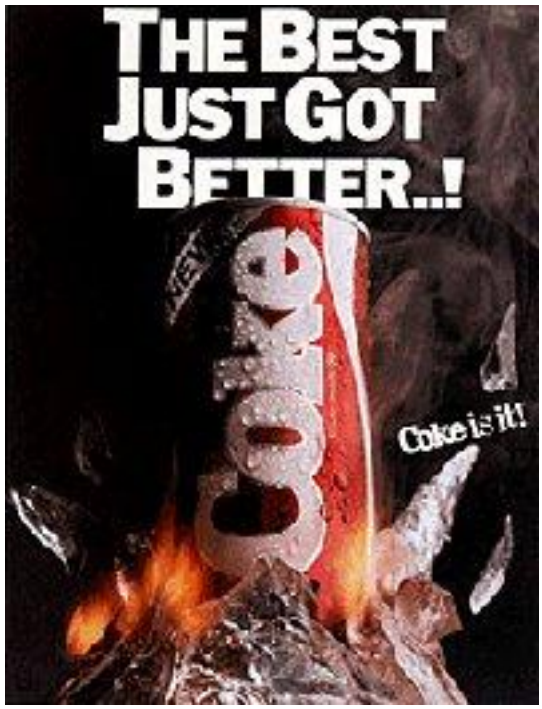


Figure 8.2: New Coke (1985), a successor of classic coca cola, is one of the most famous examples of a bad problem-solution fit.

8.4.2 Illustrative case study

The New Coke case study shows how identifying the wrong problem can result in a bad solution. In this paragraph, I will discuss a case study applied to the subject of this thesis. Note that I will draw a, somewhat caricatured, comparison between academics and customs officers to prove a point. If you ask an academic and a customs officer in the Antwerp harbor the following question: "Research has shown that color tests have a low accuracy of 68% for cocaine identification, is this a problem?". The academic will answer 'Yes!', because scientists are generally wired to strive for a high accuracy, and e.g. 68% in an academic study is simply not good enough. The customs officer, however, will explain that color tests are very valuable in many situations. They are easy-to-use, fast, portable and importantly, the officer knows the ins-and-outs, and thus, in what scenarios the color test can play an important role. However, the customs officer will also explain

some scenarios where the color tests fall short: e.g. smuggled samples. The drug cartels know that customs use color tests to confiscate drugs, and will therefore try to mislead the color tests by responding to their modus operandi: the visual color change. A famous example, which I witnessed myself at the harbor in Antwerp, is cocaine mixed with charcoal. The deep black color of charcoal makes it impossible for color tests to detect (the typically white) cocaine, which is a problem for the customs officer. They know the container they put aside probably contains cocaine, they know that the charcoal they find is probably mixed with cocaine, however their current on-site detection tool cannot prove this.

Although the difference might seem subtle, it does have implications for the problem-solution fit, and consequently also for the final application. The academic sees the low accuracy of color tests as a problem, and will subsequently try to develop an application with a very high accuracy as a solution for the problem. The customs officer sees the smuggled samples that cause problems for the color tests, and wants a solution specifically for these smuggled samples. Preferably, this solution has the same key characteristics as the color tests: easy-to-use, portable, fast and low-cost.

Again, the difference is subtle, and chances are that both problem-solution fits will eventually lead to the same solution. However, the first problem-solution fit has an important pitfall that might result in a solution that is not necessarily a good solution for the customs officer. In the hunt for the upper echelon of accuracy, compromises will be required. Every detection technology has specific samples that cause trouble, and reaching high accuracies requires finding a solution for these specific difficult samples. The academic will happily make these compromises to reach the desired high accuracy, even if this results in a final application that sacrifices e.g. ease-of-use or low-cost. A striking example here are electrochemical drug sensors for qualitative analysis, which employ expensive electrode modifications that require complex protocols.

Summarized, there is the problem the end-user experiences on the one hand, and the problem the technology provider thinks the consumer experiences on the other hand. A discrepancy between these two, no matter how small it seems, can lead to a technology solution which doesn't solve the problem of the end-user. Therefore it is of utmost importance to understand the markets and their specific problems, and develop solutions that solve those problems.

8.4.3 Problem-solution fit electrochemical illicit drug sensors

Creating a good problem-solution fit, starts by gaining a good understanding of the end-user's problem, and of the specific boundary conditions associated with the solution (e.g. price, portability,...). The most optimal way to obtain this understanding is through face-to-face interaction with end-users, i.e. bottom up research. During my PhD, I had many conversations with supply reduction practitioners, via e.g. demonstrations, trainings and meetings, which provided me with a good understanding of their practices and challenges. I had similar conversations with harm reduction practitioners through the collaborations with Sciensano. Additionally, I also performed a preferred witness interview with Nicolas Van Der Linden from Modus Vivendi to expand my understanding of harm reduction organizations and their challenges.

8.4.3.1 Supply reduction organizations

Supply reduction organizations are very well aware of the existing drug sensing technologies, and are constantly on the lookout for new emerging technologies. They have to combat organized crime, an enemy that is constantly trying everything in their power to be one step ahead of the supply reduction organizations. Specifically for drug detection, their aim is to have a broad repertoire of sensing technologies that allows them to detect illicit drugs in all different kinds of samples that organized crime throws at them. Therefore, if a new sensing technology emerges, they will initially assess if this new technology can cover illicit drugs in samples that pose difficulties for the current technologies in their sensing repertoire. The goal is to detect illicit drugs in all kinds of samples, the problem is that the current sensing technologies cannot cover all kinds of samples, and the solution is a new technology that facilitates the detection of illicit drugs in those kinds of samples that are not covered by the current sensing technologies. The problem is not that the current sensing technologies are bad, and the solution is not to develop a technology that can replace the current sensing technologies. The latter because a new technology would first be seen as an additional tool in the repertoire of sensing technologies, and not as a replacement of current sensing technologies.

The logical follow-up question is: 'What type of samples are not covered by the current technologies?'. As a start, for customs in the Port of Antwerp, the answer is very clear: mixtures. Each year, many tonnes of cocaine and heroin enter Western Europe via the port of Antwerp. In 2022, 110 tonnes of cocaine and 1.2 tonnes of heroin were confiscated by customs[218]. Organized crime organizations have many different methods to bring this product into the country, e.g. hiding it in confined spaces, bribing harbor personnel, etc. However, the vast majority of the drugs itself are not altered or masked, i.e. they appear in the form that they are intended to be consumed. This means that if customs find e.g. cocaine, this cocaine will usually appear as a white powder, and will thus easily be identified by the color test. The current sensing technologies are adequate for the large majority of drugs that enter the port. However, a smaller part of the drugs that enters the port are mixed with other agents to circumvent the current sensing technologies, such as color tests or portable Raman devices. The drug cartels specifically target the weak spots of those technologies, e.g. by mixing it with dark agents such as charcoal or fishmeal or difficult matrices such as clothes, juices or soaps. For those samples, the current technologies fall short, which is a problem for customs. They need to send suspicious samples to the lab, which takes time and slows down the decision-making process. A technology, like the voltammetric sensors described in this chapter, that allows on-site detection of these mixed samples would be a good solution for customs in the Port of Antwerp.

Secondly, I collaborated with Police Amsterdam, who informed me that a similar problem is observed for detection of MDMA in ecstasy pills. Although the intention is different, it is again the coloring of the sample that poses problems for the current sensing technologies. Ecstasy pills are typically colored to make them attractive and recognizable by consumers. Whether it is on purpose or not, MDMA is not detectable by current sensing technologies in dark-colored pills. A novel on-site technology that can detect MDMA in dark-colored pills would be a great solution. Additionally, the current on-site sensing technologies, color tests and portable Raman, cannot quantify the MDMA content in ecstasy pills. This quantification is important for monitoring trends, and is now typically done by lab-based technologies such as GC-MS or GC-FID.

It is clear that supply reduction organization have challenges associated with on-site drug testing, and that a novel on-site technology can provide solutions for these challenges. This novel on-site technology should meet several boundary conditions to make it attractive for end-users. These boundary conditions can partly be determined by looking at the current sensing technologies, i.e. what makes them attractive for end-users. Furthermore, a survey was conducted in the BorderSens project to gain more insights on these boundary conditions. Summarized, a novel on-site sensing technology should be portable, fast, easy-to-use by non-trained personnel, secure and have an easy-to-interpret result. Furthermore, the technology is preferably low-cost.

8.4.3.2 Harm reduction organizations

The goal of harm reduction organizations is to prevent harm, and for this they need information. Specifically for on-site drug testing, this can be in drug checking services, where the goal is to confirm the presence of a drug in a sample provided by a consumer, or in monitoring projects, to monitor e.g. the MDMA content in ecstasy pills at festivals or the cutting profile of heroin samples in drug consumption rooms. MDMA is the main drug of interest for harm reduction, and the same problem as for supply reduction organizations is present: dark-colored samples. Furthermore, determining the MDMA content in ecstasy pills is even more important for harm reduction organizations. Underestimating the MDMA content can lead to overdosing, and a tool that could quantify MDMA on-site would be a great solution, reducing harm by providing users at drug checking points with information on the MDMA content. This would be beneficial from a financial point of view as well, since currently samples need to be checked by other organizations for quantification, which costs money and has an impact on the limited budget of the harm reduction organizations.

Another problem that came forward during the preferred witness interview, is the lack of trained personnel. Modus Vivendi recently obtained a portable FTIR device, but interpretation of the results is cumbersome. Interestingly, the organization still uses thin-layer chromatography, an outdated technology for this application, because it gives an easy to interpret result. An easy-to-use, easy-to-interpret state-of-the-art technology would be a great solution.

Finally, PWUD that use drug checking services are interested in new psychoactive substances (NPS). The NPS market evolves rapidly, which makes it hard for sensing tools to keep up. A novel sensing technology that could rapidly be targeted towards the latest trends in NPS would be a solution for harm reduction organizations.

The specific boundary conditions for harm reduction organizations are slightly different than for supply reduction organizations. The emphasis is more on being easy-to-use and easy-to-interpret. Due to limited budget, a low-cost solution would be greatly appreciated as well. Portability and speed of analysis are still important, but less stringent than for supply reduction organizations. Specifically for drug checking services, a single drug sensor is preferred over a multidrug sensor since the customer bought a particular drug and wants to test if it is indeed that specific drug that is in the sample. Finally, non-invasive sampling (e.g. portable Raman) is preferred since customers want to consume the drug after testing. If this is not possible, the technology should use as little sample as possible.

8.5 Value proposition

Successful commercialization of a novel technology requires creating value, the end-user has to benefit from using the novel technology (product-market fit). In this chapter I described the novel electrochemical illicit drug sensor, I identified relevant market segments and I described potential problem-solution fits for those market segments. It is a matter of bringing together these elements to describe how the novel electrochemical illicit drug sensing technology can create value for supply reduction and harm reduction organizations. A great tool to visualize this, is the value proposition canvas developed by Osterwalder *et al.*[211]. I will describe the different elements that make up the canvas, and subsequently apply the canvas to both market segments.

The right part of the canvas focuses on the end-user, i.e. what are their jobs, what pains are currently experienced by them and what gains an ideal solution would provide. This part will mainly contain information from the market segmentation and problem-solution fit. On the left, the canvas starts from the novel technology, and follows up with how the technology solves pains and creates gains for the end-user. The canvas visualizes very clearly how value is created by the novel technology for the end-user (Figures 8.3 and 8.4).

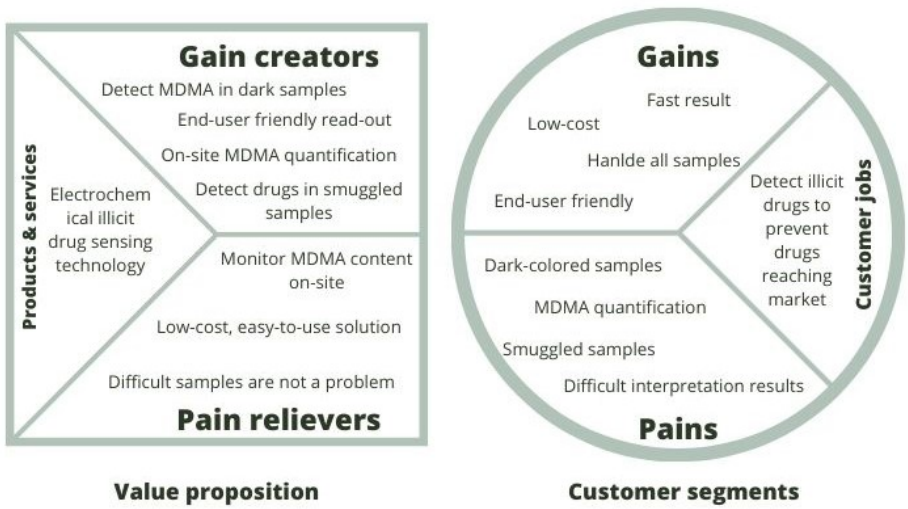


Figure 8.3: Value proposition canvas of Osterwalder *et al.* applied to supply reduction organizations[211].

Overall, it can be concluded that the novel electrochemical technology has a lot to offer, both for supply reduction organizations and harm reduction organizations. The main pain reliever is the technology's ability to handle colored samples and difficult matrices. This feature is crucial, since it offers the possibility to detect illicit drugs in samples that are not covered by the current testing technologies. The addition of the electrochemical technology to the repertoire of sensing technologies could greatly speed-up the decision-making process of supply reduction organizations. Furthermore, the quantification module in the MDMA electrochemical sensor is a great benefit as well, both for supply and harm reduction organizations. It offers a feature that is not readily available now.

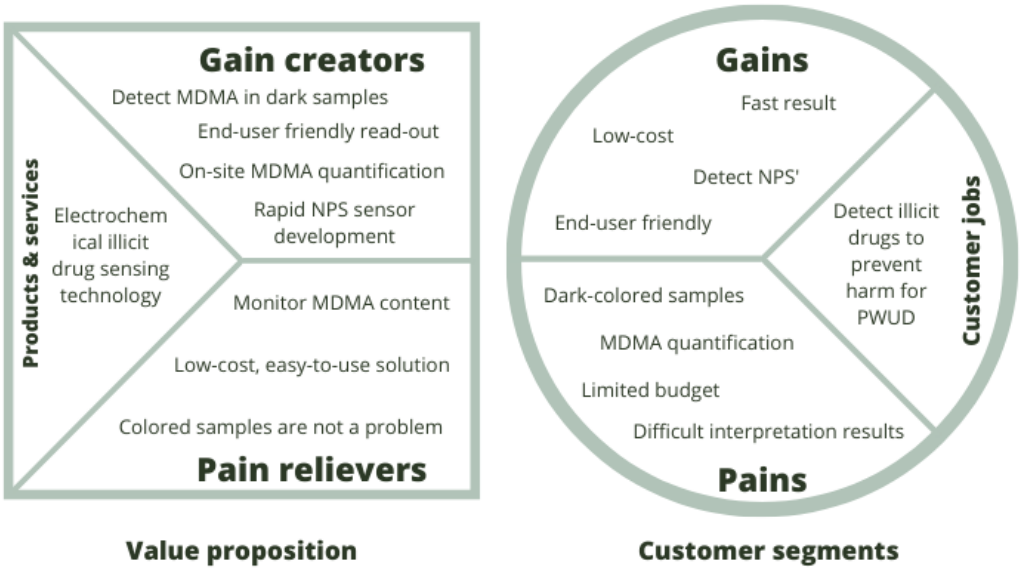


Figure 8.4: Value proposition canvas of Osterwalder *et al.* applied to harm reduction organizations[211].

Importantly, the electrochemical technology can follow-up this interesting pain relieving features with the desired boundary conditions. The technology is fast (< 1 minute), low-cost (< 5 euros), portable (< 1 kg), secure, suitable for non-experts with an easy read-out, requires little sampling amount (5 milligrams) and can easily be tuned towards new targets such as NPS. These features meet the desired boundary conditions of both supply reduction and harm reduction organizations.

It is clear from the value proposition canvas that the electrochemical on-site drug testing technology provides value for both market segments. However, it is important to realize that the true value for these market segments lies within the addition of the novel technology to the repertoire of current sensing technologies, not as a replacement of said current technologies. Keeping this in mind is crucial for the commercialization of the technology, since it has a major impact on the amount of electrochemical tests that could potentially be sold.

8.6 Commercialization strategies

Finally, I will determine the most effective commercialization strategy (business model fit) of the novel technology, based on the framework described by Gans *et al.*[212]. In this work, the authors elegantly link strategy to the commercialization environment, that is "the microeconomic and strategic conditions facing a firm that is translating an “idea” into a value proposition for customers". It is remarkable, yet not totally surprising, that commercialization strategy is typically the main challenge for technology entrepreneurs, such as ourselves, as opposed to the invention itself. The framework describes why some technology entrepreneurs better opt to enter the product market to

compete with established firms, whereas other technology entrepreneurs benefit more from cooperation with incumbents to reinforce existing market power.

It is argued that an effective commercialization strategy results from the interplay between the excludability and complementary asset environment. This results in four distinct scenario's: attacker's advantage, Greenfield competition, reputation-based ideas trading and ideas factory (Table 8.2). Before diving into the scenario that best describes this case, let us first have a look at the meaning of excludability and complementary asset environment.

8.6.1 Excludability

As the name suggests, excludability describes if the start-up can prevent development of the technology by an incumbent with knowledge of the innovation. Effective tools to secure excludability are intellectual property, trade secrets and highly specific software. The electrochemical illicit drug sensing technology has good excludability due to a strong patent portfolio and secrecy of software parameters. The software described in Chapter 3 is a crucial element of the technology, it makes the link between technology and end-user in a unique fashion, i.e. inherently different than potential competitors. The peak recognition method integrated in the software is protected by patent WO2021255230 (Interpreting an electrochemical response), with the caveat that a patent is only valuable if you can (financially) enforce it. Additionally, the parameters used in the peak recognition method are kept secret, preventing effective copying by incumbents. Overall, it can be concluded that the technology has good excludability, incumbents cannot effectively develop the innovation, even if they have knowledge of the approach.

8.6.2 Complementary assets

The term complementary assets was first coined by David Teece, whose work was very influential for the manuscript of Gans *et al.*, which forms the basis of this section[212, 219]. They can be defined as assets, infrastructure or capabilities needed to support the successful commercialization and marketing of a technological innovation, other than those assets fundamentally associated with that innovation[220]. Examples of complementary assets include e.g. marketing, sales, human resource management, office space, information technology, transportation, manufacturing, and sales channels. Gans *et al.* have a look at the complementary asset environment, more specifically, to what extent the incumbent's complementary assets contribute to the value proposition of the new technology. The incumbents of the technology are firms that currently provide drug testing technologies for the market, such as Sirchie (color tests) or Metrohm Dropsens (portable spectroscopic techniques). They possess several complementary assets that could contribute to the value proposition of the technology, such as marketing, transportation and importantly, sales channels. Metrohm Dropsens has, additionally, also manufacturing expertise with electrochemistry. I want to emphasize the sales channel as a complementary asset, since it plays an important role for our technology. The end-users of the innovation typically work within the realm of government, meaning that one has to do business to government (B2G), as opposed to the more traditional business to consumer (B2C) or business to business (B2B). Governments work with public funds,

which means that B2G requires tendering procedures. Applying for, and receiving, a tender requires a very specific (sales) expertise that is not easy to get by, especially for a starting company.

Table 8.2: Commercialization strategy environments as described by Gans *et al.*

	Do incumbent's complementary assets contribute to the value proposition from the new technology?	Can innovation by the start-up preclude effective development by the incumbent?
The Attacker's advantage	No	No
Reputation-based ideas trading	Yes	No
Greenfield competition	No	Yes
Ideas factories	Yes	Yes

8.6.3 Ideas factories

Gans *et al.* describes four different commercialization strategy environments, depending on the excludability environment and the complementary assets environment (Table 8.2). The innovation is precluded from development by the incumbent and those incumbents have multiple complementary assets that can contribute to the value proposition of the new technology, as proven in the previous paragraphs. An ideas factory environment is thus best applicable to the innovation. Summarized, this is an environment where successful invention precludes effective development by more established firms but those firms control the complementary assets required for effective commercialization. Abstractly, a university is indeed an idea factory, a place that prioritizes research and often commercializes through reinforcing partnerships with more downstream players[221]. From the technology's viewpoint, the best strategy is to contract with establishing firms since product market entry will be very costly or even impossible due to the complementary assets possessed by the incumbents. In the previous paragraph, I already mentioned the importance of a good sales channel as a complementary asset due to the B2G context and associated tendering procedures. Another implication of B2G and tendering procedures are long sales cycles, meaning that it can take a lot of time prior to receiving money. Long sales cycles are an important hurdle for starting companies, since they are typically low on cash, especially in the early phase. All of the aforementioned nods the innovation strongly to a strategy of cooperation, rather than a strategy of competition.

Since the strategy of choice is cooperation, and taking into account the strong patent portfolio, a licensing deal with an established partner in the field becomes an attractive route,

even though a licensing deal generally leads to a lower return on investment[222]. Ideally, this partner has established complementary assets (sales channels!), supplemented with expertise within the field of innovation. The innovation itself employs a scientific technique, electrochemistry, that is not yet present in the commercial on-site illicit drug sensing field, which might make it difficult to find a partner with both complementary assets and expertise within the field of the innovation. Nevertheless, there are companies that produce portable spectroscopic equipment for illicit drug detection, that also have electrochemical equipment in their repertoire (e.g. Metrohm Dropsens, Bruker, ThermoFischer Scientific). These companies are the incumbents that we, the innovators, should target for a licensing deal.

An important topic that follows is the timing, when does an innovator reach out to the incumbents to maximize the return on innovation? Ideally this is at a time when the innovation is technologically sound and convincing, while the sunk investment costs are still low. This timing is very applicable to the current status of the electrochemical illicit drug sensing technology described in this thesis. Convincing demo's can be performed to remove any technological uncertainties, while no exceptional sunk costs have been made yet. Interestingly, performing demo's, especially for potential customers, increases the bargaining power of the innovator. The effect is similar to the increase in bargaining power a car dealer receives after allowing a potential customer a test drive with the newest car model. The potential customer's desire for the new car, or the new innovation, increases, which gives more bargaining power to the car dealer, or the innovator. The only difference is that the innovator will try to license the technology to incumbents, rather than selling the innovation directly to the end user. The innovator has the opportunity to increase their bargaining power even more if two or more incumbents can be interested for a licensing deal, maybe even leading to a bidding war.

Summarized, following the Gans *et al.* framework, the electrochemical illicit drug sensing innovation developed at A-Sense Lab falls within the 'Ideas factory' commercialization strategy environment. This means that the best strategy is cooperation with incumbents, and given the strong patent portfolio and importance of good sales channels, a licensing deal seems appropriate. Several incumbents are suited, especially scientific companies that sell portable spectroscopic techniques for illicit drug detection and have electrochemical expertise, of which there are several. The timing of the innovator is crucial to maximize bargaining power.

8.7 Conclusions

The research described in this chapter has several interesting outcomes. It was found that the novel voltammetric technology is of interest for two market segments, namely supply reduction and harm reduction organizations. Both market segments have unique challenges, with a unique associated problem-solution fit, which results in a unique value proposition (product-market fit) for both market segments. As it is clear that the novel voltammetric illicit drug sensing technology can create value for end-users, it is highly relevant to pursue a valorization trajectory. Originally, the strategy was to pursue the creation of a spin-off company, fueled by the ambition of a post-doctoral researcher. However, applying the Gans *et al.* framework, it appears that a strategy of collaboration with incumbents would be more beneficial.

Chapter 9

Conclusions

"This is the end, beautiful friend."

Jim Morrison

The reader who has made it to this point in the thesis will no doubt have noticed that a conclusion is provided at the end of each chapter. In addition, in Chapters 7 and 8, a perspective on the scientific and economic future, respectively, has already been provided. The conclusion that follows will therefore be kept short and sweet so as not to bombard the reader endlessly with the same facts.

This thesis encompasses four years of work in the field of voltammetric sensors for on-site detection of illicit drugs. I focused on both methodology and software development, with a strong emphasis on generating impact. I always considered it a priority that my research realistically could provide an added value for the technology, and thus ultimately also for the end-user. In terms of methodology, I expect my most impactful achievement to be the MDMA sensor presented in Chapter 4. This sensor has all the features to provide added value for end-users within supply reduction and harm reduction. Attractive are the ease-of-use, the portability, short duration of a measurement, high accuracy, validation studies, proven worth outside of the lab and the possibility for quantification.

Furthermore, some more fundamental advances have been made within methodology development. In Chapter 5, a derivatization strategy was developed to differentiate MDMA and 2C-B. It shows that it is possible to introduce such a derivatization strategy without compromising the attractive properties of voltammetric illicit drug sensors. In Chapter 6, an important step was also made towards multidrug detection, using both an array approach and a flowchart approach. The latter was also successfully further developed and validated in Chapter 7.

Despite the added value within methodology development, as described in the previous paragraphs, I believe that the main impact in this thesis is within software development, and more specifically the data interpretation approach described in Chapter 3. It has been mentioned many times in this thesis, but I am going to allow myself to repeat it one final time. The technology is worthless to end-users without data interpretation software. The research field tends to focus heavily on methodology development, and I

am therefore happy that I could contribute with a data interpretation approach that opens up the technology to end-users. The peak recognition approach has proven throughout this thesis, and in the work of several colleagues, that it can successfully complete the tasks required of it. Furthermore, I am convinced that it can, and hopefully will, create an added value for other voltammetric sensors, within illicit drug detection, but also within other research areas.

Finally, I would like to add that a market analysis indicated that the technology described in this thesis creates value for supply reduction organizations and harm reduction organizations, and that it seems that the most effective commercialization strategy is one of co-operation with incumbents.

So, that was it. After writing down four years of work in these 172 pages, I feel fulfilled, proud and hopeful. I would like to end by thanking you, the reader, for taking the time to read this work. I leave the very last words to sports commentator William Earnest Harwell: "It's time to say goodbye, but I think goodbyes are sad and I'd much rather say hello. Hello to a new adventure!"

Appendix A

Publications

A.1 Peer reviewed journal articles

1. H. Teymourian, M. Parrilla, J.R. Sempionatto, N.F. Montiel, A. Barfidokht, **R. Van Echelpoel**, K. De Wael, J. Wang, Wearable Electrochemical Sensors for the Monitoring and Screening of Drugs, *ACS Sensors*. 5 (2020) 2679–2700.

2. **R. Van Echelpoel**, M. de Jong, D. Daems, P. Van Espen, K. De Wael, Unlocking the full potential of voltammetric data analysis: A novel peak recognition approach for (bio)analytical applications, *Talanta*. 233 (2021) 122605.

3. T. Vermeyen, J. Brence, **R. Van Echelpoel**, R. Aerts, G. Acke, P. Bultinck, W. Herrebout, Exploring machine learning methods for absolute configuration determination with vibrational circular dichroism, *Phys. Chem. Chem. Phys.* 23 (2021) 19781–19789.

4. **R. Van Echelpoel**, R.F. Kranenburg, A.C. van Asten, K. De Wael, Electrochemical detection of MDMA and 2C-B in ecstasy tablets using a selectivity enhancement strategy by in-situ derivatization, *Forensic Chem.* 27 (2022) 100383.

5. S. Thiruvottriyur Shanmugam, **R. Van Echelpoel**, G. Boeye, J. Eliaerts, M. Samanipour, H.Y.V. Ching, A. Florea, S. Van Doorslaer, F. Van Durme, N. Samyn, M. Parrilla, K. De Wael, Towards Developing a Screening Strategy for Ecstasy: Revealing the Electrochemical Profile, *ChemElectroChem.* 8 (2021) 4826–4834.

6. M. Parrilla, A. Slosse, **R. Van Echelpoel**, N. Felipe Montiel, A.R. Langley, F. Van Durme, K. De Wael, Rapid On-Site Detection of Illicit Drugs in Smuggled Samples with a Portable Electrochemical Device, *Chemosensors.* 10 (2022).

7. M. de Jong, **R. Van Echelpoel**, A.R. Langley, J. Eliaerts, J. van den Berg, M. De Wilde, N. Somers, N. Samyn, K. De Wael, Real-time electrochemical screening of cocaine in lab and field settings with automatic result generation, *Drug Test. Anal.* n/a (2022).

8. **R. Van Echelpoel**, J. Schram, M. Parrilla, D. Daems, A. Slosse, F. Van Durme, K. De Wael, Electrochemical methods for on-site multidrug detection at festivals, *Sens. Diagn.* (2022).

9. **R. Van Echelpoel**, M. Parrilla, N. Sleegers, S.T. Shanmugam, A.L.N. van Nuijs, A. Slosse, F. Van Durme, K. De Wael, Validated portable device for the qualitative and quantitative electrochemical detection of MDMA ready for on-site use, *Microchem. J.* 190 (2023) 108693.

A.2 Oral presentations conferences

1. **Van Echelpoel R. (Presenter)**, de Jong M., Daems D., Van Espen P., De Wael K. (2021, 5-9 November). Unlocking the full potential of voltammetric data analysis: a novel peak recognition algorithm [Conference presentation]. SMCBS 2021 conference, Poland (online due to Covid-19 pandemic).

2. **Van Echelpoel R. (Presenter)**, Schram J., Parrilla M., Daems D., Slosse A., Van Durme F., De Wael K. (2022, 4-6 September). Electrochemical sensor for on-site multidrug detection at festivals [Conference presentation]. Electrochem 2022 conference, Edinburgh, United Kingdom.

3. **Van Echelpoel R. (Presenter)**, Parrilla M., Sleegers N., Shanmugam S., van Nuijs L. N. A., Slosse A., Van Durme F., De Wael K. (2023, 27-31 August). Validated portable device for the qualitative and quantitative electrochemical detection of MDMA ready for on-site use [Conference presentation]. Euroanalysis 2023 conference, Geneva, Switzerland.

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