Screening Method for Methamphetamine and Amphetamine Using DART®-MS Analysis Followed by Chiral Confirmation for D-Methamphetamine

Emily Barrey, Dave Bell, and Candace Price **MilliporeSigma, Bellefonte, PA**

Introduction

Workplace drug screening methods employ immunoassay methods for the initial test for methamphetamine and amphetamine. These tests are followed by either GC/MS or LC/MS methods to confirm the presence of the analytes, as well as distinguish between the D and L forms of methamphetamine in positive samples.[1] Immunoassay methods are susceptible to cross reactivities and false positives as well as having sample storage considerations.[2] GC/MS confirmatory methods utilize a complex multistep extraction followed by derivatization to perform the enantiomeric separation.[2] These steps are necessary in order to elucidate whether a positive screen for methamphetamine comes from L-methamphetamine that is found in OTC products or the illicit form, D-methamphetamine.

In this study, Biocompatible Solid Phase Micro Extraction (BioSPME) fibers were used to determine the presence of methamphetamine and amphetamine in urine samples. Analysis of initial screening samples can be obtained in less than 60 seconds by analyzing the extracted urine samples on a DART®-MS system. Confirmation of the enantiomeric form was achieved using BioSPME with a solvent desorption step, followed by chiral separation on an LC/MS/MS system. Sample preparation via BioSPME eliminates many steps found in the GC/MS confirmatory method, which affords a reduction in preparation time, solvent consumption and laboratory costs. BioSPME preparation with DART®-MS analysis followed by enantiomeric confirmation analysis by chiral LC/MS/MS, demonstrates a fast, reproducible and accurate method for the detection of methamphetamine (D & L forms) and amphetamine.

Process

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 (Figure 1). The role of this binder is to reduce or eliminate the extraction of matrix interferences during immersion, without reducing analyte extraction. This allows for the isolation of target analytes, while minimizing the presence of matrix, resulting in a highly sensitive microextraction technique. Once the analytes have been extracted onto the fibers, they can be detected using direct analysis on a DART® system (Figure 2).

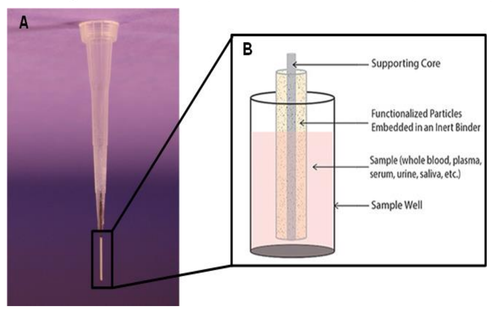

Figure 1. (A) A commercially available LC tip BioSPME device which consists of a coated fiber housed within a pipette tip. (B) A basic schematic of an extraction performed with a BioSPME fiber. The fiber is coated with functionalized particles that have been embedded within a proprietary binder. The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

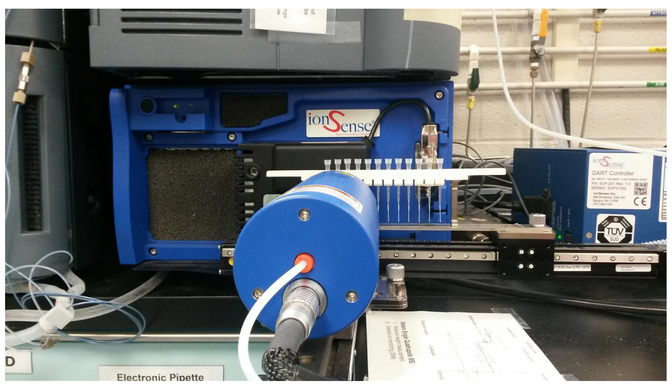

Figure 2. DART® system connected to Waters Aquity® QDa® MS analyzing BioSPME fibers

Methods

Mixed mode (C8/SCX) fibers were conditioned in 1 mL of 50:50 (MeOH/water) for 30 min with an agitation rate of 800 rpm. Followed by a water rinse for ~ 10 sec at 800 rpm.

Fibers were placed into 1 mL spiked urine samples and extracted for 10 min at 800 rpm.

Fibers screened via DART®-MS.

For chiral determination, fibers were desorbed in 0.1% ammonium hydroxide in 90:10 Methanol:water for 10 min at 800 rpm

Samples then analyzed by LC/MS/MS

DART® -MS Settings

Instrument: DART®-SVP coupled to an Acquity® QDa

Ion Mode	Positive
Temperature (° C)	300
Heater Wait Time (sec)	10
Sample Speed (mm/sec)	0.3
Contact Closure Delay (sec)	3
Shutdown State	Standby
Standby Temperature (° C)	300

Mass range: m/z 100-500
Polarity: Positive
Frequency: 10 Hz
Cone Voltage: 15V

Analyte	Precursor
Methamphetamine	150.1
Methamphetamine-d_5	155.1
Amphetamine	136.0
Amphetamine-d_5	141.0

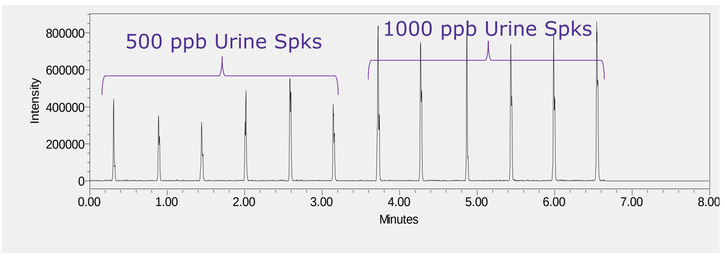

Figure 3. Example Methamphetamine DART-MS response from 12 fibers

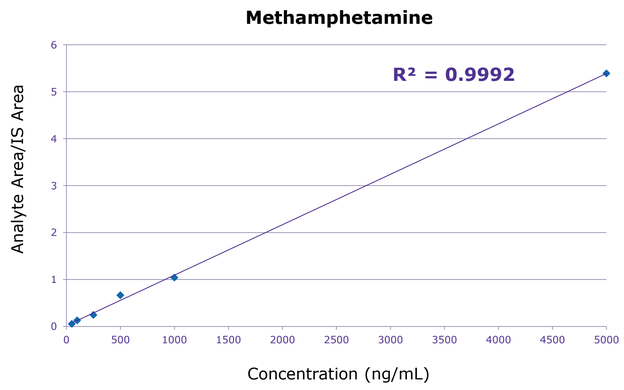

Figure 4. Calibration curve from spiked urine samples using DART(R)-MS .

LC/MS/MS Settings

column: Chirobiotic V2, 15 cm x 4.6 mm, 5 µm
mobile phase:
95:5:0.1:0.02, methanol:water:acetic acid:ammonium hydroxide
flow rate: 1 mL/min
column temp: 20 ºC
det.: MS, ESI (+), SIM=150.1 amu
injection: 1 µL
Instrument: Agilent® 1290 Infinity with Sciex 3200 QTrap

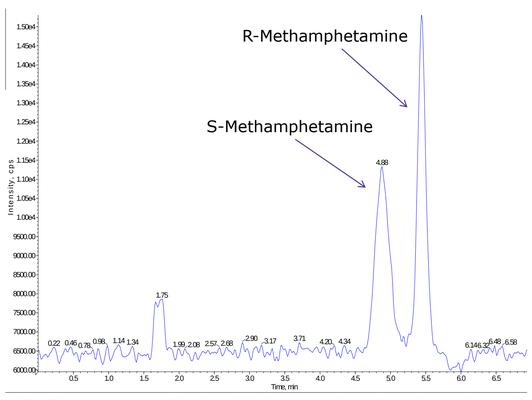

Figure 5. Chromatogram for the chiral separation of methamphetamine from a spiked urine sample.

Results

- Linear range from 50 – 5000 ng/mL was established for Methamphetamine and 50 – 1000 ng/mL for Amphetamine on the DART®-MS system.
- Recoveries ranged from 75.0% to 96.6% for the DART® screening method at all 3 concentration levels.
- Precision on the DART® was demonstrated with %RSD values ≤15% for both analytes, except for amphetamine at 100 ng/mL level which had 23.2% RSD.
- Lower MS sensitivity for amphetamine could have contributed to the higher variability at the lowest spiking level.

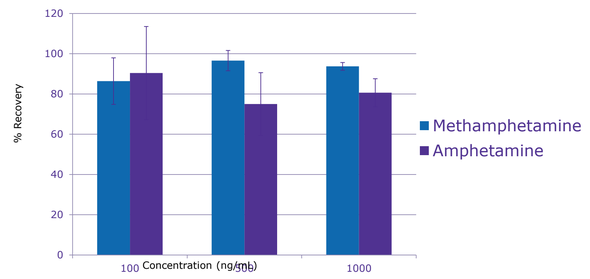

Figure 6. Average recoveries for methamphetamine and amphetamine from spiked urine samples using DART®-MS

- Recoveries for the chiral determination for methamphetamine ranged from 75.0% to 96.6% for the DART® screening method at all 3 concentration levels.
- Precision for the chiral separation was established with %RSD values ≤15% for both analytes.

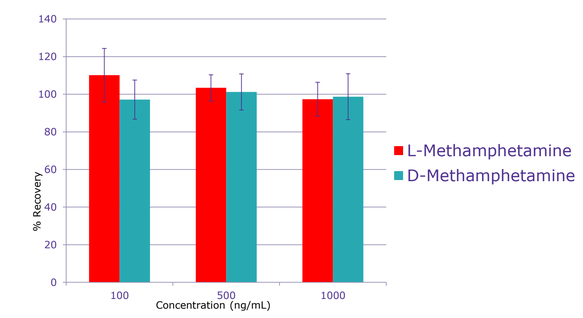

Figure 7 Average recoveries for L- & D-methamphetamine from spiked urine samples using chiral LC/MS

- The effect of matrix on analyte response was evaluated via DART® by comparing analyte response from a fiber extracted in spiked urine to a fiber extracted in spiked buffer.
- Matrix enhancement was observed for methamphetamine with matrix effects of 181%, but amphetamine was not impacted by the matrix with -2.3% matrix effects.
- Matrix effects were calculated with the following equation:

$$\% \text{ Matrix Effects} = \frac{\text{Analyte in presence of matrix}}{\text{Analyte in absence of matrix}} - 1 \times 100\%$$

- The effect of matrix on L- & D- methamphetamine response was also evaluated by spiking analyte into extracted urine blank samples and comparing the response to analyte spiked into the desorption solution.
- Desorption of the fibers reduced the matrix effects observed in direct MS analysis with % matrix effects of 9.9% for L-methamphetamine and 8.1% for D-methamphetamine.

Summary

- A fast screening method for methamphetamine and amphetamine was developed to detect these drugs in urine samples using microextraction and direct MS analysis.
- Screening of urine samples could detect positive results down to 100 ng/mL without the risk of cross reactivities or false positives.
- If a positive result is obtained, chiral confirmation can be easily performed with accurate and reproducible results down to 100 ng/mL.
- Matrix effects for methamphetamine were greater for direct MS than those seen after solvent desorption. Further investigation into the cause of these is warranted.
- Combining the simple microextraction technique with rapid direct MS detection, high throughput screening of urine samples is possible.
- Lower enatiomeric confirmation levels could be achieved by desorption of the fibers into smaller volumes.

References

1. Esposito, F. M.; Crumpton, S.; Mitchell, J.; Flegel, R. R. Evaluation of the 20% D-methamphetamine requirement for determining illicit use of methamphetamine in urine. J. Anal. Toxicol. 36(6), 2012, 399-404
2. Smith, M., Nichols, D., Underwood, P., et. al., Methamphetamine and Amphetamine Isomer Concentrations in Human Urine Following Controlled Vicks VapoInhaler Administration. J. Anal. Toxicol. 38, 524-527

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada

www.sigma-aldrich.com/biospme