

## Technical Report

# Simple, Fast and Class Selective Extraction of Amphetamine and Related Drugs from Urine using Molecularly Imprinted Polymer (MIP) SPE and LC/MS/MS

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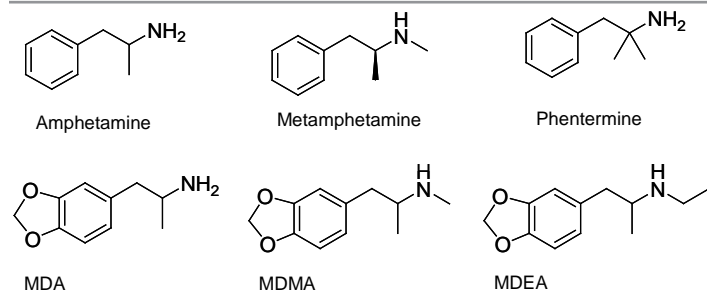
### Introduction

Amphetamine related drugs are currently among the most well-known drugs of abuse in sports, in the workplace and by recreational users. Screening and confirmative analysis is done by forensic, clinical and doping laboratories.

In this report we describe a simple, fast and class selective method for trace extraction of amphetamine related drugs from urine samples using a molecularly imprinted polymer SPE (SupelMIP™) specifically designed for selective extraction of amphetamine related drugs.

The SupelMIP extraction was compared against extraction using a conventional hydrophilic polymer SPE phase for amphetamine and related drugs, see Figure 1.

### Figure 1. Chemical Structures of Amphetamines and Related Drugs Investigated



### Molecularly Imprinted Polymers

Molecularly imprinted polymers (MIPs) are a class of highly cross-linked polymer-based molecular recognition elements engineered to bind one target compound or a class of structurally related compounds with high selectivity. Selectivity is introduced during MIP synthesis in which a template molecule, designed to mimic the analyte, guides the formation of specific cavities that are sterically and chemically complementary to the target analyte(s). As a result, multiple interactions (e.g., hydrogen bonding, ionic, Van der Waals, hydrophobic) can take place between the MIP cavity and analyte functional groups. The strong retention offered between a MIP phase and its target analyte(s) allows for the use of exhaustive wash procedures during solid phase extraction that results in superior sample cleanup prior to analysis.

### Increased Cleanliness of Extracts Using SupelMIP SPE - Amphetamines

Amphetamine and the related drugs Methamphetamine, Phentermine, MDA, MDMA and MDEA were extracted from urine using both the SupelMIP SPE and a conventional hydrophilic polymer SPE via the procedures described in Table 1. Extracts were further analyzed via LC/MS/MS (conditions, see Table 2).

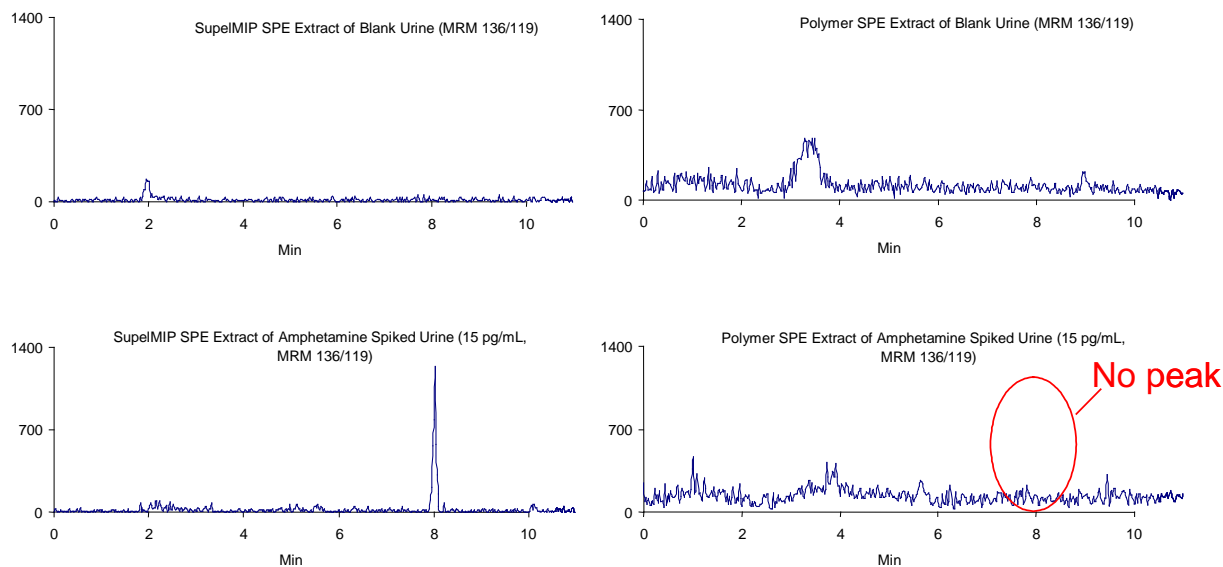
**Table 1. Comparison of SupelMIP SPE – Amphetamines Method and Conventional Hydrophilic Polymer SPE Method**

SupelMIP SPE – Amphetamines Using Conventional Hydrophilic Sample Pre-Treatment:	Published Amphetamine Method Method Polymer SPE Phase <sup>1</sup> Sample Pre-Treatment:
Human urine samples were spiked with internal standard (e.g., methamphetamine -d <sub>8</sub> and MDMA -d <sub>5</sub> ) and diluted with 1:1 (v/v) with 10 mM ammonium acetate buffer, pH 8., adjust if necessary to pH 7.5-8.5 using NH <sub>3</sub> or HAC	Human urine samples were spiked with internal standard.
<b>SPE Procedure:</b> SupelMIP SPE – Amphetamines, 25 mg/3 mL (Cat. No. 53228-U)	<b>SPE Procedure:</b> Conventional Hydrophilic Polymer SPE Phase, 30 mg/1 mL
1. Condition and equilibrate MIP phase with 1 mL methanol, and 1 mL 10 mM ammonium acetate buffer, pH 8	1. Condition and equilibrate SPE phase with 1 mL methanol and 1 mL DI water
2. Load 1 mL pre-treated sample on to the cartridge.	2. Load 1 mL pre-treated sample on to the cartridge.
3. Wash (elute interferences) using following wash scheme:	3. Wash (elute interferences) with the 1 mL 5% methanol containing 2% ammonium hydroxide and with 1 mL 20% methanol containing 2% ammonium hydroxide
<ul style="list-style-type: none"> <li>• 2 x 1 mL DI water (<b>Do not let column dry!</b>)</li> <li>• 1 mL 60/40 MeCN/DI water followed by 5-10 minute vacuum (-1 bar, -20 inHg, or -70 kPa to dry the column)</li> <li>• 1 mL 1% HAC in MeCN</li> </ul>	
4. Elute the amphetamine drugs with 2 x 1 mL 1% formic acid in methanol. Apply 0.1 bar (75 mmHg) between each fraction.	4. Elute the amphetamine drugs with 0.5 mL 20% methanol with 2% acetic acid
5. Evaporate under nitrogen to dryness and reconstitute with 100 µL LC mobile phase (90% A and 10% B) prior to LC/MS/MS analysis	5. Evaporate under nitrogen and reconstitute with 150 µL LC mobile phase prior to LC/MS/MS analysis

**Table 2. LC-MS-MS conditions**

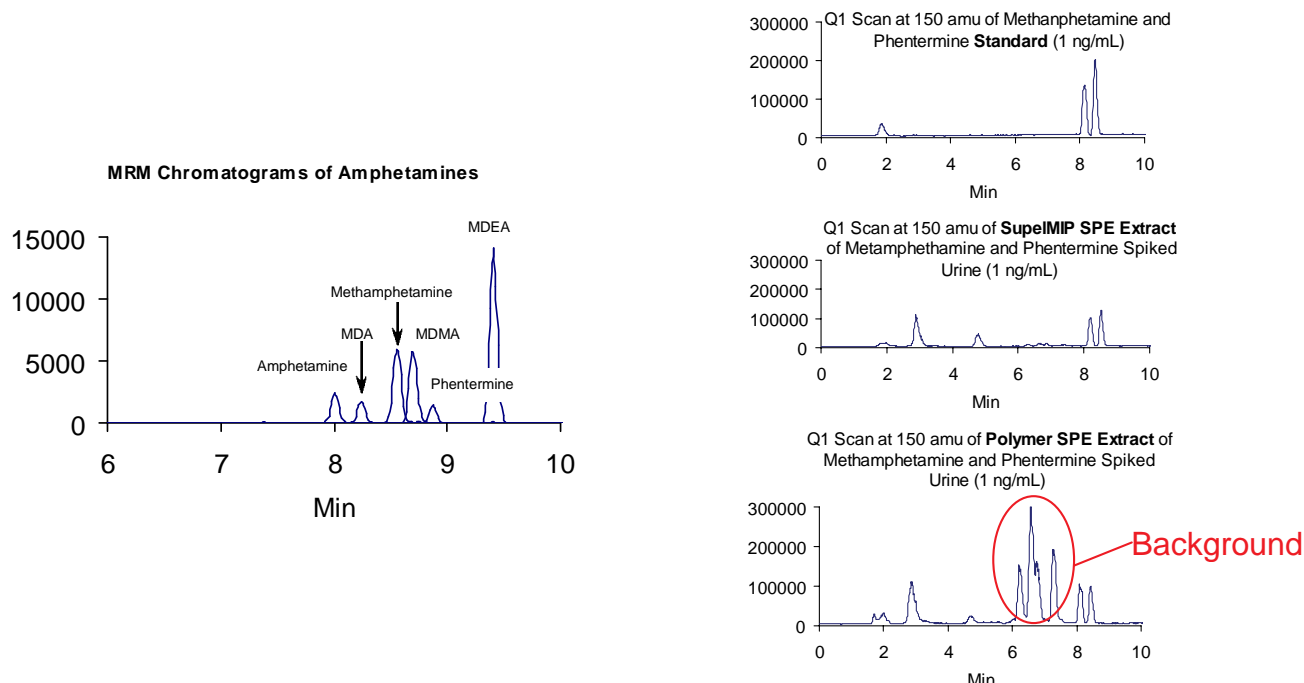
Column:	Ascentis C18, 5µm, (150x2.1 mm)	
Instrument:	Shimadzu LC-20/Applied Biosystems/MDS SCIEX API3200	
Mobile phase:	Solvent A – DI Water + 0.05% TFA Solvent B – Acetonitrile + 0.05% TFA	
Gradient:	Initial: 90% A – 10% B 7 min 70% A – 30% B 10-11 min 10% A – 90% B 11.2 min 90% A – 10 % B	
Temp.:	Ambient	
Flow rate:	0.2 mL/min	
Ion mode:	Positive	
Ion source:	Turbospray	
Ion spray voltage:	5500 V	
Source temperature:	600 °C	
Collision gas:	6 psi	
Injection:	20 µL	
Detection:	MS/MS	
MRM transition and retention times:	Compound	Rt (min)      Q1/Q3
	Amphetamine	7.8      136/119 and 136/91
	Methamphetamine	8.3      150/119 and 150/91
	Methamphetamine D <sub>8</sub>	8.3      158/124 and 158/93
	Phentermine	8.7      150/133 and 150/91
	MDA	8.0      180/163 and 180/105
	MDMA	8.5      194/163 and 194/105
	MDMA D <sub>5</sub>	8.5      199/165 and 199/136
	MDEA	9.2      208/163 and 108/105

Using the SupelMIP SPE it was possible to analyse the amphetamines at trace levels. From the LC/MS/MS chromatograms (MRM) depicted in Figure 2 it is shown that the background noise of the extracts cleaned-up on the SupelMIP SPE is much lower than the background noise of the extracts cleaned up on the conventional polymer SPE. Furthermore 15 pg/mL of Amphetamine in spiked urine could easily be detected using the SupelMIP cleanup, whereas no peak was seen when the same urine was extracted on the polymer SPE cartridge.

**Figure 2. Amphetamine Spiked Urine Samples (15 pg/mL) cleaned up with SupelMIP SPE vs. Conventional Hydrophilic Polymer SPE**

A MRM chromatogram of amphetamine and the amphetamine related drugs; methamphetamine, phentermine, MDA, MDMA and MDEA spiked at 1 ng/mL in urine is shown to the left in Figure 3. To the right in the same picture Q1 scans at 150 amu are displayed for a standard showing methamphetamine and phentermine peaks (upper chromatogram), extract after SupelMIP extraction (middle chromatogram) and extract after polymer SPE clean-up (lower chromatogram). The Q1 scans at 150 amu reveal interferences which are ionised and pass into the MS detector in addition to methamphetamine and phentermine. It is shown in the picture that in the retention time window 6-10 minutes, where all the amphetamines elute, the SupelMIP extract is much cleaner than the polymer SPE extract.

**Figure 3. MRM Chromatograms of Amphetamine, Methamphetamine, Phentermine, MDA, MDMA and MDEA, Spiked at 1 ng/mL in Urine (left) and Q1 Scans at 150 amu to the right. Upper left Chromatogram Shows Standard at 1 ng/mL, Middle Chromatogram Extract cleaned up on SupelMIP SPE and lower Chromatogram Extract cleaned up on Conventional Hydrophilic Polymer SPE**



### SupelMIP SPE Offers Decreased Detection Limits

In order to determine LOQ for the SupelMIP SPE and the conventional hydrophilic polymer SPE amphetamine drugs were spiked into urine samples at 0.015 ng/mL and the samples were extracted using both methods. It was not possible to detect the amphetamine drugs after cleanup using the polymer SPE at this concentration. LOQ was estimated based on 10 times the noise level for each analyte MRM. The LOQ for the conventional hydrophilic polymer SPE was calculated from urine samples spiked at 0.50 ng/mL. With MDA as the only exception, the LOQ was about one order of magnitude lower using SupelMIP SPE (Table 3).

**Table 3. LOQ (ng/mL) for SupelMIP SPE vs. Conventional Hydrophilic Polymer SPE**

	SupelMIP	Conventional Hydrophilic Polymer SPE
Methamphetamine	0.0066	0,052
Amphetamine	0.0073	0,138
Phentermine	0.0150	0,141
MDA	0.0430	0.261
MDMA	0.0030	0.056
MDEA	0.0025	0.052

### SupelMIP SPE improves class selectivity

The recoveries obtained with both methods for the various amphetamine drugs are summarized in Table 4. Recoveries obtained from the SupelMIP cleanup are higher than from the conventional hydrophilic polymer SPE cleanup. The increased range of amphetamine drugs with high recoveries demonstrates the improved class selectivity for the SupelMIP SPE. Internal standards were used in the calculation. Six samples of 50 ng/mL amphetamine drugs were extracted, using both the conventional hydrophilic polymer SPE and SupelMIP cleanup methods, and thereby the relative standard deviation could be determined (see Table 4). The relative standard deviations are significantly lower using the SupelMIP cleanup method than using the hydrophilic polymer SPE.

**Table 4. Recovery Comparison for Amphetamine Related Drugs from Urine using SupelMIP SPE and Conventional Hydrophilic Polymer SPE (with Internal Standard Adjustment)**

50 ng/mL spike	% Recovery		% RSD	
	SupelMIP	Hydrophilic Polymer SPE	SupelMIP	Hydrophilic Polymer SPE
Methamphetamine	101	100	1,41	5,16
Amphetamine	104	90	3,90	14,30
Phentermine	104	64	6,11	26,36
MDA	113	*	9,84	*
MDMA	97	86	2,52	8,10
MDEA	106	7	6,60	37,80

\*= No value obtained due to interference peak in chromatogram

Recoveries were also measured at lower concentrations ranging from 0.010 ng/mL up to 5 ng/mL using the SupelMIP SPE (Table 5) and the conventional polymer SPE (Table 6). For the conventional polymer SPE these measurements were only done for methamphetamine, amphetamine and MDMA as the other amphetamine related drugs all had recoveries below 80% at 50 ng/mL. Internal standards were used in the calculations. For the concentrations 0.010 and 0.015 ng/mL amphetamine and related drugs could not be detected with the hydrophilic polymer SPE, whereas using the SupelMIP SPE all analytes except MDA had high recoveries in the whole concentration range.

**Table 5. Recovery for the Amphetamine Related Drugs from Urine using SupelMIP SPE-Amphetamines (with Internal Standard Adjustment)**

Spike level (ng/mL)	Recovery (%)					
	Methamphetamine	Amphetamine	Phentermine	MDA	MDMA	MDEA
0.010	106	104	102	*	98	82
0.015	107	101	97	*	102	104
0.50	108	89	106	106	94	78
1.0	96	81	100	75	84	65
5.0	99	84	127	80	87	84

\* Not detected

**Table 6. Recovery for the Amphetamine Related Drugs from Urine using Conventional Hydrophilic Polymer SPE (with Internal Standard Adjustment)**

Spike level (ng/mL)	Recovery (%)					
	Methamphetamine	Amphetamine	Phentermine	MDA	MDMA	MDEA
0.010	*	*	**	**	*	*
0.015	*	*	**	*	*	*
0.50	102	37	**	**	81	**
1.0	99	47	**	**	82	**
5.0	100	45	**	**	91	**

\* Not possible to detect

\*\* Not evaluated

## SupelMIP SPE Reduces Ion Suppression

Blank urine samples were cleaned up according to both SPE procedures. The SPE extracts were spiked with a mixture of the amphetamine drugs post extraction. Standards for the calibration curve were prepared in the reconstitution solvent.

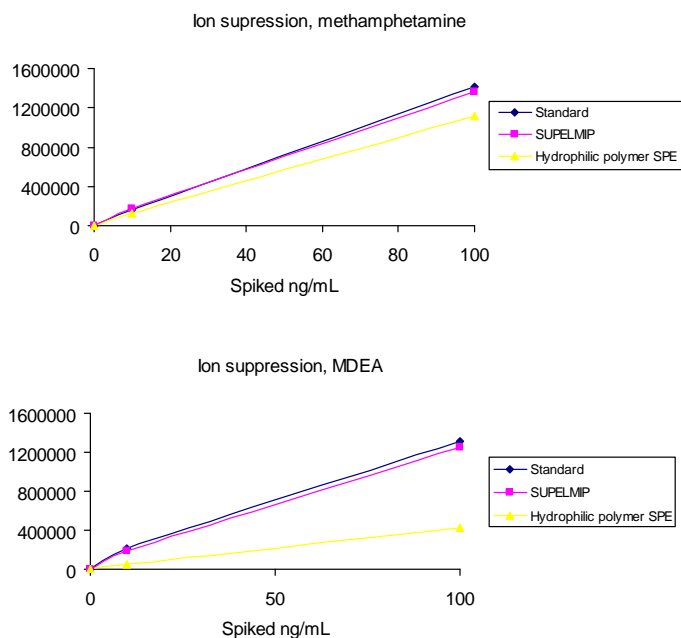
The standard calibration curves were compared to the matrix matched samples for both methods, without using internal standard corrections.

Table 7 summarizes the percentage of ion suppression at 10 and 100 ng/mL for the SupelMIP SPE and the hydrophilic polymer SPE. From the table, ion suppression is significantly less for the SupelMIP SPE. Two graphs, showing ion suppression is also given in Figure 4 for methamphetamine and MDEA. The blue line in the graphs is spiked standard in solvent, the pink line SupelMIP extract spiked post extraction and the yellow line hydrophilic polymer extract spiked post extraction. The signal responses for the SupelMIP extracts are very close to the signal responses obtained from spiked solvent, whereas the signal responses for the hydrophilic polymer extracts are much lower.

**Table 7. Ion suppression in % (without Internal Standard Adjustment)**

	SupelMIP		Conventional Hydrophilic Polymer SPE	
	10 ng/mL	100 ng/mL	10 ng/mL	100 ng/mL
Methamphetamine	0%	3%	31%	12%
Amphetamine	5%	10%	33%	28%
Phentermine	3%	2%	36%	22%
MDA	30%	23%	51%	48%
MDMA	5%	13%	45%	36%
MDEA	10%	4%	65%	52%

**Figure 4. Ion Suppression Graphs for Methamphetamine (upper) and MDEA (lower). Both Graphs show Signal Responses obtained for Standards Spiked in Pure Solvent (blue line) versus Signal Responses from Blank Urine Spiked Post-Extraction for SupelMIP Extracts (pink line) and for Hydrophilic Polymer SPE Extracts (yellow line).**



## Linear range

The linear range for the SupelMIP SPE was evaluated for the concentrations: 0.015 ng/mL, 1 ng/mL, 10 ng/mL, 50 ng/mL and 100 ng/mL. The results can be seen in Table 8. Each urine sample were spiked with 50 ng/ml of the internal standards methamphetamine- $d_8$ , and MDMA  $d_5$ . The values shown in Table 8 were obtained when the analyte peak area/IS peak area was plotted against the concentrations.

**Table 8. Linear Range for Amphetamine Drugs Extracted from Urine Samples using the SupelMIP Cleanup Method**

Compound	Slope	Intercept	R <sup>2</sup>
Methamphetamine	0.010	0.013	0.999
Amphetamine	0.006	-0.002	0.998
Phentermine	0.004	0.003	0.999
MDA	0.007	-0.005	0.984
MDMA	0.016	0.051	0.980
MDEA	0.023	0.084	0.980

## Conclusion

In this report we demonstrate that SupelMIP SPE allows highly sensitive trace analysis at ppt levels for the extraction of 6 different amphetamines. Furthermore we present a comparison of the SupelMIP SPE – Amphetamines method against a published hydrophilic polymer SPE method for the trace extraction of amphetamine drugs from urine with subsequent LC/MS/MS analysis. The SupelMIP SPE offers significantly lower LOQ values, about one order of magnitude for all amphetamine drugs studied. Furthermore recoveries within the class of amphetamine related drugs studied are higher on the SupelMIP SPE compared with the hydrophilic polymer SPE. Recoveries for the SupelMIP SPE are close to 100% for many of the amphetamine drugs at the various concentrations, while the hydrophilic polymer gave poor recoveries for MDA, MDEA and phentermine.

## References

1. M.-R. Fuh, T.-Y. Wu and T.-Y. Lin, *Talanta*, 2006, 68:987-991.

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