

Extraction of Low Concentration Levels of 6-MAM from Urine with a Mixed-mode SPE Sorbent – Strata™ Screen-C

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The screening and confirmation for drugs of abuse and their metabolites present many challenges in a toxicological analysis. The analyst routinely handles complex biomatrices such as urine or blood searching for the presence of illegal drugs, which, if present, may be at very low concentration levels. Direct analysis of these samples is often hampered by the presence of endogenous compounds in the biomatrix. These interferences are often in higher concentration than that of the target analytes and thus may mask their presence.

Solid phase extraction (SPE) is a sample preparation technique that is commonly used to purify and concentrate drug compounds and their metabolites prior to analysis.¹ This application describes how Strata Screen-C, a mixed-mode SPE sorbent, successfully extracts and concentrates 6-monoacetylmorphine (6-MAM) from urine. The Strata Screen-C sorbent is a mixed phase consisting of silica particles functionalized with C8 and benzene sulfonic acid, a strong cation exchanger (SCX). This stationary phase is excellent for the extraction of basic drug compounds and/or their metabolites. Since the pK_a of the SCX is <1 , it is always negatively charged. In acidic solutions, the basic analyte will be positively charged and thus can be retained by ionic interactions with the SCX bonded phase (in addition to the van der Waal 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 ammonia disrupts the analyte-sorbent interaction resulting in the elution of the basic compound.

Instrumentation and Equipment

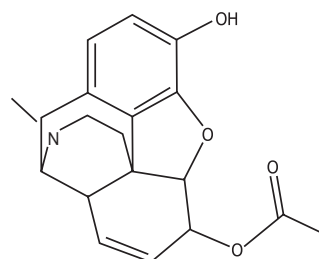
Solid phase extraction

Strata Screen-C syringe-barrel cartridges (150mg/3mL) were used for the extraction of 6-MAM from urine. **Table 1** contains information about the physical and chemical characteristics of this sorbent. Multiple SPE cartridges were processed simultaneously with a 12-position SPE vacuum manifold supplied by Phenomenex.

Gas chromatography

An HP 6890N GC system (Agilent Technologies, Palo Alto, CA) equipped with the HP 5973 Mass Selective Detector (MSD) was used for detection and quantitation of 6-MAM. The MSD was operated in SIM mode. The GC column was a Phenomenex Zebron ZB-1 (15.0m \times 0.25mm \times 0.25 μ m). The data were analyzed with HP Chemstation software

6-MAM



Morphine (IS)

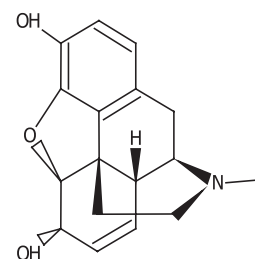


Table 1. Strata Screen-C particle characteristics

Phase	C8 + SCX
Average particle diameter:	55 μ m
Nominal pore size:	70Å
Surface area:	500m ² /g

Experimental Conditions

Specimen preparation

A 5mL urine sample spiked with 6-MAM and an internal standard (morphine) were mixed with 2mL of 100mM phosphate buffer. The pH of the solution was adjusted to 6.0 \pm 0.5 by adding 1M phosphoric acid.

SPE Method

Condition: The Strata Screen-C cartridge was first conditioned with 2mL methanol, followed by 2mL 100mM phosphate buffer (pH = 6.0). A slight vacuum (approximately 3-5 inches of mercury) was used to pull the conditioning solvents through the cartridge. The flow rate for each step of the method was 1-2mL/min.

Load: The 7mL sample was loaded onto the column in multiple aliquots.

Wash: In order to remove any weakly bound interferences, the sorbent was washed sequentially with three different solvent rinses. The solvent rinses were 2mL deionized water, followed by 2mL 100mM acetate buffer (pH = 4.5) and finally 2mL methanol. After rinsing with methanol, the column was dried for 3 minutes at a vacuum pressure >10 inches of mercury.

Elution: 6-MAM was successfully eluted with a 2mL solution of methylene chloride:isopropanol:ammonium hydroxide (78:20:2). (Note: It is recommended that a fresh elution solution be prepared daily.)



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Post-extraction derivatization

The eluent was evaporated to dryness at a temperature <40°C under a stream of nitrogen. The sample was derivatized by adding 50µL of ethyl acetate and 50µL of BSTFA and then heating the mixture at 70°C for 20 minutes. The derivatized sample was injected into the GC/MS for analysis.

Conclusion

Strata Screen-C successfully extracted low concentration levels of 6-MAM from urine. **Figure 1** shows a GC chromatogram for samples spiked with a concentration of 150ng/mL or 10ng/mL of 6-MAM. Using the morphine internal standard as a reference, the recoveries of 6-MAM were determined to be >90% for all extractions. The mixed-mode Strata Screen-C gives the analyst another sample preparation tool that can be optimized for the extraction of a specific basic drug and/or its metabolite, as shown here for 6-MAM, a metabolite of heroin.

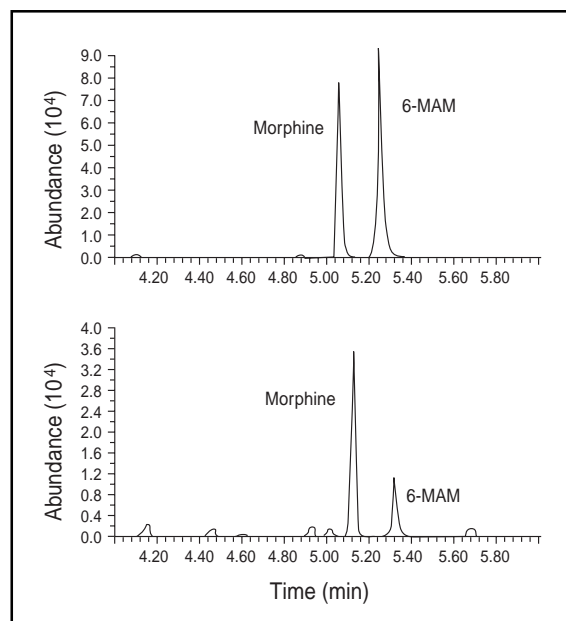
Reference

1. R. de Zeeuw and J. Franke in *Solid Phase Extraction Principles, Techniques and Applications*, 2000 Marcel Dekker, Inc. pp.243-273.

Ordering Information:

Order No.	Description
8B-S016-EAK	Strata Screen-C Tubes (100mg/1mL)
8B-S016-EBJ	Strata Screen-C Tubes (100mg/3mL)
8B-S016-SBJ	Strata Screen-C Tubes (150mg/3mL)
8B-S016-FBJ	Strata Screen-C Tubes (200mg/3mL)
8B-S016-RBJ	Strata Screen-C Tubes (300mg/3mL)
8B-S016-SCH	Strata Screen-C Tubes (150mg/6mL)
8B-S016-HCH	Strata Screen-C Tubes (500mg/6mL)
8E-S016-CGB	Strata Screen-C 96-Well Plate (25mg/well)
8E-S016-DGB	Strata Screen-C 96-Well Plate (50mg/well)
AH0-6023	12-position SPE Vacuum Manifold
AH0-6024	24-position SPE Vacuum Manifold
7EG-G001-11	Zebtron ZB-1 GC Column

Figure 1. GC chromatograms (SIM mode) for two different concentration levels of 6-MAM and morphine extracted from 5mL of urine with Strata Screen-C



Experimental conditions. Injection conditions: (top) 2µL of sample at a concentration of 150ng/mL was injected at 250°C with split ratio 5:1. (bottom) 5µL of sample at a concentration of 10ng/mL was injected at 250°C in splitless mode. GC/MS conditions: initial oven temperature was set at 175°C. The temperature was ramped to 260°C at 15°C/min and then to 320°C at 25°C/min (held for 2 minutes at final temperature). The flow rate of helium was 1.2mL/min. 6-MAM and morphine were detected in SIM mode by monitoring ions at 399 and 429, respectively.