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

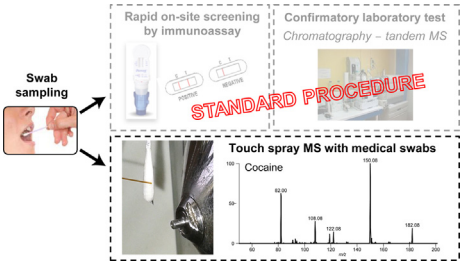
Valentina Pirro [a,b,*], Alan K. Jarmusch [a], Marco Vincenti [b,c], R. Graham Cooks [a,*]

[a] Department of Chemistry and Center for Analytical Instrumentation Development, Purdue University, 560 Oval Drive, West Lafayette, 47907 IN, USA
[b] Centro Regionale Antidoping e di Tossicologia "A. Bertinaria", Regione Gonzole 10/1, Orbassano, 10043 Torino, Italy
[c] Dipartimento di Chimica, Università degli Studi di Torino, Via Pietro Giuria 7, Torino, 10125, Italy

HIGHLIGHTS

- Direct oral fluid analysis for drug investigation at point-of-care.
- Deployment of medical swabs as sampling device and means of MS ionization.
- Qualitative detection of traditional drugs of abuse at the ng mL^{-1} level.
- MS^3 sequential product scans to confirm drug identification.

GRAPHICAL ABSTRACT



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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.

1. 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 [1–4]. 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 [1,5,6], anti-doping controls [2], and roadside or workplace drug

* Corresponding authors at: Department of Chemistry and Center for Analytical Instrumentation Development, Purdue University, 560 Oval Drive, West Lafayette, 47907 IN, USA. Tel.: +1 765 494 5263; fax: +1 765 494 9421.
E-mail addresses: vpirro@purdue.edu (V. Pirro), cooks@purdue.edu (R. G. Cooks)

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testing [4,5,7,8]. 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 [2,6]. On the other hand, drawbacks are the limited fluid volume that can be collected rapidly [4,5] and the instability of some drugs in oral fluid.

Mass spectrometry (MS) already plays a significant role in drug detection from oral fluid [9–16], 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 [6,9,10]. 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 [5,9].

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 [18,19] 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) [22–27]. 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 [28,29], 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).

2. 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