Fast quantitation of opioid isomers in human plasma by differential mobility spectrometry/mass spectrometry via SPME/open-port probe sampling interface

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Abstract

Mass spectrometry (MS) based quantitative approaches typically require a thorough sample clean-up and a decent chromatographic step in order to achieve needed figures of merit. However, in most cases, such processes are not optimal for urgent assessments and high-throughput determinations. The direct coupling of solid phase microextration (SPME) to MS has shown great potential to shorten the total sample analysis time of complex matrices, as well as to diminish potential matrix effects and instrument contamination. In this study, we demonstrate the use of the open-port probe (OPP) as a direct and robust sampling interface to couple biocompatible-SPME (Bio-SPME) fibers to MS for the rapid quantitation of opioid isomers (i.e. codeine and hydrocodone) in human plasma. In place of chromatography, a differential mobility spectrometry (DMS) device was implemented to provide the essential selectivity required to quantify these constitutional isomers. Taking advantage of the simplified sample preparation process based on Bio-SPME and the fast separation with DMS-MS coupling via OPP, a high-throughput assay (10-15 seconds per sample) with limits of detection in the sub-ng/mL range was developed. Succinctly, we demonstrated that by tuning adequate ion mobility separation conditions, SPME-OPP-MS can be employed to quantify non-resolved compounds or those otherwise hindered by co-extracted isobaric interferences without further need of coupling to other separation platforms.

Keywords

Opioid analysis; differential mobility spectrometry; open-port probe sampling interface; SPME-MS

Abbreviations

OPP: open-port probe; DMS: differential mobility spectrometry; FWHM: full-width at half maximum; SV: separation voltage; CoV: compensation voltage; SPME: solid phase microextraction

1. Introduction

Simple, robust and high-throughput sample preparation workflows capable of yielding reliable quantitative analysis results are highly desired for the determination of pain-management substances and drugs of abuse in biological fluids. Although dilute-and-shoot procedures have been evaluated for this purpose,[1] most assays still require a sample clean-up step (e.g. solid-phase extraction[2] or liquid-liquid extraction[3]) for removal of biological matrix components that could cause contamination of the chromatographic column or ionization suppression.[4] Solid-phase microextraction (SPME), a green technology that combines sampling, sample clean-up, and analyte enrichment into a single step, has been widely used for high-throughput sample preparation with minimum handling[5], including in-vivo sampling, and on-site monitoring of several substances in different environmental, clinical and food applications.[6-8] Although SPME devices are capable of performing fast sample extraction from biological matrix, chromatographic separation (i.e. GC or HPLC) still is the platform of choice for the analysis of drugs of abuse,[5, 9] because the specificity may suffer from the loss of a chromatographic dimension (e.g. interferences can be observed when analyzing isomeric species with common fragment ions such as codeine and hydrocodone). Therefore, the chromatography process has become the bottleneck to further improve the analysis throughput.

Differential mobility spectrometry (DMS) is a gas-phase ion separation technology based on the mobility difference of target ions under high- and low-electrical fields,[10-15] and it has been proven to be a powerful tool to improve the selectivity of LC-MS analysis[16] and remove endogenous chemical interferences from samples to yield significant improvements in S/N and limits of detection.[17, 18] In this study, a novel DMS device is introduced for the in-line separation and quantitation of isomeric opioids with shared fragment ions (*i.e.* codeine and hydrocodone, Figure S1) without requiring chromatographic separation. An open-port probe (OPP) sampling interface[19-21] was used to couple the high-throughput sample preparation based on SPME and the fast analysis based on DMS/MS. Employment of the OPP system allows merging the steps of analyte desorption and elution into the instrument by simply placing the

SPME device in the open-end of the system for several seconds, without employment of high-pressure parts or injection valves. Good sensitivity, accuracy, and precision were demonstrated for the targeted analytes, with total analysis time per sample ranging between 10-15 seconds when performed by high-throughput means.

2. Experimental section

2.1 Reagents and supplies

Codeine, hydrocodone and their deuterated analogues were purchased from Cerilliant (Round Rock, TX, USA), and used without further purification. HPLC grade acetonitrile and methanol were bought from Caledon Laboratory Chemicals (Georgetown, ON, Canada). Distilled deionized water (18 MΩ) was produced in-house using a Millipore (Billerica, MA, USA) Integral 10 water purification system. Plasma samples were purchased from Bioreclamation IVT (Baltimore, MD, USA). Target analytes and internal standards (deuterated analogues) were spiked in the plasma (v/v) the day before extraction. Bio-SPME mixed mode probes (*i.e.* C_{18}-SCX particles, 45 μm thickness, 15 mm coating length) were kindly provided by Millipore-Sigma (Supelco; Bellefonte, PA, USA).

2.2 Open-port probe sampling interface

The OPP sampling interface used in this work was constructed based on the design introduced by Van Berkel and co-workers[19], and the device is described in Figure S2. This OPP sampling interface uses a vertically aligned, co-axial tube arrangement enabling solvent delivery to the sampling end (open-port) through the tubing annulus (304 stainless steel, 1.75 mm i.d. and 3.18 mm o.d., Grainger, Lake Forest, IL, USA), and aspiration down the centre tube (PEEK, 255 μm i.d. and 510 μm o.d., IDEX, Lake Forest, IL, USA) into the ion source driven by the nebulizer gas (fixed at 90 psi) through a 635 μm i.d. nozzle, and the ESI electrode i.d. was 150 μm. The rate of the flow-in desorption solvent (methanol) was adjusted (Perkin Elmer 200 LC pump, Waltham, MA, USA) to achieve a dome-shaped sampling surface to maximize the contact area with SPME coatings (fixed at 200 μL min^{-1}).

2.3 Sample preparation with SPME and OPP sampling

The workflow of SPME based sample preparation consists of several steps including fibre conditioning, analyte extraction, fibre rinsing, analyte desorption and analyte elution into the mass spectrometer. By using OPP as the MS interface for SPME, the two latter steps are combined (see Figure 1), allowing for better analysis throughput and enhanced sensitivity (i.e. minimal analyte dilution). In addition, since the step of solution transfer is eliminated, chances of additional sample loss are avoided. In this study, a preconditioned SPME fibre was first inserted in a vial containing plasma sample (250 μL) for 5 min with vortex agitation (1500 rpm). Next, the fibre with extracted target analytes was rapidly rinsed in a vessel containing 0.3 mL water for 5 seconds to remove matrix components that could have potentially adhered to the coating surface. For the above two steps, multiple samples were processed simultaneously aiming to increase the analysis speed and reduce sample-to-sample variations. Finally, the SPME fibre was placed into the sampling port containing dome-shaped continuous-flow desorption solvent (i.e. methanol) for 5 seconds, and the desorbed analytes were carried to the ESI electrode for ionization.

2.4 DMS-MS system

A research grade DMS system (dimension of the DMS cell was 1.5 x 20 x 63 mm, gap height x electrode width x cell length) developed by SCIEX was mounted in the atmospheric region between the Turbo V™ ion source (ESI) and the QTRAP® 5500 system's (SCIEX, Concord ON, Canada) sampling orifice. The ESI probe was maintained at a voltage of 5500 V. A constant gas flow in the DMS cell was achieved by the curtain gas flow (N_2; 20 psi, 5.5 L min-1) and the primary stage vacuum pumping of the MS system. Nitrogen was also used as the throttle gas. The temperature of the transport gas was set at 100 °C. The analysis with conventional DMS cell was performed on a QTRAP® 6500+ system equipped with SelexION+™ technology.

The fundamental behaviour of DMS and the asymmetrical SV waveform10-15 has been thoroughly described in previous studies. For the experiments conducted in this study, the optimal compensation

voltage (CoV) for the ion transmission was determined first by scanning the CoV from 5 V to 20 V in 0.15-V increments with the separation voltage (SV) set at 6300 V (p-p). Analytes were dissolved in solution (methanol) to a concentration of 100 ng mL-1, and infused into the ESI source at a rate of 10 μL min-1. During every CoV step, multiple-reaction monitoring (MRM) signals for each analyte (Table S1) were recorded, yielding an ionogram. The optimal CoV for the transmission of each analyte through the DMS device was determined and used for the SPME-OPP sampling experiments as shown in Table S2 (fixed SV and CoV settings for each individual ions without scanning process).

3. Results and discussion

3.1 OPP as a direct sampling interface for SPME

SPME is a high-throughput sample preparation technology. Generally, the extracted/enriched analytes on SPME devices were either thermally desorbed (thermo-stable analytes) for gas-chromatography analysis, or eluted with a high affinity solvent before introduction to HPLC or LC/MS. Aiming to further simplify the analytical workflow, various coupling approaches have been developed in the past two decades to desorb/ionize analytes from SPME devices including desorption electrospray ionization (DESI)[22], direct analysis in real time (DART),[23] and dielectric barrier desorption ionization (DBDI)[24] which desorb analytes by exposing their surfaces to an ionizing medium (such as a gas or an aerosol). However, some of these techniques might require sophisticated and costly equipment, need additional changes to the front-end of the instrument, or may be amenable only for a limited class of molecules (e.g. thermally labile). Other approaches have been introduced recently for the coupling of SPME with MS through direct electrospray ionization on SPME devices including SPME-nano-ESI[25], in-tube SPME-MS,[26] and coated blade-spray.[27, 28] These techniques have been well discussed in a recent review article.[29]

Open-port probe (OPP) sampling interface[19] was designed to circumvent the usage of high-pressure pumps and injection valves, thus preventing common problems observed in LC applications, such as leaking and carryover, while combining the steps of analyte desorption and direct injection into the MS

system. As a result, OPP has shown great potential as a simple and robust tool for the analysis of multiple types of unprocessed and complex samples, including plastic polymers, marker inks, vegetable oils, and laser-cut.[19, 20, 30] In this work, the OPP is used for the direct interface of SPME fibres to MS by simply placing the coating into the sampling end of the port. In this setup, the extracted/enriched analytes were washed out by the elution solvent continuously flowing through the sampling interface, aspirated to the commercial ESI ion source and then analysed by the DMS-MS/MS analysis. Due to the short delay between the desorption and the ionization steps, minimal peak broadening was observed (e.g. FWHM was approximately 6 seconds for a 5-second elution process as shown in Figure 1).

3.2 Isomeric separation with DMS

DMS is a technology that can be used to separate gas-phase ions prior to analysis by MS.[10-15] In a DMS cell, a high frequency asymmetric waveform (SV), varying between high- and low-electric field regimes is applied across the ion transport channel between the two planner electrodes, perpendicular to the direction of the ion transport flow. The different mobilities exhibited under high- and low-electric field results in ions acquiring a "zigzag" trajectory between the electrodes. For successful transmission through the DMS cell and sampling into MS, a DC CoV can be applied to steer ions back on axis. This technology has been successfully used for both chemical noise elimination[17, 31] and isomeric ion separation (e.g. stereoisomers,[12, 17, 32, 33] structural isomers,[34-39] and even tautomers[40]).

Several approaches have been introduced aiming to enhance the resolving power of DMS, by either increasing the CoV difference (ΔCoV) or reducing the peak width in volts at half height (FWHM) in a CoV ramp scan. The modified transport gas has been widely used to improve the resolving power of DMS by adding a polar solvent (modifier) to the curtain gas.[41] Therefore, the analyte ions cluster with modifier molecules during the low-field portion of the waveform and undergo de-clustering during the high-field half-cycle, thus amplifying their differential mobilities with increased ΔCoV (*i.e.* better separation). Alcohols (e.g. methanol, isopropanol) are most frequently used as modifiers, but their high proton affinity may induce a charge stripping from the analyte, which leads to a lower sensitivity.[42] The reduction of

FWHM of a CoV peak is another approach for resolving power enhancement that is typically achieved by extending the ions' residence time within the DMS cell by reducing transport gas flow rate with the throttle gas.[12, 43] It is important to highlight that although better separation can be attained, long in-cell ion residence time also directly leads to significant ion losses. In certain applications, chemical modifier and a throttle gas can be used simultaneously to further improve DMS resolving power.[44]

In this study, an alternative approach to enhance resolving power was developed by modifying the dimensions of the DMS cell from 1 mm x 10 mm x 30 mm (gap height x electrode width x cell length) to 1.5 mm x 20 mm x 63 mm. The enlarged volume of the DMS cell extends the residence time, which reduces the FWHM of studied analytes from 3.0 V to 0.8 V (Figure S3) without application of a throttle gas. Similar FWHM could be reached with the original cell (commercial cell with smaller volume) using 34 psi of throttle gas. However, due to the small ΔCoV attained with the commercial cell (0.8 V), even when using a throttle gas of 34 psi, it was not possible to detect codeine and hydrocodone individually at their optimum sensitivity (CoV peak apex). As an alternative, the CoV can be enlarged by increasing the gap height of the DMS cell. In the presented study, owing to the larger gap between the two electrodes, CoV as well as ΔCoV were increased to 1.5x for the same field strength. Thus, the combined benefits of both reduced FHWM and increased ΔCoV enabled the complete separation of these analytes (Figure 2). As can be seen in Figure S4, the interference between codeine and hydrocodone analysis was observed to be less than one percent. Although adequate separation for these isomers was accomplished by only modifying the geometry of the DMS cell, a chemical modifier and/or throttle gas can also be implemented for more challenging applications. As an example, Figure S5 presents the separation of other opioid isomers (*i.e.* norcodeine, morphine, and hydromorphone) with shared fragment ions on the modified DMS cell with a 10 psi throttle gas.

3.3 Isomeric separation with DMS

The simultaneous quantification of codeine and hydrocodone from human plasma was chosen as the example assay to demonstrate the potential of the introduced SPME-OPP-DMS-MS platform for the fast

analysis of pain management drugs from biological matrix. As shown in Figure 3 and Figure S6, our results met the analytical requirements set by the World Anti-Doping Agency (WADA). A limit of quantification (LOQ) of 1 ng mL^{-1} (Figure S6) and linear dynamic range between 1 and 500 ng mL^{-1}), were demonstrated with good accuracy (85-106%) and linearity ($R^2 > 0.99$) (Table S3). Given that SPME devices can be arrange in a high-throughput assembly (*e.g.* 96 samples at the same time),[45] and there is no need for additional separation step other than that offered by DMS, the analysis can be as fast as 10-15 seconds per sample.

4. Conclusions

A simple, robust and high-throughput technology based on SPME and DMS-MS was introduced for quantitative analysis of drugs of abuse and pain-management drugs in biofluids. Following extraction, SPME devices with isolated and enriched target analytes were directly coupled to the MS system via an OPP sampling interface, without further requirement of injection valves or high pressure parts. A modified DMS cell, with significantly improved resolving power, successfully distinguished isomeric species with shared fragment ions such as codeine and hydrocodone, without the assistance of lengthy LC gradients. The results presented here demonstrate the great potential of coupling SPME to DMS-MS in targeted analysis where regular SPME-MS, without the assistance of chromatographic separation, might fail to provide a true picture of the sample scrutinized. Similarly, this platform could be used for the application of SPME-OPP-DMS-MS towards lipids and metabolites profiling in the future.[46, 47]

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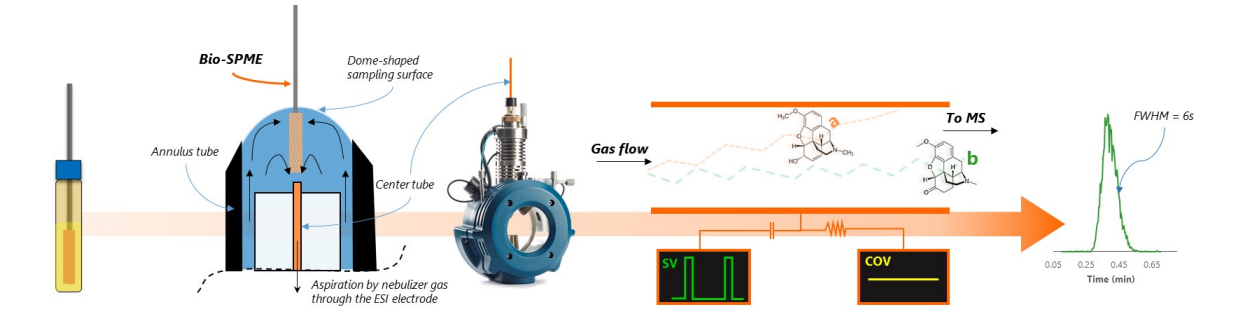
Figure Captions

Figure 1. Workflow for Bio-SPME-OPP-DMS-MS/MS analysis of controlled substances from complex matrices

Figure 2 Ionogram generated from infusion of a methanolic solution containing a mix of codeine (a) and hydrocodone (b). The SV was set as 6300 V.

Figure 3. A. Quantitative analysis of plasma spiked with codeine (1 ng ml^{-1} to 500 ng mL^{-1}) and its isotopologue d3-codeine (50 ng mL^{-1}). B. Quantitative analysis of plasma spiked with hydrocodone (1 ng ml^{-1} to 500 ng mL^{-1}) and its isotopologue d3-hydrocodone (50 ng mL^{-1}). Red triangles represent the accuracy QC levels (30, 200, and 450 ng mL^{-1}, respectively).

1 Figure 1



1. Extraction/enrichment (≤ 5 min)
300 µL of sample
Vortex agitation (1500 rpm)

2. Analyte elution/ionization (~5 s)
~ 5 µL MeOH (0.1% FA), 5.5 kV (ESI-Ion Drive™ source)
Static desorption on OPP-interface

3. DMS-separation
DMS cell (1.5 x 20 x 63 mm, gap height x electrode width x cell length)
SV= 6300 V; CoV = 13.5 V (a) and 15 V (b)

4. MS/MS analysis (~15 s)
Peak integration and statistical analysis

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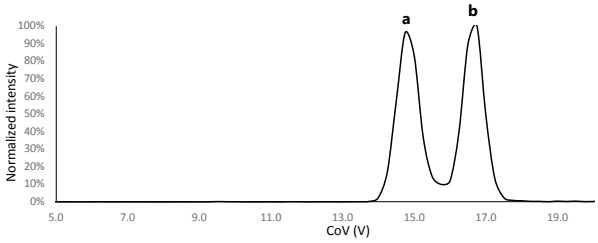
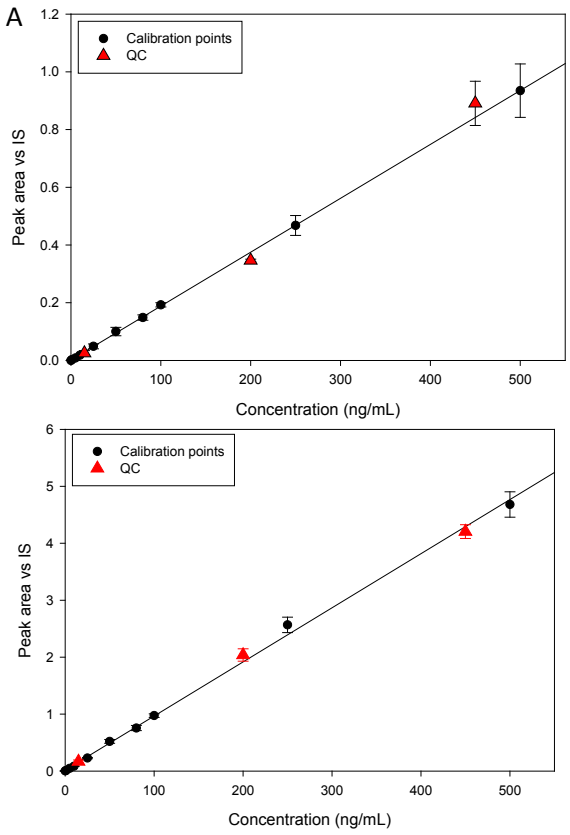
Figure 2


a
b
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Normalized intensity
5.0
7.0
9.0
11.0
13.0
15.0
17.0
19.0
CoV (V)

1 Figure 3



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1 **Graphical Abstract**

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