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Rapid detection of illicit drugs in neat saliva using desorption/ionization on porous silicon

Taryn Guinan [a], Maurizio Ronci [a,b], Hilton Kobus [b], Nicolas H. Voelcker [a,*]

[a] Mawson Institute, University of South Australia, Mawson Lakes, SA 5095, Australia
[b] School of Physical and Chemical Sciences, Flinders University, Bedford Park, SA 5042, Australia

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ABSTRACT

The ability to detect illicit drugs directly in oral fluids is of major interest for roadside, workplace and athlete drug testing. For example, roadside testing for popular drugs of abuse is being rolled out by law enforcement agencies following the introduction of legislation in several countries all over the world. This paper reports on the direct analysis of methamphetamine, cocaine and 3,4-methylenedioxy-methamphetamine in oral fluids using a hydrophobic porous silicon array as a combined drug extraction and concentration medium. Analysis by laser desorption/ionization time-of-flight mass spectrometry (MS) identified these drugs with a sensitivity in line with the suggested confirmatory cut-off concentrations, and 300 times faster. In addition, MS imaging demonstrated good spot-to-spot reproducibility of the signal. Our analytical approach is compatible with multiplexing and is therefore suitable for high-throughput analysis of samples obtained from drug testing in the field. Furthermore, the application of this analytical technology is not limited to illicit drugs or oral fluids. Indeed, we believe that this platform technology could be applied to the high-throughput analysis of diverse metabolites in body fluids.

1. Introduction

Roadside drug testing has recently been incorporated into government legislation of many developed countries including Australia [1–5]. South Australian legislation allows testing for methamphetamine (MA), 3,4-methylenedioxy methamphetamine (MDMA) and cannabis in oral fluids [6]. While not part of the legislated testing allowed in Australia, cocaine is a common recreational drug of abuse and is targeted in roadside testing in other countries [7]. The tests in use in South Australia, known as Drug Wipe® and Rapiscan®, are based on immunochemical reactions and provide a positive or negative result [8–11]. These roadside tests are not considered definitive in their own right, and samples providing positive results are submitted for laboratory analysis by liquid chromatography coupled with mass spectrometry (LC–MS) or gas chromatography (GC–MS). The European Workplace Drug Testing Society (EWDTS), for example, recommends drug cut-off concentrations for screening tests of 30 ng/mL for cocaine and 40 ng/mL for the amphetamine group. For confirmation tests, the recommended cut-off levels are 8 ng/mL for cocaine metabolites and 30 ng/mL for MA and MDMA [12].

Matrix assisted laser desorption/ionization (MALDI) is a soft ionization technique that uses a UV laser to induce desorption and ionization of analyte species deposited on a suitable substrate surface [13,14]. MALDI–MS has proven to be a powerful analytical technique, which is time efficient, highly sensitive and affords high throughput analysis. The technique has become a popular tool for the analysis of various high molecular weight analytes such as proteins, peptides, oligonucleotides and polymers [14–16]. However, detection of small organic molecules below 700 Da using MALDI–MS is often difficult due to the matrix and matrix fragment peaks appearing in the same spectral range [17]. Furthermore, the non-homogenous co-crystallization of the analyte within the matrix is a significant issue when using MALDI–MS as a semi-quantitative tool [18]. More complications arise when analyzing complex biological matrices such as blood, saliva and urine. Such analysis often requires some pre-processing techniques, which often include separation via chromatography [18], solid phase extraction [19] and ultrafiltration [20].

Desorption ionization on porous silicon (DIOS) was developed in 1999 by Shen et al. [21]. In this technique, a thin porous silicon (pSi) film attached to a silicon wafer serves as the MALDI substrate. This technique alleviates the need for a matrix due to pSi's inherent UV absorbing properties and its high surface area (up to 800 m^2/g) [22–25]. The high surface area present on pSi also allows for the

* Correspondence to: University of South Australia, Mawson Institute, GPO Box 2471, Adelaide, South Australia 5001, Australia. Tel.: +61 (08) 8302 5508; fax: +61 (08) 8302 5613.
E-mail addresses: guitm001@mymail.unisa.edu.au (T. Guinan), nico.voelcker@unisa.edu.au (N.H. Voelcker).

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loading of high concentrations of drug or analytes for a wide variety of applications including drug delivery, biomaterials, biosensing and other analytical applications [26–28]. It is particularly useful for the detection of molecules from a few hundred to a few thousand Daltons as their molecular ions. pSi is able to trap small molecule analytes through adsorption onto and absorption into the pores. The energy from the incident UV laser is absorbed by the pSi film and transferred to the analyte, which can then be detected in desorbed and ionized form using a time-of-flight (TOF) mass spectrometer. DIOS therefore combines the advantage of MALDI–MS in terms of rapid and high throughput detection with the capability of generating "clean" spectra of low molecular weight compounds. These properties make DIOS an attractive method for the detection of small molecules such as illicit drugs.

Oral fluids (saliva) can be obtained at any time in a non-invasive manner, and with low risk of adulteration or substitution. In general, the presence of the parent drug in oral fluid is attributed to, predominantly, passive diffusion [29]. To date, the main application of oral fluid collection has been the provision of specimens for testing of possible drug-affected drivers [30] and workers [31]. However, saliva is a complex aqueous mixture containing a plethora of molecules including glycoproteins, enzymes, immunoglobulins, peptides and ions such as sodium, potassium, chloride and bicarbonate [32–34]. Due to this complexity, oral fluids currently undergo extraction and derivatization procedures before conventional LC–MS or GC–MS analysis [35,36]. Therefore, the development of techniques for drug analysis without the need for extraction or derivatization processes would be highly desirable.

Recently, we reported on the use of DIOS for the detection of illicit drugs in oral samples [25]. The drugs in saliva were extracted from a proprietary buffer used in roadside drug tests (Cozart® RapiScan buffer). This study demonstrated the potential for DIOS to be used for drug testing from oral fluids in the workplace, for athlete testing or roadside drug testing scenarios providing high throughput analysis, real time analysis and a non-invasive means of testing. The present paper extends the previous study by showing that the fluorinated pSi surfaces effectively extract illicit drugs from neat saliva, allowing direct detection and quantification of drugs. There is a consensus in literature that fluorosilane modification enhances DIOS MS activity. The use of (pentafluorophenyl)propyl-dimethylchlorosilane (F_5PhPr) has been widely implemented as it performs particularly well in terms of sensitivity, stability (greater than 9 months) and low background noise [37]. This paper also includes a comparison with two other fluorosilanes. Quantitative DIOS analysis is presented for illicit drugs in both water and neat saliva using deuterated internal standards. A rinsing protocol was introduced to further improve the detection of illicit drugs in neat saliva, removing the need for the extraction and derivatization used in Ref. [25]. DIOS imaging analysis was also conducted for each drug molecule in order to investigate the homogeneity of the sample spot, which is a major drawback of conventional MALDI analysis due to random sample-matrix co-crystallization.

2. Materials and methods

2.1. Reagents

Methanol (99.9%) and HF (48%) were obtained from Merck (VIC., Australia). Ethanol (EtOH) (100% undenatured) was purchased from Chem Supply (SA, Australia). Water was purified using a Labconco water purifier (MO, USA) (referred to as milliQ water). F_5PhPr, tridecafluoro-1,1,2,2-tetrahydrooctyldimethylchlorosilane (F_{13}) and heptadecafluoro-1,1,2,2-tetrahydrodecyldimethylchlorosilane (F_{17}) were purchased from Gelest Inc. (PA, USA). α-Cyano-4-hydroxycinnamic acid (CHCA) was purchased from Bruker–Daltonics (Germany).

Certified standard solutions of MA, MDMA, cocaine, and the internal standards MA-d5, MDMA-d5 and cocaine-d3 were kindly provided by Forensic Science South Australia (SA, Australia).

2.2. Preparation of drug solutions

Stock solutions of MA, MDMA and cocaine at 0.1 mg/mL were prepared by diluting the contents of each certified standard ampoule to a final volume of 10 mL in a volumetric flask. Solutions were stored at −20 °C. Working solutions of 1000 ng/mL were obtained by diluting the stock solutions with milliQ. Working solutions were kept at +4 °C and prepared fresh every 2 weeks. Illicit drug solutions at varying concentrations (~0.5–100 ng/mL in water) containing the corresponding deuterated internal standards at 20 ng/mL, were prepared from the working solutions immediately prior to analysis.

2.3. Sample deposition method for illicit drug solutions in water

Aliquots of drug solutions (0.5 μL) were deposited onto pSi substrates using multiguard barrier tips (0.5–10 μL, Sorenson, Bioscience Inc., Utah, USA) attached to a micropipette. The solvent was allowed to completely evaporate. Upon evaporation, pSi chips were mounted on a modified MALDI target plate (MTP384, Bruker Daltonics, Bremen, Germany) and analyzed.

2.4. Sample preparation and deposition method for spiked saliva

Oral fluids were obtained according to the EWDTS guidelines [12] from a drug-free volunteer, and stored at +4 °C before use for no longer than 1 week. 40 μL of neat saliva samples was spiked with 10 μL of a solution containing both drugs and internal standards to give a final concentration in the range 10–200 ng/mL for MA, MDMA and cocaine and 100 ng/mL for the internal standards. Ammonium bicarbonate buffer (1 μL, 1 M) was subsequently added to the spiked saliva and 2 μL of the resulting solution was deposited onto the pSi chip and allowed to interact with the surface for 5 min in order to facilitate extraction. The drop was finally washed away by adding 10 μL of 10 mM ammonium phosphate, pipetting a few times and discarding the solution.

2.5. DIOS analysis

Mass spectra were collected using an Autoflex Series III Bruker MALDI-TOF-TOF mass spectrometer equipped with a SmartBeam (337 nm, Nd:YAG) 200 Hz pulsed laser, operated at 200 Hz frequency, and laser attenuator offset of 55% in reflectron positive (RP) mode. Mass spectra were generated by averaging 500 individual laser shots. Data acquisition used flexControl 3.3 (build 85) software and data analysis was performed using flexAnalysis version 3.3. Instrumental parameters for the RP acquisition were set as follows: 19.00 and 16.80 kV for ion sources 1 and 2, respectively, 8.25 kV for the lens and 21.00 and 9.40 kV for reflectors 1 and 2, respectively. Quadratic external calibration of the TOF tube was performed before each acquisition on the monoisotopic masses of CHCA adducts, namely CHCA$[M+H-H_2O]^+$, CHCA$[M+H]^+$, CHCA$[M+Na]^+$, CHCA $[2M+H-CO_2]^+$, CHCA$[2M+H]^+$ and CHCA$[3M+Na_2]^+$.

2.6. MS imaging

The Autoflex Series III Bruker MALDI–TOF–TOF, was used to perform mass imaging analysis in the RP mode in the range

20–1200 Da with a spatial resolution of 100 μm and summing 200 laser shots for each measuring point. FlexImaging 2.1 (build 25) (Bruker-Daltonics) was used to control flexControl 3.3 during the acquisition. FlexImaging was used to extract ion intensity map images, after processing the datasets by baseline subtraction, normalization and data reduction. ClinProTools 2.2 (build 83) was used as the spectra analysis and visualization tool.

2.7. Calculation of limit of detection (LOD)

The LOD was defined as three standard deviations above the average noise measured in the mass region corresponding to the mass of the protonated drugs when no drug was present. Eighteen blank replicates over three different pSi surfaces were acquired for each drug.

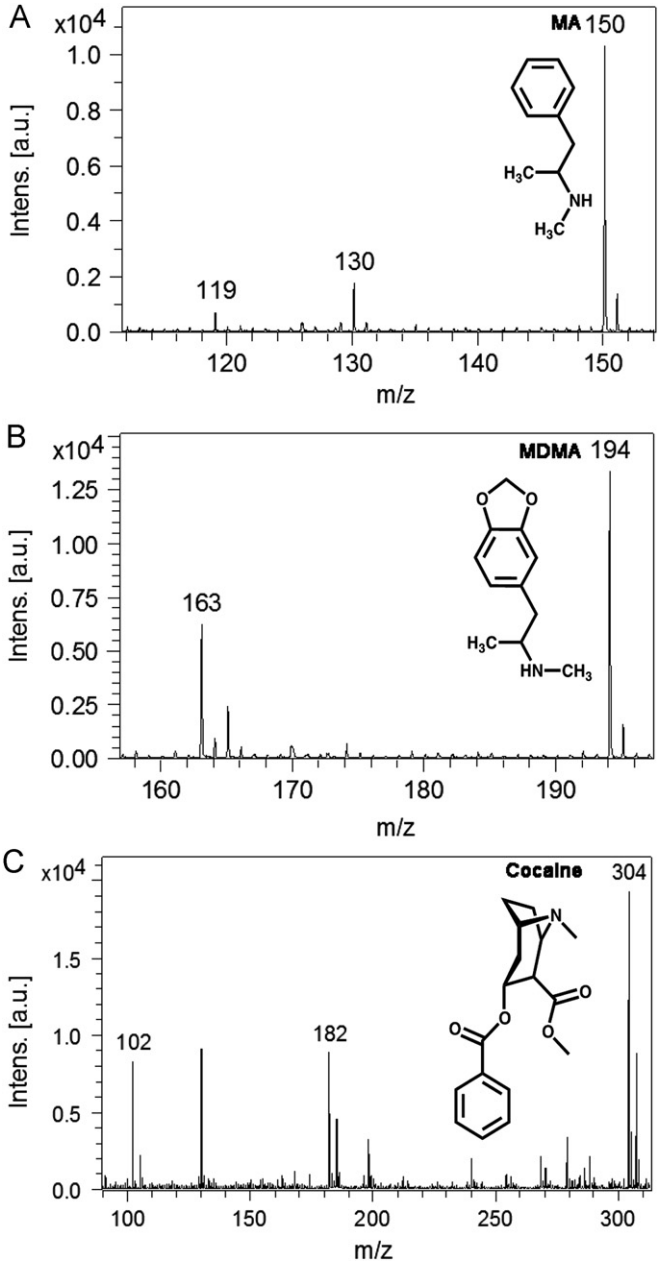

Fig. 1. Representative DIOS mass spectra and their corresponding structural formulae for (A) MA with a $MH^+ = 150$ m/z, (B) MDMA with a $MH^+ = 194/z$ and (C) cocaine with a $MH^+ = 304$ m/z.

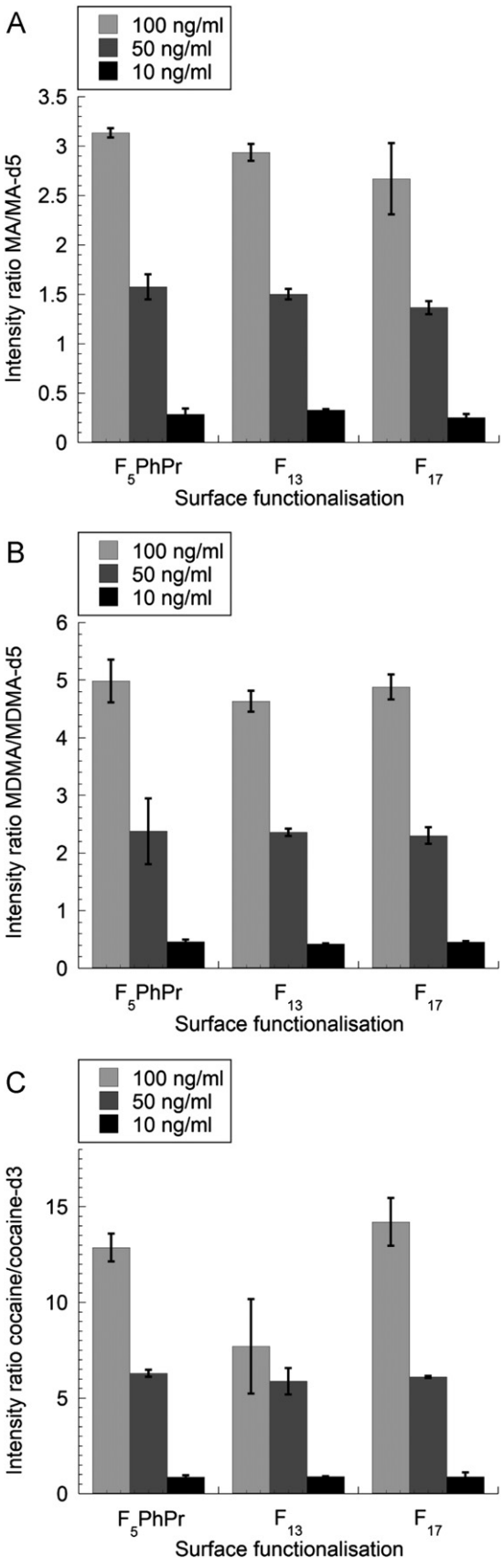

Fig. 2. Average signal intensity ratios with standard deviation for DIOS MS detection of (A) MA/MA-d5, (B) MDMA/MDMA-d5 and (C) cocaine/cocaine-d3 in milliQ water on surface-modified pSi surfaces ($n = 3$, 500 shots per spectrum).

2.8. Statistical analysis

The statistical significance of the difference between the extraction performances of the three different surface chemistries was assessed by performing a Three Way Analysis of Variance (ANOVA). The chosen factors were surface chemistry, type of drug and level of concentration and the response data was the average signal intensity ratio from three experimental replicates. SigmaStat 11 was used to run the analysis, setting the confidence to 95% and the Holm–Sidak method was used for the pairwise multiple comparison procedures.

3. Results and discussion

Scanning electron microscopy (SEM) images of the pSi surfaces are depicted in Fig. S-1a. SEM analysis showed that homogeneous pores approximately 120 nm in diameter (therefore falling into the macroporous regime) were consistently obtained by light-assisted anodization. Tapping mode atomic force microscopy (AFM) examination confirmed the SEM results (Fig. S-1b). Etching conditions produced surfaces that were consistent from etch to etch. These pore sizes are similar to those published in the reference study and have been shown to give optimal performance in DIOS [22,25]. Fig. S-1c shows an array with 100 pSi spots fabricated by etching through a photomask. This array was used for multiplexed analysis of MA, MDMA and cocaine in both water and neat saliva.

Infrared (IR) spectra of each surface modification are depicted in Fig. S-2. IR spectra of the oxidized and silanized pSi (Fig. S-2) confirmed that the anticipated surface characteristics were achieved after each chemical modification step. The freshly etched pSi surface showed vibrations at 900 cm^{-1} (attributed to the SiH_2 scissor vibration) and 2100 cm^{-1} (Si–H stretching vibrations in Si_2H–SiH or Si_3–SiH) in accordance with the expected vibrational signature for a hydride-terminated surface (I). These peaks disappeared upon oxidation and new peaks were observed at approximately 1100 cm^{-1} (O–Si–O stretching vibration) and 3500 cm^{-1} (Si–OH; II), consistent with successful surface oxidation. Upon silanization with F_5PhPr (III), F_{13} (IV) and F_{17} (V), additional peaks were observed in the region 2850–3000 cm^{-1} (see Fig. S-2b). These peaks correspond to CH_2 stretching vibrations. In addition, the peaks at 1500 cm^{-1} were attributed to benzene ring vibrations for the F_5PhPr functionalized surface. Overtones were also observed at 1150 cm^{-1} and 1220 cm^{-1} for CF_2 stretching and at 1250 cm^{-1} for CF_3 asymmetric stretching vibrations. A peak at approximately 1442 cm^{-1} was observed which could be attributed to C–H or C–F bending vibrations. Due to the monolayer nature of the silane coating and transmission mode of the IR performed, the peaks corresponding to oxidized pSi were still visible.

A key advantage of using DIOS for the detection of illicit drugs is the limited fragmentation and the presence of molecular ions in the spectra. Fig. 1A and B shows representative DIOS mass spectra for MA and MDMA in water, respectively. The major peak of MDMA attributed to the molecular ion appeared at m/z of 194. A fragment ion at 163 m/z was also observed, due to the loss of $CH_3NH_2^+$. Similarly, the molecular ion for MA (m/z=150) was observed in the MA DIOS mass spectrum with peaks at m/z=119 due to subsequent loss of $CH_3NH_2^+$. The protonated molecular ion for cocaine (m/z=304) alongside fragment peaks of lower intensity can be seen in Fig. 1C. The loss of $C_6H_5CO_2$ from the cocaine molecule resulted in the peak at m/z=182 being observed

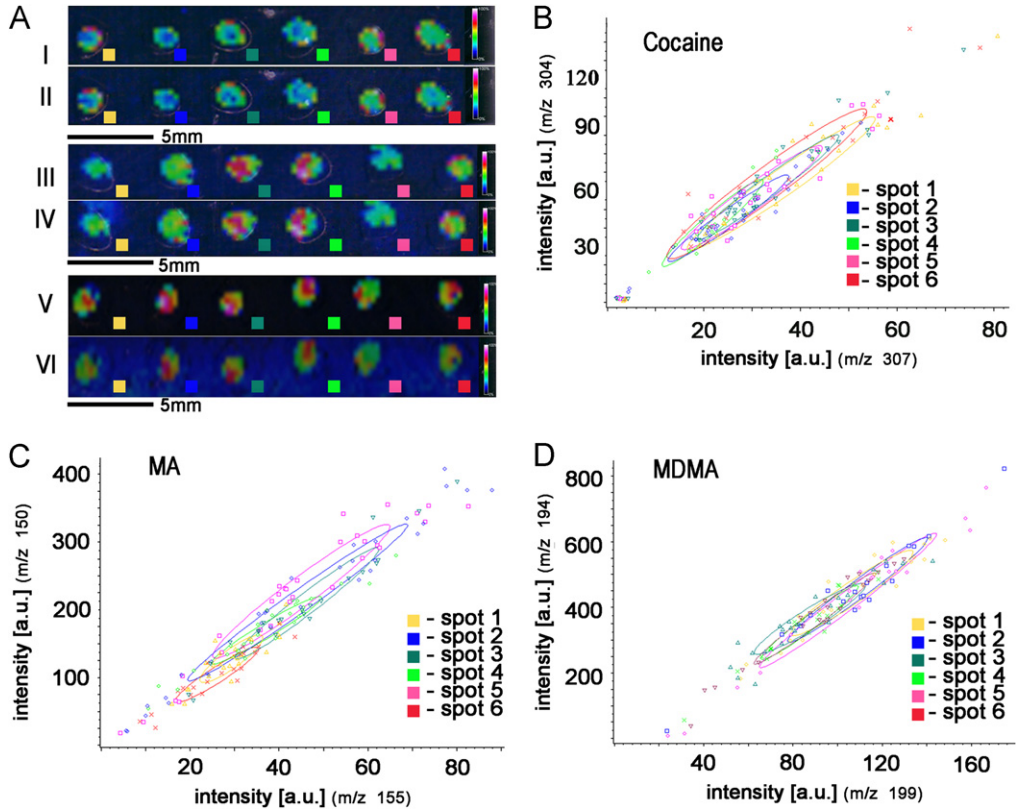

Fig. 3. DIOS imaging for A(I) cocaine (25 ng/mL) and A(II) cocaine-d3 (20 ng/mL) in water with a mass filter at=304 and 307 m/z, respectively, A(III) MA (80 ng/mL) and A(IV) MA-d5 (20 ng/mL) in water with a mass filter at=150 m/z and 155 m/z, respectively, A(V) MDMA in water (80 ng/mL) and A(VI) MDMA-d5 (20 ng/mL) with a mass filter at=194 m/z and 199 m/z, respectively. Intensity distribution comparisons for spot to spot analysis for (B) cocaine (25 ng/mL) and cocaine-d3 (20 ng/mL), (C) MA (80 ng/mL) and MA-d5 (20 ng/mL) and (D) MDMA (80 ng/mL) and MDMA-d5 (20 ng/mL).

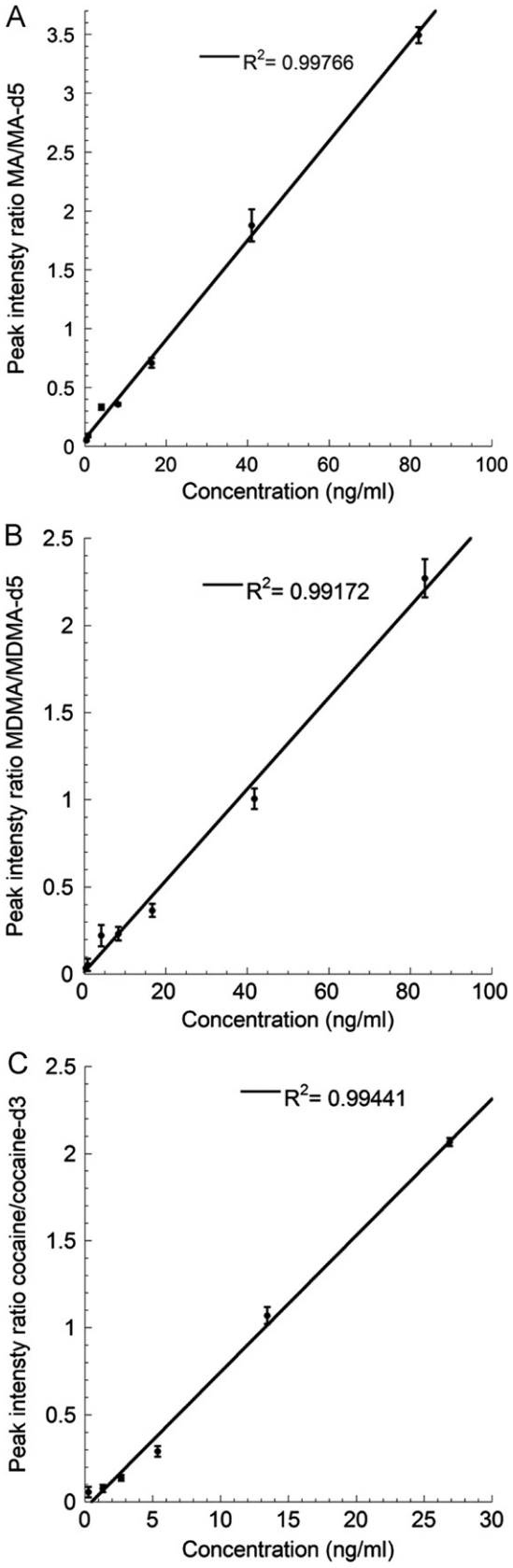

Fig. 4. Linear regression curves fitted for average peak intensity ratios for (A) MA in milliQ water for concentrations ranging 0–100 ng/mL on an F_{13} modified pSi surface, (B) MDMA in milliQ water for concentrations ranging 0–100 ng/mL on F_{13} modified pSi surface and (C) cocaine in milliQ water for concentrations ranging 0–30 ng/mL on F_5PhPr modified pSi surfaces in milliQ water. Error bars correspond to standard deviation ($n=3$, 500 shots of spectrum).

[38]. In addition, fragmentation due to α-cleavage was observed at $m/z=105$ [38]. DIOS is highly suitable for direct confirmatory analysis of illicit drugs as evidenced by the generation of unambiguous molecular ions for MA, MDMA and cocaine and the low background noise in the spectra. Although fragmentation patterns can provide clues on molecular structure, interpreting a mass spectrum can become difficult in the absence of molecular ion peaks. In addition, current MALDI–MS instruments usually have MS/MS capability, and if fragmentation information is required, they can operate in post-source-decay or the LIFT mode [23]. These results also suggest the possibility of using DIOS MS for multiplexed analysis of different target compounds in a single run.

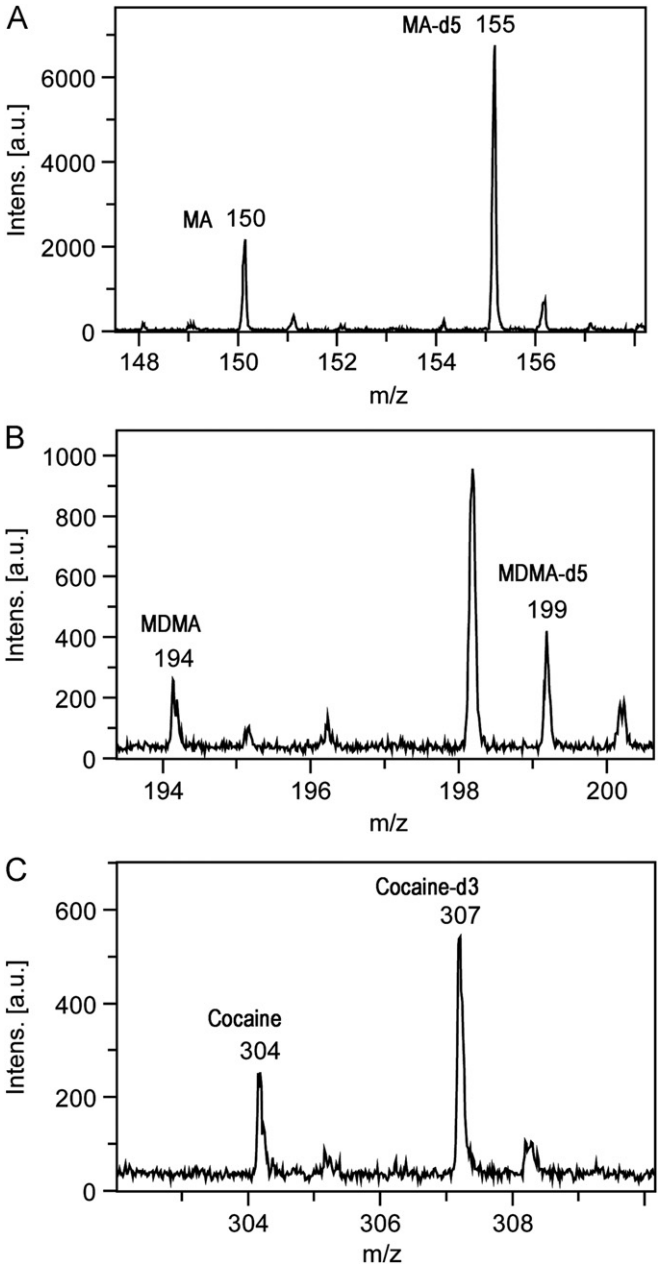

Fig. 5. Representative DIOS mass spectra for (A) MA (80 ng/mL) and MA-d5 (100 ng/mL) in neat saliva without rinsing protocol with a $MH^+=150$ m/z and $MH^+=155$, (B) MDMA (80 ng/mL) and MDMA-d5 (100 ng/mL) in neat saliva without rinsing protocol with a $MH^+=194$ m/z and $MH^+=199$ m/z, and (C) cocaine (80 ng/mL) and cocaine-d3 (100 ng/mL) in neat saliva without rinsing protocol with a $MH^+=304$ m/z and $MH^+=307$ m/z.

We next investigated the effect of pSi surface chemistry using drug concentrations of 10, 50 and 100 ng/mL (Fig. 2). This concentration range was used since all LOD analyses were conducted at concentrations less than 100 ng/mL. The ratio between raw signal intensity of each drug and its respective deuterated standard was plotted to compare the performance of each fluorosilane. Fluorosilane modification was shown to enhance signal intensities for all three drugs in comparison with freshly etched (hydride-terminated) and oxidized pSi (Fig. S-3). Indeed, hydride-terminated and oxidized pSi substrates gave only low signal intensities (Fig. S-3) in accordance with literature [37]. For MDMA and cocaine, no signals were observed below 1000 ng/mL.

Fig. 2 shows a comparison between observed intensity ratios for each drug on a commonly used fluorosilane modified surface (F_5PhPr) and two less commonly used silanes, F_{13} and F_{17}. A three way ANOVA (Table S-1) did not reveal statistically significant differences for the three silanes. Since the surface modifiers showed similar performances, the F_5PhPr surface was chosen over the F_{13} and F_{17} because the aqueous sample droplet was easier to deposit on the F_5PhPr surface than on the F_{13} and F_{17} surfaces. We reconciled this with higher water contact angle (WCA) on the F_{13} (125°) and F_{17} (131°) surfaces.

A three way ANOVA was performed to compare the differences between signal intensity ratios observed for cocaine, MA and MDMA (Table S-1). A statistically significant difference ($P<0.01$) for cocaine compared to both MA and MDMA was observed, but no statistically significant difference ($P=0.121$) was observed between MA and MDMA. This means that the ionization efficiency for the structurally related MA and MDMA (Fig. 1) is similar, probably due to the comparable proton affinity of the secondary amino group [39]. This is lower compared to cocaine, which contains a tertiary amino group that has higher proton affinity.

Non-homogeneous distribution of signal intensity from DIOS sample spots is a problem when using DIOS semi-quantitatively. Non-homogeneity causes poor shot-to-shot and sample-to-sample reproducibility [40,41]. This problem is commonly encountered in MALDI–MS with the co-crystallization of analyte and matrix. Here, DIOS MS imaging was used to investigate the distribution patterns in six adjacent spots each for MA, MDMA and cocaine solutions in water compared to their respective internal standards (Fig. 3A). Spectra recorded inside the six spots were exported in six classes and analyzed with ClinProTools, after normalization and recalibration. In Fig. 3B–D, the intensities of the signals corresponding to the three drugs versus the intensities of the signals of their respective internal standards, are reported for each replicate spot. The distribution along the diagonal indicates that the intensity ratio drug/internal standard is constant inter- and intra-spot. DIOS MS imaging analysis also confirmed that no signals corresponding to the molecular ions were present outside of the area of the sample spots for MDMA and cocaine. Conversely, for MA a weak background at 150 m/z (corresponding to the molecular ion) was observed outside the sample spots (Fig. 3A(III)). However, the observed intensity for the background peak occurring at 150 m/z was below 5% of the intensity within the spots. Small variations in signal intensities on the sample spots were observed. Only the background signal for MDMA-d5 at 199 m/z was higher than for MDMA, but the background signal intensity was still below 5% of the average signal within the spots (Fig. 3A(VI)).

For the determination of the LOD values, deuterated standards of each drug were used as internal standards at a constant concentration (20 ng/mL). The noise used to calculate the LOD for each drug was calculated from a total of 18 blanks replicated on three separate pSi surfaces to account for surface variability. The LOD for MA and MDMA were determined using concentrations within the dynamic range 0–100 ng/mL in water on a F_5PhPr modified pSi surface is shown in Fig. 4A and B. For MA and MDMA, the LOD values were calculated to be 2.88 ng/mL and 0.66 ng/mL, respectively. The LOD for cocaine was investigated over the concentration range 0–30 ng/mL on the F_5PhPr surface (Fig. 4C). For cocaine, the LOD value was calculated as 0.86 ng/mL. The three drugs showed good linearity ($R^2>0.991$) in the signal intensity ratio drug/internal standard over the whole concentration range, demonstrating that quantitation of these compounds by means of DIOS can be achieved when using internal standards.

MA, MDMA and cocaine were all detected at 80 ng/mL in neat saliva after pipetting the body fluid onto the DIOS surface and allowing the drop to dry without any subsequent rinsing (Fig. 5). However, this technique was not suitable as a thick layer of dried saliva formed on the surface of the pSi. This resulted in the three drugs only being detectable around the edges of the dried spots. For MDMA, there was also a background peak present at 198 m/z, which affected the peak intensities for MDMA-d5 (199 m/z) at lower concentrations. For more accurate intensity ratios an alternative internal standard needs to be investigated.

Currently, the recommended cut-off level for saliva screening tests for amphetamines and cocaine is 40 ng/mL and 30 ng/mL, respectively. Using the above protocol the drugs were not detected below 50 ng/mL. However, quantitation of cocaine in saliva was further investigated using a new protocol, which involved an extra rinsing step that removed the saliva before it dried onto the pSi surface. The LOD analysis for cocaine in saliva was conducted over the concentration range 200–10 ng/mL with the addition of a fixed concentration of internal standards (100 ng/mL). The pH of the saliva can vary from 5.8 to 7.6. Therefore, cocaine (pK_a 8.6) in saliva will be predominantly in its protonated form. In order to increase the hydrophobicity of cocaine and facilitate interaction with the hydrophobic pSi surface, the saliva was adjusted to pH 8 using ammonium bicarbonate buffer. Indeed, without the use of this buffer, cocaine was not detectable even after rinsing below 20 ng/mL. The results for cocaine in pH-adjusted saliva on F_5PhPr modified pSi surfaces are shown in Fig. 6. The LOD for cocaine in saliva was found to be approximately 3.79 ng/mL. For cocaine, signal intensities in neat saliva were lower than those in milliQ water at the same concentration. We attribute this discrepancy to the interfering salts and molecules present in saliva. In any case, our data showed a linear relationship between concentration in saliva and signal intensity values with $R^2=0.999$ for cocaine, demonstrating the potential for quantitative analysis. The observed LOD for cocaine is lower than the current cut-off levels for saliva swab tests involving immunoassays (30 ng/mL). In addition sample preparation is significantly

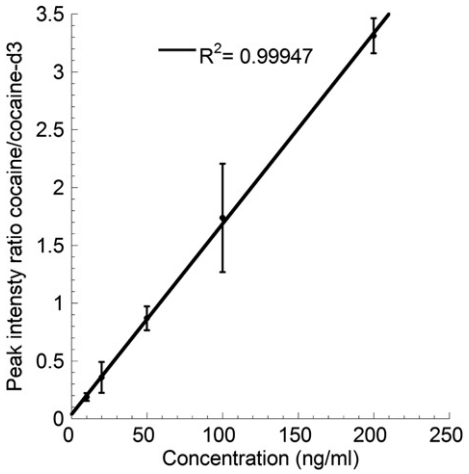

Fig. 6. Calibration curve for cocaine in saliva for concentrations ranging 10–200 ng/mL ($n=3$, 500 shots of spectrum). Internal standard: cocaine-d3 at 100 ng/mL.

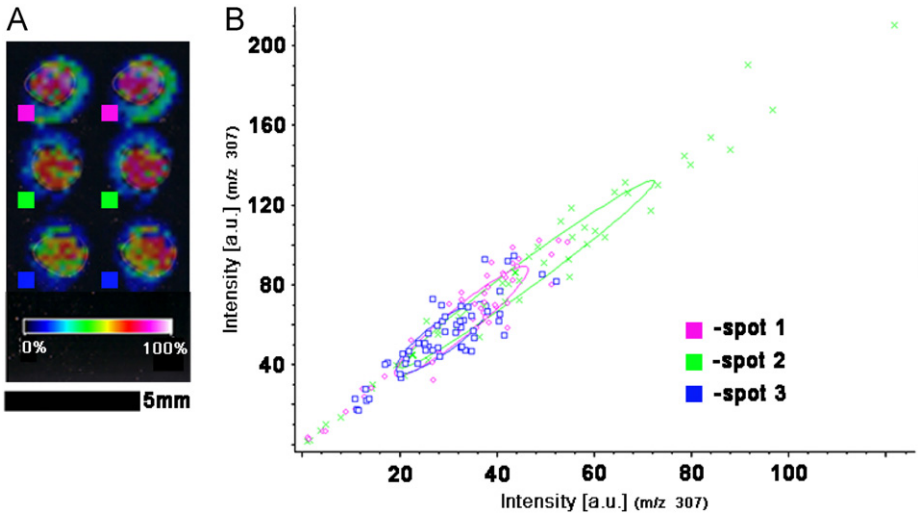

Fig. 7. DIOS imaging for cocaine in saliva at 200 ng/mL. Cocaine-d3 was used as an internal standard (100 ng/mL).

reduced using this protocol in comparison to GC–MS and LC–MS techniques, which require more complicated extractions and derivitizations. In addition to the simplicity of sample preparation, the required time for analysis of one sample was around 3 s, once prepared, while GC–MS and LC–MS analyses require 15 min analysis time per sample. So while regulations often only require the detection of the presence of an illicit drug in body fluids [42], DIOS is able to provide quantitative results as well. Furthermore, fabrication of patterned pSi is compatible with standard silicon microfabrication processes and lends itself to upscaling and cost-effective manufacture of DIOS substrates.

DIOS imaging was also conducted for cocaine in saliva (200 ng/mL) with the addition of cocaine-d3 as internal standard (100 ng/mL) (Fig. 7). Fig. 7 shows a comparison between crystallization patterns of cocaine and its internal standard (cocaine-d3). The three replicates show reasonably consistent crystallization patterns (Fig. 7A). Furthermore, the scatter plot shown in Fig. 7B shows a consistent peak intensity ratio between the signals corresponding to cocaine-d3 (m/z=307) and cocaine (m/z=304).

4. Conclusions

We have demonstrated the potential of DIOS as a rapid, high throughput method for drug detection and quantitation in oral fluids. Three illicit drugs, MA, MDMA and cocaine were detected in water using DIOS with detection limits comparable to current techniques. This study also showed that the three drugs spiked into saliva could be detected using DIOS without the need for extraction or derivatization. Quantitative DIOS analysis for illicit drugs was achieved in both water and neat saliva with the help of internal standards. Finally, DIOS imaging analysis demonstrated good spot-to-spot reproducibility of the signal. We believe that this technique holds strong potential for the high throughput and multiplexed screening of oral fluid samples from roadside, workplace or athlete testing for illicit drugs. In addition, we feel that this high-throughput screening technology is a promising tool for the analysis of other small molecules including illicit drugs and their metabolites in body fluids.

Acknowledgments

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.07.029.

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