

The Extraction of Drugs of Abuse From Viscous Sample Matrices by Gravity Flow Using a New Mixed-mode Solid Phase Extraction Sorbent with Excellent Flow Characteristics

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Abstract

In toxicological analyses, illegal drugs and their metabolites if present in complex biomatrices such as urine or blood are at very low concentrations and are often masked by the higher concentration of endogenous compounds, thus making direct analysis very difficult. Solid-phase extraction (SPE) is a sample preparation technique commonly used to purify and concentrate drugs of abuse and their metabolites from complex biomatrices prior to chromatographic analysis^{1,2}. Phenomenex's Strata™ Screen-C, a mixed-mode sorbent, is proven to be effective in the extraction of drugs from urine with higher than 90% recoveries³. However, when processing viscous samples, such as serum or whole blood, analysts often experience slow or no flow of sample through the cartridge even under vacuum, prolonging the extraction process or even making extraction impossible. Therefore, a new mixed-mode sorbent, Strata™ Screen-C GF (Gravity Flow), was developed to meet the need for extracting from sample matrices of high viscosity. Because of the excellent flow characteristics of this new sorbent, it makes extraction in the absence of vacuum or vacuum manifold possible. The extraction performance and the purity of the delivery tips, in addition to the flow properties, were characterized.

Introduction

Solid Phase Extraction is a popular method of sample clean-up and concentration technique prior to chromatographic analysis. However, most sorbents in the current market are designed for extraction from sample matrixes of low viscosity under vacuum and exhibit slower flow characteristics. This often makes the extraction of more viscous samples difficult or even impossible. Therefore, Strata™ Screen-C GF, a new mixed-mode sorbent with excellent flow characteristics and high extraction efficiency, is developed to meet the needs for the extraction from more viscous samples or the extraction under gravity flow in the absence of vacuum.

Introduction (cont.)

Key features of the newly developed Strata™ Screen-C GF sorbent:

- **Superior Flow Properties** for viscous sample matrices, even in the absence of vacuum
- **High Extraction Efficiency** comparable to conventional sorbents of smaller particle size
- **Applicable to a Wide Range of Analytes** including acidic, neutral and basic drugs
- **High Degree of Purity of Sorbent and Delivery Tips** free from contaminants

Gravity Flow Properties

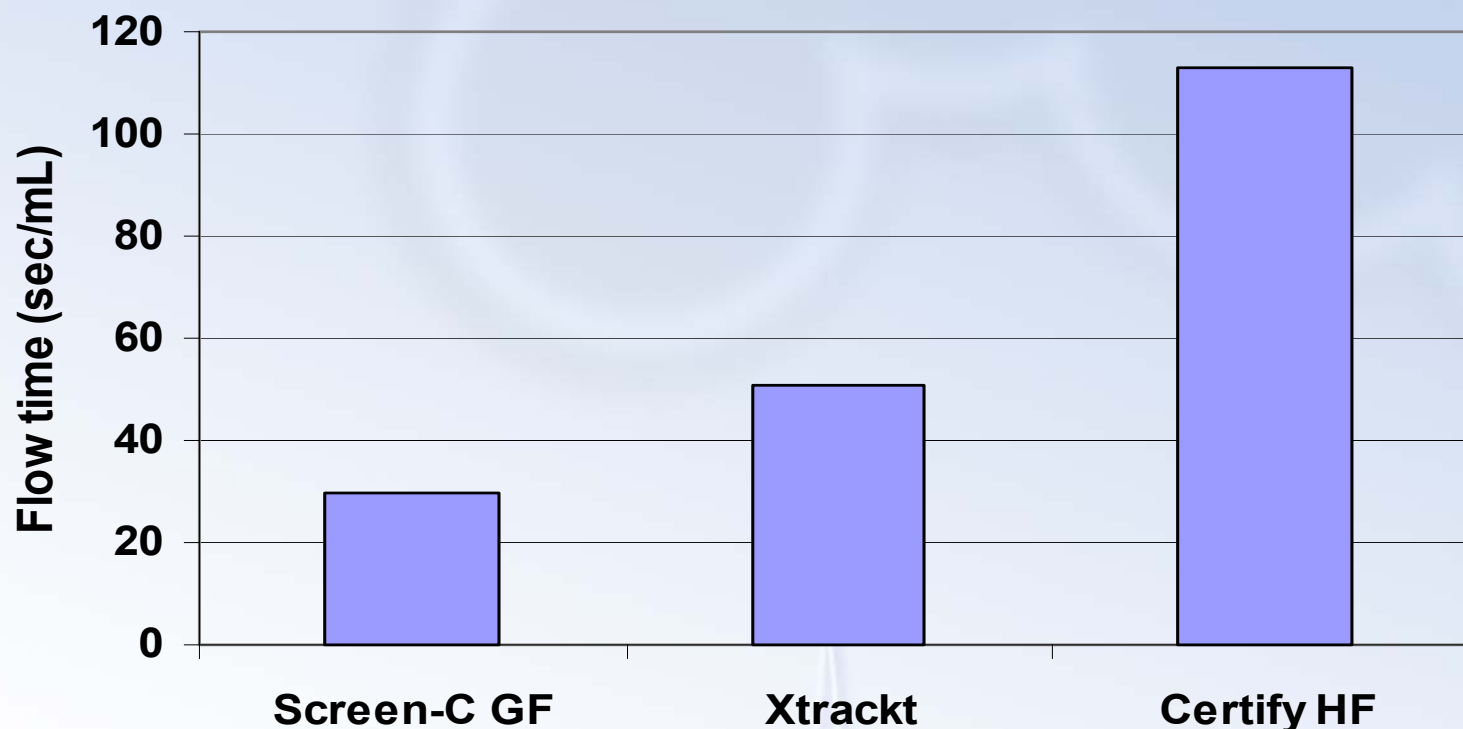
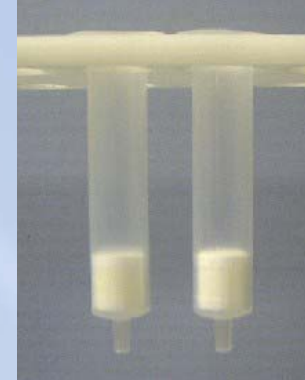


Figure 1. Average flow time of porcine serum (diluted 1:1 with PBS buffer) under gravity flow. Flow rate of Strata™ Screen-C GF is 70% and 270% faster than those of Xtrackt® and Certify HF®, respectively. Xtrackt® and Certify HF® are trademarks of UCT, Inc. and Varian, Inc. respectively.

Gravity Flow Properties (with delivery tips)

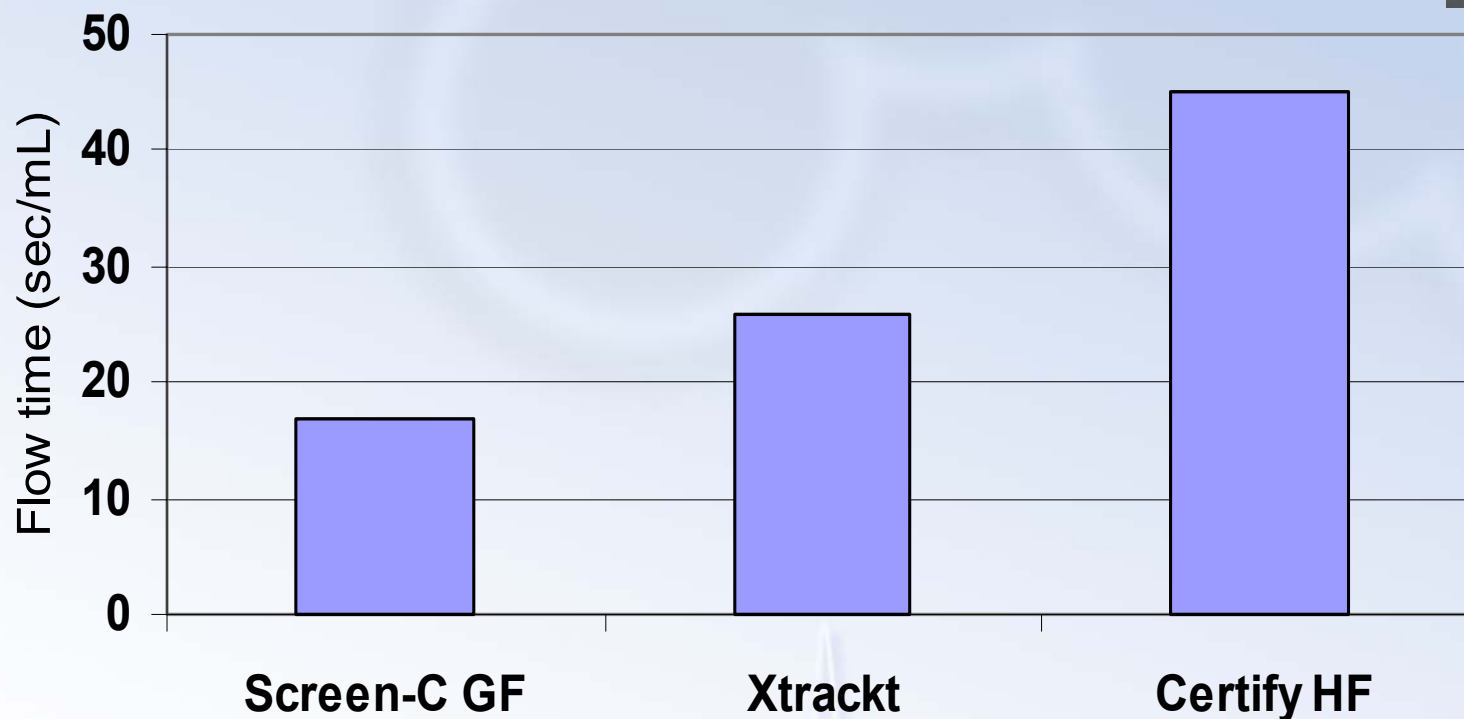


Figure 2. Average flow time of porcine serum (diluted 1:1 with PBS buffer) under gravity flow. Use of delivery tips decreases flow time by 76% to 150%. Xtrackt[®] and Certify HF[®] are trademarks of UCT, Inc and Varian, Inc. respectively.

Purity of Sorbent and Delivery Tips

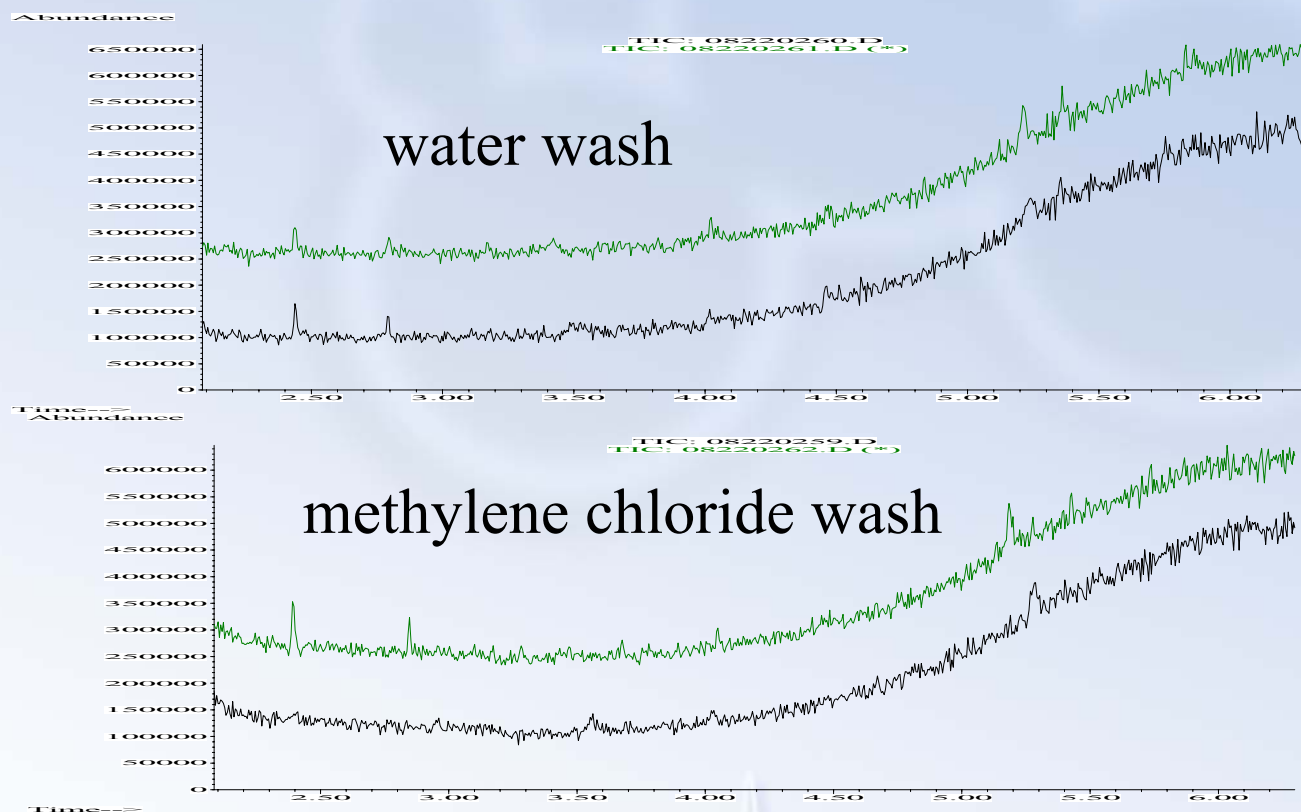


Figure 3. The purity of Strata™ Screen-C GF sorbent and delivery tip was analyzed by washing with water and methylene chloride. The wash solvents (green) and the blank solvents (black) were analyzed by GC/MS for impurities. No impurities were detected.

Experimental Conditions for Strata™ Screen-C GF (for the simultaneous extraction of acidic, neutral and basic analytes)

500mg/6mL cartridge
(gravity flow)

Conditioning

5 mL methanol and then 5 mL 100mM phosphate buffer pH 6.0



Load

3 mL analyte spiked serum diluted with 3 mL 100mM phosphate buffer pH 6.0



Wash

5 mL 5% methanol/ DI water, and then 4 mL 1M acetic acid pH 2.2.

Add 0.7 mL methanol or Dry under vacuum for 5 minutes



Elute

Acidic/Neutral Analytes: 2 x 3 mL methanol with 2% acetic acid

Basic Analytes: 2 x 3 mL EtOAc with 2% NH₄OH



Evaporate under slow stream of N₂ at < 40°C
and **Derivatize** with 50 µL EtOAc + 50 µL BSTFA at 70°C for 20 minutes

Experimental Conditions for Strata™ Screen-C GF (for the extraction of basic analytes)

500mg/6mL cartridge
(gravity flow)

Conditioning

5 mL methanol and then 5 mL 100mM phosphate buffer pH 6.0



Load

3 mL analyte spiked serum diluted with 3 mL 100mM phosphate buffer pH 6.0



Wash

5 mL 5% methanol/ DI water, and then 4 mL 1M acetic acid pH 2.2, followed by 3 mL methanol



Elute

Basic Analytes: 2 x 3 mL EtOAc with 2% NH₄OH



Evaporate under slow stream of N₂ at < 40°C
and **Derivatize** with 50 µL EtOAc + 50 µL BSTFA at 70 °C for 20 minutes.

Recoveries of a Wide Spectrum of Analytes

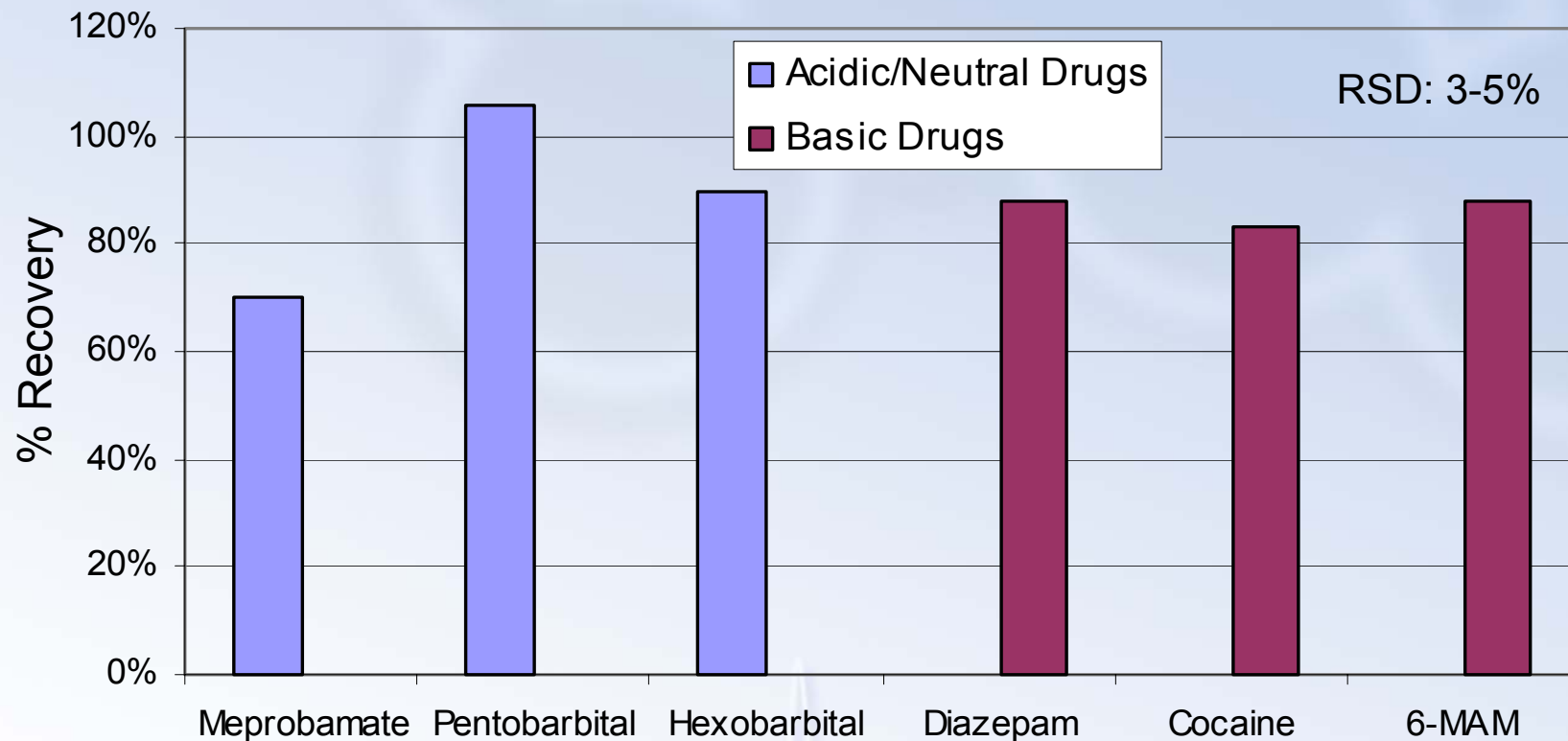


Figure 4. Percent absolute recovery of a wide spectrum of analytes by Strata™ Screen-C GF

GC-MS: Column: Zebron ZB 5, 30m x 0.25mm x 0.25 μ m
Carrier gas: Helium at 3mL/min, constant flow rate
Injection: 2 μ L splitless injection at 250°C
Oven temperature: 75°C to 320°C, at 25°C/min

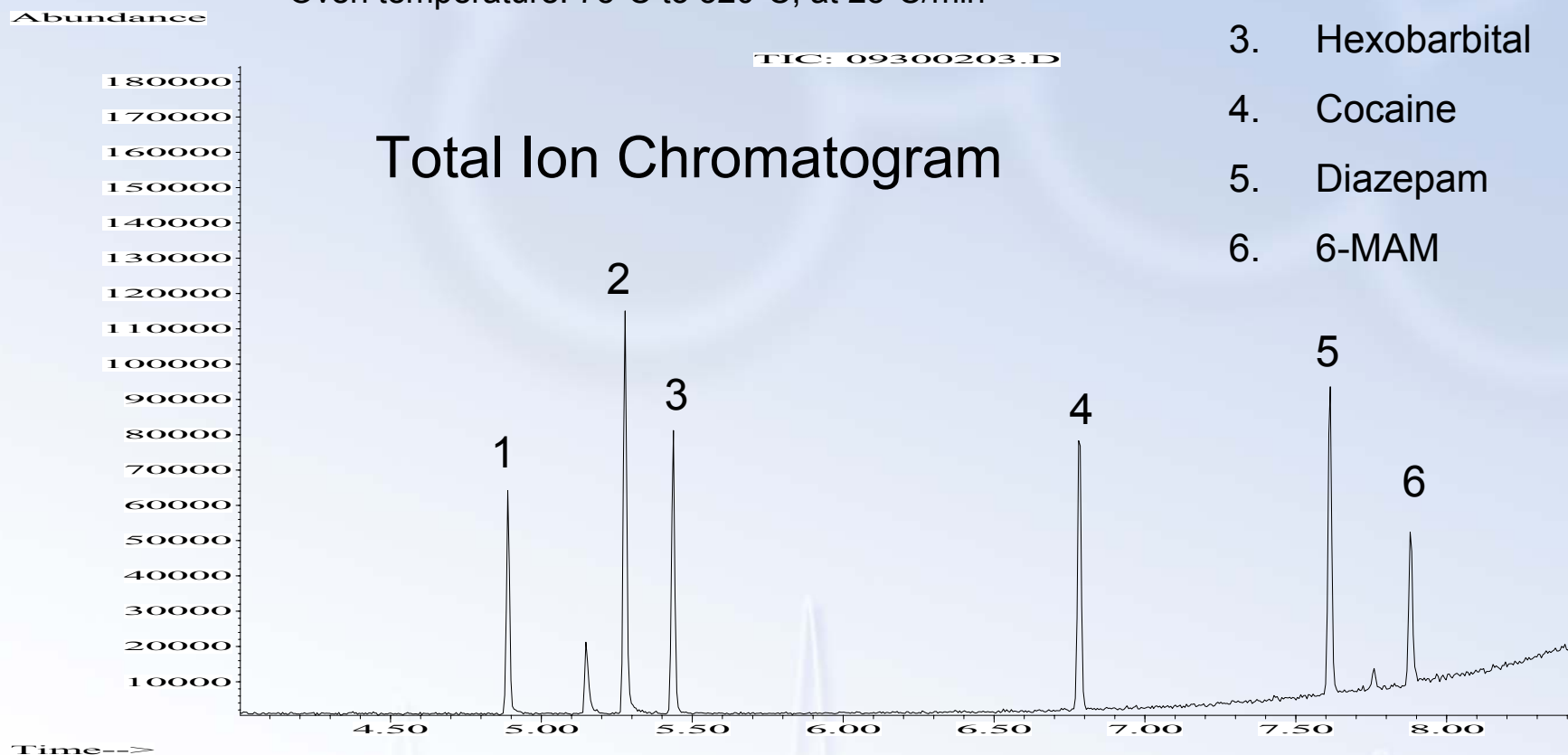


Figure 5. Drugs of abuse extracted from porcine serum using Strata™ Screen-C GF analyzed by GC/MS

Alternative Sorbent : Experimental Conditions for Strata™ Screen-C (150mg/6mL)

Conditioning (vacuum at <5" Hg unless specified)

5 mL methanol and then 5 mL 100mM phosphate buffer pH 6.0



Load

3 mL analyte spiked urine diluted with 3 mL 100mM phosphate buffer pH 6.0



Wash

3 mL DI water, and then 3 mL 100 mM acetic buffer pH 4.5, followed by 2 mL methanol
Dry under full vacuum for 3 minutes



Elute

2 mL CH₂Cl₂/IPA/NH₄OH (78:20:2)



Evaporate under slow stream of N₂ at < 40°C
and **Derivatize** with 50 µL EtOAc + 50 µL BSTFA at 70 °C for 20 minutes.

Alternative Sorbent: Experimental Conditions for Strata™-X (100mg/6mL)

Conditioning (vacuum at <5" Hg unless specified)

5 mL methanol and then 5 mL DI water



Load

3 mL analyte spiked urine diluted with 3 mL DI water



Wash

3 mL DI water, followed by 5 mL 30% methanol/DI water with 2% NH₄OH.

Dry under full vacuum for 3 minutes



Elute

3 mL methanol with 2% acetic acid



Evaporate under slow stream of N₂ at < 40°C
and **Derivatize** with 50 µL EtOAc + 50 µL BSTFA at 70 °C for 20 minutes.

Comparison of Recoveries of 6-MAM by Strata™ Screen-C GF, Strata™-X and Strata™ Screen-C

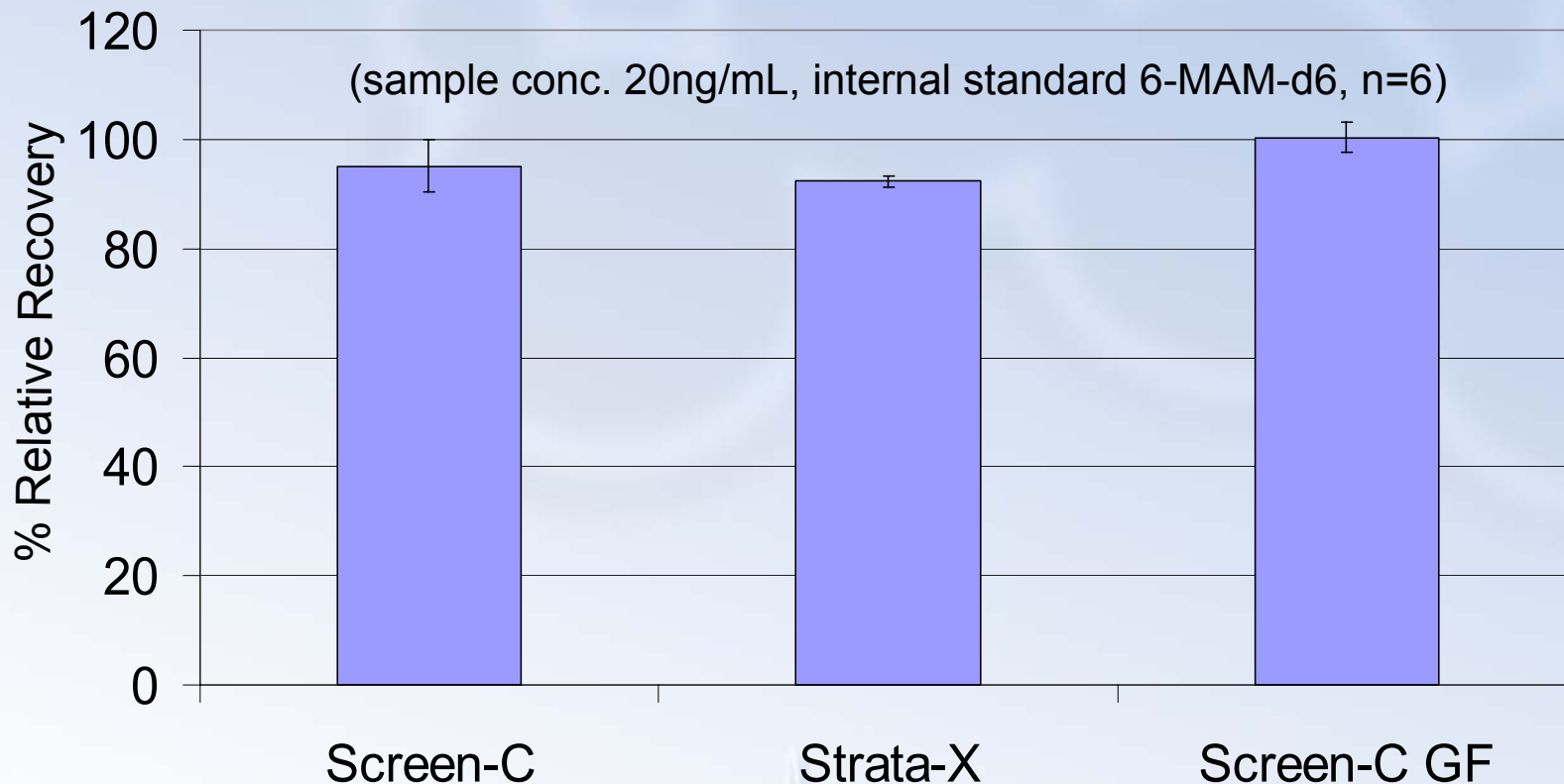
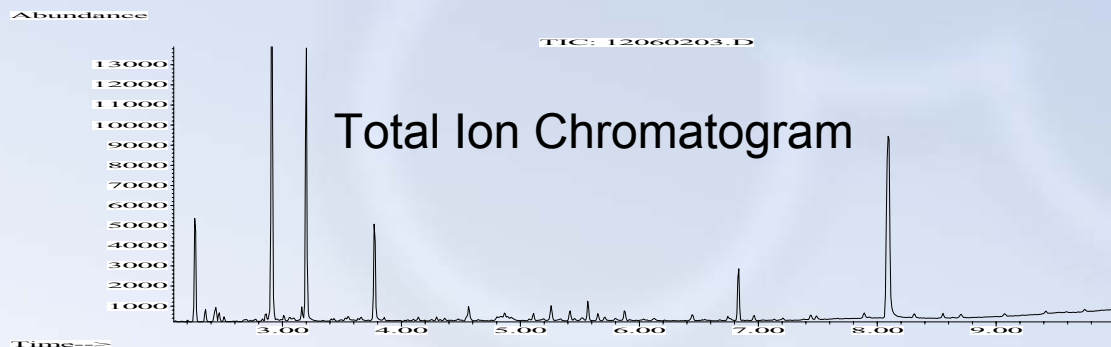


Figure 6. Recoveries of 6-MAM from urine/serum using Strata™ Screen-C GF, Strata™-X and Strata™ Screen-C.

GC/MS: Column: Zebron ZB 5, 30m x 0.25mm x 0.25 μ m
Carrier gas: Helium at 3mL/min, constant flow rate
Injection: 2 μ L splitless injection at 250°C
Oven temperature: 75°C to 320°C, at 25°C/min
SIM Mode: TMS-6-MAM: 399, 340; TMS-6-MAM-d6: 405, 343



Spiked sample: 20 ng/ml

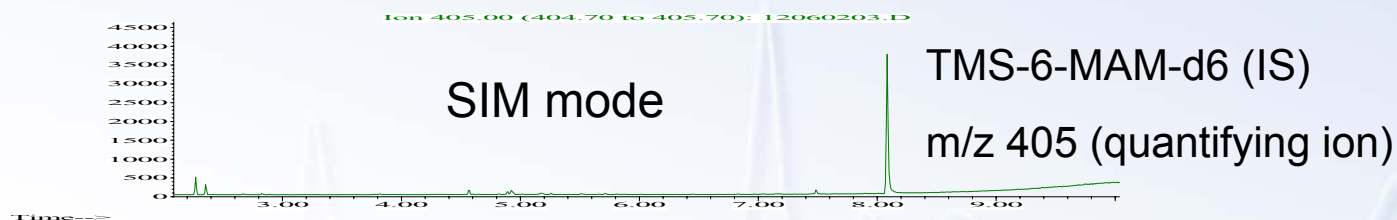
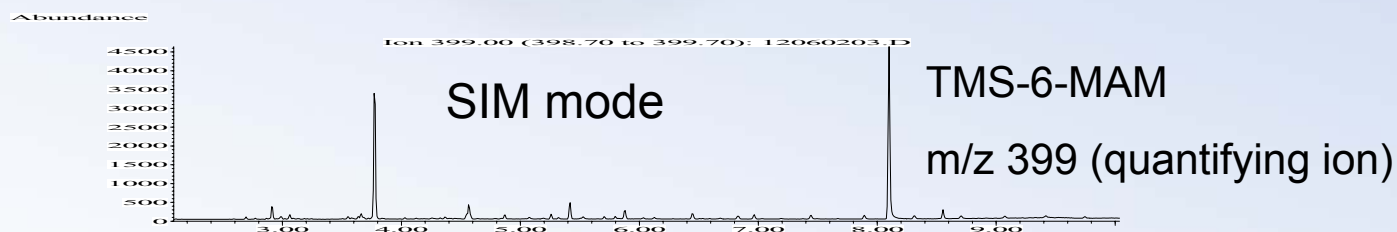


Figure 7. 6-MAM extracted from porcine serum using StrataTM Screen-C GF

Determination of Void Volume in Sorbent Bed

When vacuum is not available to dry the sorbent bed after washing, it is important to remove the washing solvents from the sorbent bed prior to the addition of immiscible elution solvent. This can be done by replacing the residual washing solvent in the sorbent bed with a “bridging” solvent, that is miscible with both the washing and elution solvents. In order to prevent the analytes of interest from eluting in the “bridging” solvent, it is important to apply only the volume enough to fill the void volume of the bed mass. The void volume can be easily determined by measuring the amount of solvent needed to elute a dye from the sorbent bed. For a 500 mg Strata™ Screen-C GF bed, a void volume of 700 μL is determined.

Discussion of Results

- Strata™ Screen-C GF exhibits superior flow characteristics even for matrices of high viscosity. It shows an improvement of up to 3X increase in flow rate over the competitors' products, reducing time for sample preparation.
- The use of the delivery tips almost doubles the flow rate.
- The gravity flow properties of the sorbent do not require the use of vacuum and vacuum manifold.
- A wide range of drugs of abuse, including acidic, neutral and basic, can be extracted from matrices of high viscosity using Strata™ Screen-C GF, with a high extraction efficiency.
- The level of impurities in Strata™ Screen-C GF and in the delivery tips proved to be insignificant when checked by the highly sensitive GC/MS.
- Multiple retention mechanisms (reversed-phase and cation exchange) allow samples to be aggressively cleaned and concentrated with high degrees of recovery for various classes of analytes and no major interferences from the matrix background were observed. Comparable recovery to other conventional smaller particle sorbents is observed.

Conclusion

Strata™ Screen-C GF, is a newly developed sorbent consisting of silica particles functionalized with C8 and benzenesulfonic acid, a strong cation exchanger (SCX). This mixed-mode sorbent exhibits excellent selectivity and provides clean, concentrated extracts of a wide range of drugs of abuse, including acidic, neutral and basic drugs from biological fluids. The gravity flow properties of this sorbent allow for extraction from matrices of high viscosity, which is usually problematic if using conventional SPE sorbents with smaller particle size. The superior flow characteristics of this sorbent also make extraction in the absence of vacuum possible. Comparable recovery of analytes from this Strata™ Screen-C GF gravity flow sorbent, makes it an excellent alternative SPE tool for the extraction of drugs of abuse from matrices of high viscosities, or for vacuum-free extractions.

References

1. R. de Zeeuw and J. Franke, Solid Phase Extraction Principles, Techniques and Applications, 2000 Marcel Dekker, Inc.
2. Nigel J. K. Simpson, Solid-Phase Extraction Principles, Techniques, and Applications, 2000 Marcel Dekker, Inc.
3. Lawrence Loo and James Teuscher, The Application Notebook – August 2002, LC/GC Magazine.