LC/MS/MS Analysis of Drugs of Abuse using Biocompatible Solid Phase Microextraction (BioSPME)

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Introduction

The field of illicit drug testing has recently become a constantly changing environment with the rapid development of unregulated designer and synthetic compounds. These compounds are reported to generate stimulating effects similar to that of methamphetamine, heroin and MDMA. The difficulty for forensic testing facilities is the fact that these compounds are not detected under normal ELISA testing methods; therefore, more specific LC/MS based approaches are necessary. This study demonstrates the benefits of Biocompatible Solid Phase Microextraction (BioSPME) over traditional "dilute and shoot" and protein precipitation methods for the enrichment of illicit drugs and drugs of abuse directly from biological matrices. The utility and unique selectivity of these sampling devices will also be explored.

BioSPME Theory

- BioSPME is an equilibrium extraction technique in which the analyte of interest partitions between the sample matrix and the coating of a BioSPME fiber.
- For this specific application, the coating consists of C18 functionalized silica particles that are embedded within a proprietary biocompatible binder.
- The purpose of the binder is to reduce or eliminate extraction of matrix interferences, without reducing analyte extraction. Therefore, this methodology allows for isolation of target analytes, while minimizing the presence of matrix, resulting in a highly sensitive microsampling technique.

Figure 1. Schematic of a BioSPME Fiber Extraction

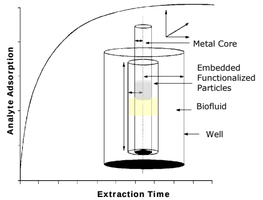
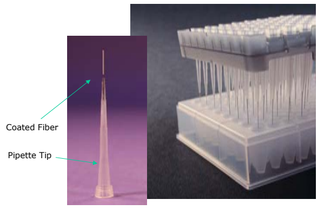


Figure 2. BioSPME LC Tip Format



Analytes of Interest

- **Model Set 1-Cathinones in Urine:** Naphyrone, α-Pyrrolidinovalerophenone (α-PVP), 3,4-methylenedioxypyrovalerone (MDPV), butylone, methylone.
- **Model Set 2-Drugs of Abuse in Urine and Plasma:** Cocaine, cocaethylene, benzoylecgonine, methadone, 2-Ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium (EDDP), norfentanyl.

Methods

Cathinones: Procedure

BioSPME Extractions:

Fibers were conditioned within 300 µL of 50:50 (MeOH:water) for 20 min with an agitation rate of 500 rpm.

↓

Following a 30 min equilibration period, spiked urine samples were extracted for 15 min at 500 rpm. Total sample volume was 300 µL.

↓

Fibers were desorbed within 300 µL of 0.125% NH_4OH in MeOH for 30 min at 500 rpm.

↓

Final extracts were diluted 1:10 with ACN (containing internal standards) and analyzed via LC/MS/MS.

Dilution:

Urine samples were spiked with analyte. A 30 min equilibration period was allowed prior to dilution.

↓

Spiked matrix samples were diluted 1:10 with ACN (containing internal standards) and analyzed via LC/MS/MS.

Drugs of Abuse: Procedure

BioSPME Extractions:

Fibers were conditioned with 1 mL of MeOH for 10 min, then 1 mL water for 10 min.

↓

Following a 30 min equilibration period, spiked urine/plasma samples were extracted for 10 min at 500 rpm. Total sample volume was 600 µL.

↓

Fibers were desorbed for 30 min at 500 rpm with 300 µL of 20 mM ammonium formate in 90:10 MeOH:water.

↓

Final extracts were vortexed and analyzed via LC/MS/MS.

Dilution (Urine):

Urine samples were spiked with analyte and IS. A 30 min equilibration period was allowed prior to dilution.

↓

Spiked matrix samples were diluted 1:10 with 20 mM ammonium formate in 90:10 MeOH:Water , vortexed and analyzed via LC/MS/MS.

Protein Precipitation (Plasma):

Plasma samples were spiked with analyte and IS. A 30 min equilibration period was allowed prior to extraction.

↓

600 µL of ACN was added to a 200 µL aliquot of spiked matrix samples.

↓

Samples were vortexed at 3000 rpm for 1 min and then centrifuged at 10,000 rpm for 10 min.

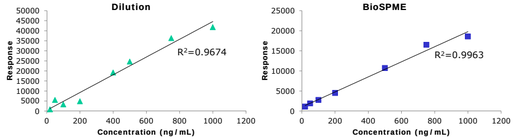
↓

The supernatant was transferred and analyzed via LC/MS/MS.

Results

Linearity

Comparison of Matrix Matched Curves for Methylone from Urine



Results (contd.)

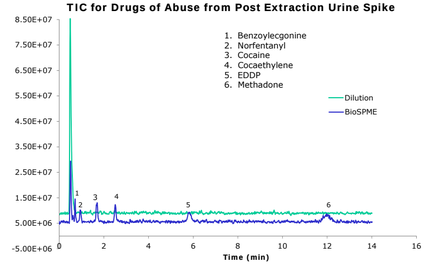
Sensitivity

These comparisons are made for the analysis of benzoylecgonine from plasma. The BioSPME method allowed for a 2X pre-concentration, resulting in a greater increase in sensitivity

Technique	Calculated Limit of Detection (ng/mL)	Calculated Limit of Quantitation (ng/mL)
BioSPME	45.4	151
Protein Precipitation	176	585

Analyte Response

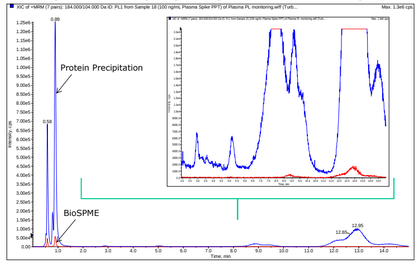
TIC for Drugs of Abuse from Post Extraction Urine Spike



The background for the "dilute and shoot" sample significantly suppressed any analyte signal.

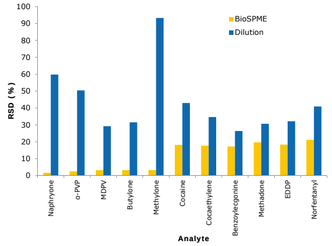
Phospholipid Monitoring

Comparison of Phospholipid Response from Plasma Samples



Matrix Factors and %RSD's - Urine

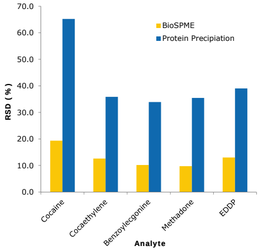
Analyte	Matrix Factor Range BioSPME	Dilution
Naphyrone	1.11-1.15	0.38-1.23
α-PVP	1.11-1.16	0.58-1.62
MDVP	1.14-1.12	1.02-1.88
Butylone	1.08-1.15	0.75-1.46
Methylone	1.16-1.23	0.21-1.08
Cocaine	0.84-1.23	0.71-1.47
Cocaethylene	0.86-1.32	0.74-1.57
Benzoylecgonine	0.86-1.33	0.48-1.38
Methadone	0.86-1.35	0.57-1.48
EDDP	0.91-1.42	0.84-1.91
Norfentanyl	0.81-1.40	0.68-1.67



Matrix Factors and %RSD's - Plasma

Analyte	Matrix Factor Range BioSPME	Protein Precipitation
Cocaine	1.03-1.06	0.71-1.47
Cocaethylene	1.08-1.37	0.74-1.57
Benzoylecgonine	1.10-1.70	0.48-1.38
Methadone	1.03-1.39	0.57-1.48
EDDP	1.13-1.43	0.84-1.91

Significant variation was observed in the matrix factors (range and %RSD) for the protein precipitated plasma samples.



Conclusions

- In conclusion, BioSPME was determined to be superior to more traditional biological sample preparation techniques such as "dilute and shoot" and protein precipitation.
- In terms of quantitative analysis parameters, BioSPME resulted in a broader dynamic range, improved linearity, and increased sensitivity.
- Due to the ability of the proprietary biocompatible binder to greatly reduce co-extraction of biological interferences, matrix effects were found to be less significant. Matrix effects were also found to be more consistent across samples. The benefit of matrix removal was especially evident when working with biological matrices containing a large concentration of phospholipids, such as plasma.
- Finally, BioSPME allowed for acceptable recoveries of all 11 analytes tested. For many analytes tested, this technique was found to have greater precision than the traditional methodologies.