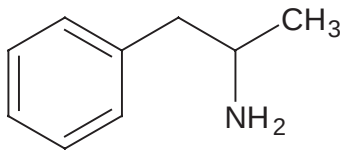
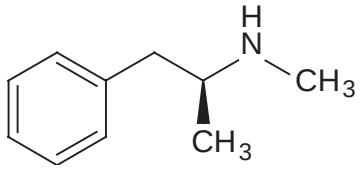
Extraction of Amphetamines from Urine using Strata™ Screen-C

This application describes how Strata Screen-C, a mixed-mode SPE sorbent, can be used to successfully extract low concentration levels of amphetamines from urine. The Strata Screen-C sorbent is a mixed phase consisting of silica particles functionalized with C8 and benzenesulfonic acid, a strong cation exchanger (SCX). This stationary phase is excellent for the extraction of basic drug compounds such as amphetamines, the recommended target analytes for SAMSHA drug confirmation. Since the pK_a of the SCX is <1, it is always negatively charged. In acidic solutions, the amine functional group will be positively charged and thus can be retained by ionic interactions with the SCX bonded phase (in addition to the Van der Waals interaction with the nonpolar C8 phase). This strong ionic retention mechanism allows the sorbent to be washed with relatively strong solvents such as methanol, which effectively remove anionic and neutral interferences without seriously affecting the recovery of the basic analyte. A mixture of organic solvent and ammonium hydroxide disrupts the analyte-sorbent interaction, resulting in the elution of the basic compound.

amphetamine



methamphetamine



Specimen preparation:
To 5mL of urine add internal standard(s) + 2mL 100mM phosphate buffer (pH 6.0). Mix/vortex. The pH of the sample should be 6.0 ± 0.5. Adjust pH with 1M potassium hydroxide, as needed.

Suggested internal standard for GC/MS: d_5-amphetamine and d_5-methamphetamine.

SPE Method:

Condition
1. 2mL methanol 2. 2mL DI water 3. 1mL 100mM phosphate buffer (pH 6)
Load
1. Apply the sample at a rate ≤2mL/min.
Wash/Dry
1. 2mL DI water 2. 1mL 100mM acetic acid 3. 3mL methanol 4. Dry column 2-5 min at full vacuum (>10" Hg)
Elute Amphetamines
1. With the vacuum turned off, apply 3mL dichloromethane/isopropanol/ammonium hydroxide (78:20:2). Allow solvent to slowly soak into sorbent for 15-30 sec before applying vacuum. Optimal flow rate of elution solvent is ≤2mL/min.

(Important: The volumes shown are for 150mg sorbent mass. For smaller or larger bed masses, solvent volumes will need to be adjusted.)

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Concentrate eluate:
To the eluate, add 100µL dimethylformamide (silylation grade). Evaporate to 30µL at ≤40°C.

Derivatize:
Add 50µL pentafluoropropionic acid anhydride. Cap and react 20 min at 70°C. Evaporate to dryness at ≤40°C. Reconstitute with 100µL ethyl acetate.

Analysis:
Inject 1 to 2µL onto GC column. (recommended: Zebron ZB-5, 15m x 0.25mm x 0.25µm).
Monitor the following ions (MSD):

amphetamine	d_5-amphetamine	methamphetamine	d_5-methamphetamine
190	194	204	208 ◀ quantification ions
91	91	118	119
118	123	160	163

Ordering Information:

Order No.	Description	Unit
8B-S016-EAK	Screen-C Tubes (100mg/1mL)	100/Box
8B-S016-EBJ	Screen-C Tubes (100mg/3mL)	50/Box
8B-S016-SBJ	Screen-C Tubes (150mg/3mL)	50/Box
8B-S016-RBJ	Screen-C Tubes (300mg/3mL)	50/Box
8B-S016-SCH	Screen-C Tubes (150mg/6mL)	30/Box
8B-S016-HCH	Screen-C Tubes (500mg/6mL)	30/Box
8E-S016-CGB	Screen-C 96-Well Plate (25mg/well)	2/Box
8E-S016-DGB	Screen-C 96-Well Plate (50mg/well)	2/Box
7EG-G002-11	Zebron ZB-5 (15m x 0.25m x 0.25µm)	1/Box

Extraction Tips!

1. Preparing solutions

100mM phosphate buffer (pH 6)
Add 13.6g of monobasic potassium phosphate to an empty 1L volumetric flask. Add 900mL DI water to dissolve the solid. Adjust the pH to 6 with 1M potassium hydroxide while stirring. Bring the volume up to the mark with DI water.

1M potassium hydroxide
Add 5.6g of potassium hydroxide to an empty 100mL volumetric flask. Add 90mL DI water to dissolve the solid. Bring the volume up to the mark with DI water.

100mM acetic acid
Add 28.6mL glacial acetic acid to 400mL DI water in 500mL volumetric flask. Bring the volume up to the mark with DI water. This makes a 1M acetic acid solution. Dilute 50mL of 1M acetic acid to 500mL with DI water. Mix.

Dichloromethane/isopropanol/ammonium hydroxide (78:20:2)
Combine 20mL isopropanol with 2mL concentrated ammonium hydroxide. Mix. Add 78mL dichloromethane. Mix.

2. Do not allow the sorbent to dry between the conditioning steps or prior to loading the sample. Excessive drying of the sorbent causes “deconditioning” which may lead to significantly lower and erratic recoveries. To ensure a properly solvated sorbent, apply each solvent immediately after the previous solvent.

3. Always condition the sorbent with the strongest solvent used in the method to ensure the cleanest extraction of the target analytes. In this method, 100mM phosphate buffer (pH 6) is used after methanol and water.

4. During the wash step, drying the sorbent removes any residual water and will ensure optimal analyte recovery.

5. Prepare the elution solvent daily, as the ammonium hydroxide rapidly dissipates in air.

Questions? Please contact your Phenomenex Technical Representative

This method is designed as a convenient starting point for further investigation. Phenomenex makes no guarantee regarding the accuracy or completeness of the method.