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Advances in the Development of a Point-of-Care Mass Spectrometer Test

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Advances in the Development of a Point-of-Care Mass Spectrometer Test

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Institute of Chemistry, Faculty of Science and Technology, University of Tartu, Estonia.

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I dwell in possibility.
Emily Dickinson
Me too, Emily, me too.

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II **M. Hecht**, H. Evard, K. Takkis, R. Veigure, R. Aro, R. Lohmus, K. Herodes, I. Leito, and K. Kipper, "Sponge Spray – Reaching New Dimensions of Direct Sampling and Analysis by MS," *Anal. Chem.*, vol. 89, no. 21, pp. 11592–11597, Nov. 2017, doi: 10.1021/acs.analchem.7b02957.

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Author's contribution

Paper I: Urine method development and review of the paper.

Paper II: The main person responsible for planning and performing the experiments as well as writing the paper.

Paper III: Conception and correction of the manuscript, collection of trifluoroethanol's MS spectra for supplementary material.

Paper IV: Responsible for planning and writing the majority of the manuscript.

Paper V: The main person responsible for the method development. Performed the majority of the experiments.

ABBREVIATIONS

5-MeO-DALT	N,N-diallyl-5-methoxy-tryptamine
BLQ	Below the limit of quantification
BZP	1-Benzylpiperazine
CSF	Cerebrospinal fluid
DAAD	Deutscher Akademischer Auslandsdienst (German Academic Exchange Service)
DBZP	1,4-Dibenzylpiperazine
EDDP	2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
ESI	Electrospray ionisation
HFIP	1,1,1,3,3,3-Hexafluoro-2-propanol
HFTB	1,1,1,3,3,3-Hexafluoro-2-methyl-2-propanol
IS	Internal standard
K_2EDTA	Dipotassium ethylenediaminetetraacetic acid
K_3EDTA	Tripotassium ethylenediaminetetraacetic acid
LC	Liquid chromatography
LC-MS	Hyphenated liquid-chromatography mass spectrometry
LLOQ	Lower limit of quantification
LOD	Limit of detection
m/z	Mass-to-charge ratio
mCPP	Meta-chlorophenylpiperazine
MDEA	3,4-Methylenedioxy-N-ethylamphetamine
MDMA	3,4-Methylenedioxymethamphetamine
MNGIE	Mitochondrial neurogastrointestinal encephalomyopathy
MRM	Multiple reaction monitoring
MS	Mass spectrometry
QQQ	Triple quadrupole
QTOF	Quadrupole time-of-flight
RNA	Ribonucleic acid
STD	Standard buffer
TIC	Total ion current
TOF	Time-of-flight
TYMP	Thymidine phosphorylase
VAMS	Volumetric absorptive microsampling

INTRODUCTION

The diagnosis of a disease is made considering any physical symptoms and, where possible, after reviewing blood test results. Once the blood sample is taken at the general practitioner's office, it may take several days for the test results to become available, due to the shipping of the sample and the process of analysing only one parameter at a time. In order to start the treatment of the patient immediately, the physical symptoms are a quicker indication, despite being less reliable.

In order to remedy this situation on-site, the so-called point-of-care tests could help, to obtain a diagnosis on the spot. The most famous versions of point-of-care tests are blood sugar quantification tools for diabetes patients. For many other diseases, the tests required are not as developed and rely on specific antibodies for detecting an analyte. In combination with lateral flow devices, the antibodies can give an affirmative or negative response to the presence of an analyte. Often a simple "yes" or "no" is insufficient, since the concentration of the analyte is a crucial piece of information.

In the laboratory, these quantification analyses are either carried out by clinical analysers, based on antibodies and enzymes, or liquid-chromatography mass spectrometry (LC-MS). While the clinical analysers are quicker and can pass samples from one analyser to the next, LC-MS has increasingly shown better sensitivity and selectivity. Furthermore, LC-MS can implement new tests for different analytes much easier.

The aim of this study was to exploit these benefits and to develop ways a mass spectrometer can be used in a point-of-care setting to quantify biomarkers and drugs, either through accelerated analysis time in traditional LC-MS methods, or through precise volume sampling. A key aspect for the determination of concentrations is the defined volume of sample, which can be obtained with volumetric absorptive microsampling devices. A new technique, called Sponge Spray Ionisation, was developed to measure the antibiotic penicillin G in blood, plasma, and urine at ambient conditions using mass spectrometry without any sample preparation. In subsequent tests another antibiotic, fosfomycin, was measured in cerebrospinal fluid and the drug methadone and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) were determined in plasma.

It became evident that the biggest challenge for the new technique was poor sensitivity. As a measure of sensitivity, the limit of detection should be use. This, however, is an underused analytical benchmark for most assays, as it is not required to be determined during method validation. Rather the lower limit of quantification (LLOQ), an arbitrary set value, is the determining restriction. In cases where the LLOQ is not set sufficiently low, valuable sample analysis results of a clinical study can be lost. An experimental limit of detection determination would allow to report values below the LLOQ, for gaining a stronger statistical analysis.

The other limitation for on-site analysis is time. Results need to be available within minutes to be relevant. For this purpose, a rapid LC-MS method with less than a 1-minute runtime was used on a miniature MS to estimate the dissolution speed of Ecstasy tablets. This method could be added into a van mounted setup to perform drug testing at clubs and literally in the fields of festivals. In addition to identifying the drug in the tablet, which is not necessarily only 3,4-methylenedioxymethamphetamine (MDMA), the dose and a time to effect estimations are needed to protect users.

A broader approach, using the same miniature instrument, was used to detect and quantify a large number of drug molecules within these tablets. While prolonging the analysis time, the amount of information that can be gathered in this manner is increased exponentially.

Taking the time to separate drugs in an LC is facilitating the MS analysis, most notably for isobaric molecules, with identical chemical formulae. In cases when standard LC stationary phases do not produce the required separation for analytes, fluorinated eluent additives may prove advantageous. Depending on the functional groups of molecules the additives shift their retention time and can further help the ionisation of the analytes to increase sensitivity.

Before the level of medication in the plasma needs to be determined, the disease needs to be identified. In order to achieve that, the analysis of biomarker molecules is crucial. Two such biomarkers are thymidine and 2'-deoxyuridine in plasma and urine, which do not appear in the plasma of healthy humans but are present in the rare mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) disorder. A confirmatory assay, complementing gene sequencing, helped identify patients and to monitor treatment progress.

In summary, the advances towards point-of-care biomedical mass spectrometry were investigated by developing a pure MS analysis with Sponge Spray Ionisation and by using fast LC-MS assays in preparation for future adoption in field-testing.

1. LITERATURE OVERVIEW

1.1. Mass Spectrometry (MS)

Mass spectrometry has rapidly progressed in the last two decades, with the invention of ionisation sources as well as instruments themselves, and the further improvement of already existing ones [1]. As it is, MS is based in the general idea of using electro-magnetism, or only magnetism, to manipulate a charged entity, with the aim of either measuring the mass to charge ratio (*m/z*) or for isolating ions with a certain *m/z* [2]. The inherent requirement for a meaningful manipulation is a high vacuum (low pressure) so no other gaseous molecules would stand in the path of the ion of interest [1], [3].

In a time-of-flight (TOF) mass analyser, the *m/z* value is calculated from the time it takes for an ion to traverse a set distance. A package of ions is accelerated, after which ions with smaller masses and higher charge numbers fly faster [4]. With very accurate time measurement the *m/z* of an ion can be calculated to 4 decimals, allowing to deduce of the chemical formula. The requirement for this precision is twofold: first, a multi-mass calibration to correlate the time and *m/z* values; and second, the parallel introduction of reference ions with the samples to maintain the calibration and correct for even small differences in pathlengths [5].

Additional information can be collected when a hybrid MS is combined from two quadrupoles and the time-of-flight (called QTOF), where, in addition to the accurate mass of the molecular ion, fragments can be generated in the quadrupoles and again accurately determined in the TOF section. The type of OTOF MS is very effective for the untargeted analysis as it can generate an accurate molecular fingerprint for the sample [6].

Quadrupoles allow a set *m/z* range to pass through, but with a far lower resolution than in the case of TOF instruments [7]. This means multiple molecules with similar *m/z* ratios cannot be separated. However, the selected molecules can be fragmented in a second quadrupole (collision cell) which functions as a guide to ensure that the ions hit a collision gas with the set collision energy, and are likely to yield different fragment ions, which can then be differentiated by the TOF, or be further filtered by a third quadrupole. The latter setup, a triple quadrupole (QQQ), is particularly well suited for quantification tests [6].

1.2. Ionisation

Prior to reaching the mass analyser, the sample must be ionised. There are multiple ionisation techniques providing that outcome, depending on the sample introduction technique and the compounds analysed. Soft ionisation techniques are considered to be those that primarily produce intact ions of complete molecules [1].

Depending on the hydrophobicity of the analyte, different ionisation options are available, with electrospray ionisation (ESI) being the most common [8]. Here, an organic solvent-water mixture, commonly supplied by a liquid chromatography (LC) pump, is introduced through a nebuliser with high voltage applied to it, generating droplets by nebulisation [9]. At the nebuliser the eluting liquid forms a Taylor-cone, as the surface tension of the liquid is deformed by the application of the high voltage and creates a jet of liquid that consequently disperses into droplets [10]. These droplets are charged, and as the solvent transitions into the gas phase (evaporates), the droplets shrink. In dedicated ESI sources, heat and gas are added from behind the sprayer to assist the process but are not required for the phenomenon of creating charged molecules. Once charges on the surface of the droplets reach a critical limit, a droplet splits into smaller droplets in a Coulomb explosion [11]. The evaporation and explosions cycle repeats itself, up to the point where charged molecules either exit a droplet or are all that is left after the solvent has evaporated.

Electrospray ionisation also occurs in the technique of paper spray where a paper triangle substitutes the electrospray nozzle [12], [13]. Here, a paper containing a sample is placed near the MS orifice and by the addition of a solvent and connecting to high voltage (between 2.5-6 kV), ions can be generated at the tip [14]. In fact, continuous flow is not used, but rather the solvent is added with a pipette to produce a burst of spray at a corner of the triangle. Paper spray is one of the many ambient ionisation techniques published so far [15], [16].

The pragmatic aspects of paper are its absorbance of liquids and the collection of samples by swiping surfaces with a flexible, renewable material. However, since paper is a natural fibre substrate, the quality of the paper, ideally chromatography paper, and the quality of the cut into a sharp corner are relevant for the spray quality and the signal obtained [17]. Applications examples of paper spray include the discrimination of bacteria [18] or coffee origins [19], the screening of pesticides on fruits and vegetables [20] and the detection of cocaine in fingerprints [21]. Nevertheless, the majority of publications around paper spray are focused on the analysis of biofluids [22].

Blood samples are a natural fit for paper spray, as dried blood spots have been used for collecting small samples of blood on paper for over 100 years [23]. Several methods have been described that analyse therapeutic and oncologic drugs from paper triangles [24]–[26]. Especially coated paper with aluminium sulphate, silica or carbon nanotubes have performed well [27]–[31]. Another ambient ionisation technique is coated blade spray, where a coated metal sheet enriches an analyte from a liquid to then spray it to the MS, providing high selectivity and low interference [32]. However, both variations require isotope labelled internal standards (IS) to ensure quantitative reproducibility [33]. The development and studies of new ambient ionisation so far however have been primarily carried out in laboratories on a stationary, benchtop mass spectrometer.

1.3. Miniaturisation of MS

Smaller, lightweight instruments are required for in-field testing and are being implemented in areas from space exploration [34]–[36], over environmental monitoring [37]–[39] to personal healthcare [24], [40]. Additionally, stationary MS in the laboratory have become more space efficient via the implementation of curved ion paths. Examples of these are reflectron time-of-flight mass analysers that allow for higher mass resolution due to the increased pathlength [41].

By forming U-shapes for the triple quadrupole MS, similar to the ABSciex 5500 [42] for example, their laboratory size footprint could be reduced. Agilent explored other options with their newest QQQ MS named Ultivo™, by implementing new technologies that reduce the length of the quadrupoles [43]. The device is small enough to be placed on a cart and be used as a mobile instrument, capable of running for 8 h on one nitrogen cylinder. (personal communications with Dr. Iain Kay, Agilent Technologies, Inc.). Due to its smaller size it also requires less time to establish the necessary vacuum and can be set up quicker in a field-setting. While smaller instruments are more mobile, they often lose to their high-end lab competition in terms of overall performance – they exhibit worse mass precision and resolution and higher detection limits [44].

One method to obtain structural information of the molecule in a mini-MS without fragmenting the ion is to actively modifying a molecule using reactive gases [45]. In addition, there are miniature ion-traps that collect, rather than filter the ions, estimating the *m/z* by the exclusive excitation of the target ions to the detector [46]. A company has even minimised the apparatus and ion optics to a degree that eliminated the need for high vacuum [47].

One of the greatest difficulties is the transition of molecular ions from atmospheric pressure to the vacuum. A high pass ion filter technique has been deployed by Agilent Technologies in the form of a dielectric capillary to bridge the gap, allowing ions to pass inside, simultaneously limiting the amount of air dragged in [48]. The capillary, however, is very sensitive to droplets, which will cause the loss of function over time, if not actively prevented.

1.4. Blood sampling

In a mature human about 5 L of blood are circulating and distributing oxygen, hormones and blood cells throughout the body [49]. Standard blood collection is conducted into tubes by a nurse or doctor using a venipuncture. Depending on the required test the tube may be plain or covered in anti-coagulant or gel [50]. The extraction of blood plasma, the most common sample matrix, requires a centrifuge [51]. The requisite for trained personnel and laboratory equipment needed only to obtain a viable sample make it far from being applicable in field analytics.

Alternatively, a few drops of blood can be collected from finger pricks, using minimally invasive lancets, onto dried blood spot cards. The downside of

dried blood spots is the haematocrit bias, which describes the variance of blood cell volume, making up between 45-70%, of the overall volume of blood [52]. It causes the lower haematocrit blood to spread further on the paper, which in turn dilutes the sample and causes discrepancies in the results [53].

Several microsampling techniques are emerging that are attempting to overcome this bias by making the collection of blood volumetric, collecting a defined volume using a capillary or a highly hydrophilic macroporous material with a precise volume. The latter is called the Volumetric Absorptive Microsampling (VAMS) devices, trademarked Mitra™ samplers [54]–[56] and works very much like a sponge. Volumetric blood sampling can also be achieved by collecting blood into a capillary and releasing the sample to dried blood spot paper [57], [58].

The small sample volumes obtained through microsampling are generally sufficient to run one required test with modern analytical instrumentation and are convenient enough to be taken by the patients themselves [59]. Another way of eliminating the haematocrit bias is to separate the blood cells from the plasma. [60]. This can be done by the centrifugation of anticoagulated blood [61], by the filtration of the cells [62], agglutination [63], or by the clotting of the sample, which yields serum instead of plasma [61]. Also, microfluidic devices can extract plasma from small samples [64].

Plasma and serum are historically the most used matrices, as the red colour of haemoglobin interfered with visual and spectroscopic tests [63]. Blood cannot be stored for long, or be frozen, as the erythrocytes break open, which changes the constitution of the sample. The standard procedure to extract plasma is by the centrifugation of an anticoagulated blood tube. There, the cells are pelleted, and the plasma can be transferred to a new vessel [64]. The diagnostic reliance on plasma, in combination with the slow process it is generated in, are the biggest obstacles in point-of-care applications of clinical testing [65].

1.5. Point-of-care testing

When medical diagnostic testing is performed at or near the patient receiving healthcare services or products, the term point-of-care testing is used. An immediate diagnostic result is immensely helpful, especially for patients and their general practitioners, and can drastically decrease healthcare costs, as an informed decision can streamline treatment [66]. Examples of currently available tests that fall in this category are blood-sugar measuring devices for diabetes patients [67], [68] and lateral-flow devices using an antibody for the detection of specific molecules or viruses, such as the human immunodeficiency virus [69]. The development and validation of new on-site tests, as well as uptake by healthcare professionals, is slow, however [70], [71].

A mass spectrometer could add flexibility to the performed tests, by virtue of being able to detect any molecule that is required. To fit the setting, a miniature MS combined with a reliable ambient ionisation technique could analyse an

array of targets [72], [73]. A short test runtime is the most crucial parameter to take any test from the laboratory to the field, providing a result during the consultation [15].

An important field of analysis that is required to leave the laboratory and extend towards point-of-care tests, is the therapeutic drug monitoring [74]. Common drugs regularly tested in the laboratory are immuno-suppressants, antipsychotics, anti-epileptics and increasingly more antibiotics [75]. In the case of such tests, no vitals monitor using visual or electric signals can give a precise measure; a blood sample is required. Yet, for such a specific analysis, only a small sample is required.

1.6. Biomarker diagnosis and medication monitoring at the patient

Currently available point-of-care tests focus on diabetes, cardiac markers, and infection detection, each with a specific test [76]. Some attempts have been made to multiplex, combining up to 10 analytes in a low complexity level system, to be accessible to non-laboratory professions [77]. However, if an indication falls outside the test capabilities at hand, performing the test becomes superfluous.

However, an MS based point-of-care device is theoretically not limited to certain diseases, it is rather limited by the user's knowledge which biomarker to look for. In the case of the rare MNGIE disease patients suffer from muscles dysfunctions, usually in the gastrointestinal tract, but also around the eye [78]. From the three diagnosis options of (1) a gene test, (2) an enzyme activity test or (3) the detection of elevated levels of thymidine and 2'-deoxyuridine, only the latter can be reasonably applied to a point-of-care test. Although there are only very few patients of this disease reported to date, a testing machine that can easily be switched to the relevant analyte, such as an MS, would allow to diagnose this and other extraordinary diseases.

The second group of analytes that are only being slowly introduced to point-of-care testing are therapeutic drugs [79]. The benefit of measuring the drug level in the blood has been recognised for immunosuppressants, antipsychotics and anti-epileptic drugs, due to the possibility to over- or underdose depending on the individual patient, especially in long term treatments [80]. Currently, samples are collected during a consultation with the general practitioner, shipped to a laboratory and the results are ideally reported before the next dose is administered [81].

The monitoring of antibiotics has become common in hospitals, as the effect of drug exposure can often be difficult to judge, especially in critically ill patients [82]. The dosing regime, which is derived from non-critically ill patients frequently fails in critically ill patients, for example due to multi-drug resistant organisms [83]. A β-lactam antibiotic, such as penicillin G, as well as antibiotics like fosfomycin that regains popularity to fight the advancing anti-

microbial resistance, would benefit from on-site tests [84]. Overall, the reduced use of antibiotics, which could be achieved with improved drug monitoring, would also decrease the risk of bacteria developing resistances [85].

Similarly to both antibiotics discussed above, the detoxification drug methadone is among the World Health Organisation's list of essential medicines [86]. This pain-relieving drug can be used to break opioid dependence or in maintenance therapy, by replacing illicit opioids. It was found to be more effective in retaining patients in therapy [87]. However, it can lead to withdrawal symptoms in the children of pregnant women [88]. A point-of-care test could help protect mothers and their children from the physiological effects of methadone treatment.

Medication manufacturers are required to scrutinise their product in a rigid quality assurance scheme [89]. However, there are illegally produced recreational drugs, such as Ecstasy tablets that reach the consumer merely with the assurance of their dealers. In order to protect the public, front- and back-of-house testing at events and festivals is becoming more common, generally identifying the active ingredient of the seized drugs [90]. In the case of tablets the usual on-site testing kit is limited to the visual identification of previously seized tablets [91], a Marquis-reagent spot test for alkaloids, which can distinguish drug subclasses [92], or a non-quantitative gas-chromatography mass spectrometry analysis, the machines for which are already available as portable instruments [93], [94]. The goal of analysing the drugs on site is to engage with the user and educating them about the risks associated with the drugs [95]. Developing a field test to determine a tablet's dose in addition to its identity would be highly beneficial. Eventually, it could be used as a point-of-care device in the ambulance, as well, to determine the cause of an overdose.

1.7. Bioanalytical Assay Validation

In order to demonstrate the robustness and accuracy of any new bioanalytical test, in or outside of the laboratory, an evaluation is carried out in accordance with one of several assay validation guidelines. This is needed to ensure that high quality reliable and reproducible data are generated by bioanalytical assays. New components have to be added to the guidelines periodically. This led to dried blood spots being included in the latest iteration (2018) of the Food and Drug Administration's document in the United States of America (USA), for the first time, acknowledging the increasing importance of dried and minimally invasive sampling [96]. Additional criteria demanded by the guideline when validating dried blood spots are homogeneity of sample spotting, haematocrit, and storage and handling temperatures.

In contrast to antibody-based assays, LC-MS analysis is depended on ionisation efficiency of molecules and the ion suppression or enhancement due to the matrix are specific to the technique [97]–[99].

1.8. The limitations and drawbacks of bioanalytical methods validation guidelines

A debatable point, only superficially addressed by the guidelines [100], is the determination of a limit of detection (LOD) for an assay, in contrast to the lower limit of quantification (LLOQ) [101], [102]. The latter is an analyst set value for the lowest concentration on the calibration line, fulfilling a ± 20% coefficient of variation and accuracy requirement for LC-MS assays [96], [103]–[106]. All other points need to be within ± 15% coefficient of variation and accuracy of their nominal concentration. Samples that are quantified below the LLOQ but have a sufficient signal can technically be extrapolated and used [107], but are regularly only reported as 'below the limit of quantification' (BLQ).

Whether an analyte can be considered detected in a sample, depends on the assays ability to distinguish said analyte from a blank sample, not containing any analyte. The level at which this distinction is made is the LOD and its value depends on the assay, the instrument, the analyte, and the matrix from which it is measured. However, it also depends on the rule one follows to determine this concentration. There are practical approaches considering three times the signal-to-noise ratio of the detector as being a sufficient difference [108], and those which demand calculating the false positive and false negative probabilities to establish a cut-off point [109].

The use of a calibration line allows for the quantification of larger amounts of samples but limits the range in which the concentration can be determined. Higher concentrated samples may be diluted [103]. However, the results from samples with an analyte concentration below the lowest calibrator (as the LLOQ) cannot be released - as the reliability of the data concerning those concentrations have not been established during method validation [110]. Such samples may occur for various reasons: during pharmacokinetic studies at late time points [111]–[115], due to the rapid degradation of the parent drug [116], [117], if the administered drug dose was too small [118]–[122], or due to the individual rate of drug degradation, absorption or excretion [123]–[126]. Over 200 articles reported the use of statistical methods for BLQ data treatment in accordance to Beal (2001) [127]. Enabling the use of numeric BLQ data in the future could potentially increase the quality of upcoming clinical studies.

2. AIMS OF THE STUDY

The aim of this dissertation was to develop a point-of-care mass spectrometer test, with sufficient sensitivity and speed of analysis.

This objective was approached via:

- The invention of a new ambient ionisation technique
  - Improvements to said new technique
  - Evaluation of several drug targets using the ambient ionisation technique
  - Combination of said new technique to a miniature MS to improve portability
  - By the addition of novel eluent additives with improve analyte signal strength intensity in MS detection
- The implementation of an analysis of biomarkers in plasma and urine using LC-MS as a pilot study for future point-of-care adaptation
- The analysis of a pharmaceutical analyte in tablets using LC in combination with a miniature MS to assess in-field application capability
  - A rapid testing for large quantities of sample
  - A comprehensive analysis of a multitude of pharmaceuticals
- The proposal of a theory on how to make data BLQ safe to report to improve the quality of pharmacokinetic studies and point-of-care testing in the future.

3. EXPERIMENTAL

3.1. Validation of thymidine and 2'-deoxyuridine [I]:

Thymidine, 2'-deoxyuridine and formic acid (LC-MS grade) were obtained from Sigma Aldrich (Poole, UK). Internal standards thymidine-1',2',3',4',5'-$^{13}C_5$ and 2'-deoxyuridine-1',2',3',4',5'-$^{13}C_5$ were purchased from Toronto Research Chemicals (Toronto, ON, Canada).

70% perchloric acid was obtained from VWR (Lutterworth, UK). Methanol (LC-grade) was supplied by Rathburn (Walkerburn, Scotland, UK). Deionised water was purified in-house (<1.0 µS/cm). Analyte-free dialysed human plasma (with K_2EDTA) was obtained from TCS Biosciences Ltd (Buckingham, UK). Analyte-free human plasma containing K_3EDTA, lithium heparin, analyte-free human serum from individual donors and whole blood (for the preparation of haemolysed plasma) were obtained from Biological Speciality Corporation (Colmar, PA, USA). Hyperlipidaemic plasma containing 247 mg/dL cholesterol was supplied by Seralab (Haywards Heath, UK). Urine was donated from in-house healthy volunteers.

An assay combining 100 µL of the plasma calibrator, QC, or the patient's sample, 100 µL IS working solution, and 100 µL of the 7% perchloric acid as the protein denaturation agent was developed. For urine, the volumes used were 50 µL, 50 µL and 125 µL for the sample, the IS solution and the perchloric acid, respectively. The samples were mixed and centrifuged before the supernatant was transferred to a vessel for LC-MS analysis with a runtime of 5 min.

The analysis was performed on a 1290 binary LC pump with a 1290 Infinity II autosampler (Agilent Technologies, Inc., Santa Clara, CA, USA), injecting 10 µL of protein precipitated sample onto a Hypercarb column (30x2.1 mm, 3 µm, Thermo Fisher Scientific, Waltham, MA, USA) held at 60 °C in an multicolumn thermostat 1290 column oven (Agilent Technologies). The 0.1% formic acid in deionised water (mobile phase A) and the 0.1% formic acid in methanol (mobile phase B) were used as mobile phases at the flow rate of 0.6 mL/min. The gradient increased the content from 30% to 100% B in 3.5 min, was kept at 100% B for 0.5 min, decreased to 30% in 0.2 min, and the column equilibrated for 0.8 min. The detection was carried out using an API 4000 (AB Sciex, Concord, ON, Canada) with Positive Heated ion spray (Positive TurboIonSpray, MH+): m/z 229.0 →m/z 113.1 (quantifier ion, Q) and m/z 229.0 → m/z 117.1 for 2'-deoxyuridine; m/z 243.0 → m/z 126.9 (Q) and m/z 243.0 → m/z 117.0 for thymidine; m/z 234.0 → m/z 113.0 for 2'-deoxyuridine-1',2',3',4',5'-$^{13}C_5$, m/z 248.0 → m/z 127.0 for thymidine-1',2',3',4',5'-$^{13}C_5$. The MS was set to: source temperature 400 °C, curtain gas, nebuliser gas and heater gas were set at 30 psig, probe voltage 5000 V, optimised collision energies for compounds ranged from 13 to 15 V and the dwell times were set to 30 ms.

3.2. Sponge Spray Ionisation 1.0 [II]:

Penicillin G and penicillin G-D_7 were from Sigma-Aldrich (Missouri, USA).

LC-MS grade acetonitrile, isopropanol, ammonium acetate, 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) and formic acid came from Sigma-Aldrich (Missouri, USA). Water was purified (18.2 MΩ·cm at 25 °C and the total organic carbon value 2−3 ppb) in-house using a Millipore Advantage A10 system from Millipore (Bedford, USA). Mitra microsampling devices (10 and 20 µL) were from Neoteryx (Torrance, CA, USA). Blood, plasma, and urine were donated by healthy volunteers and/or purchased from the Blood Bank of Tartu University Hospital (Tartu, Estonia).

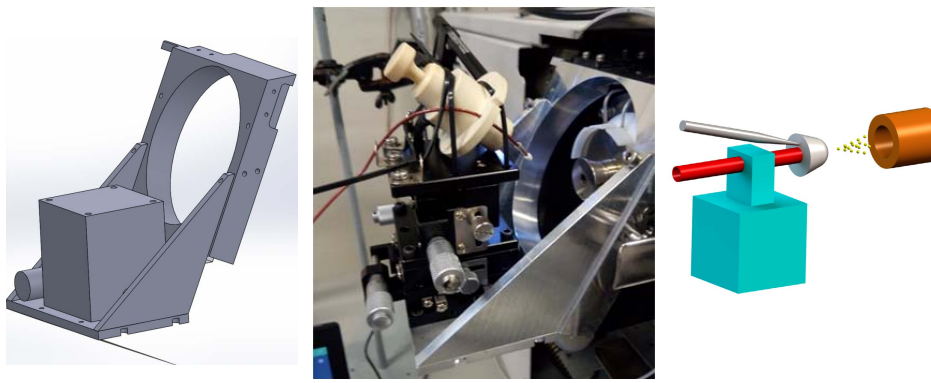


Figure 1. Sponge Spray Ionisation 1.0 setup, using an in-house build frame (left), a micromanipulation stage, an Agilent APCI corona needle (large round beige casing), with a VAMS sponge on its tip, which is connected to PEEK-tubing for adding solvent to the sponge (middle). A schematic drawing how tubing and corona needle are placed in the sponge and generate spray is shown on the bottom (right).

Sponge Spray Ionisation was performed on an MSD Trap XCT MS system (Agilent Technologies, Inc., Santa Clara, CA, USA). The arrangement for the ionisation source consisted of an Agilent APCI corona needle (providing maximally 4 mA), secured on an XYZ-3 Axis Trimming Platform Linear Stages Bearing Tuning Sliding Table 60x60 mm micromanipulator, placed on an in-house built frame connecting to the MS (Figure 1). The sponge was held by a shortened disposable pipette tip and pierced to the corona needle 23.0 mm from the MS inlet shield. A PEEK tubing (1/16 in. x 0.005 in, O.D. x I.D.) was inserted into the pipette tip and supplied eluent to the sponge from a KDS 100 infusion pump (KD Scientific, Holliston, MA, USA) at 15 µL/min. No nebulising gas was applied. The MS-transitions for penicillin G and penicillin G-D_7 were m/z 335→ m/z 160, and m/z 342→ m/z 160, respectively.

Samples of blood, plasma and urine were fortified with the analyte concentration from 0.5 to 100 mg/L. Isotopic labelled internal standard at 12.5 mg/L in methanol was pre-deposited on a VAMS device and left to dry for 2 h. Subsequently, the biological sample was collected to 10 µL or 20 µL sponges and again allowed to dry at ambient temperature (20 ± 3 °C, 2 h). Data Analysis for LC/MSD Trap Version 3.2 (Bruker Daltonik GmbH, Bremen, Germany) and Microsoft Excel were used for the interpretation of data.

3.3. Sponge Spray Ionisation 2.0:

Fosfomycin, methadone, EDDP and methadone-D_9 came from Sigma Aldrich (Poole, UK). Fosfomycin-$^{13}C_3$ was purchased from Toronto Research Chemicals (North York, Canada). Reference ions trifluoroacetic acid, Purine, HP-0321 and HP-0921 in 90% acetonitrile, 10% water solution were obtained from Agilent Technologies, Inc. (Santa Clara, CA, USA)

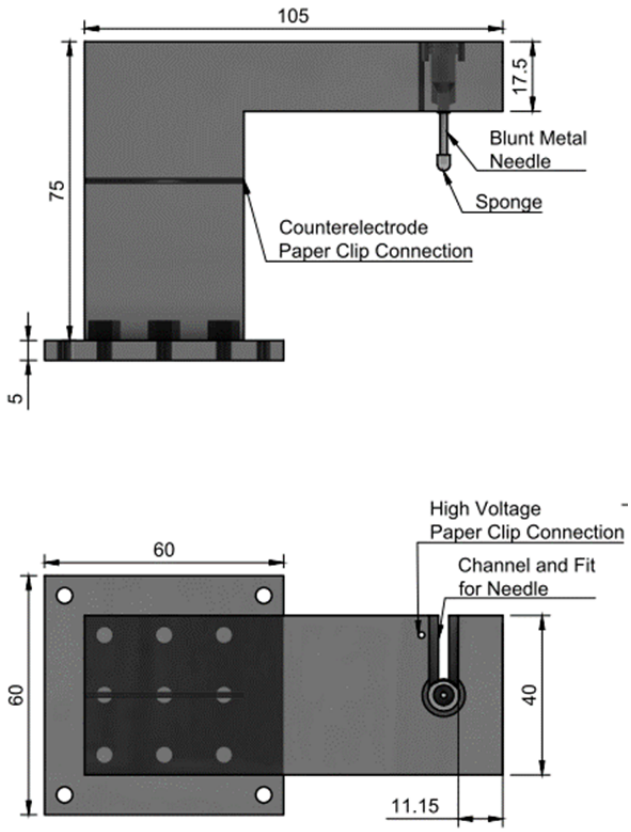
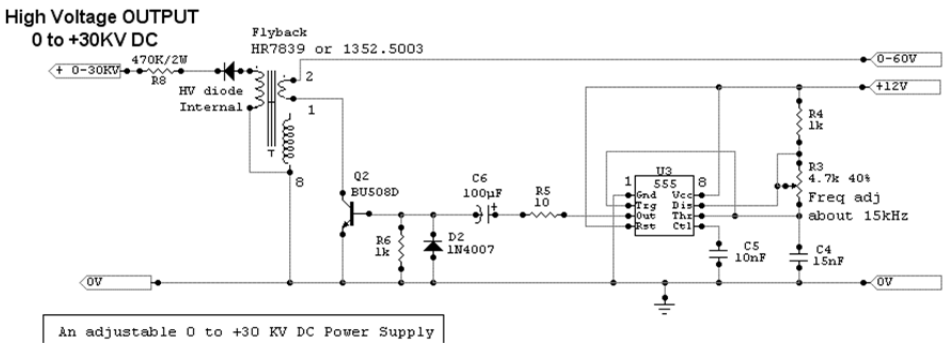


Figure 2. Scheme of Sponge Spray Ionisation 2.0 top-down holder. Top: profile view of the holder, showing the blunt metal needle fitted into the holder and the sponge. Bottom: top down view, showing the channel through which the needle can be entered. Dimensions are given in mm.

Isopropanol and hexane were purchased from VWR (Lutterworth, UK). Water was deionised in-house (> 18 MΩ·cm, Elix®, Watford, UK). LC-MS grade formic acid and the 25% ammonium hydroxide solution came from Sigma Aldrich (Poole, UK). Analyte-free human plasma containing K_3EDTA was obtained from Biological Speciality Corporation (Colmar, PA, USA). Artificial cerebrospinal fluid (CSF) was acquired from EcoCyte Biosciences (Dortmund, Germany). Mitra microsampling devices (10 and 30 µL) were obtained from Neoteryx (Torrance, CA, USA).



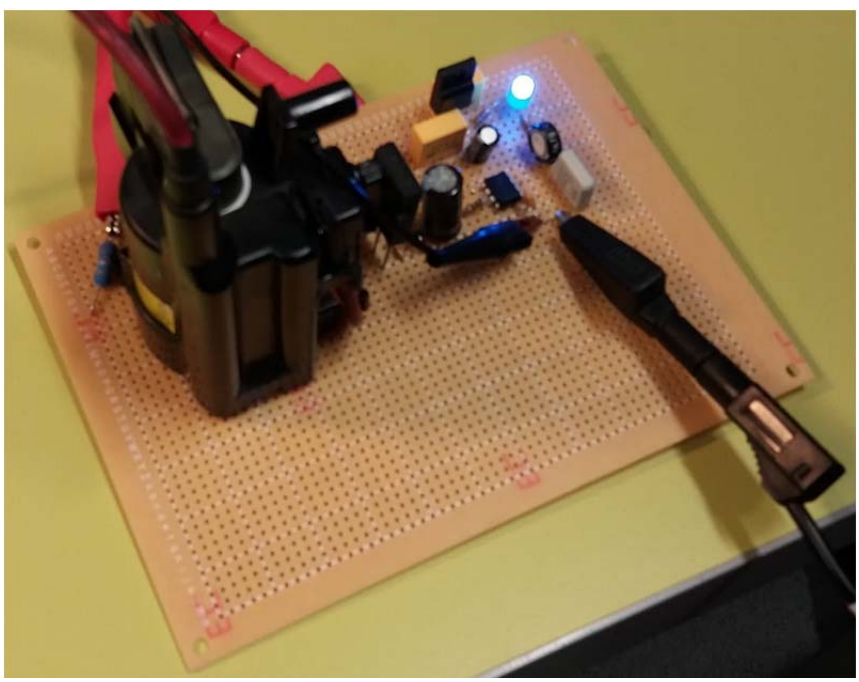


Figure 3. Scheme (top) and the powered high voltage system for positive ionisation (bottom), design from the scheme. The large black component on the left is a transformer, formerly used in a tube television set. Diagram originated from [128].

An Ultivo™ QQQ MS and for comparison a 6230 TOF-MS both using a 1290 binary LC-pump (Agilent Technologies, Inc., Santa Clara, CA, USA) were used. A new source setup was built around 14 gauge 1.5" blunt tip dispensing needles with a Luer lock (Fisnar, Germantown, WI, USA) to hold the sponge. A new arrangement was 3D printed (ColorFabb_HT filament from ColorFabb in Belfeld, the Netherlands was printed using fused deposition modelling) to point the sponge and spray direction downward, with a channel to exchange sponges on separate needles and a fit for the outside of the Luer lock (Figure 2). Bent metal paperclips connected the needle to a high voltage source and a second paper clip was tested as a counter-electrode to improve the spray stability.

The high voltage sources, both for the negative and positive ionisation, were self-build using a line output transformer to amplify low voltage (Figure 3). The low voltage was supplied by a 72-10495 bench top power supply, 0-30V 5A with Twin Outputs (Tenma, Leeds, UK). An EHG2000 heat gun (Ryobi, Anderson, USA) was tested as a substitute heated gas nebuliser but was not used in the final setup.

Isopropanol at 95% and 5% of water was used as the eluent, with additives of the 0.1% formic acid or the 0.1% ammonium hydroxide for the positive and negative ionisation modes, respectively. The flow rate was changed over the course of one run to reduce analysis time without signal at the beginning, the details can be found in **Table 1**.

Table 1. Flow rate for Sponge Spray Ionisation 2.0 experiments.

Time (min)	Flow rate (µL/min)
0	100
0.05	650
0.29	650
0.36	100
5	100

For Sponge Spray Ionisation 2.0, two sizes of Mitra™ microsampling devices were evaluated, 10 µL and 30 µL sponges. The sponges were pre-loaded with IS and dried again, methanolic solutions only required 30 min to dry, before the sample was applied.

The stock solutions of methadone and EDDP were prepared in methanol at 1,000 and 100 µg/mL, respectively, and stored at –20 °C. Two sub-stock solutions containing both methadone and EDDP were prepared for the calibrator and QC stock solutions each at 50 and 1 µg/mL in methanol. The nominal calibrator concentrations were 10, 50, 100, 500 and 1000 ng/mL, and the nominal QC concentrations were 10, 30 and 750 ng/mL for both analytes, these were obtained by diluting the stocks in human plasma. Once prepared, the calibrator and QC standards were aliquoted into 1.5 mL polypropylene micro-centrifuge tubes and stored frozen at –20 °C until they were required for the analysis. Methadone-D_9 was diluted to 100 ng/mL in methanol and stored at –20 °C.

Stock solutions of fosfomycin were prepared separately for the calibrators and QCs in methanol at 10 mg/mL and stored at –20 °C. The nominal calibrator concentrations were 1, 5, 10, 50,100 and 500 µg/mL, and the nominal QC concentrations were 1, 3, 125 and 375 µg/mL in the artificial cerebrospinal fluid. Methanolic fosfomycin$^{13}C_3$ was prepared at 50 µg/mL.

3.3.1. Sponge Spray Ionisation on Ultivo™ QQQ MS

The multiple reaction monitoring (MRM) transitions for all compounds analysed on the Ultivo™ MS are given in **Table 2**. The dwell time for multiple reaction monitoring transition was 10 ms, and the accelerator voltage was 9 V. The drying gas temperature of the MS was set to 350 °C at 9 L/min.

Ambient ionisation data collected with the Ultivo™ QQQ required signal post-processing. Single time points in each single reaction monitoring transition displayed high intensity spikes and were not consistent between the transitions of the same analyte or with the internal standard. Therefore, they were considered as noise, as a result of the assembly's instability to apply high voltage in conjunction with the mass analyser. However, when using a different mass analyser with the same assembly, this phenomenon was not observed.

Table 2. MRM transitions measured on Agilent Ultivo™. Plus and minus signs in the parenthesis correspond to the positive and negative ionisation modes used during detection.

Analyte (Ionisation mode)	Precursor (m/z)	Product (*m/z*)	Fragmentator (V)	Collision Energy (V)
Fosfomycin (-)	137.0	63.0	75	9
	137.0	79.0	75	29
	137.0	81.1	75	13
Fosfomycin-$^{13}C_3$ (-)	140.0	63.0	75	9
	140.0	79.0	75	29
Fosfomycin (+)	139.0	57.0	66	5
	139.0	121	66	5
Fosfomycin-$^{13}C_3$ (+)	142.0	44.1	63	9
	142.0	60.2	63	5
	142.0	124.0	66	5
Methadone (+)	310.2	77.1	118	60
	310.2	105.0	118	28
	310.2	265.2	118	12
EDDP (+)	278.2	186.0	118	25
	278.2	234.0	118	15
	278.2	249.0	118	15
Methadone-D_9 (+)	319.3	60.1	150	20
	319.3	105.0	150	40
	319.3	268.2	150	10

In order to extract the relevant data, the raw data were compiled into a CSV file format containing all transitions and samples of one day of experiments, containing the calibration line samples and quality controls each in duplicates. The raw data were extracted using Agilent Navigator software version B.08.00. The CSV file was imported into RStudio version 1.2.1335 and a filter function called "runmed" over 9 values was applied.

Each transition was integrated over the entire runtime of 5 min and the areas were used to calculate the analyte to the internal standard ratio, from which the calibration lines were formed. Calibration fits without weight, or 1/x and $1/x^2$ weights (x being the analyte concentration) were calculated using RStudio.

3.3.2. Sponge Spray Ionisation TOF MS

For the TOF-MS, the accurate masses of the analytes (**Table 3**) were used to extract the ion chromatograms, which were used to integrate over the whole runtime of 5 min. The reference ions, trifluoroacetic acid (113.9929 g/mol), purine (120.0436 g/mol), HP-321 (321.0408 g/mol) and HP-921 (921.0025 g/mol), required to assure that the accurate transformation of time to mass, were added to the eluent solution. The gas temperature of the MS was set to 350 °C and the flow rate at 9 L/min.

Table 3. Analytes, their chemical formulae, molecular ion constitution and theoretical mass to charge ratio for accurate mass detection.

Analyte	Chemical Formula	Molecular ion	Theoretical *m/z*
Fosfomycin	$C_3H_7O_4P$	$[M-H]^-$	137.0009
		$[M+H]^+$	139.0155
Fosfomycin-$^{13}C_3$	$^{13}C_3H_7O_4P$	$[M-H]^-$	140.0086
		$[M+H]^+$	142.0232
Methadone	$C_{21}H_{27}NO$	$[M+H]^+$	310.2165
EDDP	$C_{20}H_{23}N$	$[M+H]^+$	278.1903
Methadone-D_9	$C_{21}H_{18}D_9NO$	$[M+H]^+$	319.2730

3.4. Retention mechanism studies [III]:

Diisopropylamine, piperidine, cyclohexylamine, pyrrolidine, aniline, 4-chloroaniline, 1-naphthylamine, histamine, 4-fluoroaniline, 2,6-dimethylpyridine, 2-methylpyridine, 2-methoxypyridine were obtained from Sigma-Aldrich (Missouri, USA). All eluent additives: 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), 1,1,1,3,3,3-hexafluoro-2-methyl-2-propanol (HFTB), ammonium acetate, ammonium bicarbonate and the 25% NH_4OH solution were LC-MS grade and obtained from Sigma-Aldrich (Missouri, USA).

MeOH (LC–MS grade) was obtained from Sigma Aldrich (Missouri, USA), water was purified (18.2 MΩ·cm at 25 ºC and a total organic carbon value

2-3 ppb) in-house using a Millipore Advantage A10 system from Millipore (Bedford, USA).

The analytes were prepared as single compound standard solutions at 10 µg/mL in 1 mM ammonium acetate and the 0.1% formic acid in a water/methanol (8/2, v/v) mixture. The samples were filtered through a 0.45 µm regenerated cellulose filter (Captiva Econofilter, Agilent Technologies, Santa Clara, CA, USA) and injected onto an Agilent Zorbax RRHD Extend C18 (2.1×100 mm, 1.8 µm), resistant in the pH range from 2.0 to 11.5 (Agilent Technologies, Inc., Santa Clara, CA, USA). The column was equilibrated for 1.5 h with the investigated buffer of HFIP, HFTB, ammonium acetate and ammonium bicarbonate. Three eluent pH values of the pH 8.5, 9 and 10 were tested at similar pH values and compared to a standard buffer of 0.1% formic acid and 1 mM ammonium acetate in water at the pH 2.8 (STD).

Elution was carried out at a flow rate of 0.2 mL/min using the isocratic mode with the 25% methanol and the 75% 5 mM of additive in water or STD buffer, at 40 °C in a Shimadzu LC-MS-2020 system with binary pump LC-20AD-XR, an autosampler SIL-30AC (set at 4 °C) and a column compartment CTO-20AC (Shimadzu Corporation, Kyoto, Japan). MS analysis was carried out in the scan mode.

3.5. MDMA tablet dose and dissolution determination [V]:

MDMA and MDMA-D_5 certified reference solutions were from Cerilliant (Round Rock, USA) and MDMA hydrochloride reference standard w obtained from Sigma Aldrich (Poole, UK).

Hydrochloric acid and formic acid were purchased from Sigma Aldrich (Poole, UK). LC–MS grade methanol was acquired from Rathburn (Walkerburn, Scotland, UK). Water was deionised in-house (> 18 MΩ·cm, Elix®, Watford, UK).

The analysed MDMA tablets were collected from amnesty bins, or seized during entry searches, from a number of large music festivals and nightclubs in the UK. The tablets were crushed and dissolved in the 50% methanol in water (v/v) to estimate their dose. Intact tablets were added to a 900 mL dissolution bath of 0.05 mol/L aqueous hydrochloric acid and 11 samples were taken over 3h (the detailed procedure is described in [V]).

10 µL of dissolved samples were mixed with 20 µL MDMA-D_5 at 10 mg/L and diluted with 1.5 mL 0.1% formic acid in deionised water in a 96-well plate. From each well two injections of 2 µL were made, with one of each needle of a dual-needle autosampler of a 1290 Infinity II LC system coupled to Raptor biphenyl column (5.0 x 3.0 mm, 2.7 µm, Thames Restek, High Wycombe, UK) fitted directly to the electrospray ionisation source of an Ultivo™ MS (both Agilent Technologies, Inc., Santa Clara, CA, USA). The source conditions were set as follows: sheath gas temperature 350°C, sheath gas flow-rate 9 L/min,

nebuliser 55 psi, drying gas temperature 350°C, drying gas flow-rate 11 L/min, and capillary voltage of 2.5 kV. MDMA ion transitions (collision energy, V) were set to *m/z* 194.1→ *m/z* 104.9 (17) and *m/z* 194.1→ *m/z* 163.0 (5) and MDMA-D_5 transitions were *m/z* 199.2→ *m/z* 107.0 (21) and *m/z* 199.2→ *m/z* 165.0 (5), with a dwell time of 10 ms and an accelerator voltage of 9 V.

3.6. Drug screening in Ecstasy tablets using Ultivo™ MS

LC/MS toxicology submixes: 2, 3, 4,6,9A, 9B, 9C, 9D were obtained from Agilent Technologies, Inc. (Santa Clara, CA, USA). Stable isotope labelled internal standards for 12 drugs were purchased from Sigma Aldrich (Poole, UK).

Formic acid was purchased from Sigma Aldrich (Poole, UK). LC–MS grade methanol and acetonitrile were obtained from Rathburn (Walkerburn, Scotland, UK). Water was deionised in-house (> 18 MΩ·cm, Elix®, Watford, UK).

The instrument consisted of an Agilent 1290 Infinity high speed pump, 1290 Infinity II multisampler with cooler, 1290 Infinity II multicolumn thermostat and Agilent Ultivo™ MS system coupled with a Jet Stream electrospray ionisation source (all from Agilent Technologies, Inc., Santa Clara, CA, USA).

Tablets from the same selection as **3.5** were crushed and dissolved in 100 mL of methanol and water (50:50 v/v) and further diluted 10-fold with the 0.1% formic acid in water. 10 µL of internal standard was added to 100 µL of the calibrator or sample solution, followed by 140 µL of water. 20 µL was then injected for LC-MS analysis.

A gradient elution was carried out on a Zorbax Eclipse Plus C18 RRHD 2.1x100mm, 1.8µm column (Agilent Technologies, Inc., Santa Clara, CA, USA), with 5 mM ammonium formate with the 0.01% formic acid (aqueous) as mobile phase A and methanol with 5 mM ammonium formate and the 0.01% formic acid as mobile phase B. The gradient started at 5% B, which was held for 1 minute and then increased to 100% over 9 minutes and kept there for 2 min. The column was re-equilibrated for 1 min at 5% B, with a total runtime of 13 min. The flow rate was 0.4 mL/min.

The MRMs were selected from the MassHunter "Forensic Toxicology Triggered MRM Database and Library" (Agilent Technologies, Inc., Santa Clara, CA, USA). Five MRMs were selected for each drug, 1 quantifier, 1 qualifier, which were run in a dynamic setting around their retention time and three further MRMs, which were collected once the quantifier exceeded the set threshold of 100 counts. Two MRM transitions were monitored for the internal standards.

4. RESULTS & DISCUSSION

Modern analytical instruments, especially mass spectrometers, are capable of extracting important clinical information out of complex samples. Applying this capability to point-of-care setting will be of great benefit to general practitioners, streamlining the decision making for the future of medical care – patient-centred treatment. Identifying biological and metabolic molecules that can be attributed to physiological states is important, especially when determining the cause or expression of a disease.

4.1. Detection of biological discriminators [I]

A promising path to that end has been identifying genetic variants and mutations that lead to certain diseases. In the case of the MNGIE disease, variations in the gene encoding for the thymidine phosphorylase (TYMP), result in the accumulation of 2'-deoxyribonucleosides and subsequently mitochondrial dysfunction [78]. At that, the mere presence or absence of thymidine and 2'-deoxyuridine in plasma or urine can help to identify the disease and furthermore support the ongoing treatment, should the biomarker return to normal levels.

During method validation good accuracy of 93.83% and 104.85% in plasma and 96.14% to 107.45% in urine were observed, fulfilling the set-out acceptance criteria of 85% to 115% for the three highest QCs. The LLOQ, set for this analysis at 10 ng/mL for the plasma and 1 µg/mL for the urine, fell into these criteria as well with accuracies of 95.64% for thymidine and 103.91% for 2'deoxyuridine in plasma and 104.80% and 107.45% for the analytes respectively in urine, suggesting that the method could possibly use less sample volume or could quantify to even lower LLOQ concentration [I].

Furthermore, a degradation of all thymidine and 2'deoxyuridine in the urine when stored at room temperature and cooled at + 4 °C was observed, likely due to microbial contamination. The addition of 5% perchloric acid to the urine sample after collection improved the stability but remained overall inacceptable, as the thymidine accuracies after 14 days at 4 °C at the 3-times the LLOQ concentration was only 80.24%. Therefore, immediately freezing the sample in at least – 20 °C is suggested, as has previously been proposed as well [130].

After quantifying those two substances in the plasma and the urine with the analytical method, it was possible to diagnose two patients, whose results, that they carry the disease, were then also confirmed by gene sequencing [I]. Further samples were collected also from patients 1–3 already undergoing erythrocyte-encapsulated thymidine phosphorylase therapy (EE-TP) [131] in order to monitor the effectiveness of treatment (Table 4). The plasma concentrations of both analytes were halved for patient 3 and reduced to healthy levels in the other two. Clinical observations further confirmed the successful treatment of these patients.

Table 4. Plasma thymidine and 2'-deoxyuridine concentrations in patients with MNGIE prior to therapy and during therapy with EE-TP (patients 1–3) and in patients tested for MNGIE (patients 4 and 5).

Patient ID	*TYMP* mutation	Diagnosis concentration, ng/mL (µmol/L)		EE-TP dose (µmol/min/ 10^{10} cells)	Treatment day	In-treatment concentration, ng/mL (µmol/L)	
		Thymidine	2'-deoxyuridine			Thymidine	2'-deoxyuridine
1	Heterozygous c.866A>C c.1231_1243 del	5536.5 (22.9)	6161.3 (27.0)	18.2	117	ND	ND
2	Homozygous c.1088 delG	3029.5 (12.5)	3638.1 (15.9)	26.5	33	40.7 (0.17)	9.9 (0.4)
3	g.4009_4010insGg.4101G>A	4031.5 (16.6)	4817.6 (21.1)	27.5	34	2068.5 (8.5)	2251.0 (9.9)
4	Homozygous c.1282G>C	2380.9 (9.8)	2921.8 (12.8)	NA	NA	NA	NA
5	Homozygous c.392C>T	2892.0 (11.9)	2558.4 (11.2)	NA	NA	NA	NA

ND = Not detected; NA = Not applicable; EE-TP= erythrocyte encapsulated thymidine phosphorylase

Several different mutations can lead to the same malfunctioning enzyme and the same disease, as can be seen in **Table 4**, where the 5 patients gene sequences all displayed different mutations. Furthermore, there are regulatory gene regions, epigenetics, micro RNAs and post translational modifications to control the presence and activity of proteins and enzyme and consequently the metabolism of an individual [60]. Sequencing all possible interferences adds unnecessary complexity, where the quantification of the metabolic outcome can give the answer by itself.

Gene sequencing can highlight medical conditions, but the metabolic expression of the disease corresponds to the actual severity. Genetic disorders that are identified using their metabolic condition are among others phenylketonuria and medium-chain acyl-CoA dehydrogenase deficiency, picked up in the newborn screening right after birth [129]. The list of disorders tested for depends on the regional prevalence of diseases, but it is continuously extended [130]. The procedure only involves the collection of a few drops of blood from the baby's heel with a small prick to a dried blood spot card.

4.2. Sponge Spray Ionisation 1.0 [II]

For qualitative testing like the newborn screening, dried blood spots are sufficient. For quantity analysis the volume of the sample becomes crucial (see also **1.4**), and became possible with the invention of VAMS. As paper spray has used dried blood spots for analysing blood samples, it became the main challenge of this project to create a mass spectrometric test using VAMS sponges, adding a third dimension to the existing planar paper spray.

The uniform production of sponges is advantageous over the natural structure of paper, which has been identified as a reason for poor spray reproducibility [131], [132]. Another concern was found to be the interference of biological matrices [20], which can be compensated for by the use of IS. In paper spray, it is necessary to mix the sample with IS prior to spotting, or the IS is added to the paper using a pipette and it forms a spot with a different size than that of the sample. Using a VAMS sponge ensures that the entire sampler is fully soaked in both, IS and the sample at a defined volume.

In order to place the sponge in front of the MS, while being able conveniently change the arrangement, a frame-source was built and a micromanipulator stage was attached (**Figure 1**). A sponge was, for the lack of a better high voltage source, spiked on a corona needle, powered by the ion trap MS. With a shortened pipette tip, the connection between the sponge and a solvent capillary was made, enabling constant flow and analysis times of 30 min.

The first study using Sponge Spray Ionisation analysed penicillin G in methanol standard solution, plasma and in urine, with the intensity of signal obtained over time (the so-called "chronogram"), varying substantially (**Figure 4**). It took approximately 2 minutes to fill the sponge with the solvent to the point when electrospray was formed from a layer of solvent encompassing the

sponge. From the tips loaded with the standard solution, a slow analyte elution profile could be observed, while urine samples showed a smaller peak at 8 minutes. The intensity of the urine peak corresponded to that of the standard solutions at the same time. The response from the sample in plasma, however, only appeared like a noisy baseline, which surprisingly increased in intensity over time. It was also possible to generate spray using blood sampled sponges, resulting in similar chronograms to plasma.

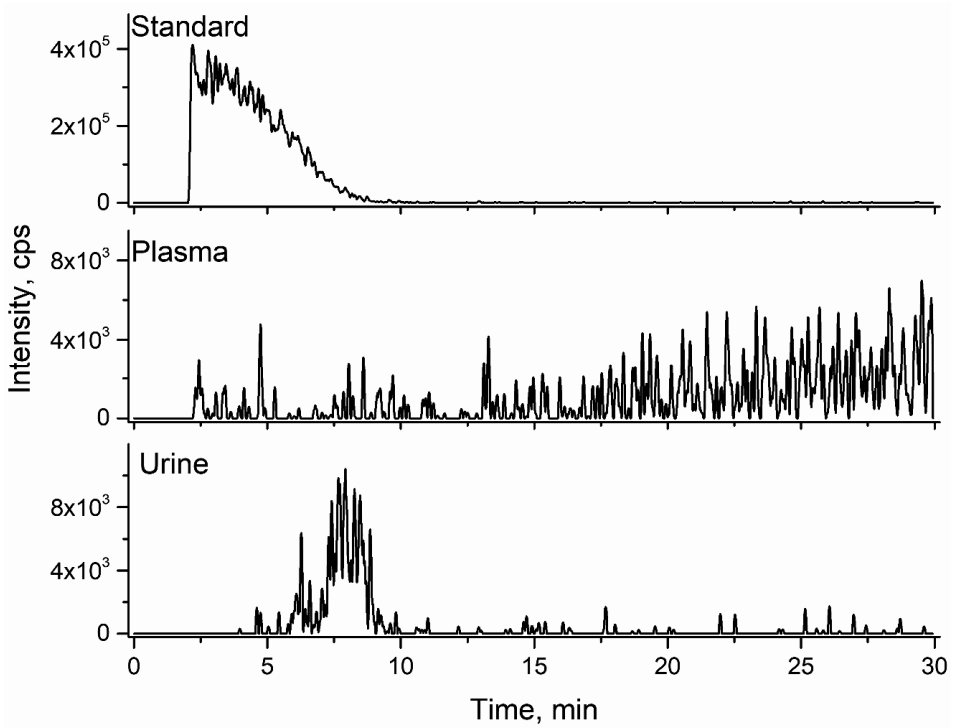


Figure 4. Chronograms of three matrices fortified with 25 mg/L penicillin G (*m/z* 335 → 160). In the standard solution, penicillin G was completely eluted from the VAMS device after 10 minutes. For plasma and urine, the suppression of the analyte signal relative to the standard solution was observed, and the analyte also eluted later. For plasma, the analyte was not completely eluted even after 30 minutes.

With the analyte in plasma and urine, sampled on 20 µL VAMS, which were pre-loaded with IS and dried, before taking up the sample, a relationship between the signal intensity and the concentration could be found, by integrating the signal from 10-20 minutes (**Figure 5**). The intensity of the IS was comparable to a similar concentration of the analyte at 10 mg/L.

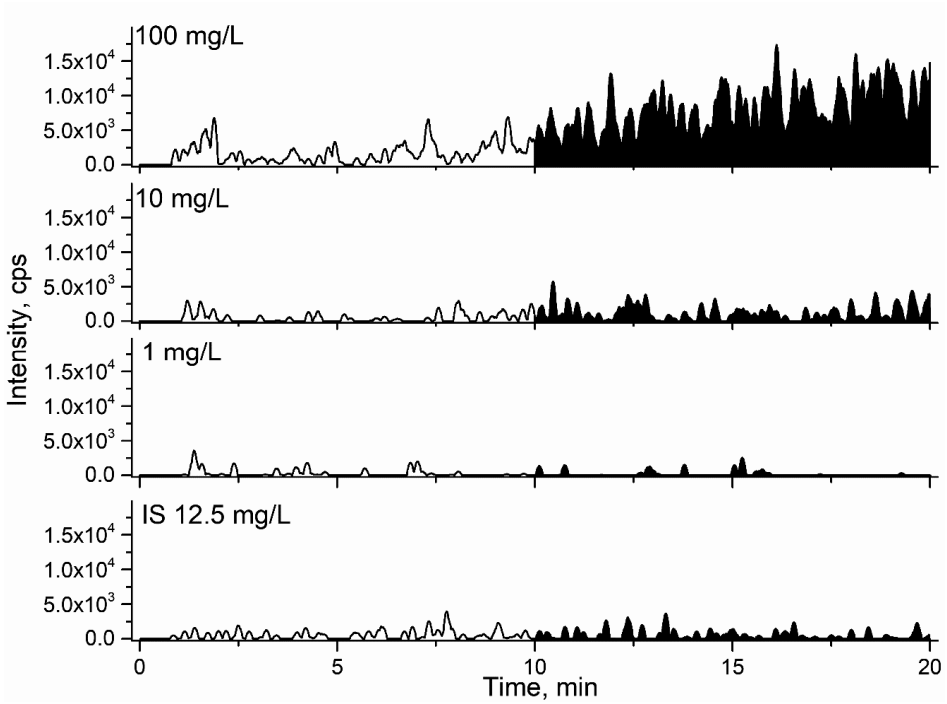


Figure 5. Extracted chronograms (*m/z* 335 → 160) for penicillin G and (*m/z* 342 → 160) for penicillin G-D_7 in plasma calibrators. The area shown in black is the 10 min integration window used for calibration.

In order to generate calibration lines for plasma and urine, the signal ratio to the internal standard was formed and a linear relationship could be established, as can be seen in **Figure 6** for plasma calibration. Along with a good reproducibility of the IS area counts gathered throughout the plasma calibration line [II Figure 5], the first experiments yielded promising results.

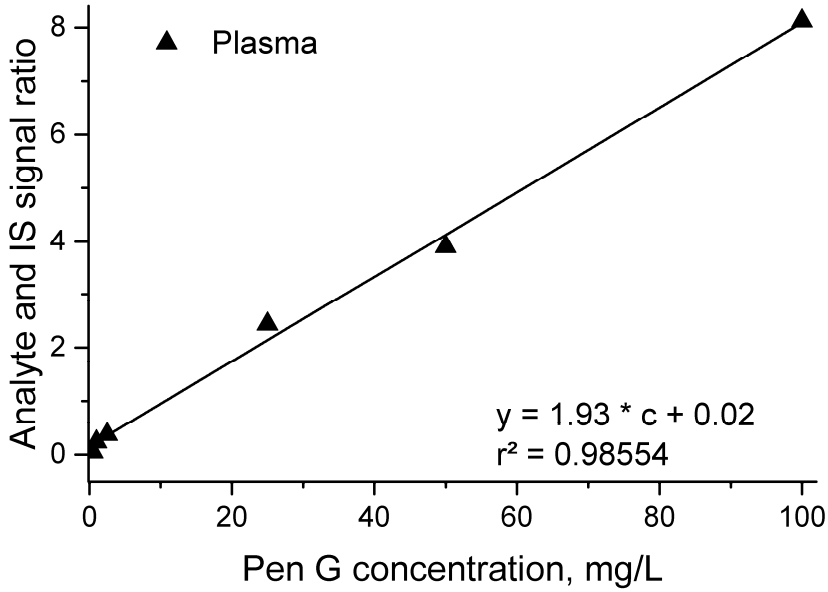


Figure 6. Calibration graphs for penicillin G in plasma using Sponge Spray Ionisation 1.0. Shown is the analyte/IS ratio versus nominal calibrator concentration.

4.3. MS signal enhancements using novel eluent additives [III]

The eluent used for the first Sponge Spray Ionisation experiments consisted of 50% (v/v) isopropanol in deionised water containing 0.1% (v/v) HFIP, 0.1% (v/v) formic acid and 1 mM ammonium acetate. The decision to include the novel eluent additive HFIP was made after promising results were obtained, showing an increase in the ionisation efficiency of model drug compounds [III]. The largest effect was observed for protonated bases, with signals intensities increasing up to 12 times for pyrrolidine compared to the standard buffer at the pH 2.8 (STD) in **Table 5**.

For the subsequent progressions of Sponge Spray Ionisation, variations in eluent compositions were tested. In order to simplify the experimental setup, HFIP or other novel eluent additives were not included in the eluent for further studies.

Table 5. MS peak areas in arbitrary units, of analytes, depending on the eluent additive and *pH of the eluent*.

Buffers	STD	Ammonium acetate			Ammonium bicarbonate		
pH	*2.8*	*8.5*	*9*	*10*	*8.5*	*9*	*10*
Diisopropylamine	74858392	259827791	282304764	325962012	748165281	983435198	617220544
Piperidine	36951200	31829927	435841644	412055740	737456548	874537709	487681984
Cyclohexylamine	43343684	21857296	11579111	84293752	53177938	26238147	172813113
Pyrrolidine	19871073	124472464	225801955	214569904	424734254	489621143	319021394
Aniline	14028476	23930212	10369357	7485969	12633819	8677645	4872316
4-Chloroaniline	3693034	1433397	1623281	663150	1180256	710624	0
1-Naphthylamine	37571474	9192311	11054570	17838959	5829051	4248195	11219666
Histamine	45640498	19966293	37233899	71218374	94795867	21389881	206610641
4-Fluoroaniline	17323060	824948	880179	152739236	704823	24661	666824
2,6-Dimethylpyridine	34076735	18709230	14002765	12784909	15008685	12270164	8648053
2-Methylpyridine	18745688	12801912	11330318	9158476	12105982	8185669	6455491
2-Methoxypyridine	1261010	7494630	4839327	4500942	0	2358877	1679280

Buffers	HFIP			HFTB		
pH	*8.5*	*9*	*10*	*8.5*	*9*	*10*
Diisopropylamine	704997132	615654076	496892865	333312822	337862293	288311488
Piperidine	331672124	421579291	320686646	218435887	191120905	234928250
Cyclohexylamine	14993795	421579291	15199699	4507696	24881479	92735730
Pyrrolidine	235068562	245899209	227365482	115431127	119385673	75687968
Aniline	3438511	4061713	6456661	8667960	3349964	5203304
4-Chloroaniline	453822	35633	544774	131011	325143	714582
1-Naphthylamine	10369670	1364609	33798754	297066	1581832	51123196
Histamine	10374512	14636993	12412524	6326567	28825185	192703709
4-Fluoroaniline	65598	29274	40312	24872	44896	1125172
2,6-Dimethylpyridine	7969485	6714204	9648835	110484905	6642802	8427954
2-Methylpyridine	5556821	5442703	7304771	127683	3369076	5692894
2-Methoxypyridine	2232143	1338285	3943342	1569807	2267639	2524737

4.4. Alterations in the Sponge Spray Ionisation setup

The slow, and in the case of plasma, incomplete elution of the analyte in Sponge Spray Ionisation 1.0 was identified as a key aspect that required improvement. For that purpose, the sponge was attached to a blunt metal needle, which could easily be connected to an LC, instead of a syringe pump, to deliver the solvent to the sponge. This needle also coupled the sponge to the high voltage power source, which increased the ease of use and throughput. Given that the voltage output of the corona needle used in [II] was not adjustable and the casing of the corona needle was very bulky (**Figure 1**), the new blunt needle made it less complicated to experiment with the position of the sponge relative to the MS inlet. One of two self-built high voltage sources can be seen in **Figure 3**, including the diagram schematic for the positive mode source and the final product. The second source for negative mode ionisation was similar, except the input and output of the transformer were switched.

A list of parameters adjusted to improve the spray stability during Sponge Spray Ionisation and MS signal for detection, were:

a. the eluent composition,
b. the flow rate of the solvent,
c. the possibility to apply a gradient elution scheme,
d. the voltage strength at the sponge,
e. a circular counter electrode,
f. the position and distance of the sponge from the MS,
g. the temperature and gas flow from the MS,
h. the addition of heated air from behind the sponge
i. a sponge rinsing step with hexane before the eluent was added and sprayed

To remove lipids from the sponge (i), a syringe pump supplying hexane was connected to the "divert-to-waste" valve, so that after being set to waste position, hexane was supplied to the tip. As an alkane the interaction of hexane with hydrophilic molecules should be minimal, while lipids should, in theory, be washed off. This rinsing step lasted for 30 seconds, forming 2 drops, and was followed by the 95% isopropanol in deionised water (v/v) eluent at 100 µL/min, through switching the valve again. The eluent mixture was miscible with, and substituted, hexane from the sponge during the sample run. An astonishing electrospray formed at the bottom of a large droplet, but in contrast to a regular Taylor-cone, the overall shape of a droplet persisted, as can be seen in **Figure 7**. The standard isopropanol-water eluent forms either drops, when no voltage is applied, or sheds excess liquid instantly once the voltage is applied and only a very thin film of solvent remains around the sponge. The gradual change in the solvent's composition on the tip led to an unstable spray, which could hardly be maintained by manually adjusting the voltage on the sponge.

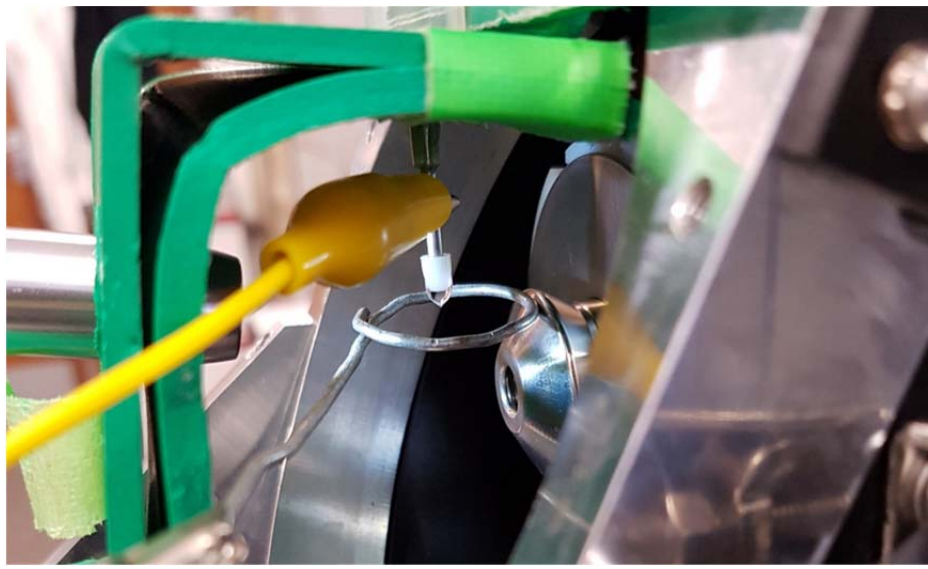


Figure 7. Sponge Spray Ionisation using a hexane rinsing step. An unusual combination of droplet and electrospray formation can be seen as, the bottom of a large droplet narrows, where an electrospray is produced. Also, the round counter electrode setup can be seen. The holder displayed here is an intermediate top-down arrangement, before the 3D printed holder was introduced.

Similarly, continuously increasing the organic solvent's proportion (c), much like in a gradient elution using an LC pump, produced an unstable spray, which could only be sustained, by decreasing the voltage gradually. Manual adjustments were performed, once the spray had ceased, trying to recreate a stable spray. The resulting gaps in the recorded data, caused by the lack of spray, and the overall missing reproducibility between samples made this approach unfeasible. For fixed water to organic solvent ratios (a), comparable to isocratic elution, the voltages can be adjusted well, and a continuous spray over the analysis runtime could be achieved. The voltage strength (d) was very depended on the distance to the MS inlet cone (f), the flow rate and eluent mixture used, and had to be checked and, if necessary, adjusted for every new sponge.

The implementation of a focusing counter electrode (e) in front of the sponge (as seen in **Figure 7**), much like in the cartridge of Salentijn et al. [133], created multiple smaller sprays directed towards the counter electrode rather than one spray towards the MS orifice, decreasing the obtained signal. Also, a heat gun emitting 400 °C was placed approximately 15 cm behind the sponge (h), but this only led to the evaporation of the solvent from the large surface of the sponge, without achieving a stable spray. To find a balance between drying the droplets and not blowing the droplet away from the MS, the gas supplied by the MS (g) was set to the highest possible temperature of 350 °C at a moderate flow rate of 9 L/min.

Increasing the flow rate (b) from 15 µL/min in [II], it was possible to generate spray and signal at 500 µL/min, but the spray quality was poor, containing large droplets ejecting from the spray. At similar flow rates in LC-MS, the spray desolvation is supported by heat and gas, which were not feasible for Sponge Spray Ionisation. When droplets enter into the ion transfer capillary, as fitted in the used MSs from Agilent, these droplets can reduce or block the ion transmission rate. A flow rate of 100 µL/min was selected for further studies, as is demonstrated a good balance of speed and spray quality.

In order to protect the MS from these droplets, a similar arrangement as seen in the orthogonal spray configuration of many LC-MS ESI interface was attempted – by focusing the spray downwards (g) and collecting the ions, rather than directing the droplets directly into the MS. For that purpose, a 3D printed holder (see **Figure 2**) was assembled, with the goal to switch between samples in less than 1 minute. The blunt needle holding the sponge was connected to a LC via a Luer lock and slid into the holder (**Figure 2 bottom**). The power for the high voltage source was interrupted during the exchange of sample sponges and connected to the needle via a touching bent metal paperclip. With a short flush of solvent to fill the hollow needle and sponge (**Table 1**), the initiation time for when the spray is formed could be reduced from 2 in **4.2** to 0.5 min.

The lack of a sharp tip on the sponges is beneficial in generating a recognisable electrospray Taylor cone, but it is not good for the ionisation as the ejected droplets should be as small as possible at the tip of the cone [134]. Without the addition of a nebulising gas flow and heaters to aid the process, low flow rates and a high organic eluent content are beneficial.

Concluding the experiments, the top-down arrangement (**Figure 2**) was considered preferable to safeguard the MS, while it continued to be perceived less sensitive. The 3D printed holder allowed for an increase in sample throughput, as the samples could be exchanged quickly. Unfortunately, it also allowed for small wiggle variance in sponge placement, which in turn required frequent voltage adjustments. A higher flow rate using a higher percentage organic solvent was employed, focusing the method on analysis speed.

4.5. Analysis of fosfomycin in cerebrospinal fluid with Ultivo™ QQQ MS using Sponge Spray Ionisation 2.0

The antibiotic called fosfomycin could greatly benefit from a rapid measurement and therapeutic drug monitoring due to its aggressive interaction with bacteria and the patient alike. With a molecular mass of 138 g/mol, fosfomycin is small enough to cross the blood brain barrier in patients and can be found in CSF as well. As a matrix, CSF generally provides little ion suppression, as it consists mainly of water, salts, vitamins and peptides/proteins [135]. Fosfomycin can be ionised both in the positive and negative modes, enabling a comparison of both modes used in this measurement technique. Additionally, the expected concentrations of fosfomycin in CSF are rather high (see **3.3**), which

makes it into a promising compound to analyse with Sponge Spray Ionisation, which so far had demonstrated low sensitivity.

It was possible to compare three parameters in the experimental set-up of Sponge Spray Ionisation 2.0 using fosfomycin as a model compound. First, both positive and negative ionisation modes were tested, second, a comparison between dried samples and freshly sampled and immediately "sponge sprayed" tips could be made, and third, 1 year over stated shelf life VAMS devices were compared with recently purchased devices.

Table 6. Parameters and area counts of fosfomycin at 250 µg/mL in the CSF test and IS pre-deposited at 50 µg/mL to determine the preferable ionisation polarity mode, sampling time and sponge age.

Ionisation mode	Sample form	Sponge age	Fosfomycin (area count)	Fosfomycin-$^{13}C_3$ (area count)
negative	wet	old	79.35	24.86
negative	wet	new	50.62	25.15
negative	dry	old	36.15	28.48
negative	dry	new	49.82	23.05
positive	wet	old	173.58	320.50
positive	wet	new	231.24	277.20
positive	dry	old	404.50	302.63
positive	dry	new	333.73	654.23

The older VAMS devices filled with the sample considerably slower, taking more than 30 s, than the new ones, which only required 5 s to fill completely. The VAMS itself can be produced from different materials, including sintered glass, sintered steel, sintered ceramics, and sintered polymers of plastic, preferably sintered polyethylene. Some of the mentioned materials, such as polyethylene, then require surface modification to achieve hydrophilicity [56]. This can be achieved by adding a surfactant to the VAMS but is limited to amounts that do not foster the haemolysis of the blood cells collected. Alternatively, oxygen gas plasma, can add polar groups to surfaces and increase their hydrophilic properties [139]. Judging by the decreased speed of absorption in old VAMS, this treatment loses effect with time, and while VAMS devices as old as 3 years are still considered functional, their handling requires more patience to ensure volumetric sample collection. Using Sponge Spray Ionisation, the integrated chronograms calculated from spraying the aged sponges did not differ much from the newer ones, as can be seen in **Table 6**.

The freshly sampled, wet sponges produced an unstable spray during the first minute of analysis, requiring a manual adjustment of the high voltage to

improve and keep the spray consistent. The continuous addition of the solvent changed the ratio of water to the organic solvent on the sponge, which required less voltage to spray, as discussed above for the hexane and gradient elution experiments. The interruption in the spray is visible in **Figure 8 A** after 0.5 min and again, after 1 min. The signal is slightly stronger for wet samples in negative mode than that of the dried sample, but this is not the case in positive mode. The dry sample also had a short period of no spraying, which could be recovered shortly after.

The fastest possible way to analyse a biological sample is to use a freshly sampled and still wet sponge to spray and quantify a compound from it. The spray could be generated with a constant high percentage organic solvent diluting the sample, requiring, again, a manual adjustment of the voltage. These adjustments were very subjective and focused on the formation of a Taylor cone, rather than the signal at the detector and the always looming possibility of not recording any reliable signals for a sample (**Figure 8**).

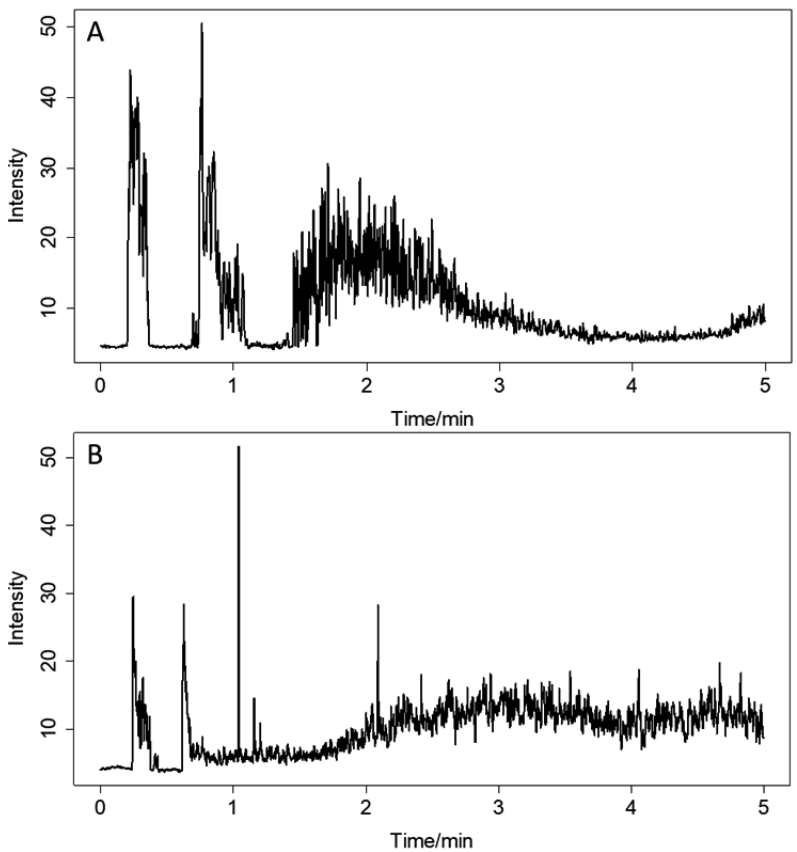


Figure 8. Chronograms of fosfomycin in CSF (summed MRMs) from a new wet sponge (A) and a new dry sponge (B) in the negative ionisation mode.

With the progression of mass spectrometers becoming a standardised instrument, which can also be used by untrained analysts, limitations are implemented into the software of the instrument. An opportunity to improve ambient ionisation techniques to work with QQQs would require the option for experienced users to lift those limitations. Being able to open the selection window for Q1, to select the analyte and the isotopically labelled internal standard simultaneously, followed by a narrow window scan of the fragments, a true representation of the ion suppression for every MS cycle affecting the analyte and the internal standard identically, could be obtained [136], [137].

4.6. Analysis of fosfomycin in CSF with TOF MS using Sponge Spray Ionisation 2.0

A further test of ionisation modes was performed using an Agilent TOF MS, after the area of fosfomycin obtained for the positive ionisation mode in **Table 6** on the QQQ MS was much higher than that obtained when the negative ionisation mode was used. An accurate mass mass-analyser like a TOF or an Orbitrap MS inevitably detect both ions (analyte and IS) simultaneously and are simply, for that feature, better detectors for ambient ionisation techniques [138]. Furthermore, they do not require the fragmentation of the analyte molecule for identification, which results in better sensitivity [139].

The spectra of the respective m/z region for both modes can be found in **Figure 9**. In the negative mode, an ion can be found at m/z 137.0007, with mass accuracy - 1.46 ppm. In contrast, the positive mode spectrum shows three mass-to-charge ratios in close proximity, ranging from - 372.62 to 827.25 ppm around the actual m/z of the proton charged fosfomycin of m/z 139.0155. The three ions are technically far enough apart for a TOF mass analyser to separate and distinguish the analyte of interest.

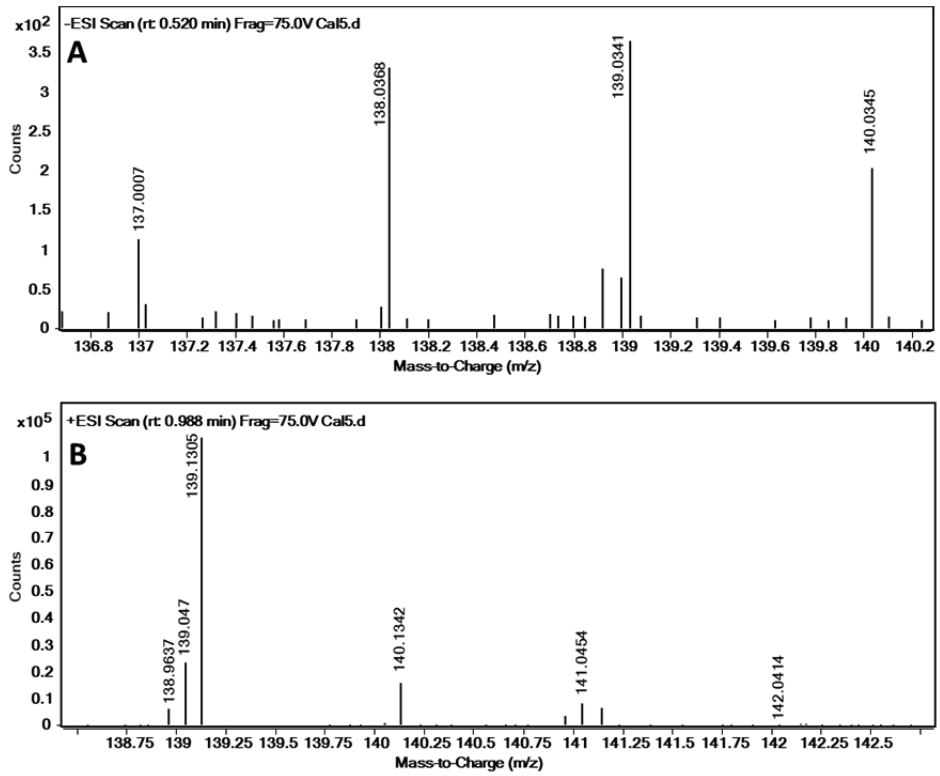


Figure 9. Mass spectra for fosfomycin and the internal standard fosfomycin-$^{13}C_3$ in the negative (A) with the theoretical *m/z* 137.0009 and *m/z* 140.0086 and the positive (B) ion mode with the theoretical *m/z* 139.0155 and *m/z* 142.0232, respectively, recorded on an accurate mass TOF MS.

However, the same three ions will be selected in the first quadrupole of a QQQ MS, and since the fragments monitored have very low mass and are common fragments (**Table 2**), the high intensities in the positive mode for fosfomycin with Ultivo™ MS can be explained. In fact, when a set of calibrators was measured, no change in the signal with an increase in concentration was seen when using either MS.

Another important lesson from using the positive detection mode in the experiment with TOF MS was the necessity to use reference ions with m/z close to that of the target analyte. In the experimental set-up of TOF instruments, ions of a known exact mass are being continuously introduced into the MS in addition to the sample, in an LC-TOF assembly this happens via a dual-nebuliser ESI-source. The reference ions are provided in a mixture of compounds, which in turn produce ions on the lower and higher border of the mass detection. The recorded times of those two ions are used to form a two-point calibration to convert other ions time-to-detector into the mass-to-charge ratio. For Sponge Spray Ionisation such an external reference ion introduction setup was not

available. A workable solution, used for Sponge Spray Ionisation TOF experiments, was to add these compounds to the eluent used during the experiment.

For the test in **Figure 9 B**, the regular reference ions chemicals used with the Agilent TOF instrument, purine at *m/z* 121.0509 and HP-921 at *m/z* 922.0098 (both not shown) were added to the eluent. However, due to the lack of signal when the spray was forming at the beginning of each measurement, the software did not recognise those ions as reference ions. This resulted in shifting *m/z* ratios over the course of one Sponge Spray Ionisation run, making it impossible to extract the same analyte ion from multiple runs. A re-calibration of each recorded spectrum can be performed manually in post-processing, but since each 5 min run contains 900 spectra, such re-calibration was not done.

For further TOF experiments, the reference ions were selected as close to the analyte *m/z* as possible. In the negative mode (**Figure 9 A**), the ions used for reference were trifluoroacetic acid at *m/z* 112.9856 and purine at *m/z* 119.0363 resulting in easier result processing. However, the sensitivity was insufficient for the calibrators to construct a full calibration range of fosfomycin, starting from 1 µg/mL in CSF at the low end.

Overall, when assessing the more favourable ionisation mode, the positive mode resulted in a stronger signal, which could be consequently attributed to interfering ions close in mass-to-charge to the analyte (**Figure 9**). The negative mode, however, did not yield sufficient sensitivity for the target analyte of fosfomycin. It can generally be assumed that positive ionisation charges more molecules, which in addition to the interference seen here, also enables ion suppression, mostly from phospholipids [140]. In a traditional LC-MS analysis this fact is being addressed by sample preparation and the separation of the interfering compounds using chromatography, both options are not available for ambient ionisation as used here. However, a tool that can be applied is an ion-mobility spectrometer, separating ions according to their collision cross section, possibly overcoming the interference [141]–[143].

4.7. Analysis of methadone and EDDP in plasma with TOF MS using Sponge Spray Ionisation 2.0

Another target analyte measured using the TOF-MS was methadone, which has a molecular mass of 309.45 g/mol. Therefore, for methadone detection in the positive ionisation mode, the upper reference ion was selected with HP-321 at *m/z* 322.0485 (R2), while the lower one was kept with purine at *m/z* 121.0509 (R1). Additionally, the methadone metabolite EDDP and the internal standard methadone-D_9 were analysed (see **Table 3**).

This experimental set-up provided an interesting opportunity to evaluate the use of the reference ions themselves as substitutes for the internal standard. This would also help to exclude the time-consuming step of preparing (sampling and air-drying) sponges with internal standard prior to sampling. A method of using reference ions as IS would be more suited for an untargeted analysis because it

would enable a relative interpretation to any ion, which can be observed in the scan generated by the TOF.

An exemplary concentration to area calibration for methadone was created from the calculated area of methadone extracted ion chronogram, as well as the ratio to the internal standard and both reference ions. Linear and quadratic fits were evaluated, using different weightings. The weighting of the calibration lines increases the impact of lower concentrations, helping to maintain the accuracy requirements (see **1.7**), as the absolute deviation at a higher concentration is generally smaller. The best fits for methadone calibration curves were obtained using quadratic fits with $1/x^2$ weighting for the area count and the ratio to the internal standard, and 1/x weighting for both reference ions, as can be seen in **Figure 10**.

The selection of calibration lines was made by excluding a maximum of two points, but not at the same calibrator concentration, and allowing a difference of ± 20% for accuracy. These criteria were set to mimic the European Medicines Agency's bioanalytical method validation guideline [103], while allowing a slightly larger imprecisions to the set ± 15% accuracy of the guideline [108] therein for all calibration points and QCs except for the LLOQ. For calibration fits that did not fulfil these requirements, the closed viable fit was selected to enable comparison of all variation investigated. Additionally, failing calibrators were denoted with underlined values in **Table 7**.

The fits closest to fulfilling the set out conditions for the methadone area, the ratio of methadone to the IS, the reference ion 1 (R1) and to reference ion 2 (R2), are displayed in **Figure 10**, respectively, and the values used and accuracies are shown in **Table 7**. The accuracies were calculated as the percentage of the calculated area or the area ratio value to the theoretical value obtained from the fit at the same concentration. One sample of each of the highest and second highest calibrators were excluded from all fits (italic denotation in **Table 7** and crosses in **Figure 10**). This resulted in the remaining points passing the criteria for two of the options investigated, the area ratio to the IS and area ratio to reference ion 1. Interestingly, for passing calibration lines, the quality control samples are outside the given tolerance. As for the fits that have an additional one or two non-duplicate calibrations points outside the ± 20% accuracy boundary (underlined values in **Table 7**), in the methadone area and the area ratio to R2 fits, the quality controls would pass, as no duplicates and a maximum of two QCs of the measured 6 fail.

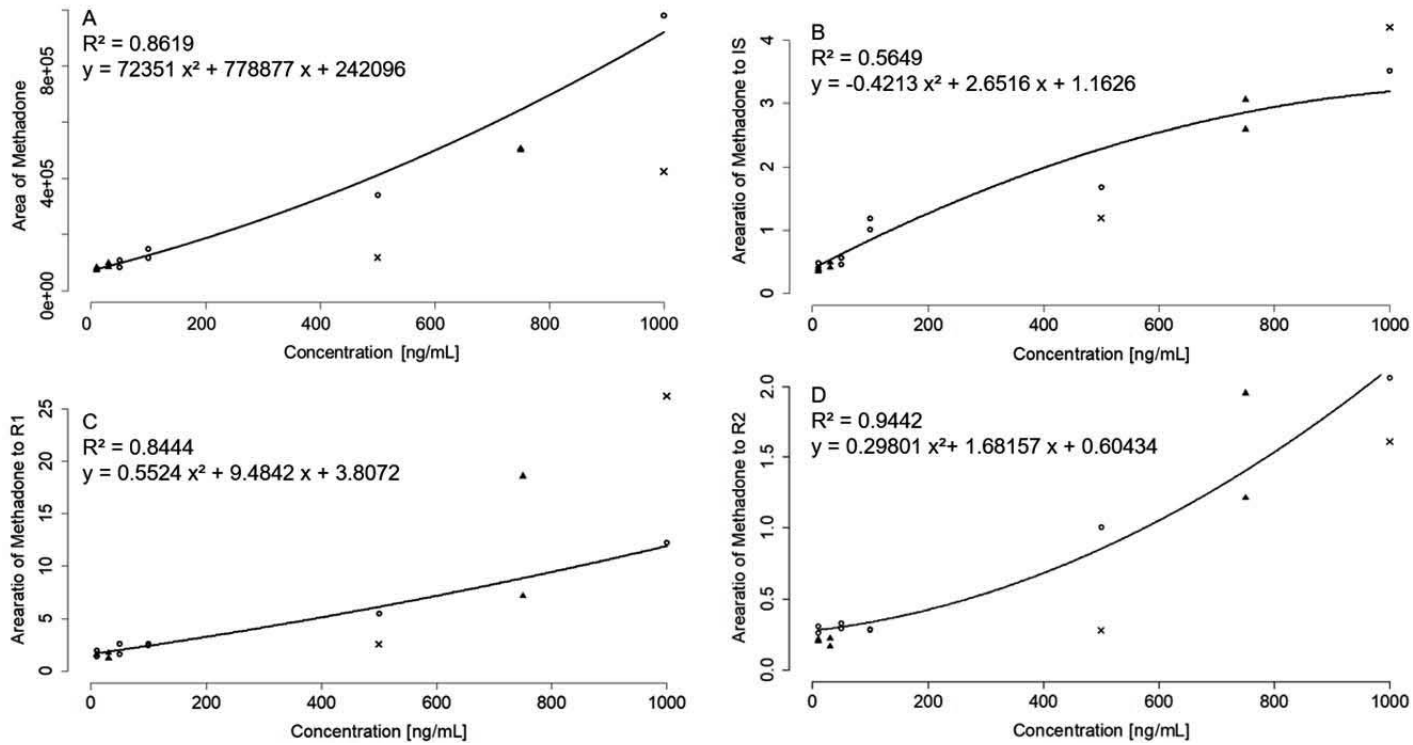


Figure 10. Quadratic calibration lines of methadone using TOF-MS and 10 µL VAMS sponges. A) Calibration was performed using the methadone area count, while in B) the area ratio to the internal standard was used. The ratios to either reference ion can be seen in C) and D) for R1 and R2, respectively. Empty circles express included points, crosses are excluded points from the calibration line samples measured in duplicates. Triangles express quality control samples.

Table 7. Area counts and accuracies of methadone in plasma calibration lines and quality controls measured from 10 µL sponges on a TOF-MS. The areas and ratios to the internal standard and reference ions are also shown. Italic values have been excluded from the calculation of the calibration line; underlined values exceed the ± 20% accuracy threshold.

Sample	Concentration [ng/mL]	M	IS	R1	R2	M/IS	M/R1	M/R2	Accuracy M	Accuracy M/IS	Accuracy M/R1	Accuracy M/R2
C1	1000	980355.4	278333.7	80121.4	475290.6	3.5	12.2	2.1	102.5%	96.9%	106.5%	110.3%
	1000	425620.8	101141.6	16212.9	264736.2	4.2	26.3	1.6	*220.0%*	*75.5%*	*46.2%*	*131.8%*
C2	500	340819.5	203516.0	62243.1	338216.9	1.7	5.5	1.0	89.2%	117.9%	82.8%	73.3%
	500	281102.6	96172.8	25593.4	248024.1	2.9	11.0	1.1	*178.8%*	*132.6%*	*68.3%*	*127.9%*
C3	100	149120.5	147224.7	60047.8	516173.7	1.0	2.5	0.3	101.8%	85.5%	117.9%	119.4%
	100	118477.1	99323.4	45348.6	421329.9	1.2	2.6	0.3	107.1%	83.2%	93.7%	<u>140.6%</u>
C4	50	110784.0	242484.9	67273.3	375811.2	0.5	1.6	0.3	81.0%	96.8%	112.6%	<u>73.1%</u>
	50	82805.3	147717.8	31653.7	252267.5	0.6	2.6	0.3	<u>128.6%</u>	107.8%	84.1%	89.8%
C5	10	79136.7	197700.1	56219.3	255384.2	0.4	1.4	0.3	81.9%	109.5%	102.5%	91.3%
	10	75270.0	156568.4	38018.1	287970.0	0.5	2.0	0.3	115.2%	92.4%	97.5%	109.7%
QC1	750	499472.0	192537.6	69498.2	411958.4	2.6	7.2	1.2	81.0%	86.3%	<u>77.4%</u>	90.6%
	750	505097.3	164908.3	27149.0	258797.8	3.1	18.6	2.0	<u>209.6%</u>	<u>138.9%</u>	<u>78.3%</u>	107.0%
QC2	30	97780.0	240527.6	78220.1	436080.5	0.4	1.3	0.2	<u>66.7%</u>	<u>76.5%</u>	111.5%	<u>76.4%</u>
	30	83014.8	168420.6	47075.7	503704.8	0.5	1.8	0.2	94.0%	<u>56.2%</u>	94.7%	92.6%
QC3	10	73853.3	193531.3	45791.0	366020.7	0.4	1.6	0.2	93.8%	<u>71.3%</u>	95.7%	87.1%
	10	81456.7	232005.7	51930.2	378657.1	0.4	1.6	0.2	91.2%	<u>76.0%</u>	105.5%	80.1%

M: methadone, IS: methadone-D_9, R1: Reference Ion 1 (*m/z* 121.0509), R2: Reference Ion 2 (*m/z* 322.0485)

Using the TOF-MS for this analyte presented two features: first, the potential of the technique to be adopted to any target molecule, possibly even without using an internal standard, only using the reference ions required to run the instrument by itself. Second, it showed the inconsistency of this particular ambient ionisation technique, working as desired for the quality controls, while not producing a viable calibration line to go with it.

4.8. Analysis of methadone and EDDP in plasma with Ultivo™ QQQ MS using Sponge Spray Ionisation 2.0

An identical set of calibrators and QCs (described in **3.3** and **4.7**) of methadone and EDDP was measured with QQQ MS for two different sponge volumes of 10 µL and 30 µL. The areas reported in **Table 8** are for the calibrators and quality controls used on 10 µL sponges and in **Table 9** for 30 µL sponges. The areas differ drastically between the duplicates of the same calibrator in both volumes. Additionally, the smaller volume sponge showed higher area counts for both analytes. However, a change in the areas of methadone-D_9 ensured that the ratio between the analyte and internal standard yielded reasonable results.

Linear calibration lines for EDDP and methadone from the 10 µL sponges could be constructed using a 1/x weighting, by excluding one of the highest and second highest calibrators from each, as they did not fit the curve, and only one QC failing (italic values in **Table 8** were excluded, underlined values in **Table 8** and **Table 9** failed).

This being said, for 30 µL sponges, a calibration line without weight adjustment resulted in the best linear fit and fulfilled the set-out accuracy requirements for the calibration mentioned above. No points needed to be excluded and the lower limit of quantification was within the 20% deviation rule. The described calibration fits can be seen in **Figure 11**. One theory is that, although the analyte signal was lower, the noise was reduced as well, possibly being hold back in the larger sponge.

The differences in the area for the same concentrations are associated with the spray quality during the measurement. Even though the improvements implemented included the sponge being in a fixed position to the MS, the spray generated was still inconsistent. Partially it was due to the high voltage generator (**Figure 3**), which had to be adjusted for every sample and the spray quality was evaluated visually.

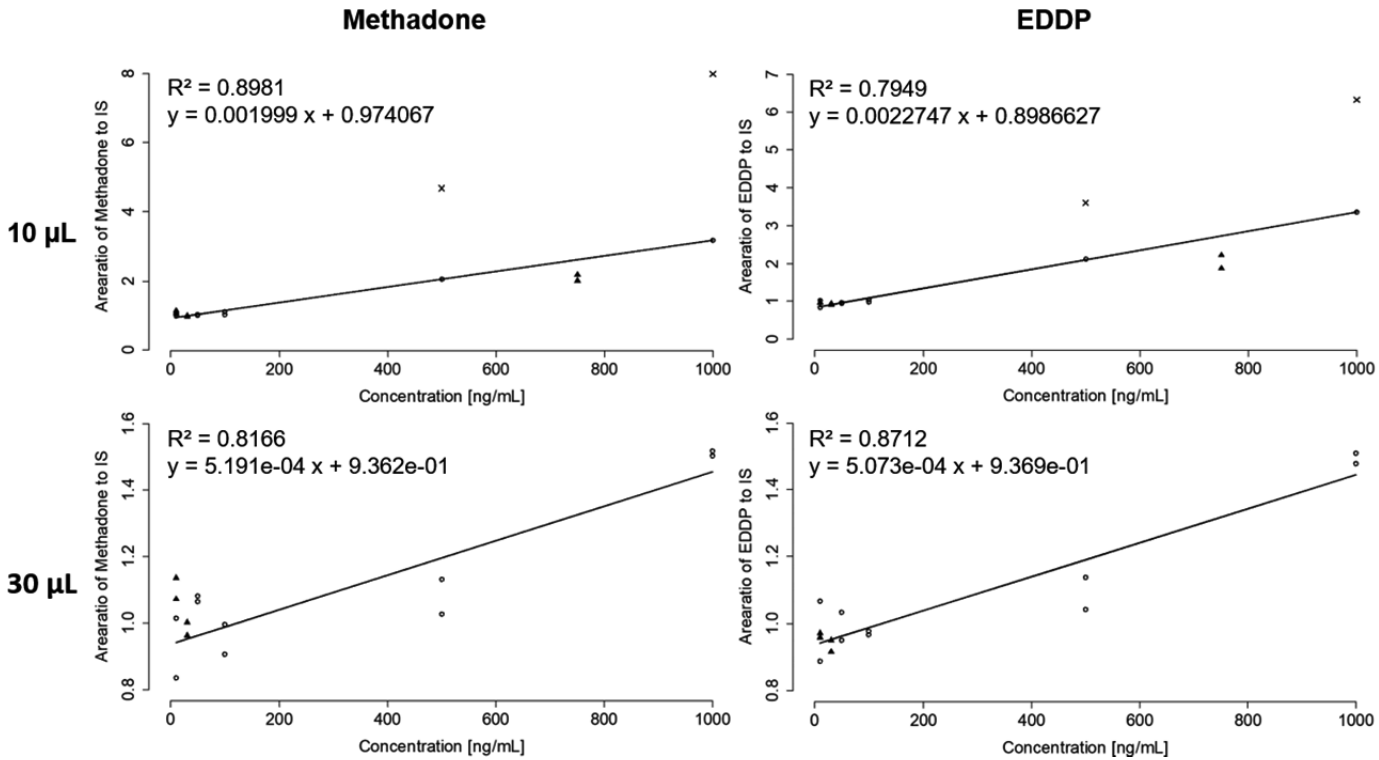


Figure 11. Calibration lines for methadone and EDDP obtained from 10 and 30 µL sponges on the QQQ MS. Empty circles express included points; crosses are excluded points from the calibration line samples measured in duplicates. Triangles express quality control samples.

Table 8. Areas of integrated chronograms and accuracies of EDDP and methadone measured from 10 µL sponges in the plasma on Ultivo™ using a 1/x weighting. Italic script indicates excluded calibrators and underlined are failing QC samples.

Sample	Concentration [ng/mL]	E	M	IS	E/IS	Accuracy E/IS	M/IS	Accuracy M/IS
C1	1000	552.06	695.58	87.21	7.98	*251%*	7.98	*268%*
	1000	101.38	95.69	30.08	3.37	106%	3.18	107%
C2	500	451.23	586.10	125.29	4.68	*415%*	4.68	*398%*
	500	60.78	58.97	28.70	2.12	104%	2.05	104%
C3	100	140.68	156.96	141.69	0.99	108%	1.11	111%
	100	34.70	34.08	33.22	1.04	93%	1.03	87%
C4	50	378.95	411.50	399.61	0.95	94%	1.03	96%
	50	246.86	253.94	253.10	0.98	96%	1.00	93%
C5	10	1020.80	975.51	990.42	1.03	112%	0.98	99%
	10	44.55	54.69	52.39	0.85	92%	1.04	105%
QC1	750	187.51	201.87	100.12	1.87	<u>72%</u>	2.02	<u>82%</u>
	750	117.92	115.94	53.16	2.22	85%	2.18	88%
QC2	30	490.26	496.89	515.75	0.95	98%	0.96	93%
	30	203.41	222.67	222.03	0.92	95%	1.00	97%
QC3	10	175.65	205.38	180.75	0.97	105%	1.14	114%
	10	545.45	609.91	568.46	0.96	104%	1.07	108%

E: EDDP, M: methadone, IS: methadone-D_9

Table 9. Areas and accuracies of EDDP and methadone measured from 30 µL sponges in the plasma using a calibration line without weighting. Underlined are failing QC samples.

Sample	Concentration [ng/mL]	E	M	IS	E/IS	Accuracy E/IS	M/IS	Accuracy M/IS
C1	1000	205.62	209.04	128.24	1.60	111%	1.63	110%
	1000	42.11	44.56	28.67	1.47	102%	1.55	101%
C2	500	655.10	721.24	629.84	1.04	87%	1.15	87%
	500	247.87	228.22	220.46	1.12	94%	1.04	94%
C3	100	529.43	487.19	547.82	0.97	98%	0.89	98%
	100	549.64	567.80	570.95	0.96	97%	0.99	97%
C4	50	509.39	529.30	493.41	1.03	107%	1.07	107%
	50	73.50	82.30	77.33	0.95	99%	1.06	99%
C5	10	47.96	47.37	59.32	0.81	86%	0.80	86%
	10	42.36	40.74	38.34	1.11	117%	1.06	117%
QC1	750	61.76	63.44	54.15	1.14	87%	1.17	86%
	750	234.79	241.86	194.06	1.21	92%	1.25	91%
QC2	30	26.70	27.22	28.33	0.94	99%	0.96	99%
	30	360.94	377.95	350.01	1.03	108%	1.08	108%
QC3	10	27.49	28.19	29.78	0.92	98%	0.95	98%
	10	52.59	48.79	45.91	1.15	122%	1.06	122%

E: EDDP, M: methadone, IS: methadone-D_9

In order to calculate these calibrations, extensive data post-processing was required, as can be seen from a representative chronogram of the highest calibrator of methadone in **Figure 12 A** – the signal recorded was very noisy. Intense singular spikes presented themselves throughout the chronogram, which did not co-occur with the other monitored transitions or the internal standard transitions. From that, it can be concluded that these spikes are a form of noise, and the actual signal was extracted using a noise filter function (see **3.3.1**) in R (**Figure 12 B**). The sum of the three MRM chronograms was then integrated over the entire measurement time (5 min), as can be seen in **Figure 12 C**.

In contrast, the TOF analyser did not show such noise spikes (**Figure 12 D**). In comparison, the same noise reduction which was used for the data obtained with Ultivo™ MS was also applied to the TOF chronogram and can be seen in **Figure 12 E**, but it was not used for further calculations. Instead, the original trace was integrated (**Figure 12F**).

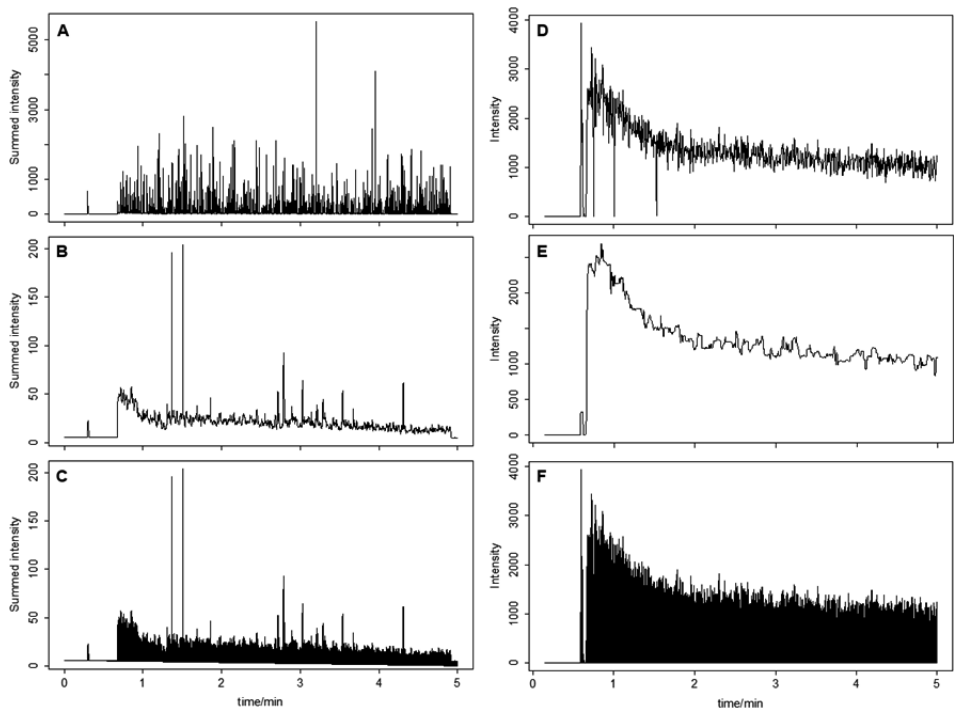


Figure 12. Signal over time (chronogram) of Sponge Spray Ionisation from the dried plasma from the highest calibrator (1 µg/mL) for methadone, recorded on a QQQ (A), the noise reduced with the filter function in R(B) and the noise reduced and integrated of the sum of three MRMs for methadone (C), as well as the record on a TOF-MS (D), noise reduced (E) and integrated, but not noise reduced (F).

The length of analysing one sponge could be reduced from 20 min in **Figure 5** to 5 min using Sponge Spray Ionisation 2.0 (**Figure 12**), with a total of 36 sponges being processed in one workday, including pre-depositing IS, drying for 30 min, sampling and again drying for 60 min. Future improvements of the method to reduce the time necessary to generate a result would be: pre-prepared sponges containing IS; analysing wet samples, given an automatic voltage adjustment; and a dedicated ambient ionisation analysis software. Increasing sensitivity has been an ongoing struggle, but presumably it depends largely on the analyte in question. It is likely that the small and polar antibiotic fosfomycin could not be eluted, ionised, or both to its full extent, due to a suboptimal eluent composition, whereas methadone, a far less polar compound, displayed a strongly tailing "peak" (**Figure 12**) with the 95% isopropanol in deionised water eluent. Therefore, a library or prediction software of suitable solvent mixtures for target molecules, much like an LC-retention time prediction model using the chemical structure dependent parameters for chromatographic separation [144], [145], could prove beneficial. A better understanding of the sponge-sample-eluent interaction is required to ensure analyte extraction, while avoiding the ion suppression of coeluting matrix components.

In order to move forward and make Sponge Spray Ionisation a part of a viable point-of-care test, the drawbacks detailed above need to be addressed. The mass spectrometer used should ideally be an accurate mass variant, or a triple quadrupole with a linear ion trap functionality. The solvent supply from an LC-pump was beneficial and could be miniaturised using a portable LC-platforms [146], [147]. The signal strength did not increase by using a three-times larger sample volume sponges. The methods lack in sensitivity is its biggest weakness.

4.9. Makin data below the limit of quantification usable [IV]

The sensitivity of any bioanalytical assay is dependent on the sample preparation technique used, the instrumentation available and, in the best case, by the selection of the analyst, the lowest concentration point of which it is necessary to quantify. The chosen limit is not the LOD, but the LLOQ, required by method validation guidelines to fulfil a 20% of coefficient of variance criterion for LC-MS assays.

Establishing an LOD is not required during validation; however, it would be beneficial for all assays. It would disambiguate the ability of a method to detect an analyte from the background of a blank sample and the arbitrary set limit of quantification in the form of the calibration line.

In the case of a significant number of samples during a study fall BLQ, the LOD of the method could help to make use of these samples [IV]. The ideal solution would be to revalidate the method with an adjusted calibration range to include the BLQ samples and to analyse the samples again, which usually is not possible, due to limited sample volumes and lack of resources available.

A post sample analysis estimation of the LOD could be achieved with a minimal workload at the laboratory if an LOD-sample would be fortified and quantified in three consecutive measurements [IV]. In this manner, a coefficient of variance for the LOD-sample can be calculated and, in a conservative designation, be assigned to all values below the LLOQ samples of the study, enabling the analyst to report the data.

4.10. Rapid-LC-MS/MS determination of MDMA content in Ecstasy tablets [V]

There are only a few cases where illicit recreational drugs such as MDMA, are investigated in a clinical study to estimate pharmacokinetic, pharmacodynamic and toxicologic parameters. It has been researched in the treatment of post-traumatic stress disorder, alcoholism and anxiety associated with autism, in combination with psychotherapy [148]. Recreationally consumed Ecstasy tablets vary substantially in their dose, with tablets purchased online in the Netherlands containing a slightly higher dose, than those purchased on the street [149].

In order to protect users, the practice of on-site testing to identify illicit drugs at events such as festivals has been implemented through the police, security, and non-governmental organisations. The next step for improving the testing effort would be the estimation of the dose in a tablet, which is not viable with the spectroscopic detection or portable gas-chromatography MS instrumentation currently available. As a foundation for an LC-MS based test, the dose in MDMA tablets, from a collection of tablets from the United Kingdom since 2001, was measured in a retrospective study [V]. Additionally, the determination of the dissolution profiles of the 247 tablets of Ecstasy at 11 time points was performed.

For that purpose, a rapid-LC-MS, with an analysis runtime of 36 seconds per sample, was employed. This very short runtime was achieved by drastically increasing the flow rate to 1.25 mL of eluent per minute and decreasing the column length (instead of an analytical column, a 5 mm pre-column was used in its place) to minimise backpressure, while still achieving chromatographic separation. Post-column diffusion needed to be eliminated as much as possible to keep a good peak shape, which was accomplished by connecting the “analytical-pre-column” used for separation, directly to the ESI-source.

The results obtained showed large differences in the dissolution speed, which can be grouped into three groups of fast (n = 95), intermediate (n = 67), and slow (n = 85) releasers, which could be distinguished at the 15 min time point (**Figure 13**). These differences in dissolution profiles could not be attributed to the colour or shape of the tablet, thus are impossible to determine for the user. However, the MDMA dose in the tablets does not significantly differ between the different dissolution groups (V Fig. 5A). This indicates that there are low dose MDMA tablets, which release the MDMA fast, but also and more dangerously, there are high dose tablets, which release the MDMA content

slowly. The latter type is especially dangerous for oblivious user as the tablet taken will not achieve the desired effect before the user decides to ingest the next tablet, thus leading to an eventual overdose.

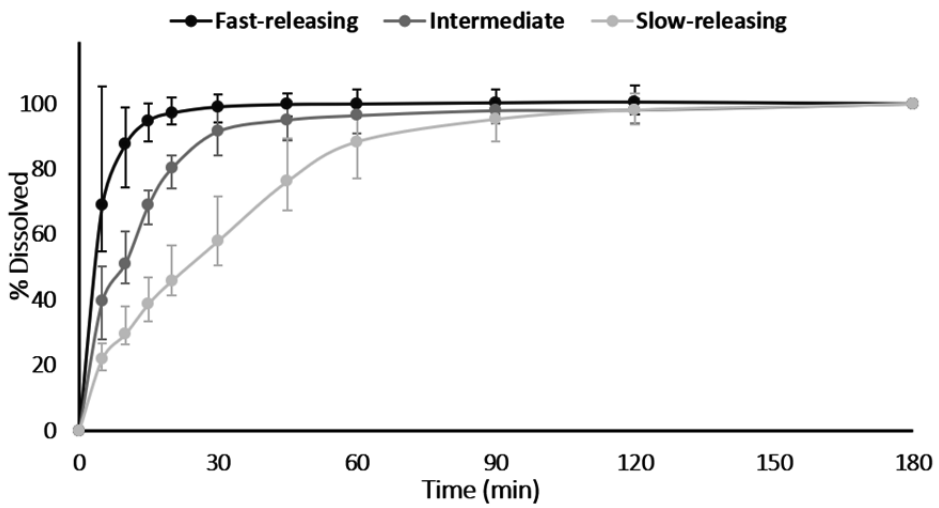


Figure 13. Dissolution profiles showing the median (25th and 75th percentile) % dissolved vs time, for tablets classified as fast- releasing, intermediate-releasing, and slow-releasing at the 15-minute time point.

The poor consistency in content and non-existing quality control for these counterfeit drugs found in the study, combined with the disproportional individual responses of the human body, show exactly how risky drug use is. Testing illicit tablets and informing society of dangers connected to drug use is in many ways easier than analysing a sample of human origin, even more so in the field, and it can also prevent the harm, whereas a blood or saliva sample only helps in the diagnosis of what already went wrong. By applying the method used in this study to the field, for example in a mobile laboratory, the turnaround time for analysis is just right to inform and engage the user concerning the risks.

4.11. A comprehensive analysis of a multitude of pharmaceuticals in Ecstasy tablets

Some of the MDMA tablets contained more than just one drug, be it substituting missing ingredients or to "enhance" the experience for the user. As a pilot project tablets known to contain mixtures were analysed on the Ultivo™ QQQ MS studying over 100 drugs and their metabolites.

In order to quantify a number of analytes previously detected using gas-chromatography MS and to screen for further common toxicological compounds, a 10 min routine method from an in-house QTOF-MS toxicology screening assay was adopted on the Ultivo™ QQQ-MS. Using 12 isotopically labelled IS, 107 compounds were analysed in dissolved tablet samples, while several drugs were merely detected but not quantified due to the lack of an appropriate standard in the calibration mixture.

An example chromatogram from a calibrator (1 mg/L) can be seen in **Figure 14**. The unintegrated total ion current (TIC) chromatogram appears noisy, due to additional conformation MRMs being triggered by a threshold exceeding the quantifier signal, contributing to the TIC line.

Within 33 samples (tablets), collected in the period 2004–2017, MDMA, MDEA and caffeine were quantified (see **Figure 15**). Furthermore, amphetamine and methamphetamine were quantified in trace levels, likely being impurities arising during the production of tablets, but only denoted as detected in **Figure 15**. The calibration ranged from 0.01–1 mg/L and was linear with $R^2>0.993$ for all analytes. A weighting of 1/x was used for the calibration.

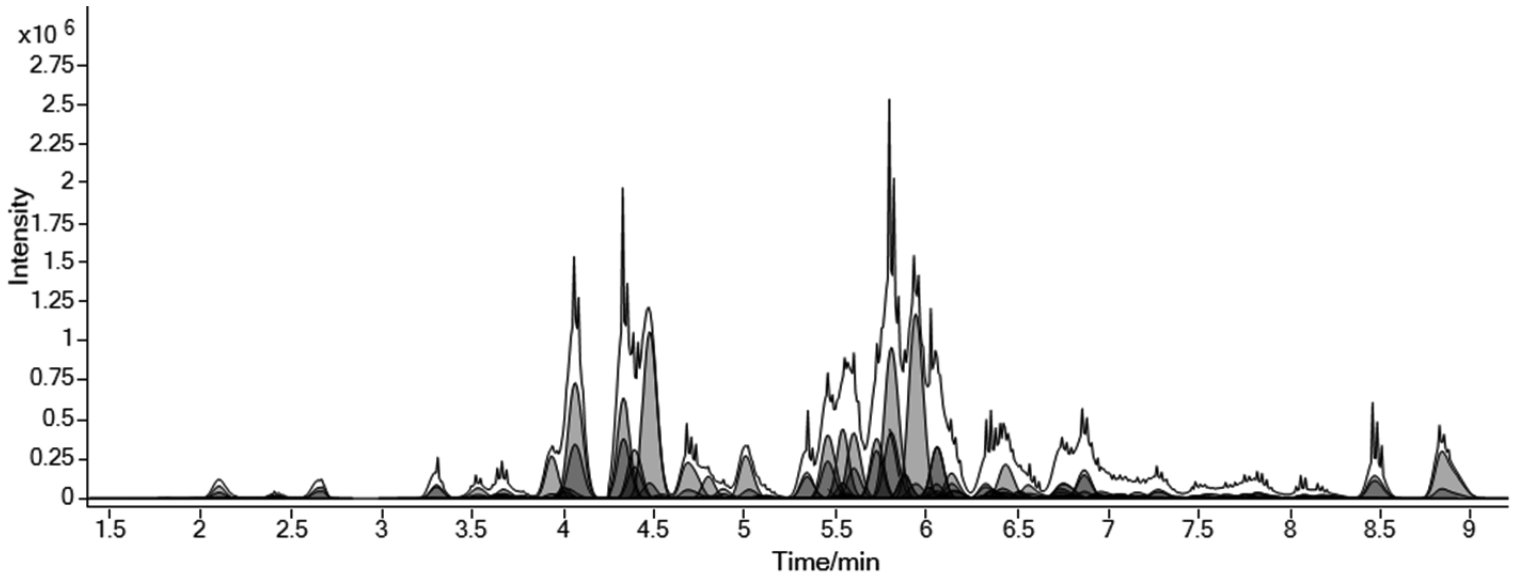


Figure 14. Chromatogram obtained from a calibrator (1 mg/L) using the described method for 107 drugs and 12 different IS (integrated peaks). The unintegrated line shows the TIC.

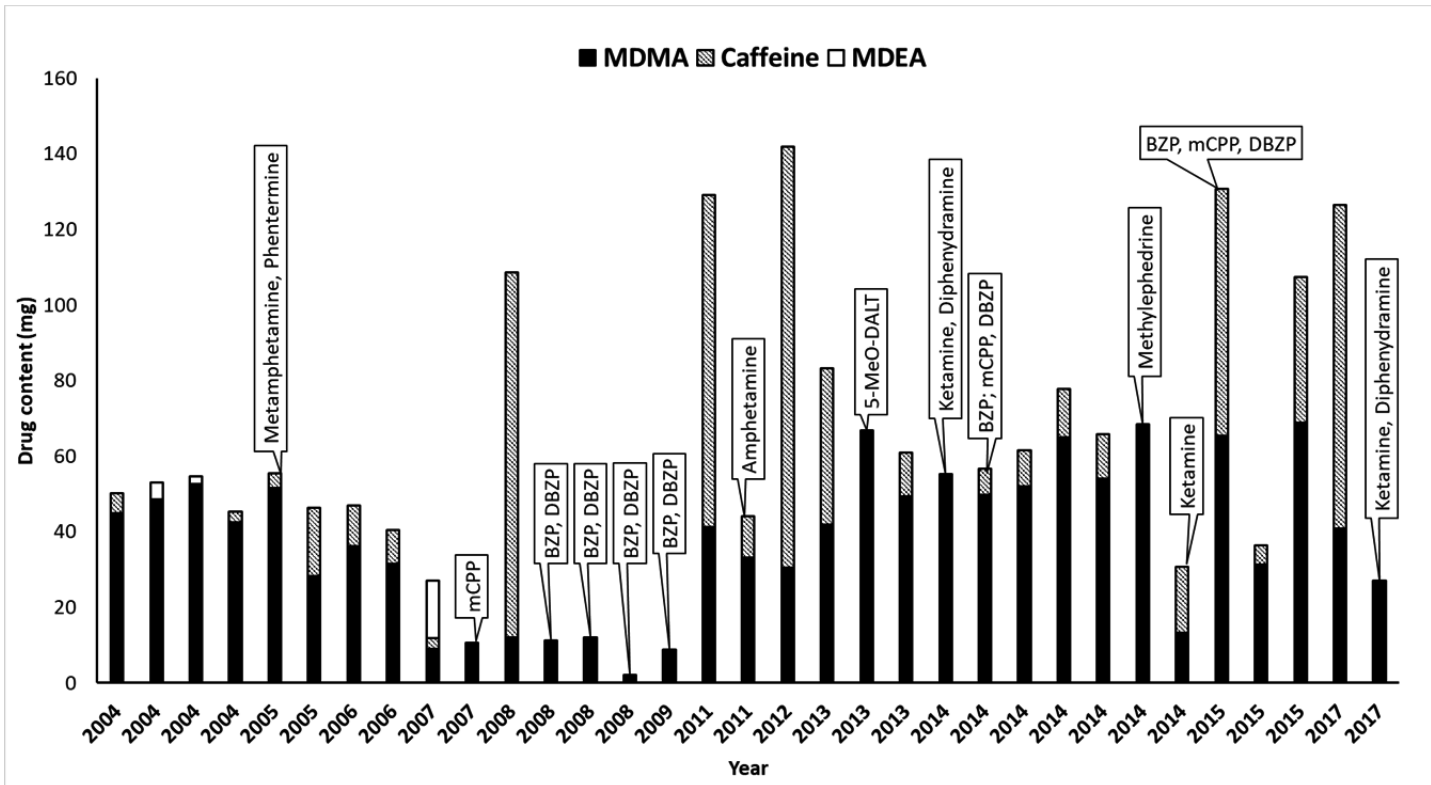


Figure 15. Drug content of 33 Ecstasy tablets known to contain a secondary drug alongside MDMA. Quantification was carried out for MDMA, MDEA, caffeine, amphetamine, and methamphetamine, while a confirmation of identity was determined for others.

Until 2006, the initial occurrence of a second drug in tablets as indicated in **Figure 15** shows how it was only added to "fill" the desired dose of the tablet to reach a total dose of approximately 50 mg, as was a common dose during that time [150]. Overall, every second tablets analysed had an addition of caffeine (to lower the costs and complexity, while still producing an effect on the user).

Other compounds detected, with number of occurrences in parenthesis, were cocaine (1), ketamine (3), methylephedrine (1), phentermine (1), 1-benzylpiperazine (BZP) (6), 1,4-dibenzylpiperazine (DBZP) (6), meta-chlorophenylpiperazine (mCPP) (3), diphenhydramine (2), and N,N-diallyl-5-methoxytryptamine (5-MeO-DALT) (1). In 2008 and 2009 a number of tablets containing the novel psychoactive substances BZP and DBZP could be identified. Tablets containing these piperazine were not limited to these two years and could be found in 2014 and 2015 as well (**Figure 15**). The dose of MDMA in tablets containing co-formulated drugs was significantly lower than in MDMA-only tablets ($p < 0.001$).

The production of Ecstasy tablets is not regulated, and no quality control steps are undertaken. On-site testing becomes even more important for the safety of an event and the consumers. Portable Raman and infra-red spectrometer are capable of determining the identity of powders and crystals, but the binding material of tablets may interfere.

Truck-mounted LC-MS systems have been used for the analysis of cannabis in Canada [151] and a laboratory van in Austria was able to determine approximately 40 samples per hour on-site at events and raves [V]. The currently used visual identification of previously encountered shapes and colours and the reagent kit colouring test, would be well supplemented by on-site screening using MS [152].

The rapid analysis of illegal drugs, prior to ingestion, as well as the biomarkers and hormones influenced after the consumption of psychoactive and therapeutic drugs is a continuously growing field of interest. The cost and outcome benefits of an immediate result are tremendous, for the patient and society.

Applying mass spectrometry to the field promises: sensitivity and specificity; effortless adjustability to new analytes; and a fast speed of testing. All these are good arguments for using MS, but the ability to multiplex is what makes MS stand out. It has the ability to distinguish quantitatively hundreds of molecules in a short period, given some separation through gas, liquid, supercritical fluid, or paper chromatography and it is what sets MS apart.

However, transforming an expensive piece of equipment, such as a mass spectrometer, into a point-of-care device does not enable to use the technology to its full capabilities. Instead, using the differentiation power of an MS on the cumulative information of biomarkers and drugs from one sample, is where mankind can finally understand human physiology, treat patients accordingly and act upon informed decision.

The last step necessary to increase the speed of such tests is to develop better sample preparation techniques.

SUMMARY

The diagnosis of diseases and the monitoring of drug treatments requires taking large blood volumes and includes long waiting times until the result is available. Point-of-care diagnostics take the testing to the patient, reporting the result within the consultation time with the physician and aiding the decision on the prescription. Unfortunately, non-laboratory tests are slow to develop, require extensive validation and suffer from slow uptake by doctors.

A perspective to solve this conundrum is the use of mass spectrometry and simple, quick and dirty ambient ionisation techniques to measure samples rapidly outside a laboratory. Generating a charged analyte from a small, complex biological sample with minimal to no sample preparation is assembled under the term: ambient ionisation technique. The generated ions are then distinguished in a mass spectrometer, which is a great quantification tool.

It was demonstrated that volumetric absorptive microsampling devices, sponge-like blood collection devices, with a defined volume, can be used to quantify several medications by using a technique named Sponge Spray Ionisation. No sample treatment between sample collection and analysis was required. The fixed volume sponge was prepared by depositing an isotopically labelled analyte analogue and dried, before the sample was collected. By doing so, the relative interpretation of the results for the quantification was enabled. Different Sponge Spray Ionisation setups on several mass spectrometers, including a miniature QQQ instrument, were evaluated. The sample analysis time could be reduced from an initial 20 min to 5 min, and the antibiotic penicillin G and the detoxification agent methadone were successfully quantified. Without the need for sample handling and preparation, the need for a laboratory is omitted and the test required for whichever circumstance can be performed at the point-of-care.

The time until the result is available is a crucial aspect of point-of-care analytics. As a basis for quick-testing for future adaptation to the field, a 36 seconds per sample method was set up, using a more traditional LC-MS system. The dissolution profiles of ecstasy tablets were investigated using a gradient elution at a high flow rate through a short column generating retention. Groups of slow, intermediate, and fast releasing tablets were identified, and it was determined that there was no correlation to the shape, colour, or dose of the tablets. Only comprehensive testing on-sites where the tablets are consumed, in clubs and at festivals, can reduce the large risks to the users. An analysis of the presence and dose of co-formulated drugs in these tablets was also performed.

In addition to the analysis of drugs, the quantification of biomarkers is increasingly required, ideally also as in point-of-care diagnostics. To that end, an LC-MS assay for the identification and treatment monitoring of MNGIE-patients via thymidine and 2-deoxyuridine was developed, confirming two suspected cases, and allowing the evaluation of the effectiveness of a new kind of

treatment. Using this test in quick response to the treatment, it could be seen that the two biomarkers returned to healthy levels.

The observation of samples falling below the lower limit of quantification is a somewhat frequent phenomenon, to which only statistical methods limit the loss of important information. By determining an experimental limit of detection and evaluating its reliability, this data might become useful again. As quick, on-site test generally struggle with a lack in sensitivity, the limit of detection should become a benchmark parameter evaluated.

Advances towards a multitarget quantitative point-of-care device utilising mass spectrometry are described within this dissertation. Strength and weaknesses of combining different methods are addressed, and the most promising way forward is formulated.

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SUMMARY IN ESTONIAN

Patsiendilähedaste massispektromeetriliste mõõtmismetoodikate arendamine

Haiguste diagnoosimine ja ravimi efektiivsuse kinnitamine nõuab suhteliselt suure ruumalaga vereproovide võtmist ja sisaldab pikki ooteaegu kuni analüüsitulemused on kättesaadavad. Kohapeal patsiendi vereproovide testimine ning arsti visiidi ajal tulemuste kättesaamine aitks kaasa kiirele ravi määramisele. Mittelaboratoorsete testide arendamine võtab kahjuks kaua aega, nõuavad põhjalikku valideerimist ja ei ole sageli arstide poolt kiirelt omaksvõetud.

Selle probleemi lahendamiseks võib massispektromeetria koos atmosfäärirõhulise ionisatsioonimeetodiga pakkuda kiiret, lihtsat ning robustset lahendust proovide analüüsiks väljaspool laborit. Atmosfäärirõhuline ja toatemperatuuril ionisatsioonimeetod hõlmab ilma prooviettevalmistuseta keerulisest bioloogilisest proovist analüütide ioniseerimist ning nende eristamist, detekteerimist ning analüüdi kvantiseerimist massispektromeetriga.

Käesolevas töös näidati, et proovi võtmiseks rakendatud maht-absobeeriv proovivõtuseade ehk käsna sarnane kindla ruumalaga vere kogumise seade on kasutatav mitmete ravimite kvantitatiivseks määramiseks kasutades töös leiutatud käsnapihustus ionisatsiooni (*Sponge Spray Ionisation*) tehnikat. Proovi eeltöötlus proovi võtmise ja analüüsimise vahel ei olnud eelnevalt vajalik ning proove sai otse käsnade pealt otse mõõta. Enne proovi võtmist koguti määratud ruumalaga käsnale isotoop-märgistatud sisestandard, mille mõõtmine aitas saada korduvaid kvantitatiivseid tulemusi. Töös tutvustati erinevaid käsnapihustus ionisatsiooni võimalusi erinevatel massispektromeetritel, sealhulgas miniatuursel kolmekordsel kvadrupoolil. Esialgset proovi analüüsimise aega vähendati 20 minutilt 5 minutile ja kvantiseeriti edukalt antibiootikumi penitsilliin ning metadoon inimeste erinevatest kehavedelikest. Käesolevas töös käsitletud kiire proovi analüüs ilma eelneva proovi ettevalmistuseta aitab analüüsi viia patsientidele lähemale ning eemaldada vajaduse mõõtmisi laboris korraldada.

Analüüsi tulemusteni kuluv aeg saab määravaks juhul kui analüüsimeetod tuuakse patsiendini. Selleks, narkootikumide tarvitajatele pakkuda kiiret analüüsi nende *ecstasy* tablettide mõõtmiseks, töötati välja 36 sekundi pikkune vedelikkromatograafia-massispektromeetria (LC-MS) metoodika. *Ecstacy* tableti lahustuvusprofiile uuriti nimetatud metoodikaga ja kõrged voolukiirused ning lühike kolonn aitasid saada kiireid tulemusi. Lahustuvusprofiilidest tuvastati aeglased, keskmised ja kiiresti lahustuvad tabletid ja näidati, et puudub selge korrelatsioon tableti välimuse, värvi ja doosi vahel. Ainult laiaulatuslik testimine kohtades, kus tablette tarvitatakse (näiteks ööklubides ja muusikafestivalidel), võimaldab vähendada tarbimisriske narkootikumide kasutajatele. Analüüsiti ka teiste ravimite esinemist ja kogust nimetatud tablettides.

Lisaks ravimite analüüsile on kasvanud ka vajadus biomarkerite sisalduse määramiseks inimeste proovidest ning seeläbi parem haiguste diagnoosimine.

Biomarkerite tümidiin ja 2-deoksüuridiini määramiseks arendati LC-MS metoodika, et määrata neid aineid MNGIE-patsientidel. Sellega kinnitati 2 arvatavat haigusjuhtu ning võimaldati hinnata uut tüüpi ravi efektiivsust. Väljatöötatid metoodika rakendamine aitab ravist taastuvate patsientide tervislikku seisukorda jälgida.

Üsna sageli saadakse analüüsitulemuseks sisaldus, mis on allpool madalaimat määramispiiri. Olgu see siis tingitud näiteks analüütilise instrumendi tundlikkusest või ravimkontsentratsioonide valest hinnangust bioloogilistes proovides. Samas võivad need allpool määramispiiri mõõdetud tulemused anda olulist infot ravimite farmakokineetika kohta ja parandada modelleerimise tulemusi. Määrates eksperimentaalselt analüüdi avastamispiiri ja hinnates selle usaldusväärsust, saaks neid andmeid kasutada paremate farmakokineetiliste mudelite tegemiseks. Määramispiiri hindamine ja valideerimine võib olla samuti kasulik ka kiiretele, kohapeal tehtavatele analüüsidele, millel puudub vajalik tundlikkus.

Käesolevas töös on kirjeldatud edasiarendusi kohapealseks analüütide kvantitatiivseks määramiseks nii haiglavoodi kõrval kui ka vabas õhus (näiteks muusikafestivalidel) mitmete oluliste analüüdide näitel kasutades massispektromeetriat. Erinevate meetodite kombineerimise tugevused ja nõrkused on välja toodud ja kõige paljulubavamad analüüsivõimalused lugejani toodud.

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2008–2009	Fontane Klinik Motzen, Motzen (Saksamaa), ametnik

Teaduspublikatsioonid

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