

# Simultaneous Analysis of Seven Amphetamine Class Drugs in Urine for Forensic Toxicology

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## GOAL

To demonstrate that the use of an online SPE system (ACQUITY® Online SPE Manager) efficiently removes sample matrix effects and improves the LC/MS analysis of amphetamines in urine

## BACKGROUND

Amphetamines increase activity related to the neurotransmitters dopamine and norepinephrine in the brain. Well known in popular culture (as Speed) and often abused in the 1960's and 70's, current usage of amphetamines is strictly regulated and monitored.

However, in the past few years, amphetamine and its many derivatives (metamphetamine, MDMA, MDA, etc.) have become extraordinarily popular as recreational and illicit drugs around the world. Unfortunately, these psychoactive drugs are commonly manufactured by illegal drug manufacturing operations and often find their way onto the market in very potent form. Higher doses of amphetamine class drugs used recreationally to achieve a sense of euphoria or highly energetic state are potentially very toxic, habit forming, and even fatal. Some compounds in this class, such as methamphetamine, are particularly

## Increased Analytical Sensitivity for Analysis of Amphetamines Enabled with Sample Preparation

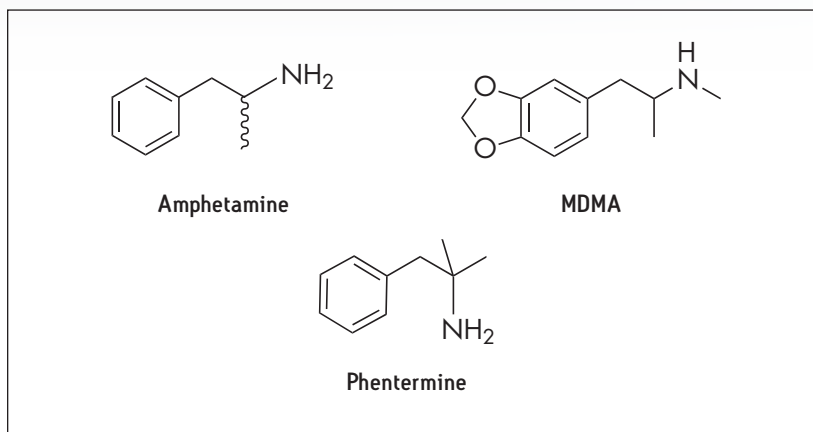


Figure 1: Structure of Some Amphetamine Class Drugs.

dangerous both to manufacturers and users. A number of deaths, or cases of serious overdose, related to these drugs has led to a greater need for detection in some populations. In many parts of the world, use of these types of drugs at parties and in nightclubs has reached epidemic proportions and the need for effective testing to identify these compounds in criminal or forensics cases has become crucial.

In many drug testing laboratories, amphetamines and their derivatives are screened for, and identified by, testing a urine sample from a suspected user. The analysis of these compounds can be done by GC or LC/MS. In the case of LC/MS analysis, simple dilution of the urine sample is typically the only sample preparation method employed. However, as urine is a sample matrix containing many potential interfering compounds, this type of relatively crude sample preparation can lead to issues in analysis. Potential matrix interferences from urine can compromise method sensitivity, and allow potential cross contamination contributing to an increased instrument maintenance burden. These interfering compounds can also compromise the effectiveness of an analytical LC column very quickly.

## THE SOLUTION

In this effort, a forensic toxicology method incorporating an online SPE system (ACQUITY Online SPE Manager-OSM) with LC/MS for analysis of a group of amphetamine class compounds in urine was developed. Results from the method utilizing SPE for sample preparation were compared with those obtained from results obtained using urine samples that were simply diluted before LC/MS analysis. Results were compared in terms of background signal, matrix interferences and analytical sensitivity for amphetamine drugs.

## EXPERIMENTAL

### Sample Preparation Method

Urine samples were spiked with the appropriate concentrations of standards and then diluted 1:10 in 10mM ammonium formate. Samples were directly injected after dilution for comparison with online SPE methods using the analytical conditions described below.

### Analytical System Configuration:

System:	ACQUITY UPLC®
Mass spectrometer:	Xevo® TQD
Column:	ACQUITY UPLC HSS T3, 100Å, 1.8 µm, 2.1 mm x 50 mm (p/n 186003538)
UPLC flow rate:	0.5 mL/min
SPE sample preparation:	ACQUITY Online SPE Manager (OSM)
SPE:	ACQUITY Online SPE Manager C <sub>18</sub> Cartridge (p/n 186005672)

### Chromatography Conditions:

Mobile phase A:	H <sub>2</sub> O + 5% NH <sub>4</sub> OH		
Mobile phase B:	Acetonitrile		
Flow rate:	0.5 mL/min		
Gradient:	Time	%A	%B
	Initial	70	30
	2.0	5	95
	2.5	5	95
	2.6	70	30
	3.5	70	30
Injection Volume:	25 µL onto OSM cartridge		

SPE was performed by the Online SPE (OSM) system as follows:

Step	Solvent	Volume (mL)
Cartridge conditioning	MeOH	1.00
Cartridge equilibration	5% NH <sub>4</sub> OH	1.00
	H <sub>2</sub> O	1.00
Sample load	5% NH <sub>4</sub> OH	0.5
Cartridge wash	5% NH <sub>4</sub> OH	0.5
Cartridge wash 2	H <sub>2</sub> O	0.5
Clamp flush	0.2% Triethylamine	0.5
	ACN	0.5
	H <sub>2</sub> O	0.5

OSM elution time: 1 min

### Mass Spectrometry Conditions:

MS System:	Waters Xevo TQD
Acquisition mode:	ESI +ve
Capillary voltage:	2.0 kV
Sampling cone:	20.0 V
Source temperature:	120°C
Desolvation temperature:	500°C
Desolvation gas flow:	1000 L/Hr
Cone gas flow:	20 L/Hr

### Multiple Reaction Monitoring Conditions:

Compound	Parent m/z	Daughter m/z	Cone (V)	Collision (V)
Amphetamine	136.01	91.08	22	20
		119.05	22	8
Methamphetamine	150.09	91.04	20	16
		119.1	20	10
Phentermine	150.09	91.04	20	16
		119.1	20	10
Propylamphetamine	178.18	91.04	32	18
		119.02	32	12
MDA	180.06	105.03	18	20
		135.54	18	20
MDMA	194.08	105.04	24	22
		163.09	24	12
MDEA	208.16	105.03	26	24
		163.05	26	14

RESULTS

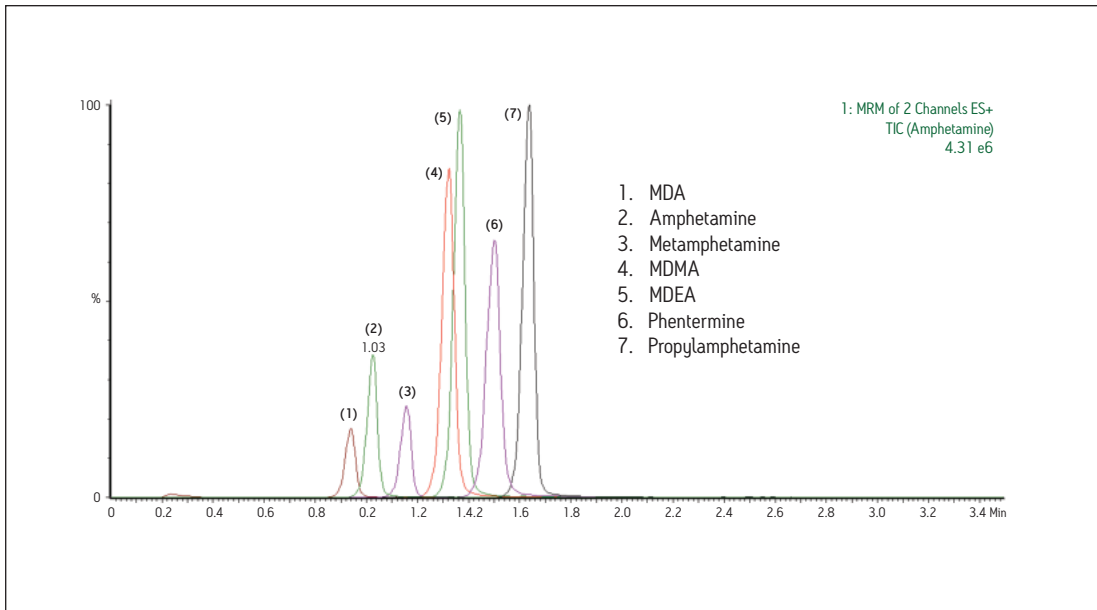


Figure 2: Separation of a 7 amphetamine class drug mix spiked into urine at 10 ppb using an OSM-LC/MS system.

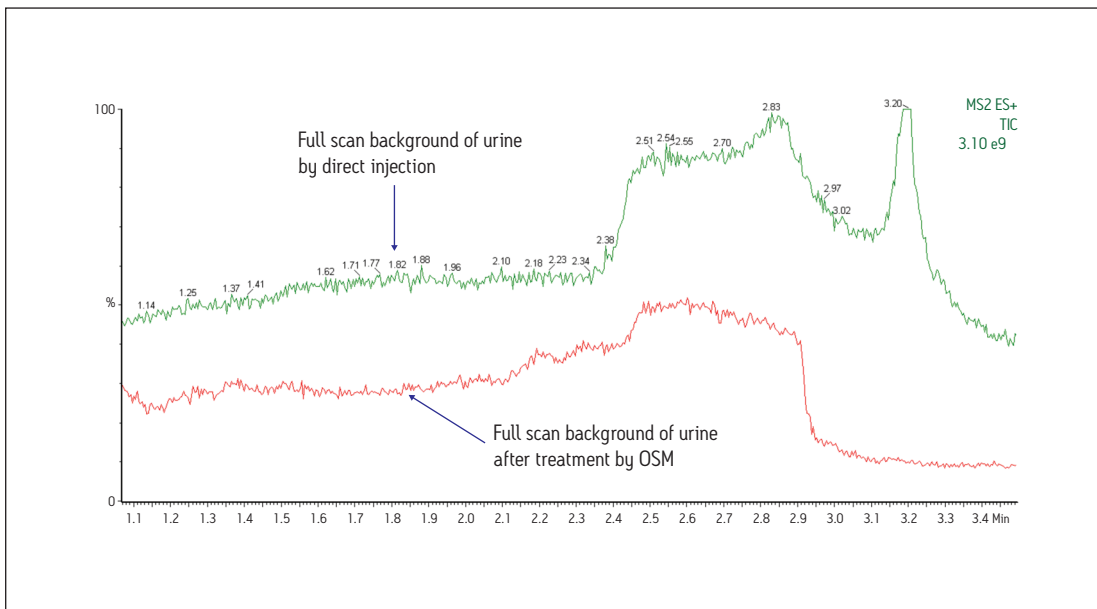


Figure 3: Removing background signal with SPE. This figure compares the difference in background signal from urine that is either directly injected (green) or prepared using online SPE (red). The treatment of the urine by online SPE removes much of the background signal from potential interfering matrix compounds found in urine samples.

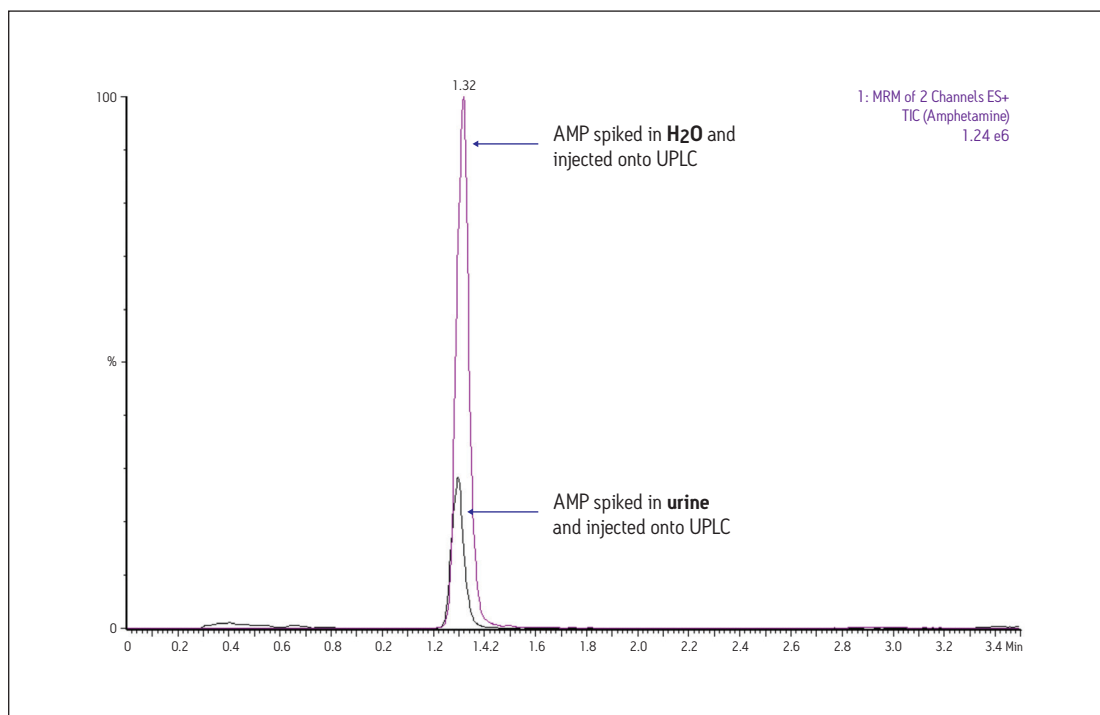


Figure 4A: Ion Suppression effect of Urine. Analysis of identical concentrations of amphetamine spiked into water or urine. Note the significant loss in signal from the amphetamine in the urine sample indicative of matrix interferences from urine leading to ion suppression effects.

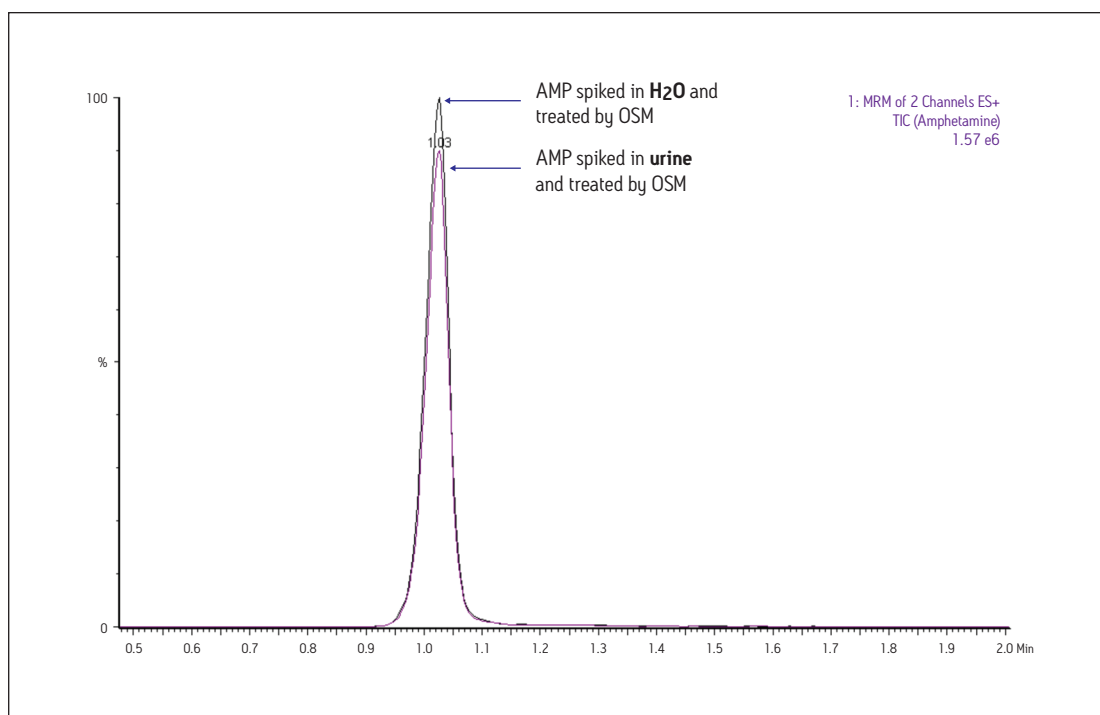


Figure 4B: Removal of Matrix Effects from Urine. Signal from identical concentrations of Amphetamine in water and urine compared after treatment of the sample by online SPE. Little difference in signal is observed in contrast to the matrix effects on amphetamine signal in diluted urine measured in Figure 4A.

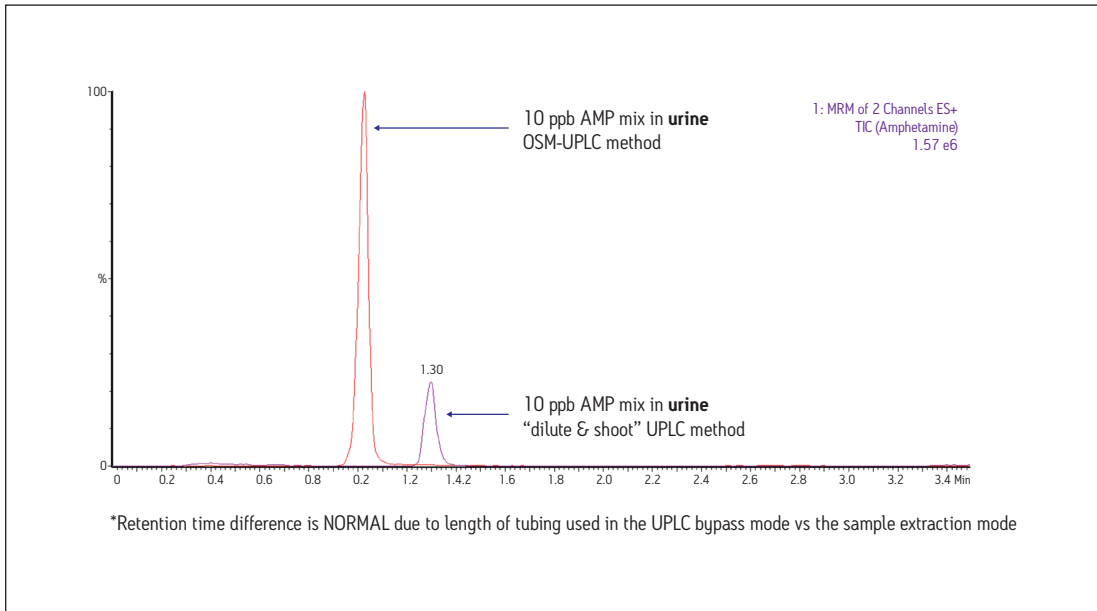


Figure 4C: Amphetamine analysis method sensitivity depends on Sample Preparation Comparison of 10 ppb amphetamine analyzed by either an OSM-UPLC method utilizing online SPE or direct analysis using simple dilution instead of online SPE. Note the significant difference in signal from the online SPE method. The signal intensity is several fold greater than the simple “dilute and shoot” method.

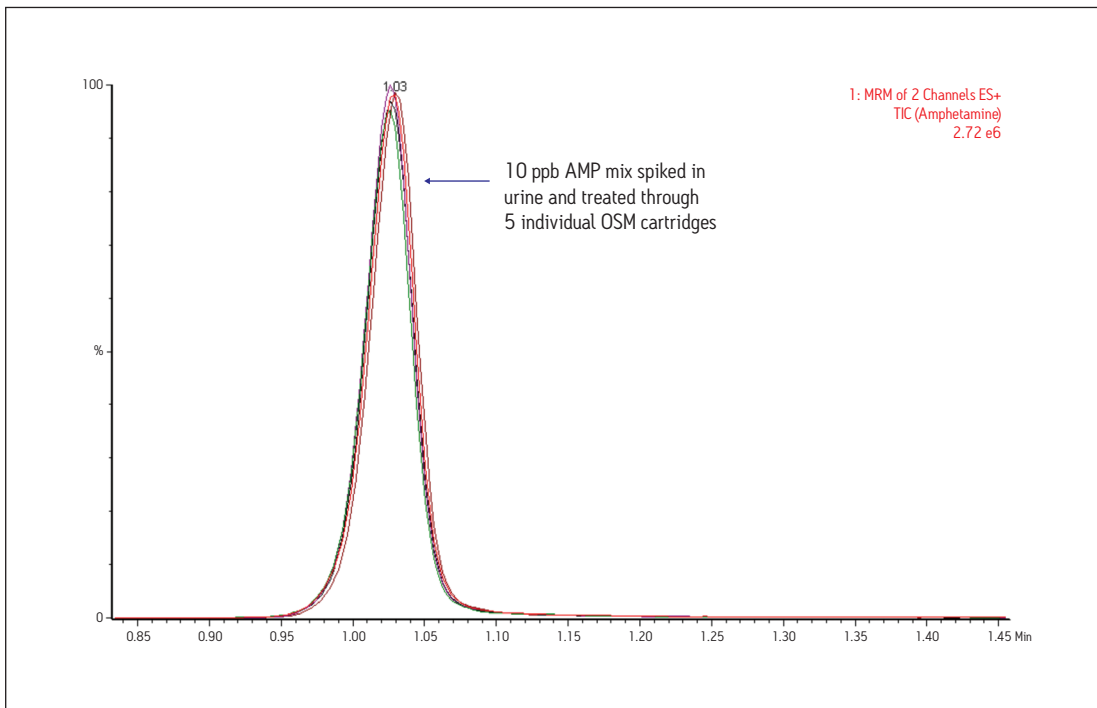


Figure 5: Variability of amphetamine measurements with different OSM cartridges. The online SPE system (OSM) utilizes disposable SPE cartridges. This figure compares the signal for 10 ppb amphetamine in urine analyzed with 5 different OSM cartridges. The signal from all 5 cartridges is nearly identical.

## SUMMARY

In this study, an assay has been developed for the simultaneous measurement of amphetamine class drugs from urine. The method utilizes LC/MS and an online SPE system. This combination of SPE sample preparation coupled with the analytical power of LC/MS is able to deliver an efficient assay for this commonly abused class of drug compounds.

It was seen that urine contains a significant level of matrix interferences that cause ion suppression in the analysis of amphetamines. To optimize the sensitivity of methods for analyzing amphetamines using urine as a sample matrix, these interferences must be removed. The use of the online SPE sample preparation in the method was found to greatly reduce the matrix effects from urine, and allowed for measurement of amphetamines at significantly lower levels than simple urine “dilute and shoot” methods.

The reproducibility of sample analysis with online SPE was judged to be very good even when injecting samples onto different disposable online SPE cartridges. The LC/MS system utilized in this work completely automated, and effectively integrated SPE sample preparation and LC/MS into a single platform.

The forensic toxicology method for measuring amphetamines in urine incorporating online SPE sample preparation with LC/MS developed herein provides:

- Analysis of a variety of amphetamine class drugs (> 10 ppb) in urine
- Removal of matrix interferences and ion suppression effects seen in urine
- Increased analytical sensitivity for amphetamine analysis compared to “dilute and shoot” methods
- Excellent sample prep reproducibility from multiple online SPE cartridges (n=5)
- Highly efficient online SPE sample preparation integrated with LC/MS

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December 2013 720004881EN IH-PDF

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