Open-Port Probe Sampling Interface for the Direct Coupling of Solid-Phase Microextraction to Atmospheric Pressure Ionization Mass Spectrometry

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ABSTRACT

Solid phase micro-extraction (SPME) is a green technology that combines sampling, sample preparation and extraction of analytes in one step thus simplifying the analytical process to a minimum and making it suitable for on-site use.[1-3] During the last two decades, SPME has been developed to couple to different analytical instruments. However, the desorption step is often necessary for non-thermally labile analytes to elute the compounds from SPME device to a solvent. To take the full advantage of the enrichment potential of SPME devices, several approaches have been developed for direct SPME-MS coupling.[4] However, substantial modifications to the instrument front-end are typically required. In this study, we introduce an electrospray based open-port probe (OPP) as a direct sampling interface for SPME devices.[5]

INTRODUCTION

Open-port probe (OPP) sampling interface used a vertically aligned, co-axial tube arrangement enabling solvent delivery to the sampling end (open-port) through the tubing annulus and aspiration down the center tube into the ion source driven by the nebulizer gas. In this study, the SPME device (after extraction and washing) was inserted into the open-port for 5 seconds to touch the continuous flowing stream that transports the desorbed material into the ion source directly. The transit time between sampling and signal was less than 5 seconds. In this SPME-OPP setup, steps of desorption and sampling are combined, simplifying the workflow. Sensitivity is improved because of the rapid and efficient extraction of the sample from the fiber and the highly concentrated plug of sample entering the source. The LOQ in the ng/mL range can be achieved.

Here, various assays in different biological matrix have been tested, including clenbuterol in urine, opioids isomers (codeine and hydrocodone) and antifungal medication (variconazole) in plasma. The analysis speed is about 10-15 seconds per sample.

The selective sample extraction on to SPME fibers and the flowing rinsing steps remove salts, and non-specific bound species (e.g. proteins) from the biological matrix. Additional selectivity gains other than MRM can be achieved with other in-line techniques including MRM^3 and differential mobility spectrometry (DMS) without requiring any additional sample analysis time. In MRM^3, 1st fragment ions are trapped in Q3, followed by excitation to perform the 2nd fragmentation step. The unique 2nd generation product ions allow the differentiation of clenbuterol from interference. Alternatively, DMS is a gas-phase ion separation technique based on the mobility difference,[6] which was placed in between the ion source and the sampling orifice. In this study, MRM^3 was used to effectively eliminate the interference from urine, and DMS was used for the successful discrimination of opioid isomers with shared fragment ions.

MATERIALS AND METHODS

Sample preparations:
A mix-mode coated SPME fibre (*i.e.* C18-SCX particles) was immersed in biological samples (300 µL urine for clenbuterol analysis, 100 µL plasma for the voriconazole quantification, and 250 µL plasma for the analysis of codeine and hydrocodone) spiked with standard and extracted for 2 minutes followed by water wash for 5 seconds. The SPME fibre coating was inserted into the open port sampling interface for 5 seconds to transfer into the ESI ion source.

OPP-MS system:
An Sciex QTRAP® 5500 or 6500+ MS/MS system with Turbo V™ source was used in this study. The electrospray ionization (ESI) nozzle was modified as 635 µm i.d. and the ESI electrode i.d. was 150 µm. The rate of the OPP flow-in desorption solvent (methional) was adjusted to achieve a dome-shaped sampling surface to maximize the contact area with SPME coatings (fixed at 200 µL/min) A research grade DMS cell (1.5x20x63mm) was used as a high-resolution alternative of the standard commercial cell (1.0x10x30mm). For all DMS analysis, nitrogen was used for the transport gas as well as the resolving gas.

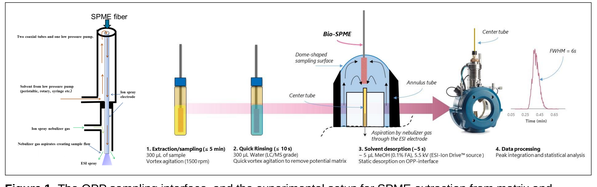

Figure 1. The OPP sampling interface, and the experimental setup for SPME extraction from matrix and desorption-ionization via OPP.

RESULTS

Analysis of variconazole from palsma with SPME-OPP-MRM

Voriconazole is a triazole antifungal medication that is generally used to treat serious and invasive fungal infections. Its concentration in plasma needs to be monitored to determine the time for a second dose. The required LOQ is 100 ng/mL, and the necessary linear dynamic range is up to 20 µg/mL. In this study, we used the mix-mode (C18 & SCX) SPME fiber for a 1 min extraction from 100 µL plasma containing spiked-in voriconazole and internal standard (1 µg/mL voriconazole-D3), followed by the 5 seconds wash with water and 5 seconds sampling in the OPP.

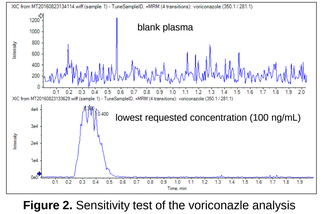

Figure 2. Sensitivity test of the voriconazle analysis

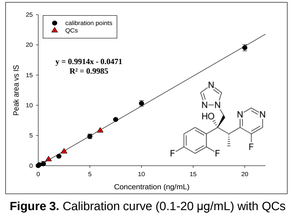

Figure 3. Calibration curve (0.1-20 µg/mL) with QCs

Table 1. Precision and accuracy of the voriconazole analyss in human plasma (N=3)

Concentration (µg/mL)	Mean	SD	RSD (%)	Concentration (µg/mL)	Range	Accuracy (%)
1.0	1.08	0.03	3.3	1.0	1.07-1.13	107-113
2.5	2.33	0.12	5.3	2.5	2.25-2.50	90.2-100
6.0	5.79	0.04	0.8	6.0	5.85-5.94	97.4-98.9

Analysis of clenbuterol from urine with SPME-OPP-MRM^3

Significant interference in the MRM analysis of clenbuterol in human urine have been bound to raise the LOQ for real samples to unacceptable levels that are substantially higher than those achieved in standard solutions. In this study, we used the highly selective MRM^3 technique for quantification.

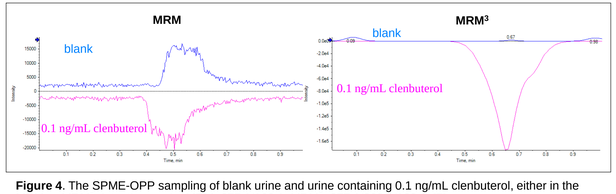

Figure 4. The SPME-OPP sampling of blank urine and urine containing 0.1 ng/mL clenbuterol, either in the MRM or MRM^3 detection mode. MRM: 277.1/168.1. MRM^3: 277.1/203.1/168.1. [7]

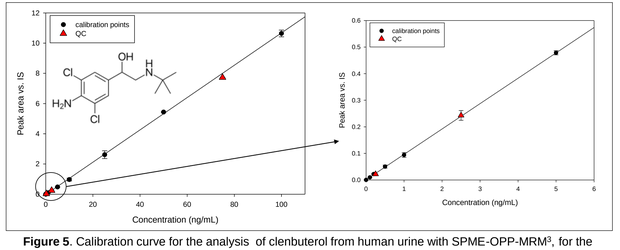

Figure 5. Calibration curve for the analysis of clenbuterol from human urine with SPME-OPP-MRM^3, for the concentration range of 0.1-100 ng/mL. [7]

Simultaneous quantification of codeine and hydrocodone from plasma with SPME-OPP-DMS-MRM

Codeine and hydrocodone are isomers with shared fragments, and it is challenging to differentiate them by MRM. Their analysis typically requires LC separation to ensure unique detection and avoid interferences. Here, we demonstrate the high-throughput and sensitive analysis of these compounds with the SPME-OPP-DMS-MRM setup.

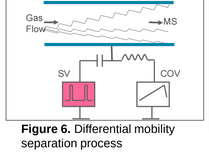

Figure 6. Differential mobility separation process

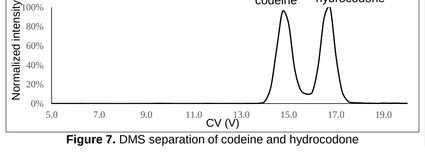

Figure 7. DMS separation of codeine and hydrocodone

Good accuracy and linearity was observed with the linear range of 1-500 ng/mL (Figure 8).

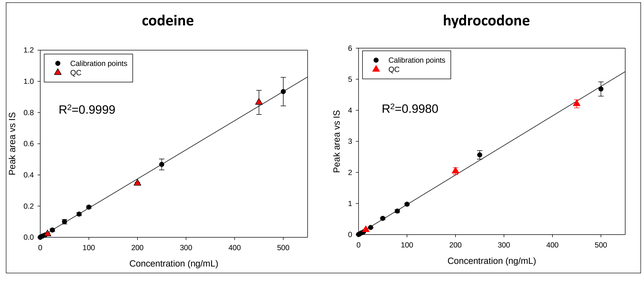

Figure 8. Calibration curve for simultaneous quantification of codeine and hydrocodone from plasma with SPME-OPP-DMS-MRM, for the concentration range of 1-500 ng/mL.

CONCLUSIONS

Here we demonstrated the successful coupling of SPME device with MS via an open-port probe sampling interface for the high-throughput and sensitive quantifications. The direct immersing of the SPME coatings into the OPP device combines the steps of elution and sampling, potentially improves the analysis speed to about 10-15 seconds per sample. High-throughput techniques including MRM^3 and DMS have been used to provide sufficient speed and selectivity. Good accuracy and linearity was observed over a wide linear range. The robustness and the ease of use makes this system valuable for routine high-throughput analysis.

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