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Direct Analysis of Drug of Abuse by BioSPME

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Introduction

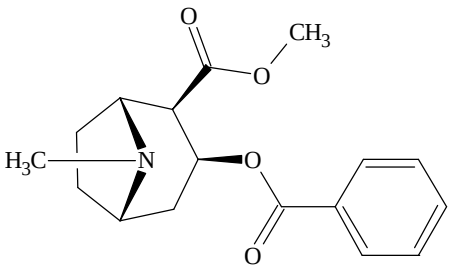
Urine testing for the presence of drugs of abuse requires methodology sensitive enough to monitor well below therapeutic levels and selective enough for identification of specific drugs without false positives. Current methodologies for urine analysis consist of ELISA reagent kits. While not specific to an individual drug, samples often must also be subject to confirmation testing by HPLC-MS/MS or GC-MS. In this study, newly developed biocompatible Solid Phase Microextraction (SPME) fibers are utilized for the extraction of drugs of abuse from urine samples.

Introduction (contd.)

With the recent development of the SPME LC Tips with C18 fibers chemistry, reversed phase liquid chromatographic techniques can be employed for the extraction of polar and non-polar compounds from aqueous matrices. The SPME LC Tips consist of functionally bonded silica particles with a C18 stationary phase. The particles are then bonded to a metal fiber core using a proprietary binder that is inert to common organic solvents. The SPME LC Tips have been developed to address limitations of current commercially available coatings, making them suitable for extraction in biological matrix and for direct solvent desorption

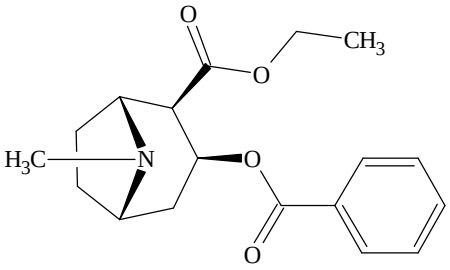
In this study, the SPME LC Tips were investigated as an alternative method for analysis of illicit drugs in urine. Cocaine, benzoylecgonine, coca-ethylene, norfentanyl, methadone and EDDP were selected as representative drugs and metabolites. Matrix matched standards with isotopically labeled internal standards were prepared for calibration. Urine samples that had tested positive by ELISA techniques for the presence of cocaine metabolites were then analyzed using the SPME LC Tips extraction technique. The C18 fibers were then directly desorbed and analyzed by LC-MS/MS technique. Extraction technique and chromatographic conditions are described.

Structures



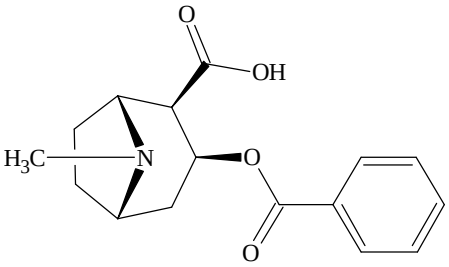
Cocaine

Monoisotopic Mass = 303.147058 Da



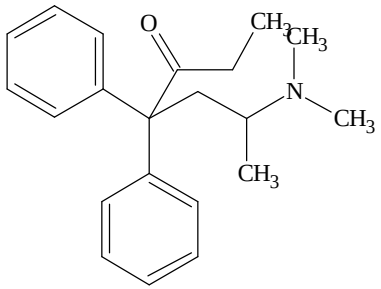
Cocaethylene

Monoisotopic Mass = 317.162708 Da



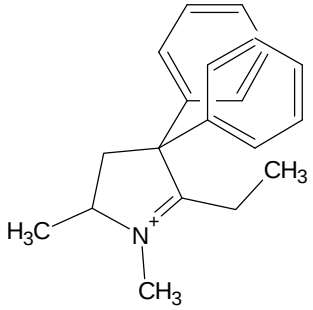
Benzoylecgonine

Monoisotopic Mass = 289.131408 Da



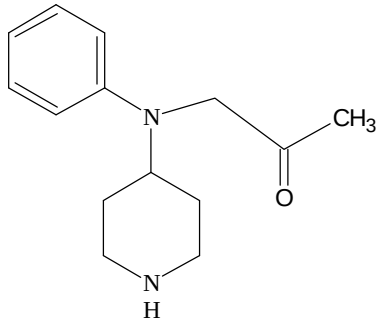
Methadone

Monoisotopic Mass= 309.209264 Da



EDDP

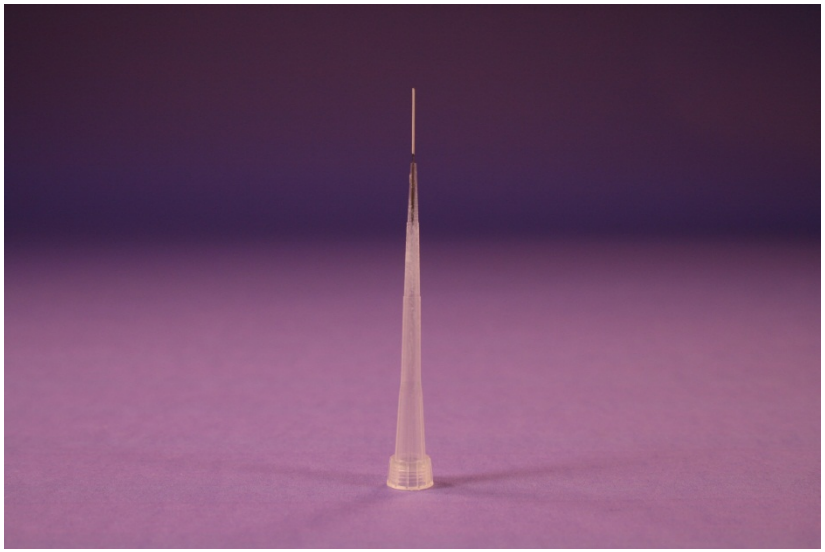
Monoisotopic Mass = 278.190326 Da



Norfentanyl

Monoisotopic Mass= 232.157563 Da

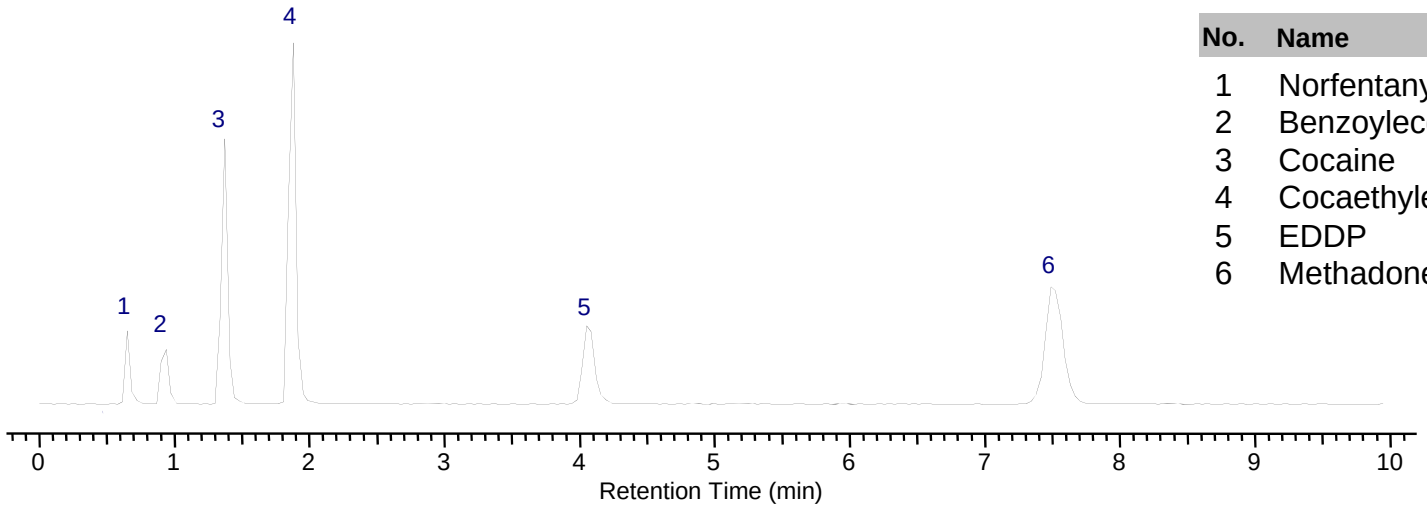
Figure 1. SPME C18 Fibers, Tip Configuration



SPME LC Tips consist of a functionally bonded C18 silica particle coated onto a 0.008” core. Fiber is affixed into a 20 µL pipette tip. This tip configuration aids in handling and allows for automation using existing robotic handling systems.

Figure 2. Chromatographic Conditions

column:	Ascentis® Express RP Amide, 10 cm x 2.1 mm I.D., 2,7 µm (53913-U)
mobile phase:	10 mM ammonium formate (75:25 water:acetonitrile)
flow rate:	0.2mL/min
temp.:	35 ºC
det.:	ESI+, MRM
injection:	2 µL
instrument:	Agilent® 1100, ABI-3200Qtrap



No.	Name	tR
1	Norfentanyl	0.7
2	Benzoylecgonine	0.9
3	Cocaine	1.4
4	Cocaethylene	1.9
5	EDDP	4.1
6	Methadone	7.5

Table 1. MS Parameters for Analysis of Drugs and Metabolites

	MS/MS Transitions	
Compound	**Precursor**	**Product**
Norfentanyl	233	84
d_5 Norfentanyl	238	84
EDDP	278	234
d_3 EDDP	281	234
BZE	290	168
d_3 BZE	293	171
Cocaine	304	182
d_3 Cocaine	307	185
Methadone	310	265
d_3 Methadone	313	268
Cocaethylene	318	196
d_3 Cocaethylene	321	

Sample Preparation

Calibration standards were prepared at 20,50, 100, 200, 500, and 1000 ng/mL in matrix matched urine.

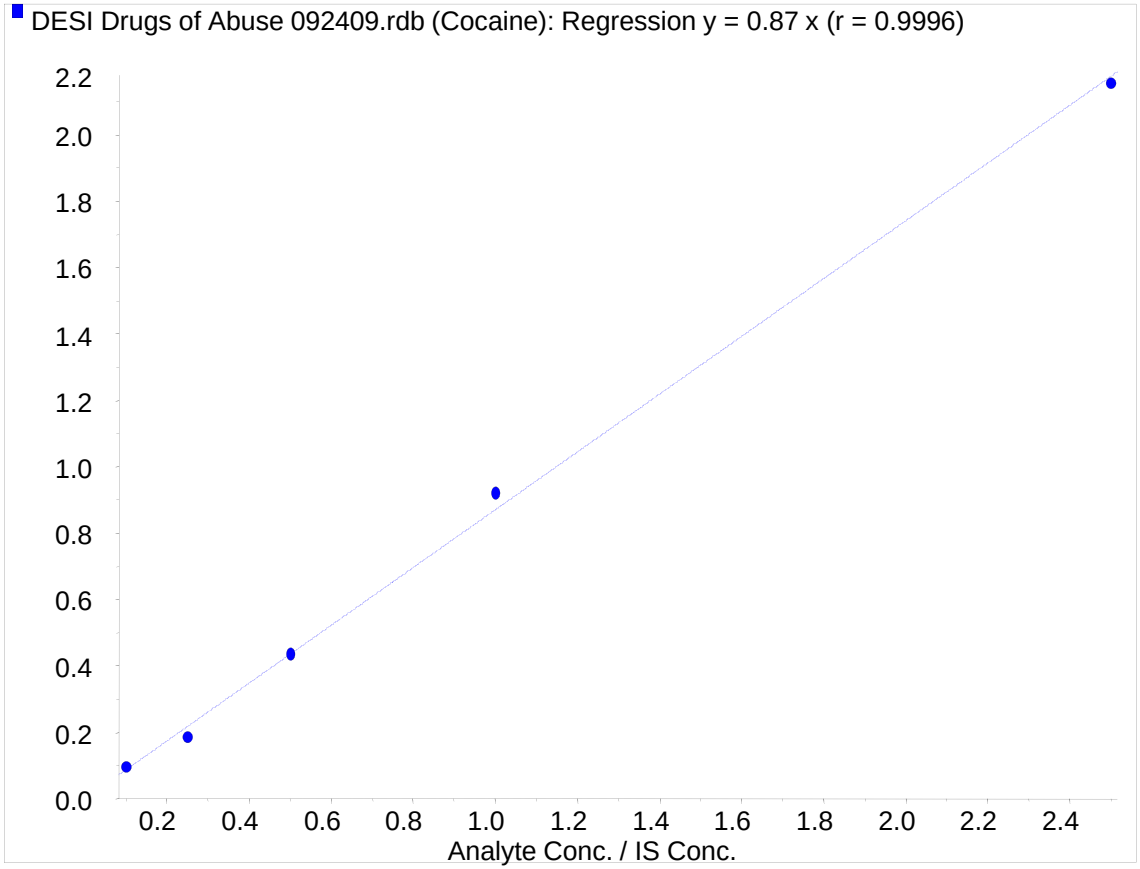
Deuterated internal standards were added at 200 ng/mL concentration for all calibration standards and samples.

Aliquots of 500 µL of calibration standards and sample were placed into micro centrifuge vials, 50 µL of 2 mM ammonium formate pH 3.7 buffer was added to each sample. Because the urine samples were from different subjects, the addition of the ammonium formate buffer was necessary to control the pH variability within the sample population. This ensured consistent extraction efficiencies for all samples.

SPME C18 Extraction Protocol

1. Fibers were conditioned by soaking in methanol for 10 minutes
2. Fibers were then equilibrated by soaking in water for 10 minutes
3. Sample extraction was conducted by placing the SPME C18 fiber into the microcentrifuge vial and allowing to soak for 10 minutes
4. Fibers were then removed from urine samples and placed into HPLC vial that had been pre-filled with 200 µL of 20 mM ammonium formate (90:10 methanol:water). Vials were then agitated on a rotating table for 30 minutes.
5. Fibers were removed and vials were capped and analyzed directly.

Figure 3. Calibration Data of Standards Extracted by SPME C18



Compound	Correlation
Norfentanyl	0.9978
EDDP	0.9999
BZE	0.9908
Cocaine	0.9996
Methadone	0.9936
Cocaethylene	0.9996

Figure 3 depicts the calibration curve for extraction of cocaine. Similar curves were obtained for all other analytes. Good correlation was demonstrated for all analytes across the concentration range of 20 ng/mL to 1000 ng/mL. The high degree of linearity demonstrates the quantitative ability of the SPME LC Tips extraction technique. SPME is not an exhaustive extraction technique, but an equilibrium between the analyte concentration in the sample and the absorbed concentration onto the fiber. Variations in sample pH, temperature and ionic strength can affect the extraction rates of analytes, but simple control of these variable results in highly linear, quantitative extraction technique.

Urine samples were then extracted using the described protocol and quantitated against the calibration table. All samples had previously tested positive for cocaine metabolites using the ELISA reagents, but actual concentration levels were not determined.

Figure 4. Detection of Cocaine and Metabolites in Urine Samples

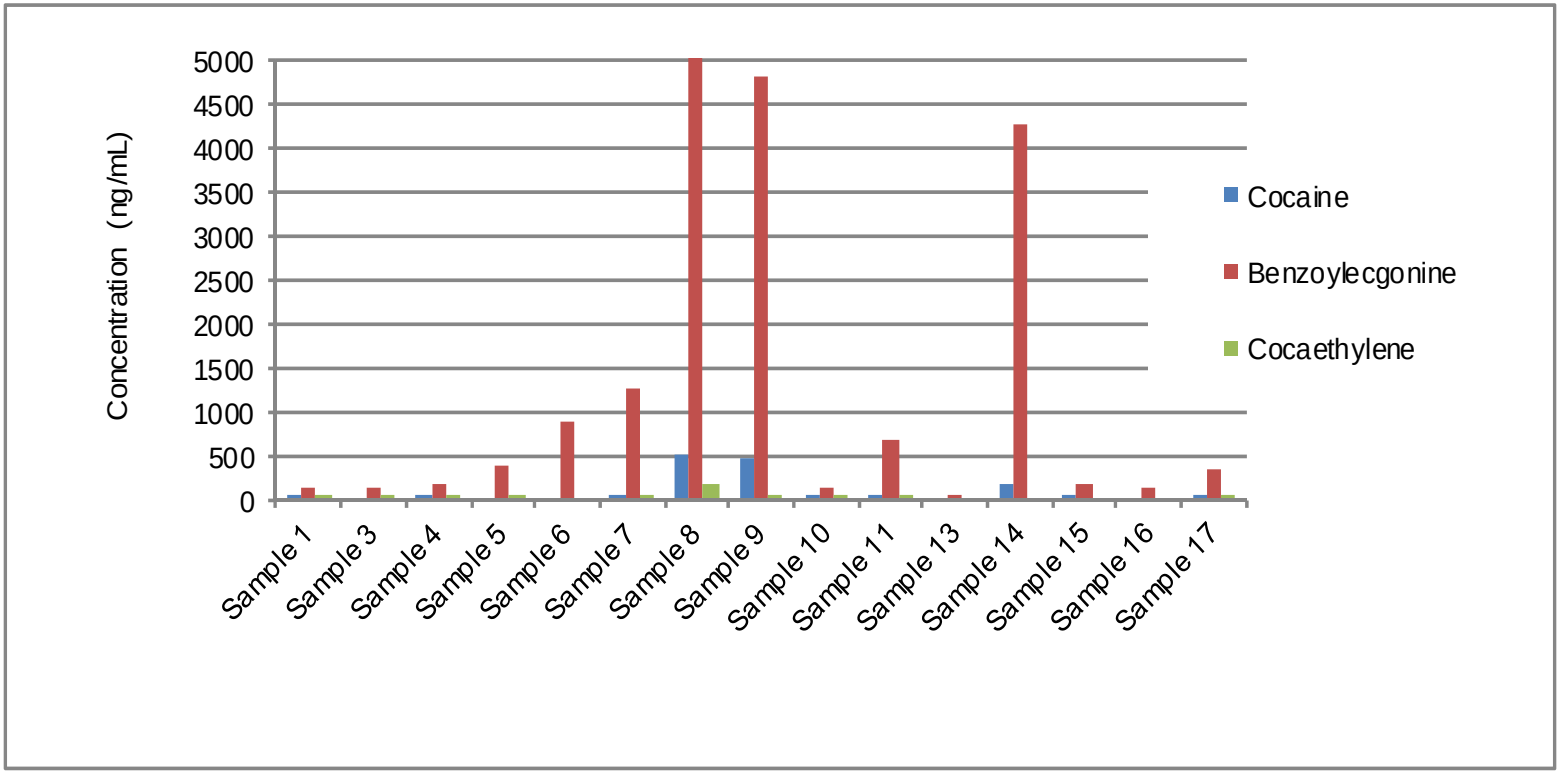


Figure 5. Detection of Methadone and Metabolites in Urine Samples

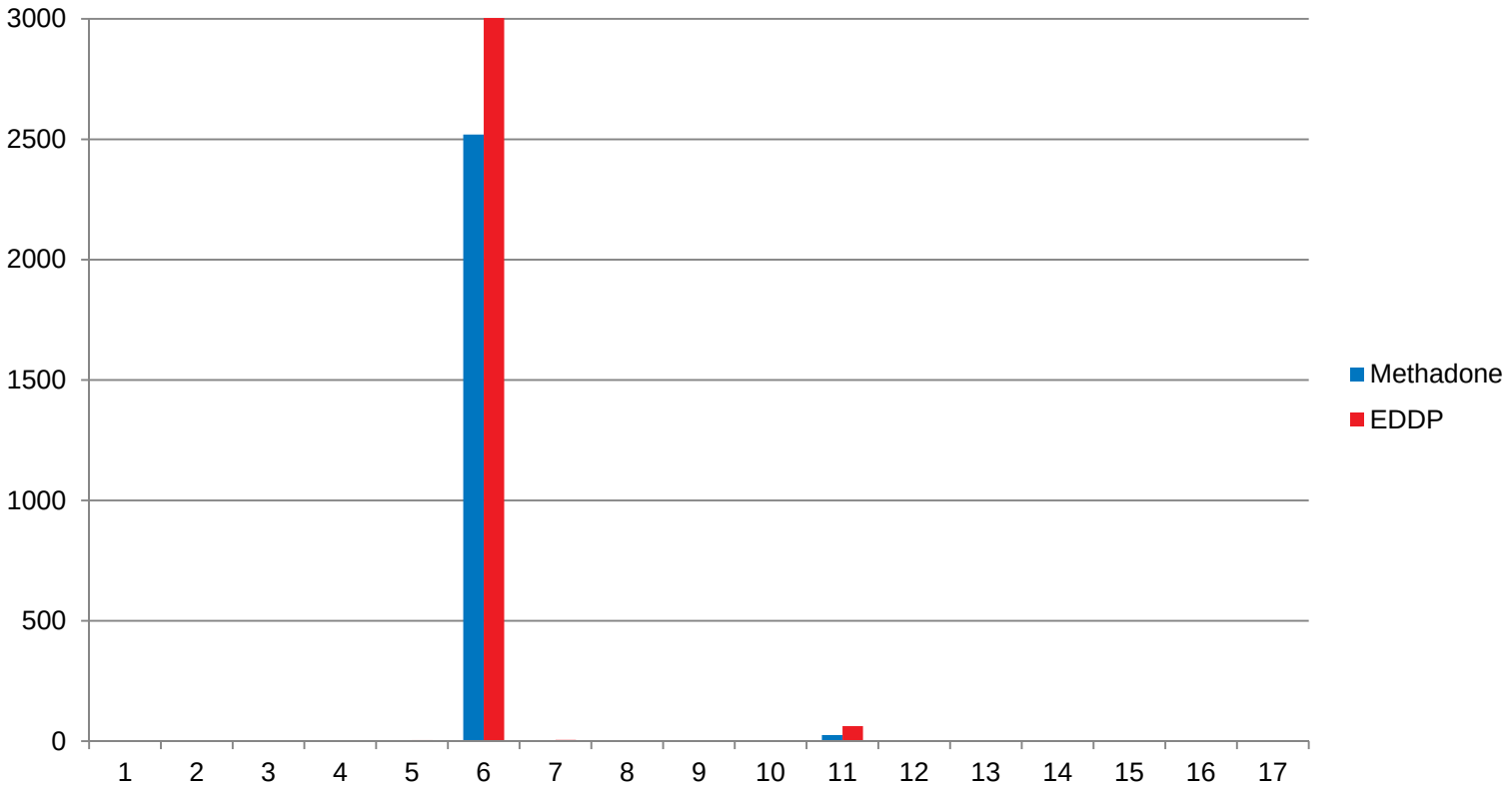


Figure 2. Table of Detected Drugs of Abuse

	Methadone	EDDP	Cocaine	Benzoylecgonine	Cocaethylene	Norfentanyl
Sample 1	0	No Peak	0.904	120	0.438	0
Sample 3	0	0.697	0	119	0.122	0
Sample 4	0	0.359	0.391	162	0.581	0
Sample 5	0.634	4.93	0	381	0.473	0
Sample 6	2520	25200	0	896	0	0
Sample 7	0.752	5.36	0.697	1260	0.334	0
Sample 8	0.46	1.27	493	33200	167	0
Sample 9	0.816	2.45	455	4850	27.8	0
Sample 10	0	2.78	1.96	146	0.871	0
Sample 11	25.6	62.5	3.19	685	0.514	0
Sample 13	0	1.06	0	39.1	0	0
Sample 14	0	0	163	4280	0	0
Sample 15	0	0	18.3	154	0	0
Sample 16	0	0.518	0	116	0	0
Sample 17	0	1	5.15	347	2.28	0

As depicted in Figure 4 and 5, the range in analyte concentration across the samples was very broad. Analyte concentration ranged from none detected to higher than 30,000 ng/mL. The benzoylecgonine content in Sample 8 was outside the highest calibration level, results estimated at more than 33,000 ng/mL. Because Sample 8 was outside the range, the graph on Figure 5 was scaled to 5000 ng/mL for better visualization of the data.

The content of methadone and metabolites in most of the samples was low with the exception of Sample 6, at a level of 25,200 for EDDP well outside the calibration range. Norfentanyl was not detected in any of the samples, the summary of all data is compiled in Table 2.

The benefit of this technique is the speed of the process along with the limited sample volumes needed. The total amount of time necessary to process all of samples was 60 minutes including condition time of the fibers. Because multiple samples can be extracted simultaneously, the technique is not sample quantity rate dependent. Extracting 100 samples take the same amount of time as 20. This technique also only required minimal amount of sample, only 500 µL of sample was necessary from each sample for this study. In most cases less than 100 µL volume is all that is necessary to perform the SPME LC Tips extraction technique.

Summary

- The benefits of the SPME LC Tips technique to extract biological samples are four-fold. First, the entire process is fast. The total amount of time necessary to process all samples was 60 minutes, including time to condition the tips. Because the SPME LC Tips are supplied in a 96-well array, multiple samples can be extracted simultaneously, maintaining high lab throughput.
- Second, it uses very small sample volumes. In most cases, less than 100 µL is all that is required.
- Third, it is selective. The SPME LC Tips extraction technique demonstrated the ability to extract drug and polar metabolites from the biological matrix. Urine was shown here, but the Tips have been successfully applied to serum, plasma, saliva, and whole blood.
- Fourth, it is quantitative. A high degree of extraction linearity was demonstrated across the range of 20 ng/mL to 1000 ng/mL for all analytes tested.