Direct drug analysis from oral fluid using medical swab touch spray mass spectrometry

Abstract

Fourteen common drugs of abuse were identified in spiked oral fluid (ng mL^{-1} levels), analyzed directly from medical swabs using touch spray mass spectrometry (TS-MS), exemplifying a rapid test for drug detection. Multiple stages of mass analysis (MS^2 and MS^3) provided identification and detection limits sought by international forensic and toxicological societies, Δ^9-THC and buprenorphine excluded. The measurements were made using a medical swab as both the sampling probe and means of ionization. The adaptation of medical swabs for TS-MS analysis allows non-invasive and direct sampling of neat oral fluid. Data acquisition was rapid, seconds per drug, and MS^3 ensured reliable identification of illicit drugs. The reported data were acquired to investigate (i) ionization of common drugs from commercial swabs, (ii) ion intensity over spray duration, and (iii) dynamic range, all as initial steps in development of a quantitative method. The approach outlined is intended for point-of-care drug testing using oral fluid in clinical applications as well as *in situ* settings, *viz.* in forensic applications. The proof-of-concept results presented will require extension to other controlled substances and refinement in analytical procedures to meet clinical/legal requirements

Graphical abstract

Keywords

Drug testing; Medical swab; Ambient ionization; Touch spray ionization; Tandem mass spectrometry; Toxicology

Introduction

Over the past 10 years, oral fluid has gained consideration as a useful biological matrix – specifically as an alternative to blood – for the investigation of recent drug usage [1], [2], [3] and [4]. Oral fluid analysis is of interest for situations in which blood sampling is legally difficult or the assessment is ideally performed *in situ* (*i.e.*, not in a clinical–laboratory setting). Examples include therapeutic drug monitoring, pain management programs [1], [5] and [6], anti-doping controls [2], and roadside or workplace drug testing [4], [5], [7] and [8]. Major advantages of oral fluid analysis include these features: (i) non-invasive, (ii) sex-neutral (*i.e.*, common to male and females), (iii) directly observable sample collection (unlike urine testing), and (iv) reduced biohazard risks [2] and [6]. On the other hand, drawbacks are the limited fluid volume that can be collected rapidly [4] and [5] and the instability of some drugs in oral fluid.

Mass spectrometry (MS) already plays a significant role in drug detection from oral fluid [9], [10], [11], [12], [13], [14], [15] and [16], providing high specificity, selectivity, and sensitivity in molecular identification of a wide range of small analytes, especially illicit and pharmaceutical drugs. However, its use – almost ubiquitously coupled with gas-chromatography (GC) or liquid-chromatography (LC) – has been confined to the analytical laboratory and is relatively slow when coupled to chromatography. Indeed, despite the advances in the development of rapid GC–MS/LC–MS technology, measurement costs remain great, pretreatment of biological fluids can be laborious, and analyses require dedicated work areas [6], [9] and [10]. In most cases, *in situ* screening is highly advantageous and this consideration has led to wide use of immunoassay devices for on-site testing. These devices are portable, cheap, and fast but their specificity is poor, resulting in additional samples being required for confirmation by established hyphenated MS techniques [5] and [9].

The transfer of laboratory MS techniques to *in situ* screening methodology would aid in testing for drugs of abuse. Schwab et al. [17] recently stated that "…a series of revolutionary developments in MS is turning this complex technique into a model of simplicity…," a vision of MS utilization which the authors share. Prospectively, the adoption of transportable mass spectrometers [18] and [19] and ambient ionization techniques – which allow the generation of ions under atmospheric conditions and require minimal to no sample preparation [20] – holds the potential for the development of *in situ*, rapid and straightforward analysis of intact oral fluid without significantly compromising the selectivity, sensitivity, and wide applicability that MS offers [17].

Among the ambient ionization techniques, touch spray (TS) is a recently developed spray-based technique in which sample is transferred to a substrate with subsequent ionization occurring as a result of charged droplet emission from a point at which an applied potential creates an electric field of sufficient strength [21]. The use of the same substrate (*i.e.*, probe) for sample collection and ion generation is an ideal feature for direct oral fluid analysis. When the substrate can serve both

as the means for sample collection and ionization, straightforward handling and analysis of either solid or liquid samples can occur without pretreatment [21]. Initial TS-MS experiments [21] used metallic substrates, an attribute of probes used in some other ambient ionization methods, *e.g.*, probe electrospray ionization (PESI), although not ubiquitous (*i.e.*, other ambient techniques use non-metallic probes, such as wooden tips) [22], [23], [24], [25], [26] and [27]. While metallic probes are advantageous for MS analysis, they do not meet the requirements for direct oral fluid testing, which requires non-invasive and safe sampling. Medical swabs satisfy these requirements and allow ready ionization that is believed to occur similar to paper spray ionization [28] and [29], in that ions are generated from a porous surface *via* electrospray-like mechanisms. The use of medical swabs as a substrate for ionization was recently tested for the detection of strep throat causing bacteria in oral fluid [30].

Medical swabs are widely used in clinical microbiology, cytology, and DNA testing to sample body orifices and surfaces. Their design is specific to each application, with appropriate shape and materials being chosen for each type of application. Commonly, the swab tip is made of cotton, rayon, or polyester in brush, rounded, squared or fused shapes. The shaft can be made of plastic, wood, rolled paper or metallic wire. Notably, the use of swabs to collect biological fluids is soundly established in clinical toxicology. Many on-site drug screening tests have been designed with swabs as specimen collectors (*e.g.*, STATSWAB®, Oratect®), but their shape and/or composition are not ideal for TS-MS (*i.e.*, to serve the dual purpose as collection devices and ionization substrates); therefore, commercially available medical swabs were used.

The aim of this study was to investigate the potential of TS-MS using medical swabs for the (*in situ*) detection of drugs from oral fluid. The work presented herein is the first evaluation of TS-MS in this type of application. The results presented are the initial steps in the development of a rapid and semi-quantitative MS method for direct oral fluid drug testing. Traditional drugs of abuse were investigated, allowing comparison with requirements for oral fluid analysis in forensic settings (*e.g.*, roadside and workplace testing).

Materials and methods

2.1. Chemicals and reagents

All standards were purchased from Cerilliant (Rendon, USA). Acetonitrile was purchased from Sigma–Aldrich (St. Louis, MO, USA). Formic acid (LC–MS grade) was obtained by Fisher Scientific (Geel, Belgium). Five deuterated compounds were used as internal standards (IS): cocaine-d_3 (COCA-d_3), amphetamine-d_6 (AMP-d_6), MDMA-d_5 (MDMA-d_5), morphine-d_3 (MORPH-d_3), Δ^9-tetrahydrocannabinol-d_3 (THC-d_3). Fourteen illicit drugs were monitored: cocaine (COCA),

benzoylecgonine (BZE), ketamine (KETA), amphetamine (AMP), methamphetamine (MAMP), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyethamphetamine (MDEA), morphine (MORPH), codeine (COD), 6-*O*-monoacetylmorphine (6-MAM), methadone (METADO), buprenorphine (BUPRE), Δ^9-THC.

Stock standard solutions were prepared in acetonitrile at 10 mg L^{-1} and stored at −20 °C until use. For each class of compounds, working solutions were prepared by dilution in acetonitrile to a final concentration of 1 mg L^{-1}. Working solutions of the internal standards were prepared by dilution in acetonitrile to 250 ng mL^{-1}.

Rayon straight wire swabs possessing an aluminum wire handle and rayon tip were used (Copan Diagnostics, Inc., Brescia, IT). The swabs are sterile, ready to use, and packaged in individual tubes for easy transport and storage. The swab is mounted in a plastic cap (the opposing end of the swab) which serves as a convenient holder, so there is no need for direct handling of the swab. Each tube and cap assembly is sealed with a tamper proof label for assurance of sterility. After sample collection, the swab can be stored into the same tube, conveniently sealed and signed to assure chain of custody, critical for clinical and forensic investigations.

2.2. Touch spray mass spectrometry with medical swabs

Negative controls, pooled human oral fluid specimens, were not spiked or otherwise altered. Positive controls were prepared by spiking oral fluid (1 mL in Eppendorf tubes) with the target substances at the desired concentrations, and then dipping the swabs once (complete submersion of swab) into the solution for ~2 s. If not otherwise stated, specimens were spiked at the cut-off concentrations, as set by Italian guidelines (Table 1) [31]. The average amount of oral fluid absorbed by the swab when dipped was estimated as 40 µL. Whenever quantitative information was sought from the TS-MS experiments, a precise volume of oral fluid was spiked *via* pipette onto the swab tip (40 µL).

Before TS-MS testing, the swabs were dried for ~10 min using an electric vacuum desiccator (VWR Desi-Vac Container 3164, Radnor, PA, USA). Subsequent to the drying period, 20 µL of the internal standard solution at 250 ng mL^{-1} was spiked onto the swab tip, and then the swabs were dried for a further 5 min.

Table 1. Target illicit drugs. Cut-off concentrations in oral fluid and settings for MS2 and MS3 detection.

Compounds[a]	Cut-off (ng mL^{-1})	Fragmentation (*m/z*)	Collision energy (V) (MS^2; MS^3)	Capillary (V)	Tube lens (V)
Cocaine	8	304.3 > 182.0	23; 30	28	75
BZE	8	290.3 > 168.0	25; 31		
Cocaine-d3	–	307.3 > 185.2	22; 30		
Ketamine	–	238.2 > 207.0	25; 25	30	70
MDA	50	180.2 > 163.0	20; 28		
MDMA	50	194.2 > 163.0	20; 28		
MDEA	50	208.2 > 163.0	23; 28		
MDMA-d5	–	199.2 > 165.0	20; 28		
AMP	50	136.2 > 119.0	25; 25		
MAMP	50	150.2 > 119.0	25; 25		
AMP-d6	–	142.2 > 125.0	25; 24		
Morphine	40	286.2 > 201.0	30; 30	49	85
Codeine	40	300.2 > 215.0	28; 30		
6-MAM	4	328.2 > 211.0	28; 30		
Morphine-d6	–	292.2 > 201.0	28; 30		
Methadone	–	310.5 > 265.2	20; 25		
Buprenorphine	–	468.4 > 414.2	22; 22		
Δ^9-THC	2	315.5 > 259.1	26; 30	47	80
Δ^9-THC-d_3	–	318.5 > 262.1	25; 30		

[a]For each substance and internal standard, the cut-off value in oral fluid samples, the selected MS and MS/MS masses, with the corresponding collision energies for fragmentation, are listed. Tuning capillary voltages and tube lens potentials are reported as well.

The swab was affixed to a ring stand *via* a three-finger clamp, and held in front of the MS inlet vertically, approximately 5–8 mm from and 5–6 mm above the inlet (Fig. 1). Acetonitrile with formic acid 0.1% (v/v) was applied to the swab tip using the instrument syringe pump (500 mL Hamilton syringe) and a fused silica capillary (i.d. 250 µm, o.d. 360 µm). The syringe pump flow rate was set at 50 µL min^{-1} for about 30 s, accounting for dead volume and wetting the swab tip. After pumping solvent for 30 s, high voltage (6 kV) was applied to the metallic handle *via* the instrument's high voltage cable and using a copper clip. The syringe pump was then slowed to 19 µL min^{-1} to

produce and maintain a stable electrospray plume. Data acquisition was started concurrently with high voltage application and formation of a Taylor cone at the end of the swab tip, as shown Fig. 1.

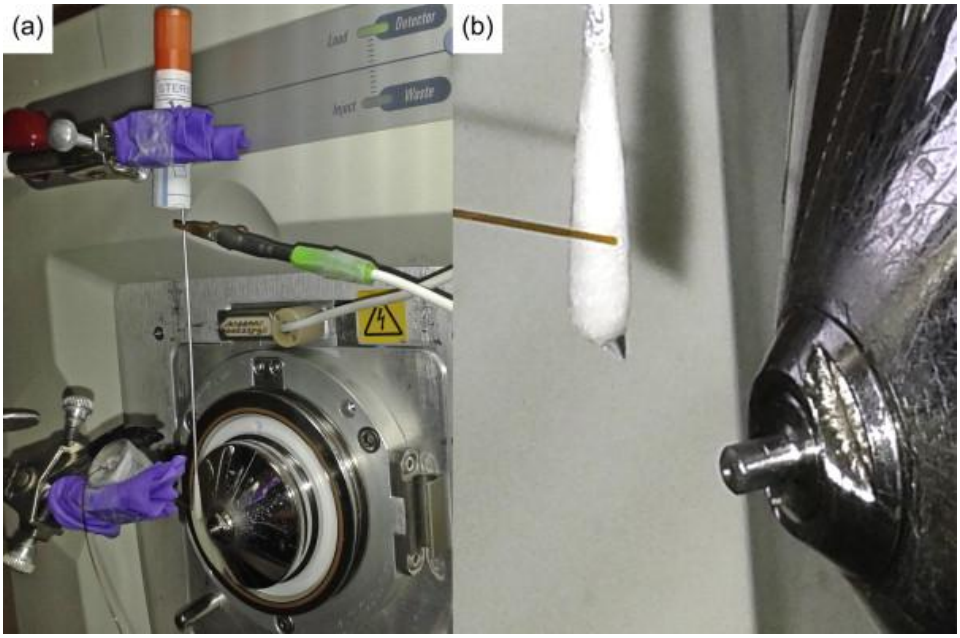

Fig. 1. (a) Photograph of the medical swab TS-MS experimental setup. High voltage is delivered via the copper clip and cable, marked in green. Solvent is delivered via the fused silica capillary pictured in the bottom-left corner. The syringe pump using for solvent delivery is not visible. (b) Zoom-in of swab tip, note the visible Taylor cone. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.3. MS analysis

All experiments were performed using a linear ion trap mass spectrometer (LTQ, Thermo Scientific, San Jose, CA). Spectra were collected in the positive ionization mode with automatic gain control (AGC) on. Sequential product scans were acquired for confirmatory identification of drugs [31] and [32]. A series of sequential product scans (MS^3) was performed by fixing the parameters for the MS and MS^2 events and acquiring data (as schematically represented with the common system of dots and arrows, ●→ ● →○) [33]. Capillary voltages, tube lens values, and collision energies are reported in Table 1. The collision energies for the MS^3 scans were selected in order to maintain residual MS^2 product ion intensity (the precursor for the MS^3 scan) at around 1–10% intensity relative to the highest MS^3 fragment. To maximize the structural value of the fragmentation data, MS^3 precursors due to losses of H_2O were avoided whenever possible. Cocaine, MDMA, 6-MAM and Δ^9-THC were used to tune the instrument, assumed to be representative of each class investigated. Other parameters were held constant: injection time, 500 ms; number of microscans, 2; range for sequential product scan, *m*/*z* 50–500; capillary

temperature, 250 °C. For automatic acquisition of the MS^3 scans for all the target compounds, four methods (one per class of compounds) were built using the generic MS^n function of the software Xcalibur (Thermo Scientific, San Jose, CA) and run consecutively from a single swab. The total analysis time was about 4 min to detect 14 compounds and 5 internal standards, with ~0.2 min of detection time (windows of the chronogram shown in Fig. 2 a) per substance.

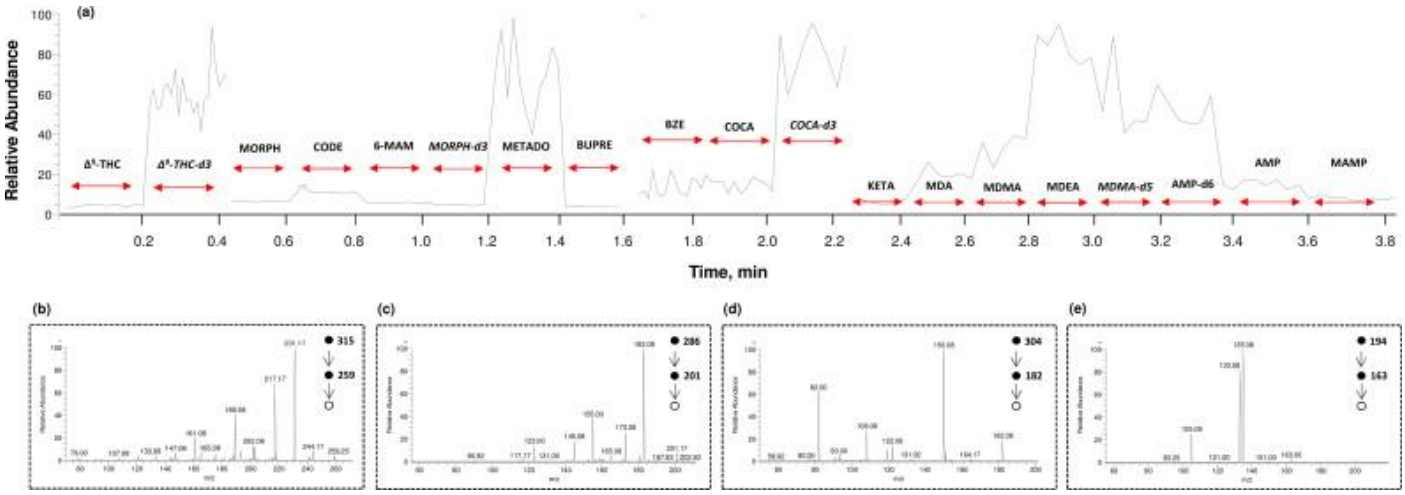

Fig. 2. Detection of the targeted illicit drugs by sequential precursor scans. (a) Compiled total ion current chronogram over a 4-min period in which a swabs was sequentially analyzed using tune methods matching particular analytes (breaks in the chronogram reflect switching between tune methods). (b) Sequential product scan for Δ9-THC in oral fluid spiked at 500 ng mL−1. (c) Sequential product scan for morphine in oral fluid spiked at 40 ng mL−1. (d) Sequential product scan for cocaine in oral fluid spiked at 8 ng mL−1. (e) Sequential product scan for MDMA in oral fluid spiked at 50 ng mL−1. Note, MS3 spectra displayed in (b–e) were averaged (~12 scans) over a time span of ~0.2 min.

The length of acquisition used greatly exceeded what was required for compound detection, but the extended experiment allowed concurrent measurement of the variation of absolute ion intensity and acquisition of systematic information needed for protocol development.

Results and discussion

3.1. Touch spray mass spectrometry using medical swabs

The detection of drugs from oral fluid by TS-MS was performed *in vitro* using a medical swab with a sample consisting of pooled human oral fluid spiked with illicit drugs. Testing was performed using neat oral fluid absorbed into the swab, without addition of buffer solutions [5] and [6]. Drying

is the only step that precedes MS analysis, as it is beneficial for stable electrospray formation likely the result of reducing water content. Although untested in this initial study, drying could also reduce the biological degradation of the sample [34], thus increasing specimen stability and facilitating swab transport and storage.

The medical swabs used were unaltered from their original construction. Swabs with rayon tips and metallic handles gave superior performance in comparison to those with paper and plastic handles [30]. The hemispherical shape of the swabs required higher voltages (6 kV) for the electrospray formation, as seen in a previous report [30]. The necessity for increased voltage is simply the result of the lack of consideration of the MS application in the design of swabs tip. For example, 3.5 kV is enough to generate a stable and efficient electrospray in paper spray ionization experiments as the paper is cut to a sharp tip [29].

Multiple factors that influence data quality were explored. Swab orientation and distance from the MS inlet significantly affect signal quality, primarily signal intensity, thus affecting the reproducibility of the technique. However, drug identification proved robust *via* reproducible fragmentation patterns observed in MS^3. As shown in Fig. 1, swabs oriented vertically provided the best reproducibility, as the variability in emitted droplet direction was reduced. Manually positioning the swabs between ~5 mm and ~8 mm above the MS inlet provided an average relative standard deviation (percentage) in absolute ion intensity of 33% (range 10–68%) and proved to be independent of target analyte and concentration. The absolute signal intensity and quality was also affected by the manufacturing tolerance and variability of the commercial swabs. Minute differences in tip dimension and shape affected the quantity of solvent necessary to form a reproducible spray plume (*viz.* the second and third swab tips from the left shown in Fig. S1). Indeed during method development, the solvent flow rate was slightly modified (16–22 μL min^{-1}) to compensate, producing an optimal spray plume. The solvent flow rate must be optimized to maintain a stable spray plume. A plume that was visually too small provided low spray current and signal, whereas a plume that was too large produced larger primary droplets, reducing ionization efficiency, and increased chance of discharge. A small fraction of swabs possessed significant deformation of the tip shape (as shown for the first swab tip in Fig. S1) which completely compromised the formation of a spray plume, yielding no signal. Improvements are expected with the use of swabs manufactured specifically for MS and better control in their position in front of the MS inlet.

The capillary temperature also affected signal intensity, with higher temperatures (up to 300–350 °C, the limit for the instrumentation) increasing the signal for cocaine and opiates while decreasing those of AMPs and MAMPs. Lower temperature (about 150 °C) provided the opposite results and therefore 250 °C was chosen as a compromise between signal intensity and thermal degradation. While this condition was not optimal for any one analyte, the use of different capillary

temperatures would increase analysis time, introducing equilibration times between sequential product scan acquisitions.

The solvent used for analysis was selected empirically; experiments were performed with methanol and acetonitrile, these two solvents in combination and with water (10% v/v) or formic acid (0.1% v/v). Acetonitrile with 0.1% (v/v) of formic acid proved to be the best combination, in terms of signal intensity and stability of the spray. Unsurprisingly, the presence of water in the solvent system decreased spray stability, resulting in greater currents (>1.5 μA) and in cases a discharge that adversely affects spectral quality. The same effect was noted when analyzing oral fluid directly from the swab without any period of drying; however, after a short period of drying spray stability and spectral quality was adequate. Under the ionization conditions tested, no significant formation of common salt adducts (*e.g.*, sodium or ammonium) was observed for any of the drugs or in-source fragmentation phenomena. A full scan mass spectrum from blank oral fluid spiked on the swab tip is shown in Fig. S2. In the high mass range (m/z > 500), the background chemical noise is consistent and likely related to polymers extracted from the polymer tip and the glue used to attach it to the handle. Background chemical noise in the low mass range (m/z < 500) is compensated for by multiple stages of mass analysis (MS^3) which provided satisfactory analytical specificity.

In this study, a continuous infusion of solvent onto the swab was used. Although a discrete addition of solvent in TS-MS has previously been used in complex mixture analysis [21], including the detection of bacterial lipids in oral fluid [30], screening for a large number of potential drugs requires a longer data acquisition period that would require multiple discrete additions of solvent. Initial experiments with repetitive discrete additions *via* pipetting proved drastically inconsistent in generating a stable plume. In contrast, the continuous infusion of solvent *via* syringe pump allowed a stable spray to be maintained for over 9 min (a window much longer than that required to analyze the entire set of drugs). The signal intensity for the target analytes was exhausted after 5 min on average, as shown later in the time profiling investigation. The period of data acquisition is dictated by the MS type used, the data acquisition mode, and the number of analytes to be screened. Factors that would dictate the sequence of data acquisition include the evolution of signal intensity over time and analyte specific detection limits (not evaluated herein).

Whether or not a sequential product ion scan experiment is performed affects the final analysis time, which is a compromise between the number of analytes detected, the injection time (related to analyte concentration), and the number of microscans per measurement. With the conditions used in this study (500 ms of injection time and the average of two microscans), the detection of 14 illicit drugs and 5 internal standard was done in 4 min, as shown in the chronogram of the total ion current (TIC) in Fig. 2a.

The average MS^3 spectra for Δ^9-THC, morphine, cocaine, and MDMA are also shown in Fig. 2(b–e), each spectrum is normalized to the most intense peak. As many as ~0.2 min of acquisition was set per analyte (~12 scans), greatly exceeding the minimum requirements for compound detection

and was used in monitoring stability and absolute intensity changes over time. The duty cycle can be substantially shortened if desired by reducing microscans or injection time, at the expenses of detection limits for low abundance compounds; *e.g.*, the analysis time would be ~20 s with only one scan per analyte.

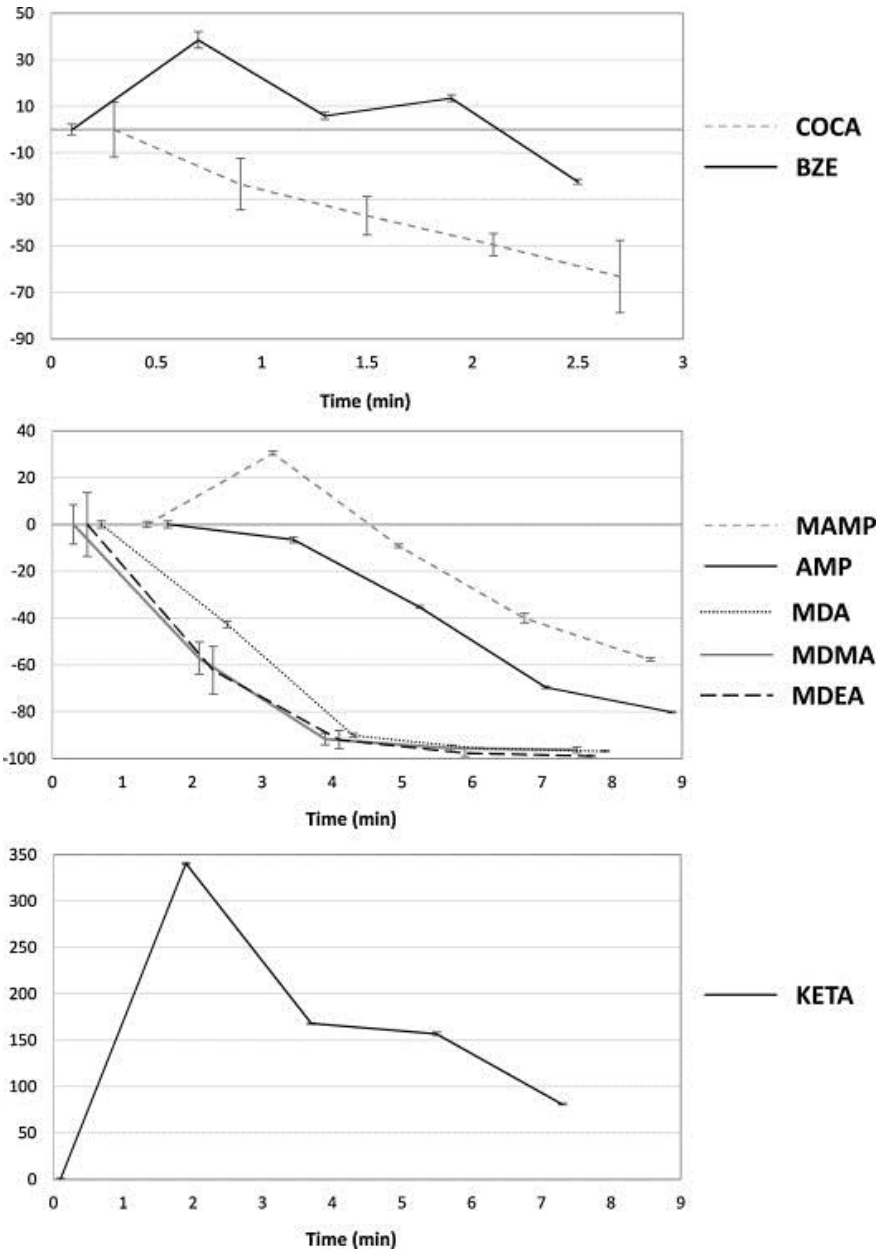

Fig. 3. Decrease of the total ion current (TIC) vs. spray time: (a) cocaine and BZE; (b) AMPs and MAMPs; (c) ketamine. Error bars represent the average standard deviation in TIC over _0.2 min of ion acquisition.

3.2. Time profiling for drug detection

To investigate any interaction that might occur between analyte and swab tip, and how signal evolves over time, the same swab was analyzed five times consecutively. The decrease in the total

ion current (TIC) was monitored using sequential product ion scans, as described in Section 2 . One swab, analyzed sequentially, was used for each class of drugs: one for cocaine and BZE; one for ketamine, AMPs and MAMPs; one for opiates and opioids; and one for Δ^9-THC. With the exception of ketamine and BZE, the target compounds showed a continuous decrease in signal intensity, indicating little to no interaction with the swab material (and likewise no inter-drug interactions). Nearly all the compounds were detectable after about 5 min of continuous spraying, absolute intensity having diminished with time. For example, cocaine showed a 70%-decrease of signal intensity after 3 min (see Fig. 3) and a 90%-decrease was observed for MAMPs after 5 min. By contrast, ketamine and BZE showed an increase in signal intensity of about 400% and 40%, respectively, after about 1 min of spraying. After which a decreasing trend of intensity over time was noted, suggesting ketamine and BZE interact to a greater degree with the rayon substrate than the other tested analytes.

A detection window of 5 min is great enough to assure flexibility in the way the ions are acquired, allowing the prioritization of those compounds with lower ionization efficiencies and faster rates of signal exhaustion. Data dependent (DDA) and data independent acquisition (DIA) systems can be also implemented to automate the entire methodology and allow untargeted real time decision-making strategies [35]. The implementation of DDA and DIA systems on a stable long-lasting signal by direct MS analysis may also result in more efficiency than its utilization on transitory chromatographic peaks.

3.3. MS^3 structural information for drug confirmation

Screening for drugs requires molecular identification and is typically performed *ex situ* using hyphenated chromatography/MS techniques to increase the certainty of identification. Without chromatographic separation prior to MS testing, two to three stages of mass analysis are desirable (*e.g.*, a sequential product ion scan) to provide the necessary structural information and confirm the detection of known illicit drugs when using low resolution MS. We used a commercial ion trap mass analyzer to develop a TS-MS protocol for oral fluid drug detection, with the intention of transferring the same methodology to miniaturized ion trap mass spectrometers capable of performing MS^n experiments [18] and [19]. However, the experiment is not limited to portable equipment and MS offers greater flexibility in the ion acquisition modes that can be adopted to develop confirmatory methods by TS-MS. Triple quadrupole, time-of-flight, ion mobility and various hybrid mass analyzers could also be used to acquire low or high mass resolution data *via* multiple stages of mass selection and subsequent fragmentation. For example, a single fragmentation step might be sufficient if performed using high mass resolution; likewise ion mobility spectrometry (IMS) [36], [37] and [38] provides an additional dimension of information for drug identification.

For each compound investigated in this study, the characteristic MS^3 fragments are reported in Table 2, while the description of their structures and modes of fragmentation can be found elsewhere [39] and [40]. The target compounds in simulated oral fluid swabs were detected in most cases at concentrations equal to or lower than limits suggested from official guidelines [31]. There are three exceptions, BZE, Δ^9-THC and buprenorphine. Remarkably, 6-MAM can be detected at single ng mL^{-1} levels, which is important for the identification of heroin users [5] and [41]; ketamine and methadone are detectable at relatively low concentrations (50 ng $mL^{-1}$and 40 ng mL^{-1}), MAMPs can be detected at concentrations below 25 ng mL^{-1}. BZE can be detected from concentrations of ~40 ng mL^{-1} and upwards. The detection of BZE aids in the interpretation of positive results as indicating the presence of cocaine when a passive contamination is claimed. Although an official limit has been set at 8 ng mL^{-1}[31], we envision that a LOD close to 40 ng mL^{-1} does not compromise screening, since much higher concentrations are found in actual oral fluid samples [42] and current immunoassays do not always reach the official values.

Table 2. Repeatability of relative ion intensities in MS3 spectra.

Compound[a]	**Ion (*m/z*)**	**mean**	**Ion (*m/z*)**	**Mean ± SD (RSD%)**	**Ion (*m/z*)**	**Mean ± SD (RSD%)**
Morphine	183	100	155	39 ± 3 (7)	123	11 ± 2 (15)
Codeine	183	100	155	18 ± 2 (15)	137	7 ± 1 (12)
6-MAM	193	100	183	79 ± 1 (1)	165	36 ± 8 (23)
Methadone	223	100	247	95 ± 3 (4)	105	73 ± 5 (6)
Ketamine (50 ppb)	179	100	125	31 ± 10 (32)	163	15 ± 4 (27)
MDA	135	100	133	73 ± 7 (9)	105	21 ± 2 (9)
MDMA	135	100	133	73 ± 4 (5)	105	24 ± 1 (5)
MDEA	135	100	133	74 ± 4 (5)	105	25 ± 2 (6)
Amphetamine	91	100	119	30 ± 2 (7)	–	–
Methamphetamine	91	100	119	10 ± 2 (19)	–	–
BZE (40 ppb)	150	100	82	87 ± 9 (10)	119	37 ± 3 (9)
Cocaine	150	100	82	55 ± 10 (18)	108	30 ± 8 (28)
Cocaine (50 ppb)	150	100	82	64 ± 5 (8)	108	34 ± 5 (16)
Cocaine (250 ppb)	150	100	82	63 ± 1 (2)	108	34 ± 1 (3)

[a]For each compound, the relative ion intensities in the MS3 spectra are reported as mean ± standard deviation (SD). The relative standard deviation (RSD%) is reported in brackets.

By contrast, Δ^9-THC and buprenorphine are detectable at concentrations in the range of hundreds of ng mL^{-1} and improvement is required to meet official cut-off values, despite the fact that oral fluid concentrations >1 µg mL^{-1} have been reported for Δ^9-THC shortly after smoking marijuana

[43] and [44] and for buprenorphine during opiate withdrawal treatment periods. In situations in which the analytical performance for a particular analyte is not satisfactory, a wide portfolio of off-line reactions and on-line derivatization reactions can be used to increase the ionization efficiency and reach lower limits of detection [29], [45] and [46].

Table 2 lists mean, standard deviation, and relative standard deviation (RSD%) of relative ion intensities for target analytes, which were calculated by analyzing five blank oral fluid samples spiked at the following drug concentrations: cocaine, 8 ng mL^{-1}; BZE, 40 ng mL^{-1}; ketamine, AMPs and MAMPs, 50 ng mL^{-1}; morphine, codeine and methadone, 40 ng mL^{-1}; 6-MAM, 4 ng mL^{-1}. The RSD% values satisfy the maximum permitted tolerances for relative ion intensities [31] and [32] in all cases expect for cocaine and ketamine (RSD% of 28% and 32% at 8 and 50 ng mL^{-1}, respectively). For ions with intensities relative to the base peak within the range 20–50%, a tolerance of ±25% is indicated for ESI-based MS methods, while the values for ketamine and cocaine are slightly higher. However, RSD% values decrease at higher concentrations (as shown in Table 2 for cocaine) following the Hortwitz equation [47] and substantiate that at levels exceeding the cut-off, the measured RSD% are adequate. The specificity of the medical swab TS method also proved satisfactory: blank oral fluid samples provided no or little signal in which case fragmentation patterns differing than those expected for the target analytes were noted.

The strength of TS-MS using swabs lies in the fact that the swabs are both the sampling tool and the substrate for ionization. There will be no information available in real clinical swabs in regards to recovery since the oral fluid volume adsorbed while swabbing a person's mouth is not controlled. Semi-quantitative analysis has been achieved as indicated in Fig. S3. As one would expect, ion intensities of the MS^3 fragments increase in proportion to the quantity of drug retained on the swab tip, while the fragmentation pattern is maintained. Note that in this case, 40 µL of oral fluid was spiked on the swab tip, thus the analytical measurements are subject to less sampling error (other than that associated with pipetting). There exist swabs designed so that sampling reproducibility is increased by visually indicating (*e.g.*, colored line) when a certain amount of oral fluid has been absorbed [5] and [6], potentially of use for a quantitative protocol. In any case, poorer quantitative performance is expected if compared with developed hyphenated chromatographic and mass spectrometric methods (usually with triple quadrupoles or hybrids as mass analyzers) – which are preceded by sample clean-up, pre-concentration or derivatization. The use of triple quadrupoles in the SRM mode instead of ion traps surely would improve quantitative performance [29]; however, absolute quantitation is not always necessary for *in situ* oral fluid drug detection. The potential of multiple stages of mass analysis with ion traps to increase the analytical specificity may be of greater benefit. For example in roadside drug testing, several countries have introduced zero-tolerance legislation for drug-caused impairment, making it sufficient to merely prove that a suspect has detectable illegal substances rather than quantify drug concentrations to estimate the actual impairment. It has been recently asserted that "…determination of impairing concentrations is

unattainable and that withholding drug legislation pending the acquisition of such data is tantamount to plan for inaction with regard to an important and growing public health and safety problem…" [43]. This suggests that the application of straightforward *in situ* ambient MS methods on intact oral fluid samples might be ideal.

Conclusions and perspectives

The development of innovative methods for drug detection in oral fluid is a current need in bioanalysis, particularly in clinical and forensic toxicology. In this study, a TS-MS method using medical swabs has been developed, allowing the rapid detection of several traditional illicit drugs from neat oral fluid. Currently, the drying step represents the main time-consuming part of the analytical protocol.

Direct analysis from swabs enormously simplifies oral fluid sampling and testing, particularly by MS, with a single device used for collection and direct analysis. Performance appears to be competitive with that of screening immunoassays in respect to time required for analysis, but it has the potential for superiority in terms of analytical specificity, selectivity and sensitivity. The qualitative detection of several illicit drugs proved reliable using a benchtop mass spectrometer; however, the transfer of the methodology on a transportable scale, with the entire clinical-forensic assessment being performed *in situ*, represents a future endeavor. Further research is necessary to advance the methodology to a more quantitative level and to validate performance, such as measurement uncertainty, LOD values, dynamic ranges, matrix effects, repeatability, robustness, *etc*.

The challenge for drug testing is to develop and update untargeted and flexible analytical protocols that can deal with the actual phenomenon of drug abuse, characterized by poly-consumption and continuous appearance of new synthetic drugs on the black market. The traditional workflow with *in situ* immunoassay screening and hyphenated MS-based confirmatory analysis has proved to be inefficient in shifting with dynamics of synthetic drug manufacture, with commercial *in situ* immunoassay devises vulnerable to poor selectivity against emerging synthetic drugs [48], [49] and [50]. By contrast, a one-step screening-confirmatory analysis based on ambient MS represents a feasible strategy to address this problem and is a future endeavor.

Supplementary material

http://www.sciencedirect.com/science/article/pii/S0003267015000112

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors thank Copan Diagnostics® (Brescia, Italy) for providing the medical swabs used in this study. Kevin S. Kerian (Department of Chemistry and Center for Analytical Instrumentation Development, Purdue University, IN, USA) and Alberto Salomone (Centro Regionale Antidoping e di Tossicologia “A. Bertinaria”, Italy) are acknowledged for their contributions. V.P. acknowledges the Foundation L’Oreal/UNESCO for Women in Science for financial support (National Award L’Oreal/UNESCO for Women in Science 2013). Support from the National Science Foundation (CHE 1307264) is gratefully acknowledged.

References

[1]D. Di Corcia, S. Lisi, V. Pirro, E. Gerace, A. Salomone, M. Vincenti Determination of pharmaceutical and illicit drugs in oral fluid by ultra-high performance liquid chromatography–tandem mass spectrometry
J. Chromatogr. B, 927 (2013), pp. 133–141

[2]S. Anizan, M.A. HuestisThe potential role of oral fluid in antidoping testing Clin. Chem., 60 (2014), pp. 307–322

[3] H. Gjerde, K. Langel, D. Favretto, A.G. Verstraete Estimation of equivalent cutoff thresholds in blood and oral fluid for drug prevalence studiesJ. Anal. Toxicol., 38 (2014), pp. 92–98

[4] C. Moore Oral fluid and hair in workplace drug testing programs: new technology for immunoassays Drug Test. Anal., 3 (2011), pp. 166–168

[5] W.M. Bosker, M.A. Huestis Oral fluid testing for drugs of abuse Clin. Chem., 55 (2009), pp. 1910–1931

[6] M.A. Huestis, A. Verstraete, T.C. Kwong, J. Morland, M.J. Vincent, R. de la Torre Oral fluid testing: promises and pitfalls Clin. Chem., 57 (2011), pp. 805–810

[7] G. Cooper, C. Moore, C. George, S. Pichini Guidelines for European workplace drug testing in oral fluid
Drug Test. Anal., 3 (2011), pp. 269–276

[8] O.H. Drummer Introduction and review of collection techniques and applications of drug testing of oral fluid
Ther. Drug Monit., 30 (2008), pp. 203–206

[9] M. Vincenti, A. Salomone, E. Gerace, V. Pirro Application of mass spectrometry to hair analysis for forensic toxicological investigations Mass Spectrom. Rev., 32 (2013), pp. 312–332

[10] H.H. Maurer What is the future of (ultra) high performance liquid chromatography coupled to low and high resolution mass spectrometry for toxicological drug screening? J. Chromatogr. A, 1292 (2013), pp. 19–24
[11] F. Musshoff, E.G. Hokamp, U. Bott, B. Madea Performance evaluation of on-site oral fluid drug screening devices in normal police procedure in Germany Forensic Sci. Int., 238 (2014), pp. 120–124
[12] P. Amaratunga, B. Lorenz Lemberg, D. Lemberg Quantitative measurement of synthetic cathinones in oral fluid J. Anal. Toxicol., 37 (2013), pp. 622–628
[13] S. Strano-Rossi, L. Anzillotti, E. Castrignanò, F.S. Romolo, M. Chiaretti Ultra high performance liquid chromatography–electrospray ionization–tandem mass spectrometry screening method of direct analysis of designer drugs, spice and stimulants in oral fluid J. Chromatogr. A, 1258 (2012), pp. 37–42
[14] N. Badawi, K.W. Simonsen, A. Steentoft, I.M. Bernhoft, K. Linnet Simultaneous screening and quantitation of 29 drugs of abuse in oral fluid by solid-phase extraction and ultra performance LC–MS/MS Clin. Chem., 55 (2009), pp. 2004–2018
[15] K. Langel, T. Gunnar, K. Ariniemi, O. Rajamäki, P. Lillsunde A validated method for the detection and quantitation of 50 drugs of abuse and medicinal drugs in oral fluid by gas chromatography–mass spectrometry
J. Chromatogr. B, 879 (2011), pp. 859–870
[16] A. de Castro, B. Piñeiro, E. Lendoiro, A. Cruz, M. López-Rivadulla Quantification of selected synthetic cannabinoids and Δ9-tetrahydrocannabinol in oral fluid by liquid chromatography–tandem mass spectrometry
J. Chromatogr. A, 1295 (2013), pp. 99–106
[17] N.V. Schwab, M.N. Eberlin Mass spectrometry made easy: the quest for simplicity Drug Test. Anal., 5 (2013), pp. 137–144
[18] L. Li, T.C. Chen, Y. Ren, P.I. Hendricks, R.G. Cooks, Z. Ouyang Mini 12, miniature mass spectrometer for clinical and other applications. Introduction and characterization Anal. Chem., 86 (2014), pp. 2909–2916
[19] P.I. Hendricks, J.K. Dalgleish, J.T. Shelley, M.A. Kirleis, M.T. McNicholas, L. Li, T.C. Chen, C.H. Chen, J.S. Duncan, F. Boudreau, R.J. Noll, J.P. Denton, T.A. Roach, Z. Ouyang, R.G. Cooks Autonomous in situ analysis and real-time chemical detection using a backpack miniature mass spectrometer: concept, instrumentation development, and performance Anal. Chem., 86 (2014), pp. 2900–2908
[20] R.G. Cooks, N.E. Manicke, A.L. Dill, D.R. Ifa, L.S. Eberlin, A.B. Costa, H. Wang, G. Huang, Z. Ouyang New ionization methods and miniature mass spectrometers for biomedicine: DESI imaging for cancer diagnostics and paper spray ionization for therapeutic drug monitorino Faraday Discuss., 149 (2011), pp. 247–267

[21] K.S. Kerian, A.K. Jarmusch, R.G. Cooks Touch spray mass spectrometry for in situ analysis of complex samples Analyst, 139 (2014), pp. 2714–2720
[22] K. Yoshimura, L.C. Chen, M.K. Mandal, T. Nakazawa, Z. Yu, T. Uchiyama, H. Hori, K. Tanabe, T. Kubota, H. Fujii, R. Katoh, K. Hiraoka, S. Takeda Analysis of renal cell carcinoma as a first step for developing mass spectrometry-based diagnostics J. Am. Soc. Mass Spectrom., 23 (2012), pp. 1741–1749
[23] J. Liu, R.G. Cooks, Z. Ouyang Biological tissue diagnosis using needle biopsy and spray ionization mass spectrometry Anal. Chem., 83 (2011), pp. 9221–9225
[24] B. Hu, P.K. So, H. Chen, Z.P. Yao Electrospray ionization using wooden tips Anal. Chem., 83 (2011), pp. 8201–8207 View Record in Scopus
[25] K. Hiraoka, K. Nishidate, K. Mori, D. Asakawa, S. Suzuki Development of probe electrospray using a solid needle Rapid Commun. Mass Spectrom., 21 (2007), pp. 3139–3144
[26] P.K. So, T.T. Ng, H. Wang, B. Hu, Z.P. Yao Rapid detection and quantitation of ketamine and norketamine in urine and oral fluid by wooden-tip electrospray ionization mass spectrometry Analyst, 138 (2013), pp. 2239–2243
[27] S. Saha, M.K. Mandal, K. Hiraoka Direct detection of trace level illicit drugs in human body fluids by probe electrospray ionization mass spectrometry (PESI-MS) Anal. Methods, 5 (2013), pp. 4731–4738
[28] N.E. Manicke, P. Abu-Rabie, N. Spooner, Z. Ouyang, R.G. Cooks Quantitative analysis of therapeutic drug in dried blood spot samples by paper spray mass spectrometry: an avenue to therapeutic drug monitoring J. Am. Soc. Mass Spectrom., 22 (2011), pp. 1501–1507
[29] R.D. Espy, S.F. Teunissen, N.E. Manicke, Y. Ren, Z. Ouyang, A. van Asten, R.G. Cooks Paper spray and extraction spray mass spectrometry for the direct and simultaneous quantification of eight drugs of abuse in whole blood Anal. Chem., 86 (2014), pp. 7712–7718
[30] A.K. Jarmusch, V. Pirro, K.S. Kerian, R.G. Cooks Detection of strep throat causing bacterium directly from medical swabs by touch spray-mass spectrometry Analyst, 139 (2014), pp. 4785–4789
[31] GTFI, Linee guida per le strutture dotate di laboratori per gli accertamenti di sostanze d'abuso con finalita` tossicologico-forensi e medico-legali, 2012 http://tossicologia-forense.unina2.it/dwn/LINEEGUIDA%20-%20REV.3_2010.pdf (accessed 03.06.13).
[32] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002).
[33] J.C. Schwartz, A.P. Wade, C.G. Enke, R.G. Cooks Systematic delineation of scan modes in multidimensional mass spectrometry Anal. Chem., 62 (1990), pp. 1809–1818

[34] C.P. Lødøen, L.E. Eng Eibak, K.E. Rasmussen, S. Pedersen-Bjergaard, T. Andersen, A. Gjelstad
Storage of oral fluid as dried spots on alginate and chitosan foam. A new concept for oral fluid collection Bioanalysis, 5 (2013), pp. 317–325
[35] Thermo. Application note. Data-Dependent™ Neutral-Loss Mass Spectrometry for the Identification of Protein Phosphorylation. http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/cmd-support/ltq-xl/scientific-resources/application-notes/App-Note-332-Data-Dependent-Neutral-Loss-Mass-Spectrometry-for-the-Identification-of-Protein-Phosphorylation.pdf (accessed 25.09.14).
[36] A.J. Midey, A. Patel, C. Moraff, C.A. Krueger, C. Wu Improved detection of drugs of abuse using high-performance ion mobility spectrometry with electrospray ionization (ESI-HPIMS) for urine matrices Talanta, 116 (2013), pp. 77–83
[37] S. Armenta, J. de la Guardia, M. Alcalà, M. Blanco, C. Perez-Alfonso, N. Galipienso Detection and characterization of emerging psychoactive substances by ion mobility mass spectrometry Drug Test. Anal. (2014) http://dx.doi.org.offcampus.dam.unito.it/10.1002/dta.1678
[38] J.R. Verkouteren, J.L. Staymates Reliability of ion mobility spectrometry for the qualitative analysis of complex, multicomponent illicit drug samples Forensic Sci. Int., 206 (2011), pp. 190–196
[39] E. Castiglioni, C. Zuccato, R. Chiabrando, R. Fanelli Mass spectrometric analysis of illicit drugs in wastewater and surface water Mass Spectrom. Rev., 27 (2008), pp. 378–394
[40] L. Bijlsma, J.V. Sancho, F. Hernández, W.M. Niessen Fragmentation pathways of drugs of abuse and their metabolites based on QTOF MS/MS and MS(E) accurate-mass spectra J. Mass Spectrom., 46 (2011), pp. 865–875
[41] M. Garnier, C. Coulter, C. Moore Selection of an immunoassay screening cutoff concentration for opioids in oral fluid J. Anal. Toxicol., 35 (2011), pp. 369–374
[42] C.R. Crooks, S. Brown Roche DAT immunoassay: sensitivity and specificity testing for amphetamines, cocaine, and opiates in oral fluid J. Anal. Toxicol., 34 (2010), pp. 103–109
[43] A.G. Verstraete More reliable on-site detection of cannabis in oral fluid Clin. Chem., 58 (2012), pp. 1389–1391
[44] D. Lee, R. Vandrey, D.R. Mendu, S. Anizan, G. Milman, J.A. Murray, A.J. Barnes, M.A. Huestis
Oral fluid cannabinoids in chronic cannabis smokers during oral Δ9-tetrahydrocannabinoltherapy and smoked cannabis challenge Clin. Chem., 59 (2013), pp. 1770–1779
[45] N.D. Danielson, P.A., Gallagher, J.J. Bao. Chemical Reagents and Derivatization Procedures in Drug Analysis in Encyclopedia of Analytical Chemistry. in: R.A. Meyers (ed.) (2008) pp 7042–7076.

[46] R.D. Espy, M. Wleklinski, X. Yan, R.G. Cooks Beyond the flask: Reactions on the fly in ambient mass spectrometry Trends Anal. Chem., 57 (2014), pp. 135–146
[47] W. Hortwitz, R.J. Albert The Hortwitz ratio (HorRat): a useful index of method performance with respect to precision Assoc. Off. Anal. Chem., 89 (2006), pp. 1095–1109
[48] M. Nieddu, L. Burrai, C. Trignano, G. Boatto Evaluation of commercial multi-drug oral fluid devices to identify 39 new amphetamine-designer drugs Leg. Med. (Tokyo), 16 (2014), pp. 106–109
[49] A. Arntson, B. Ofsa, D. Lancaster, J.R. Simon, M. McMullin, B. Logan Validation of a novel immunoassay for the detection of synthetic cannabinoids and metabolites in urine specimens J. Anal. Toxicol., 37 (2013), pp. 284–290
[50] R. Kronstrand, L. Brinkhagen, C. Birath-Karlsson, M. Roman, M. Josefsson LC–QTOF–MS as a superior strategy to immunoassay for the comprehensive analysis of synthetic cannabinoids in urine Anal. Bioanal. Chem., 406 (2014), pp. 3599–3609