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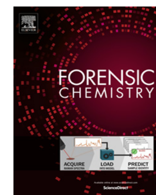

Full length articles

Rapid confirmation and quantitation of drugs-of-abuse in oral fluid using a low cost, small footprint mass spectrometer

Emily Schepens, Sophie Inman, Bryan J. McCullough *, Chris Hopley

Organic Analysis, LGC, Queens Road, Teddington TW11 0LY, UK

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ABSTRACT

Recent developments in the miniaturisation of mass spectrometry have led to a new generation of low-cost instruments which are making MS a more accessible technology in all areas of analytical science. While the utility of these instruments for qualitative analysis has been demonstrated, their usefulness for quantitative analysis – particularly in complex matrices – has not been proven. Here we describe the development of a rapid LC-MS method for the analysis of drugs-of-abuse in oral fluid using the Waters QDa, a small, low-cost, single quadrupole MS. The method involves direct injection of oral fluid samples followed by a five minute chromatographic run with MS detection. In order to improve the specificity of the method the instrument cone voltage was used to generate in-source fragments of the analytes, these were combined with retention time to confirm the presence of each compound. The final method was tested using synthetic saliva spiked with a mix of twelve drugs-of-abuse at 5–250 ng/mL; LODs were $\leq$5 ng/mL for all analytes studied except THC and MDA which had LODs of 10 and 25 ng/mL respectively. LOQs for the method were at or below the required cut-off limits for the analysis of oral fluid for drug driving with the exception of THC. The accuracy and repeatability of the method were demonstrated using repeat injections of spiked saliva at 10, 25 and 50 ng/mL; in all cases the repeat injections showed excellent repeatability (typically within 5% RSD) and excellent accuracy (bias typically within ±10%).

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1. Introduction

Mass spectrometry has long been considered the “gold standard” technique for quantitative and qualitative chemical analysis thanks to its specificity, speed, sensitivity and robustness. Testing of biological fluids for the presence of drugs-of-abuse (DoA), for example, is typically carried out by GC–MS [1–5] and LC-MS [6–10]. The analysers used in such analyses are typically high-end mass analysers such as triple quadrupole (QqQ) [6–8], quadrupole time-of-flight (Q-ToF) [9] and Orbitrap [10]. The complexity, cost and size of such instruments can often be a barrier to their implementation, particularly for screening applications, and their use is therefore typically limited to secondary confirmation and quantitation. The size, weight, power consumption and services required for such MS instrumentation typically also limit their use to larger laboratories.

To address some of these issues it has long been a goal of instrument developers to miniaturise and simplify MS instrumentation and thereby open up new application areas for the technology. Cooks and co-workers at Purdue University have been at the forefront of such development designing a series of ever-smaller mass spectrometers [11–14] such as the Mini-11, a rectilinear ion trap-based instrument weighing around 5 kg [13]. This, and similar, technology has been commercialised by a number of manufacturers including Griffin [15], Torion [16] and 908 Devices [17]. However, the vacuum requirements for successful MS operation mean that such instruments have been restricted to analysis of gaseous samples [17] or use of pulsed ion sources (e.g. the pinch-valve device employed on the Mini-11) [13,14]. One area where these MS systems are beginning to show real promise is when combined with so-called ambient ionisation sources such as desorption electrospray ionisation (DESI) [18] and direct analysis in real time (DART) [19]. Using such techniques these instruments have been used for a variety of rapid analysis applications such as drug detection [20–22], food analysis [23,24] and trace explosive detection [25,26].

A result of this academic development of miniaturised MS has been the development of small-footprint, single quadrupole mass spectrometers (QMS) aimed at the HPLC and synthetic chemistry markets notably from Microsaic Systems [17–29], Advion [29] and Waters [30,31]. While these instruments are typically larger than the ion trap-based instruments discussed above they are all smaller than standard lab MS, with the Waters and Microsaic

* Corresponding author.
E-mail address: bryan.mccullough@lgcgroup.com (B.J. McCullough).

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instruments being designed to fit within an HPLC stack in a manner similar to a UV detector [29,31].

The comparatively simple nature of a QMS means the range of experiments which can be performed on such an instrument is limited when compared to more complex instrumentation; this in turn limits the information which can be obtained in order to provide confident analyte identification. On a QqQ, for example, analyte confirmation is typically carried out by use of selected reaction monitoring (SRM) experiments where the instrument monitors analyte-specific precursor>product fragmentation – such an experiment is not possible using QMS. The question therefore is: with this compromise in selectivity does this new technology have a place in routine analytical testing?

The UK government estimates that during the year 2013–14 in England and Wales 8.8% of adults (aged 16–59) had taken an illicit drug with 3.1% of this population reported to be frequent drug users (i.e. they use illicit drugs on more than one occasion per month) [32]. As a consequence of this high level of reported drug use there is interest in testing individuals for signs of drug use from a number of organisations including employers, law enforcement agencies and the military [33]. Driving while impaired by drugs – both illegal and prescription – is of particular concern for traffic enforcement officers with DoA reported to be a contributing factor in 3% of fatal traffic accidents during 2011 [34]. Historically, driving under the influence of drugs (DUID) was established by a complicated and lengthy process involving a series of roadside field impairment tests followed by a medical examination and eventually an LC/MS urine or blood analysis [35]. However, as of April 2015 the law in England and Wales has been changed to include prescribed cut-off concentrations in blood for a series of drugs and drug-related substances [36]. This new legislation has therefore given rise to the need of new techniques for the rapid screening of drug driving suspects both at the roadside and in police stations.

This new legislation covers 8 illegal drugs and drug-related substances (benzoylecgonine, cocaine, delta-9-tetrahydrocannibinol (THC), ketamine, lysergic acid diethylamide (LSD), methylamphetamine, methylenedioxymethamphetamine (MDMA) and 6-monoacetylmorphine (6AM, a heroin metabolite)), 8 prescription drugs (clonazepam, diazepam, flunitrazepam, lorazepam, methadone, morphine, oxazepam and temazepam) and amphetamine which is considered separately due to its use as both an abused and prescribed drug. The threshold limits for these drugs vary a great deal between drugs ranging from 1 μg/L for LSD up to 1000 μg/L for temazepam. While these thresholds are defined in blood there is good evidence to suggest that the concentration of these drugs found in oral fluid can be directly correlated to their concentration in blood [37]. The relatively non-invasive nature of oral fluid sampling compared with blood sampling therefore makes oral fluid the preferred candidate matrix for rapid screening of drug driving suspects [37]. There are currently two immunoassay-based devices approved for drug testing in oral fluid by UK police forces both of which are currently only approved for cocaine and cannabis (THC) detection [38]. While these immunoassays are relatively quick and easy to perform they tend to have high false positive and false negative rates due to their comparatively low specificity and sensitivity [39]. They are also somewhat inflexible requiring the development of a new detection kit (at considerable cost) for each additional analyte.

A large number of LC-MS and GC–MS based methods for the analysis of DoA in oral fluid have been reported in the literature [1,6,7,9]. The size and cost of the instruments used in these works combined with the sample preparation requirements (particularly for GC–MS) mean that these methods are confined to dedicated analytical laboratories. The emergence of cheap, small footprint MS, therefore, presents an opportunity to provide MS analysis in smaller forensic labs and perhaps even non-laboratory settings such as police custody suites (although the prospect of using LC/MS technology at the roadside seems unlikely). Here, we describe the development and testing of a rapid LC-MS method for the detection of DoA in oral fluid using a low cost, miniaturised QMS.

2. Experimental

2.1. Chemicals

The full suite of seventeen compounds outlined in the DUID legislation [40] was not studied in this current work, however, a sub-set of compounds chosen does cover the full range of included compound classes (4 amphetamines, 1 benzodiazepine, 2 opiates (codeine was included as an additional analyte), cocaine and methadone) apart from LSD. 1 mg/mL Certified Reference Materials of the following drugs were obtained from Sigma Aldrich (Poole, UK): Amphetamine, Methamphetamine, Oxazepam, Cocaine, Benzoylecgonine, 6-monoacetylmorphine (6-AM), MDMA, MDA, Methadone, Codeine, Delta-9-Tetrahydrocannabinol (THC) and Ketamine.

Solvents were obtained from LGC Standards (Teddington, UK) and formic acid was obtained from Fisher Scientific (Loughborough, UK).

Synthetic saliva was prepared according to the specifications provided by the Centre for Applied Technology Solutions (see Table S1 in Supplementary data) for testing DoA in oral fluid measurement devices [40] and was obtained from LGC Standards.

2.2. Sample preparation

A mixed standard of the twelve drug solutions was prepared at 1 μg/mL in water for use in method development. For spiking, mixed standards of the 12 analytes were prepared in methanol at 1, 5, 10 and 25 μg/mL. These stock standards were then used to create calibration samples by spiking 1 mL of synthetic saliva with the appropriate volume of standard. The total organic solvent composition of each sample was kept below 1% v/v in all cases. Synthetic saliva samples were kept at 4 °C when not in use and discarded 48 h after preparation.

2.3. LC-MS

Measurements were carried out on a QDa interfaced with an Acquity H-Class UPLC both controlled by MassLynx software version 4.1 (Waters, Wilmslow, UK). Before use the MS m/z scale was calibrated and the mass resolution was optimised using the on-board, proprietary calibrant.

The MS was operated in full scan and selected ion monitoring (SIM) modes using the following conditions: Source temperature: 120 °C; desolvation Temperature: 600 °C; capillary voltage: +/− 800 V; cone voltage: variable (see below); scan rate: 5 Hz (full scan); 3.571 Hz (SIM).

UPLC separation was carried out on a Waters C18 BEH column (2.1 mm × 50 mm, 1.7 μm) over a 3 min water/acetonitrile/formic acid gradient (5 min total run time) with a 0.7 mL/min flow rate using a 5 μL injection. The LC flow was directed to waste for the first and last 30 s of each HPLC run. The full details of the LC method can be found in Supplementary data (Table S2).

3. Results and discussion

3.1. MS method development

The standard procedure in many laboratories (including ours) for the identification of analytes using LC-MS involves a set of instrument-specific criteria which are some combination of expected retention time, minimum signal-to-noise, detection of multiple ions and/or fragment ions, signal detected for multiple SRM transitions, ion ratios within an certain tolerance, mass resolution and mass accuracy. These criteria are typically weighted depending on how specific they are to the analyte for that particular instrument. Mass accuracy, for example, is treated as a more specific metric on a high resolution mass spectrometer (e.g. Orbitrap) than on a low resolution mass spectrometer (e.g. QMS) [41–43]. For LC-MS measurement on a QMS such as the QDa the available criteria are signal-to-noise, mass resolution, mass accuracy and retention time. The mass resolution and accuracy of such an instrument mean that confident identification at the trace levels required for drug driving testing would not be achievable. It was necessary therefore to move away from a standard mode of

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