

## SupelMIP™ SPE – Amphetamines

### Product Description:

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 target compounds with high selectivity. Selectivity is introduced during MIP synthesis in which a template molecule, designed to mimic the analyte, guide the formation of specific cavities or imprints that are sterically and chemically complementary to the target analyte(s). It is therefore critical for analysts to use the methodology described below when using this phase. Conventional generic methodologies employed with conventional SPE chemistries (e.g., reversed-phase C18) will yield sub-optimal results when employed with this phase.

The following method(s) have been developed for the class-selective extraction of Amphetamine, Methamphetamine, Phentermine, MDA, MDMA, MDEA from human urine. The method is highly reproducible and offers an average recovery greater than 80%. Lower limits of detection and quantitation from 1 mL urine using the described SupelMIP SPE and LC-MS-MS procedures are as follows:

	LOD (ng/mL)	LOQ (ng/mL)
<b>Methamphetamine</b>	0.0020	0.0066
<b>Amphetamine</b>	0.0022	0.0073
<b>MDA</b>	0.0129	0.0430
<b>MDMA</b>	0.0009	0.0030
<b>MDEA</b>	0.0008	0.0025
<b>Phentermine</b>	0.0044	0.0150

**Extraction Procedure:** Flow rate at  $\leq 0.5$  mL/min. is recommended during sample load, 0.5-1 mL/min. during wash steps, and  $\sim 0.2$  mL/min. for elution. If possible use gravity flow during the sample load step. A gentle vacuum ( $-0.4$  bar or  $-12$  inHg for 5-10 s) should be applied between each wash step and between the elution fractions unless described otherwise.

Application Name:	Extraction of Amphetamines from Urine
Analyte:	Amphetamine, Methamphetamine, Phentermine, MDA, MDMA, MDEA
Sample Matrix:	Human urine
General Comments:	The method is optimized for the class-selective extraction of trace levels of multiple amphetamines in human urine. Using the described procedure, ion-suppression is reduced thereby allowing lower limits of quantitation relative to traditional SPE procedures.
SupelMIP SPE – Amphetamines:	25 mg/3 mL (Cat. No. 53228-U)
Sample Pre-treatment:	Dilute up to 5 mL urine with 10 mM NH <sub>4</sub> Ac pH 8.0 (1:1, v/v). Adjust to pH 7.5-8.5 with NH <sub>3</sub> or CH <sub>3</sub> COOH. For particulate laden samples, centrifuge at 3000 g for 10 minutes and isolate supernatant for SPE preparation. Apply deuterated internal standard as necessary.
1. Condition/equilibrate cartridge with:	<ul style="list-style-type: none"> <li>◆ 1 mL methanol</li> <li>◆ 1 mL 10 mM NH<sub>4</sub>Ac buffer pH 8.0</li> </ul>
2. Load sample:	Apply 1 mL diluted urine sample. Note that up to 10 mL diluted urine sample can be applied. Note: Flow rate at $\leq 0.5$ mL/min. is recommended during sample load. If possible use gravity flow during the sample load step.
3. Wash (interference elution):	<ul style="list-style-type: none"> <li>◆ 2 x 1 mL DI water (elution of salt and matrix components). <b>Important:</b> Do not let the cartridge dry after the water wash steps</li> <li>◆ 1 mL 60% acetonitrile in DI water (elution of hydrophobic matrix components) <b>Important:</b> Apply vacuum through cartridge for 5-10 min. to remove residual moisture from cartridge (<math>-1</math> bar, <math>-20</math> in Hg, or <math>-70</math> kPa).</li> <li>◆ 1 mL 1% acetic acid in acetonitrile Apply a gentle vacuum (<math>-0.4</math> bar or <math>-12</math> inHg) for <math>\sim 30</math> sec. to the cartridge before elution.</li> </ul> <p><b>Note:</b> The wash steps should be performed in the prescribed order. A flow rate of 0.5-1 mL/min. is recommended for each wash step.</p>
4. Analyte elution:	<p>Elute amphetamines with 2 x 1 mL 1% formic acid in methanol. Apply a gentle vacuum (<math>-0.4</math> bar or <math>-12</math> inHg) for <math>\sim 30</math> sec. to the cartridge between each elution fraction.</p> <p><b>Note:</b> recommended flow rate <math>\sim 0.2</math> mL/min.</p> <p>Evaporate the elution solvent to dryness and reconstitute in 100 <math>\mu</math>L mobile phase (90% A and 10% B) prior to analysis.</p> <p>For GC-MS analysis, reconstitute and derivatize according to the selected method.</p>

<p><b>Recommended Analytical Technique:</b></p> <p><b>Note</b> that the original LC-MS-MS method developed and validated using TFA as a mobile phase pH modifier/ion-pairing agent was based on a method described by Fuh et al (1). Under these run conditions, excellent peak shape/efficiency and minimal ion-suppression was observed allowing for maximum sensitivity during chromatographic analysis.</p> <p>However, for analysts concerned with using TFA as a mobile phase ion-pairing agent, an alternative method was developed using ammonium acetate buffer. Please note that when using the ammonium acetate method, instrumental LOQs for the amphetamine compounds will be inferior relative to the TFA method, and a run time of at least 17 minutes will be required to achieve proper re-equilibration of HPLC column.</p> <p>1. <i>Determination of amphetamine and methamphetamine in urine by solid phase extraction and ion-pair liquid chromatography electrospray-tandem mass spectrometry, Fuh M, Wu T, Lin T, Talanta 68 (2006) 987-99.</i></p>	<p>column: Ascendis C18, 15 cm x 2.1 mm I.D., 5 µm particles (581304-U)</p> <p>instrument: Sciex API 3200</p> <p>mobile phase A: 0.05% TFA in DI water <b>OR</b> 13 mM ammonium acetate, pH 7</p> <p>mobile phase B: 0.05% TFA in acetonitrile <b>OR</b> 13 mM ammonium acetate in acetonitrile (when using ammonium acetate buffer as mobile phase A)</p> <p>temp.: 22 °C</p> <p>flow rate: 0.2 mL/min.</p> <p>gradient: <b>Time (min.) %A %B</b></p> <p>0.0 90 10</p> <p>7.0 70 30</p> <p>10.0 70 30</p> <p>11.0 10 90</p> <p>11.2 90 10</p> <p>15.0 90 10</p> <p>det.: <b>MS/MS, MRM transitions:</b></p> <table border="1"> <thead> <tr> <th>Compound</th> <th>Rt (min.)</th> <th>Q1/Q3</th> <th>DP</th> <th>EP</th> <th>CEP</th> <th>CE</th> <th>CXP</th> </tr> </thead> <tbody> <tr> <td rowspan="2">Amphetamine</td> <td rowspan="2">7.80</td> <td>136 / 119</td> <td rowspan="2">20</td> <td rowspan="2">7.5</td> <td rowspan="2">12</td> <td>13</td> <td>2</td> </tr> <tr> <td>136 / 91</td> <td>30</td> <td>2</td> </tr> <tr> <td rowspan="2">Methamphetamine</td> <td rowspan="2">8.33</td> <td>150 / 119</td> <td rowspan="2">25</td> <td rowspan="2">5</td> <td rowspan="2">12</td> <td>14</td> <td>2</td> </tr> <tr> <td>150 / 91</td> <td>29</td> <td>2</td> </tr> <tr> <td rowspan="2">Methamphetamine D<sub>5</sub> (IS)</td> <td rowspan="2">8.33</td> <td>158 / 124</td> <td rowspan="2">30</td> <td rowspan="2">5</td> <td rowspan="2">12</td> <td>14</td> <td>2</td> </tr> <tr> <td>158 / 93</td> <td>25</td> <td>2</td> </tr> <tr> <td rowspan="2">Phentermine</td> <td rowspan="2">8.66</td> <td>150 / 133</td> <td rowspan="2">25</td> <td rowspan="2">5</td> <td rowspan="2">12</td> <td>13</td> <td>2</td> </tr> <tr> <td>150 / 91</td> <td>29</td> <td>2</td> </tr> <tr> <td rowspan="2">MDA</td> <td rowspan="2">8.04</td> <td>180 / 163</td> <td rowspan="2">25</td> <td rowspan="2">5</td> <td rowspan="2">12</td> <td>14</td> <td>2</td> </tr> <tr> <td>180 / 105</td> <td>33</td> <td>2</td> </tr> <tr> <td rowspan="2">MDMA</td> <td rowspan="2">8.48</td> <td>194 / 163</td> <td rowspan="2">27</td> <td rowspan="2">5</td> <td rowspan="2">15</td> <td>18</td> <td>2</td> </tr> <tr> <td>194 / 105</td> <td>33</td> <td>2</td> </tr> <tr> <td rowspan="2">MDMA D<sub>5</sub> IS</td> <td rowspan="2">8.48</td> <td>199/ 165</td> <td rowspan="2">25</td> <td rowspan="2">4</td> <td rowspan="2">10</td> <td>17</td> <td>4</td> </tr> <tr> <td>199/ 136</td> <td>29</td> <td>4</td> </tr> <tr> <td rowspan="2">MDEA</td> <td rowspan="2">9.18</td> <td>208 / 163</td> <td rowspan="2">27</td> <td rowspan="2">5</td> <td rowspan="2">15</td> <td>18</td> <td>2</td> </tr> <tr> <td>208 / 105</td> <td>35</td> <td>2</td> </tr> </tbody> </table> <p>polarity: Positive</p> <p>ion source: Turbospray</p> <p>ion spray voltage: 5500 V</p> <p>source temp: 600 °C</p> <p>collision gas: 6 psi</p> <p>curtain: 10 psi</p> <p>lon source gas 1: 60 psi</p> <p>lon source gas 2: 60 psi</p> <p>dwll time: 100 msec.</p> <p>run time: 15 min.</p> <p>inj.: 20 µL</p>	Compound	Rt (min.)	Q1/Q3	DP	EP	CEP	CE	CXP	Amphetamine	7.80	136 / 119	20	7.5	12	13	2	136 / 91	30	2	Methamphetamine	8.33	150 / 119	25	5	12	14	2	150 / 91	29	2	Methamphetamine D <sub>5</sub> (IS)	8.33	158 / 124	30	5	12	14	2	158 / 93	25	2	Phentermine	8.66	150 / 133	25	5	12	13	2	150 / 91	29	2	MDA	8.04	180 / 163	25	5	12	14	2	180 / 105	33	2	MDMA	8.48	194 / 163	27	5	15	18	2	194 / 105	33	2	MDMA D <sub>5</sub> IS	8.48	199/ 165	25	4	10	17	4	199/ 136	29	4	MDEA	9.18	208 / 163	27	5	15	18	2	208 / 105	35	2
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**Product Information:**

Description	Pkg. Qty.	Cat. No.
<b>SupelMIP SPE - Clenbuterol</b> 25 mg/10 mL (LRC)	50	53201-U
<b>SupelMIP SPE - Beta-agonists (class selective)</b> 25 mg/10 mL (LRC)	50	53202-U
25 mg/3 mL	50	53225-U
<b>SupelMIP SPE – NNAL</b> 25 mg/10 mL (LRC)	50	53206-U
25 mg/3 mL	50	53203-U
<b>SupelMIP SPE - Riboflavin (Vitamin B2)</b> 25 mg/10 mL (LRC)	50	53207-U
<b>SupelMIP SPE - Triazine 10</b> 25 mg/10 mL (LRC)	50	53208-U
<b>SupelMIP SPE - Chloramphenicol</b> 25 mg/10 mL (LRC)	50	53210-U
25 mg/3 mL	50	53209-U
<b>SupelMIP SPE - Beta-blocker (class selective)</b> 25 mg/10 mL (LRC)	50	53218-U
25 mg/3 mL	50	53213-U
<b>SupelMIP SPE - TSNA's (NNK, NNN, NAB, NAT)</b> 50 mg/10 mL (LRC)	50	53221-U
50 mg/3 mL	50	53222-U
<b>SupelMIP SPE - Full Beta-receptors (beta-blockers &amp; beta-agonists)</b> 25 mg/10 mL (LRC)	50	53223-U
25 mg/3 mL	50	53224-U
<b>SupelMIP SPE – Amphetamines (class selective)</b> 25 mg/3 mL	50	53228-U

*SupelMIP SPE developed by MIP Technologies AB*