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 for and confirmation of 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 new mixed-mode SPE sorbent from Phenomenex, successfully extracts and concentrates ppb concentration levels of 6-monoacetylmorphine (6-MAM) 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 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 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 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 (150 mg/3 mL) were used for the extraction of 6-MAM from urine. Table I contains information on the physical characteristics of Strata Screen-C. 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, California) 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 (15.0 m × 0.25 mm × 0.25 μm). The data were analyzed with HP Chemstation software.

Experimental Conditions

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

SPE extraction method:

Condition: The Strata Screen-C cartridge was first conditioned with 2 mL methanol, followed by 2 mL of 100 mM phosphate

Table I: Strata Screen-C particle characteristics

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

buffer (pH = 6.0). A slight vacuum (approximately 3–5 in. of mercury) was used to pull the conditioning solvents through the cartridge. The flow rate for each step of the method was 1–2 mL/min.

Load: The 7-mL 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 2 mL of DI water, followed by 2 mL of 100 mM acetate buffer (pH = 4.5) and finally 2 mL of methanol. After rinsing with methanol, the column was dried for 3 min. at a vacuum pressure >10 in. of mercury.

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

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

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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 150 ng/mL or 10 ng/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* (Marcel Dekker, Inc., New York, 2000), pp. 243–273.

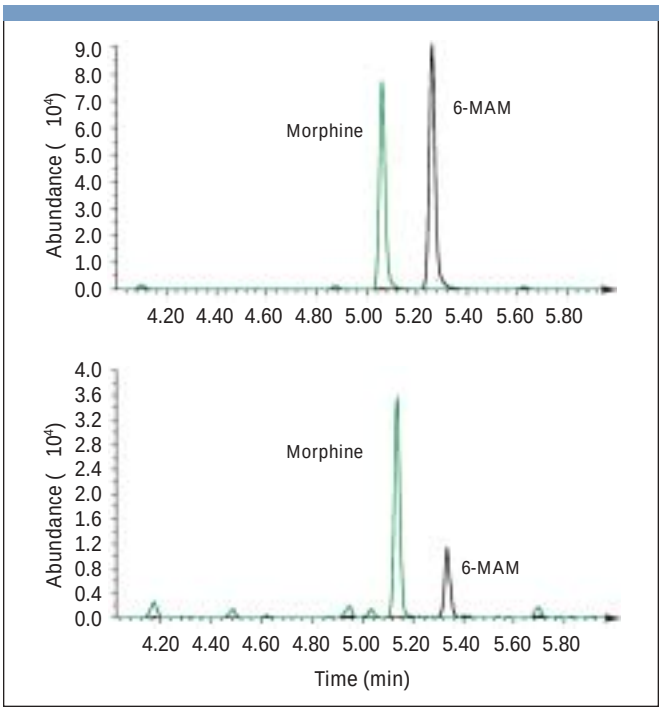

Figure 1: GC chromatograms (SIM mode) for two different concentration levels of 6-MAM and morphine extracted from 5 mL of urine by Screen-C. Experimental conditions: Injection conditions: (top) 2 µL of sample at a concentration of 150 ng/mL was injected at 250 °C with split ratio of 5:1; (bottom) 5 µL of sample at a concentration of 10 ng/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 min at final temperature). The flow rate of helium was 1.2 mL/min. 6-MAM and morphine were detected in SIM mode by monitoring ions at 399 and 429, respectively.

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