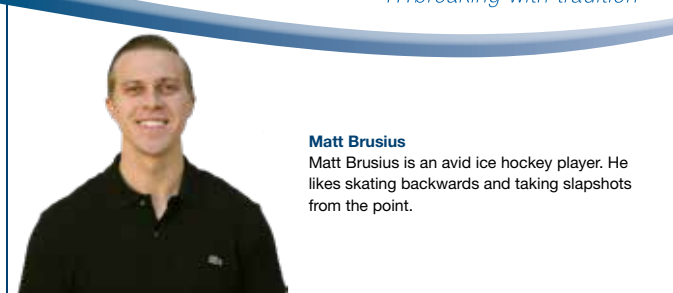


APPLICATIONS

Comparison of Tradition Solid Phase Extraction (SPE) and Strata[®]-X PRO SPE for the Extraction of Acids, Neutrals, and Bases from Plasma

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Matt Brusius
Matt Brusius is an avid ice hockey player. He likes skating backwards and taking slapshots from the point.

Overview

Solid Phase Extraction (SPE) is a widely used technique for the preparation of samples prior to analysis by HPLC or GC. The procedure is used to simplify complex sample matrices, removing components whose presence may cause significant issues in the following chromatographic method. It is commonly applied to the extraction and clean-up of small molecule drugs from biological samples such as serum, plasma, silica, CSF, and urine. As well as providing a means of sample clean-up, SPE also allows analytes present at low concentration in samples to be concentrated and facilitates solvent switching.

While SPE does provide benefits for analysts, it is often perceived as complicated and time-consuming, these issues advert some potential users from adopting the technique. Traditionally SPE consists of 5 or more steps:

1. Conditioning of the SPE sorbent
2. Equilibration of the SPE sorbent
3. Load Sample
4. A wash step to remove weakly bound impurities
5. A drying step (this can be skipped in some methods)
6. Elution of the desired analyte fraction

In this technical note a fast and efficient SPE sorbent, Strata-X PRO, was chosen to extract a panel of acids, neutrals, and bases from plasma. Strata-X PRO is a reversed phase SPE sorbent combined with a matrix removal component/device specifically designed to reduce the number of steps in the protocol while providing high recoveries, low variability, and clean samples. This simple method eliminates time-consuming method development surpassing traditional SPE in ease of use, matrix factor response, and recovery for extremely polar analytes.

Strata-X PRO Protocol

96-Well Plate: Strata-X PRO, 30 mg/well
Part No.: 8E-S536-TGA
Load: 400 μ L Plasma/0.1% Formic acid in Water (1:1)
Wash: 1 mL 5% Methanol in Water
Dry: 1 minute at 5" Hg
Elute: 1mL 0.1% Formic acid in Acetonitrile/Methanol (90/10)
Dry Down: 1 minute at 5" Hg
Reconstitute: 200 μ L 5% Methanol in Water

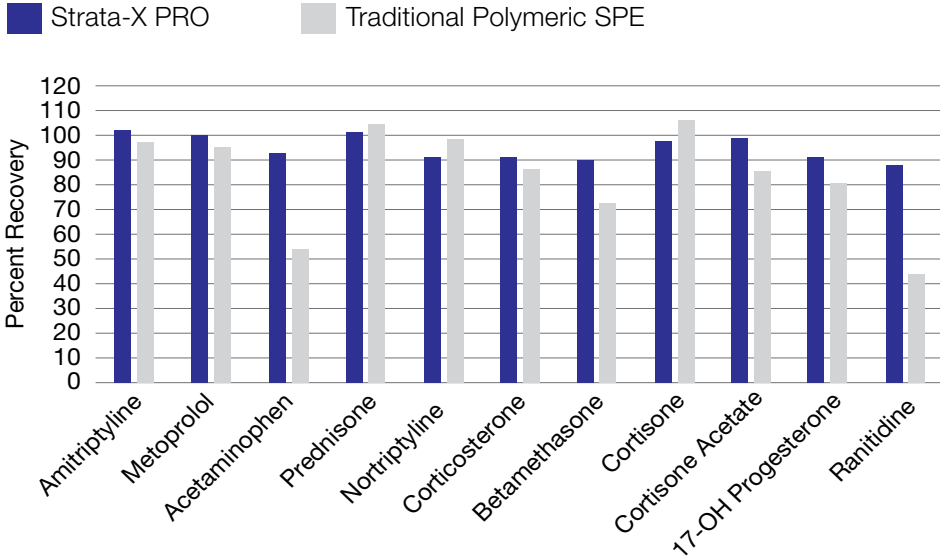
Polymeric SPE Protocol

Condition: 1 mL Methanol
Equilibrate: 1 mL Water
Load: 400 μ L Plasma/0.1% Formic acid in Water (1:1)
Wash: 1 mL 5% Methanol in Water
Dry: 1 minute at 5" Hg
Elute: 1 mL 0.1% Formic acid in Acetonitrile/Methanol (90/10)
Dry Down: 1 minute at 5" Hg
Reconstitute: 200 μ L 5% Methanol in Water

Table 1. Percent Recovery for a Panel of Acids, Neutrals, and Bases

Analyte Name	Strata-X PRO % Recovery	Polymeric SPE% Recovery
Amitriptyline	102	97
Metoprolol	100	94
Acetaminophen	92	54
Prednisone	101	104
Nortriptyline	90	98
Corticosterone	91	86
Betamethasone	90	73
Cortisone	97	106
Cortisone Acetate	99	85
17-OH Progesterone	90	81
Ranitidine	88	44

Figure 1.
Recovery of Acid, Neutrals, and Bases for Strata[®]-X PRO vs Polymeric SPE



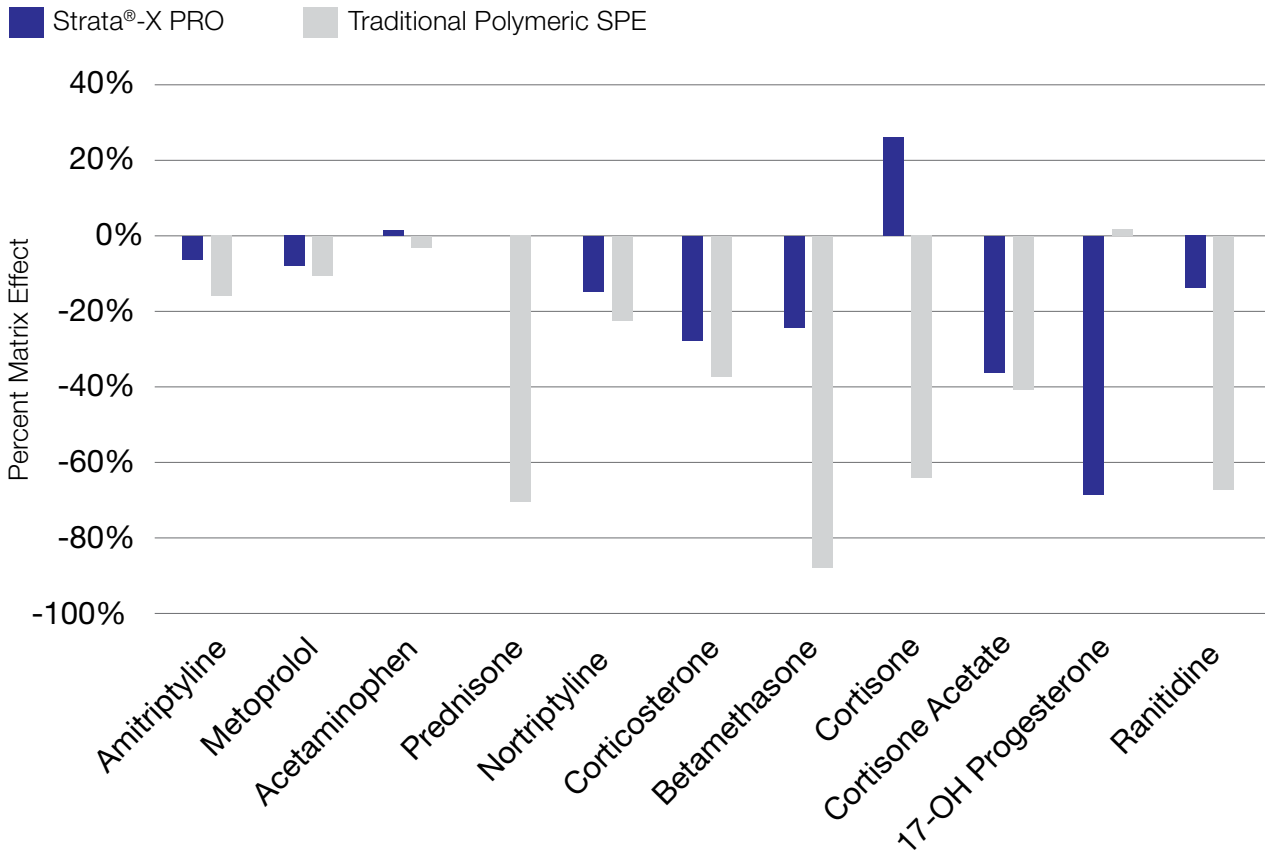
LC Conditions

Column: Kinetex[®] 2.6 μm C18
Dimension: 50 x 2.1 mm
Part No.: 00B-4462-AN
SecurityGuard[™] ULTRA Cartridge: AJ0-8782
Guard Holder: AJ0-9000
Mobile Phase: A: 0.1% Formic acid in Water
 B: 0.1% Formic acid in Methanol
Gradient:

Time (min)	% B
0	5
3	95
4	95
4.01	5
6.76	5

Flow Rate: 0.4 mL/min
Temperature: 40 °C
Detector: SCIEX[™] API 4000[™]

Figure 2.
Percent Matrix Effects of Strata-X PRO vs Polymeric SPE



Results and Discussion

While traditional SPE sorbents require a condition and equilibration step, Strata[®]-X PRO eliminates the need for this part of the protocol. Using the pre-treated samples, it is then possible to load them directly onto the Strata-X PRO cartridge for extraction. The elimination of these two previously required steps in traditional SPE reduces method complexity, sample processing time, and also solvent consumption. The data in **Figure 1** displays that analyte recovery is not compromised by using this approach and even provides better recovery for metoprolol, acetaminophen, and ranitidine, which are the most polar analytes in the suite.

Choice of wash solvent will influence both analyte recovery and sample cleanliness, and an ideal method will provide high recovery and added cleanliness, but often the conditions are compromised to allow for either cleaner samples or the highest recovery. With Strata-X PRO, the generic 5% methanol wash provides better matrix factor response for the majority of analytes in the panel, indicating that the plasma sample is cleaner when compared to traditional polymeric SPE processed with the same method (**Figure 2**). In addition, the 5% methanol wash does not compromise recovery and shows either equivalent or better recovery in the same comparison (**Figure 1**).

The difference in analyte recovery (**Figure 1**) is most noticeable for acetaminophen and ranitidine. Moving from a traditional SPE material to Strata-X PRO significantly increases recovery for both analytes. These two analytes are the most polar in the panel and

they are the most challenging to retain during a reversed phase extraction. An increase in recovery suggests that more polar analytes are retained more strongly during both loading and washing, areas where losses would be anticipated using traditional SPE materials. Acetaminophen is a weak acid, and ranitidine is a moderate base, indicating that Strata-X PRO is effective at retaining both polar acids and bases using the same simple load conditions and the fixed elution solvent of 0.1% formic acid in acetonitrile/methanol (90:10) proves to be strong enough to elute these analytes back off the cartridge.

The data in **Figure 2** shows that in the case of most analytes there is less impact from any residual matrix than could be seen from a traditional SPE material. This is indicative of an SPE material that allows for more of the matrix to be removed during the wash step, or alternatively being selectively retained even during the elution step. Since Strata-X PRO contains added matrix removal technology, the matrix effects are greatly reduced, leading to a reduction in ion suppression that could affect analytes response. Using Strata-X PRO removes phospholipids more efficiently and ultimately can lead to a more sensitive analysis.

Conclusions

Strata-X PRO's simplified method reduces sample processing and method development time, while also providing results with high recovery and reduced matrix effect when compared to traditional solid phase extraction analyzed by LC-MS/MS.

APPLICATIONS

Ordering Information

Strata[®]-X PRO SPE

Format	Sorbent Mass	Part Number	Unit
Tube			
	10 mg	8B-S536-AAK	1 mL (100/box)
	30 mg	8B-S536-TAK	1 mL (100/box)
	30 mg	8B-S536-TBJ	3 mL (50/box)
	60 mg	8B-S536-UBJ	3 mL (50/box)
	200 mg	8B-S536-FBJ	3 mL (50/box)
	100 mg	8B-S536-ECH	6 mL (30/box)
	200 mg	8B-S536-FCH	6 mL (30/box)
	500 mg	8B-S536-HCH	6 mL (30/box)
96-Well Plate			
	10 mg/well	8E-S536-AGA	ea
	30 mg/well	8E-S536-TGA	ea
	60 mg/well	8E-S536-UGA	ea
96-Well Microelution Plate			
	2 mg/well	8M-S536-4GA	ea

Kinetex[®] Core-Shell LC Columns

2.6 µm Minibore Columns (mm)						SecurityGuard [™] ULTRA Cartridges [†]
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4462-AN	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782 for 2.1 mm ID

2.6 µm MidBore [™] Columns (mm)						SecurityGuard [™] ULTRA Cartridges [†]
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775 for 3.0 mm ID

2.6 µm Analytical Columns (mm)						SecurityGuard [™] ULTRA Cartridges [†]
Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768 for 4.6 mm ID

1.7 µm Minibore Columns (mm)						SecurityGuard [™] ULTRA Cartridges [†]
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1		3/pk
C18	00A-4475-AN	00B-4475-AN	00D-4475-AN	00F-4475-AN		AJ0-8782 for 2.1 mm ID

1.7 µm MidBore Columns (mm)				SecurityGuard [™] ULTRA Cartridges [†]
Phases	50 x 3.0	100 x 3.0		3/pk
C18	00B-4475-Y0	00D-4475-Y0		AJ0-8775 for 3.0 mm ID

[†]SecurityGuard Ultra Cartridges require holder, Part No.: AJ0-9000



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Presston[™] 1000 Positive Pressure Manifold

Part No.	Description
AH1-7033	Presston 1000 Positive Pressure Manifold, 96-Well Plate

Phenomenex warrants the Presston 1000 will be free of defects in materials and workmanship under normal installation, use, and maintenance for a period of 12 months following delivery. Please visit www.phenomenex.com/Presstonwarranty for complete warranty information.

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