Biocompatible Solid Phase Microextraction (BioSPME) of Drugs of Abuse from Urine

By Emily Barrey, Senior R&D Scientist & Sara Smith, PhD
MilliporeSigma, Bellefonte, PA, USA

Biocompatible solid phase micro extraction (BioSPME) is an expansion of the traditional sample preparation technique that has allowed for direct sampling from biological matrices, without significant co-extraction of matrix interferences. In this study, BioSPME was used to extract several common drugs of abuse and their metabolites from urine. A comparison was also drawn to an established sample preparation technique: simple dilution ('dilute and shoot'). Due to the ability of BioSPME devices to repel matrix interferences and pre-concentrate the sample prior to analysis, an increase in sensitivity was observed in comparison to the traditional method. Matrix effects were found to be minimal and consistent, and a decrease in analyte response was not observed after repeated analysis of BioSPME extracts. The combination of these benefits results in a more robust analytical method, while still employing a simple sample preparation procedure.

Introduction

Preliminary or presumptive screening of urine for the presence of drugs of abuse is typically accomplished using less specific methods such as ELISA testing kits. However, samples with a positive indication are required to be submitted for confirmatory testing. These methods need to be sensitive enough to monitor values well below therapeutic levels, yet selective enough for identification of specific drugs without false positives. Therefore, GC-MS and/or LC/MS/MS are commonly used for confirmation. Although selective, the sensitivity of a mass spectrometer (MS) is highly dependent on the quality of the sample. The presence of matrix interferences such as salts, proteins, and phospholipids can affect analyte response, a phenomenon known as matrix effects. Therefore, the sample preparation procedure can have a great impact on the accuracy, precision, and robustness of the analytical method.

BioSPME is an equilibrium extraction technique in which the analyte of interest partitions between the sample matrix and the extraction coating on a BioSPME device. For this study, the BioSPME device was a LC tip format, which consists of a coated fibre housed within a pipette tip. This tip allows for the device to be easily manipulated via liquid handlers or robotics, and is therefore high throughput amenable. The extraction coating contains C18 functionalised silica particles that are embedded within a proprietary biocompatible binder (Figure 1). The role of this binder is to reduce or eliminate the extraction of matrix interferences, without reducing analyte extraction. This allows for the isolation of target analytes, while minimising the presence of matrix, resulting in a highly sensitive micro extraction technique. The binder also allows for the extraction and desorption steps to be performed via immersion. This allows the technique to be compatible with LC/MS/MS analysis.

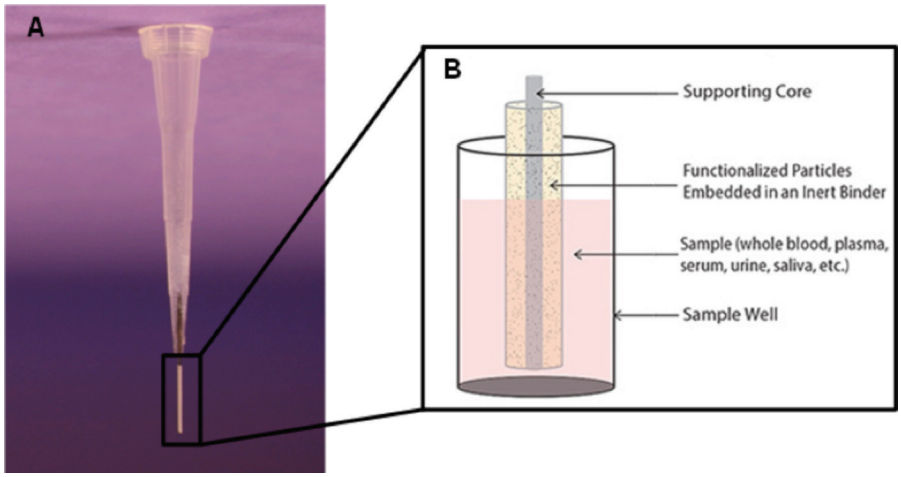

Figure 1. (A) A commercially available LC tip BioSPME device which consists of a coated fibre housed within a pipette tip. (B) A basic schematic of an extraction performed with a BioSPME fibre. The fibre is coated with functionalised particles that have been embedded within a proprietary binder. The binder allows the fibre to be placed directly within a biological fluid for sampling.

Experimental

SPME LC Tips, C18 (Part No. 57234-U), LC/MS ultra-grade acetonitrile, ammonium formate, and LiChrosolv® methanol were purchased from MilliporeSigma (Darmstadt, Germany). LC/MS grade water was obtained from a Milli-Q® Integral water purification system with a LC-Pak® polisher,

Table 1. MS source settings.

Parameter	Setting
Scan Type	MRM
Polarity	Positive
IS Voltage (V)	3200
Source temperature (°C	450
Gas source 1 (arbitrary units)	50
Gas source 2 (arbitrary units)	55
CAD	Medium
Collision exit potential (V)	4
Dwell time (ms)	100

Table 2. Compound specific MS parameters.

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Declustering Potential (V)	Collision Energy (V)	Retention Time (min)
Methadone	310.3	265.3	26	19	10.4
Methadone-d_3	313.3	268.0	56	41	10.4
EDDP	278.3	234.0	56	41	5.26
EDDP-d_3	281.3	234.0	56	41	5.26
Cocaine	304.2	182.3	36	25	1.56
Cocaine-d_3	307.2	185.3	36	25	1.56
Benzoylecgonine	290.2	105.2	41	39	0.67
Benzoylecgonine-d_3	293.2	171.2	41	39	0.67
Cocaethylene	318.2	196.3	41	25	2.27
Cocaethylene-d_3	321.2	199.3	41	25	2.27
Norfentanyl	233.2	84.2	70	50	0.85
Norfentanyl-d_5	238.2	84.2	70	50	0.85

also purchased from MilliporeSigma (Darmstadt, Germany). Certified analytical reference standards of methadone, methadone-d_3, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) perchlorate salt, EDDP-d_3 perchlorate, cocaine, cocaine-d_3, benzoylecgonine, benzoylecgonine-d_3, cocaethylene, cocaethylene-d_3, norfentanyl, and norfentanyl-d_5 were purchased from Cerilliant® (Round Rock, TX). Synthetic urine (Surine™) was purchased from Cerilliant® (Round Rock, TX).

BioSPME extraction procedure. SPME LC Tips were conditioned within 1 mL of methanol for 10 min, followed by equilibration within 1 mL of water for 10 min. The fibres were then placed in 600 µL of spiked synthetic urine and agitated for 10 min at 500 rpm using an orbital shaker. The fibres were then removed from the samples and placed in 300 µL of 20 mM ammonium formate in 90:10 (methanol:water) containing 200 ng/mL of deuterated internal standards, for 30 min with agitation at 500 rpm.

Spiked synthetic urine standards were prepared in a concentration range of 20 to 1000 ng/mL in order to prepare an extracted curve. In addition, six replicate extractions were performed at an analyte concentration of 100 ng/mL. A 30 min equilibration time was allowed after matrix spiking. Unspiked matrix samples were also extracted in order to prepare post-extraction spikes. All samples were analysed by LC/MS/MS.

Simple dilution procedure. Spiked samples of synthetic urine (100 µL) were aliquoted into an appropriate vial and then diluted with 900 µL of 20 mM ammonium formate in 90:10 (methanol:water). Samples were vortexed and analysed by LC/MS/MS. A matrix matched standard curve was prepared in a concentration range of 20 to 1000 ng/mL and six samples were spiked at an analyte concentration of 100 ng/mL prior to dilution. The final internal standard concentration was maintained at 200 ng/mL. Unspiked samples were also diluted in order to prepare post-extraction spikes.

LC/MS/MS analysis. Samples were analysed using an Agilent 1290 Infinity UPLC coupled to a Sciex 3200 QTrap mass spectrometer. Separations were achieved with an Ascentis Express® RP-Amide (10 cm x 2.1 mm; 2.7 µm) column, purchased from MilliporeSigma (Darmstadt, Germany). An isocratic method was used with a mobile phase consisting of 10 mM ammonium formate in 75:25 (water: acetonitrile) at a flow rate of 0.4 mL/min. The column temperature was maintained at 40°C with an injection volume of 2 µL. The MS source and compound dependent parameters are displayed in tables 1 and 2.

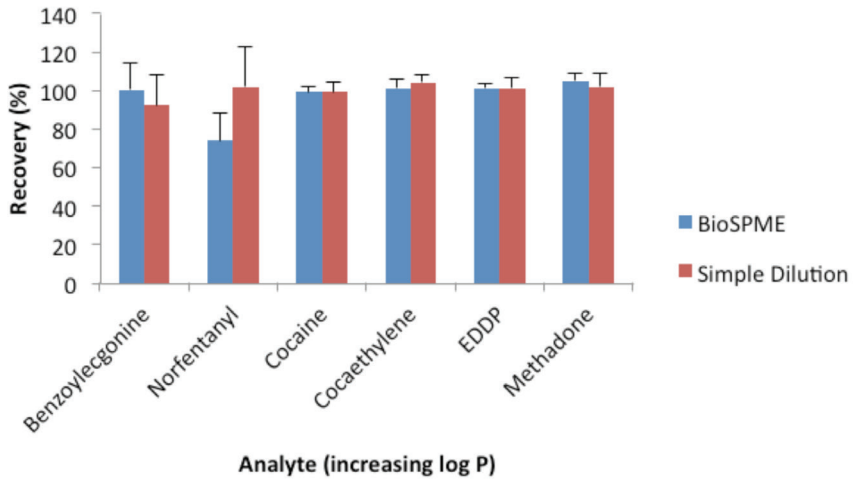

Figure 2. Comparison of analyte recoveries of 100 ng/mL spiked synthetic urine samples using BioSPME and simple dilution, n=6.

Results and Discussion

As the BioSPME technique only extracts the free portion of drug within a sample, extracted standard curves were prepared for each analyte, spiked within synthetic urine. These curves were used to determine the average recovery of each analyte within the 100 ng/mL spiked samples. The developed BioSPME extraction method resulted in comparable analyte recoveries as the accepted simple dilution technique (Figure 2). For both methods, the relative standard deviation (RSD) was elevated for the two most polar analytes, benzoylecgonine and norfentanyl. The loss in precision for these analytes with the BioSPME technique may be due to the use of a C18 phase within the fibre coating. This phase has a reduced selectivity towards more polar analytes, resulting in a lower response. Overall, the average RSD (%) across the six analytes was determined to be 6.6% and 9.2 % for the BioSPME and simple dilution methods, respectively

In addition to analyte recovery, the

Table 3. Average matrix factors and matrix factor ranges for BioSPME and simple dilution preparations of synthetic urine. The matrix factor range represents the lowest and highest value calculated for each set of six replicates.

	Average Matrix Factor (n=6)		Matrix Factor Range	
Analyte	BioSPME	Simple Dilution	BioSPME	Simple Dilution
Benzoylecgonine	1.06	0.79	0.86-1.3	0.5-1.4
Norfentanyl	1.08	1.04	0.82-1.4	0.7-1.7
Cocaine	1.03	1.14	0.84-1.2	0.71-1.5
Cocaethylene	1.08	1.09	0.86-1.3	0.74-1.6
EDDP	1.17	1.26	0.91-1.4	0.84-1.9
Methadone	1.10	1.05	0.86-1.4	0.57-1.5

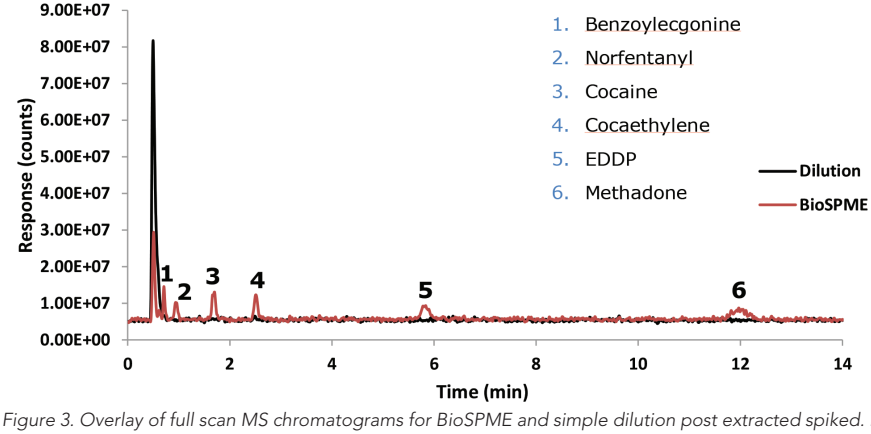

Figure 3. Overlay of full scan MS chromatograms for BioSPME and simple dilution post extracted spiked. Matrix suppression was more significant for the dilution samples indicating that BioSPME offered improved matrix removal over the traditional technique.

effect of any remaining matrix on analyte response following sample preparation was also evaluated. Comparisons were drawn by calculating the matrix factor. For each clean-up procedure, extractions were also performed using blank (unspiked) matrix. Following processing, these matrix blanks were then spiked with a standard solution of analyte.

The matrix factor is then determined by comparing the analyte response of a pure solvent standard containing no matrix to a post-extracted spike sample containing the same concentration of analyte but also any matrix that has not been removed by sample preparation. A matrix factor of one would indicate that any matrix remaining within the post-extracted spike sample had no significant impact on analyte response. A matrix factor greater than one would indicate that matrix present within the sample resulted in an increase in analyte response. This is often referred to as signal enhancement. Finally, if a matrix factor is less than one, it indicates that matrix present within the sample resulted in a decrease in analyte response, or ion suppression. Table 3 displays the average matrix factor and the matrix factor range obtained for six replicates using each sample preparation method. These calculations were performed without internal standard normalisation.

The average matrix factors calculated for BioSPME were found to be comparable to the accepted technique of simple dilution. However, upon closer investigation it was noticed that the range of matrix factors differed between the two sample preparation techniques. The ability of BioSPME to selectively extract analytes without co-extracting matrix interferences led to less variation in analyte response, thus increasing the robustness of this method over simple dilution.

Full scan MS data was also collected for matrix blank samples that were fortified post-extraction using each sample preparation method (Figure 3). All six analytes were clearly detected for a BioSPME post extracted spike. However, for a sample prepared by simple dilution, no detectable signal was obtained. This is likely due to matrix induced ion suppression, which was indicated by the lower matrix factors that were calculated for some simple dilution samples.

Conclusions

BioSPME resulted in comparable analyte recoveries to the more traditional method of simple dilution. An improvement in precision was also noticed in the BioSPME extraction of some of the analytes evaluated during this study. However, the significant advantage of BioSPME was revealed when evaluating matrix removal. By calculating matrix factors, it became apparent that BioSPME removes matrix interferences in a consistent manner. Ion suppression was a common issue when analysing samples prepared by simple dilution. This suppression was significant enough to completely mask the response of all six analytes when full scan MS data was collected on post-extracted spikes. The improved matrix removal and higher level of precision suggests that BioSPME offers a more robust and reproducible method for the detection of drugs of abuse in urine.