

TIME BY AT LEAST

REDUCE PROTOCOL
TIME BY AT LEAST

WITH A REVOLUTIONARY
3- AND 2-STEP SPE

NO EQUILIBRATION NEEDED. NO CONDITIONING NEEDED.

HIGH RECOVERIES



APPLICATION BOOK





Advantages



www.phenomenex.com/sampleprep

Getting Started



Prepare Your Sample

Plasma/Serum

If the analyte of interest is an acid, 2% phosphoric acid can be used ($20 \mu L$ 85% H_3PO_4 to 1 mL of plasma (or serum) to disrupt the drug-protein interaction. If the analyte of interest is basic, 0.1M sodium hydroxide can be used to disrupt the drug-protein interaction. After addition of acid or base, the sample should be vortexed for 20-30 seconds followed by centrifugation. The supernatant is now ready for further analysis. Alternatively, a zinc sulfate or strong organic solvent can be used to disrupt the protein interaction.

Whole Blood

There are several pre-treatment strategies that can be followed for whole blood. If the target analyte is present in red blood cells, a hemolysis step is necessary.

a. Hemolysis: To 0.2 mL whole blood (spiked with analytes and internal standard) in a 1.2 mL centrifuge tube, add 400 µL of 2 % zinc sulfate/80 % methanol. Vortex for 10-20 seconds followed by centrifugation at 14,000 rpm for 10 minutes. Collect the supernatant for further analysis.

Preparation of zinc sulfate/methanol: Into a 100 mL volumetric flask add 20 mL water and $3.6 \,\mathrm{g}$ ZnSO₄ $7 \, ^{\bullet}\text{H}_2\text{O}$. After the solution is clear and the salt crystals have dissolved, add $100 \, ^{\circ}\text{M}$ methanol. Refrigerate the solution at $2 - 8 \, ^{\circ}\text{C}$ for 7 days.

- b. Osmotic breakdown: To 1 mL of whole blood add internal standard and 4 mL of distilled water. Mix/vortex and let stand for 5 minutes. Centrifuge at 670 g for 10 minutes and discard the pellet. Adjust the pH of the supernatant accordingly with the addition of a buffer solution
- **c. Sonication:** Add 3-6 mL of appropriate pH buffer (such as potassium phosphate) to 1 mL of whole blood and sonicate for 15 minutes at room temperature.

Urine

Enzymatic hydrolysis is necessary in case of conjugated forms (sulfated or glucuronide) of the analye present. Enzymatic hydrolysis requires specific pH (pH 4-5) and temperature ranges. An acid or base hydrolysis can be performed as well, depending on the stability of the compound.

a. Enzymatic hydrolysis: To 500 μL sample (spiked with analyte and internal standard) add 100 μL acidic buffer (see below) and 100 μL β-glucuronidase. Vortex 5-6 seconds. Incubate in a water bath at 63 °C for 30 minutes. Transfer sample to a 96-well collection plate or autosampler vial. Seal and centrifuge for 10 minutes at 2,000 rpm.

Preparation of acidic buffer (1.0 M acetate buffer, pH 4.0): Dissolve 3.0 g of glacial acetic acid and 4.1 g of sodium acetate in a 1L volumetric flask.

- b. Base hydrolysis: To 1 mL urine (spiked with analyte and internal standard) add 100 μL 10 N KOH. Mix, vortex, and hydrolyze for 20 minutes at 60 °C. Cool and adjust pH to 3.5- 4.0 (by adding 200 μL glacial acetic acid).
- c. Acid hydrolysis: To 1 mL urine add 0.25 mL HCl in a screw capped test tube. Screw the tube top on loosely and heat in a boiling water bath for 60 minutes. Adjust to pH 7 (or as needed) with 1.0 N NaOH.

Saliva

No hydrolysis is required for oral fluids and the generic protocol used for plasma/serum pretreatment may be followed.

Tissue

Homogenize with organic or aqueous solvent depending upon analyte solubility. Settle, decant, centrifuge or filter supernatant. Perform direct Matrix Solid Phase Dispersion (MSPD) extraction on tissue.



Getting Started (cont'd)



Find the Correct Volumes

Sample Matrix	Strata-X PRO Sorbent Mass	Sample Size
Blood, serum, plasma	30 mg	250 µL
Urine	30 mg	1 mL
Filtered tissue homogenates	60 mg	100 mg
Oral Fluid	30 mg	500 μL
Environmental Samples	Strata-X PRO Sorbent Mass	Sample Size
Water (particulate-free) drinking	500 mg	100 - 400 mL
Water (particulate-laden) rivers, runoff, etc.	500 mg	100 - 400 mL
Soil extracts	500 mg	100 g

Recommended Sorbent Was	h and Elution Volumes	
Strata-X PRO Sorbent Mass	Practical Minimum Wash and Elution Volume	Recommended Wash and Elution Volumes
	4 bed volumes	8 bed volumes
10 mg	100 µL	200 µL
30 mg	300 µL	600 µL
60 mg	600 µL	1.2 mL
100 mg	1 mL	2 mL
200 mg	2 mL	4 mL
500 mg	5 mL	10 mL



Determine Your Method



2-Step Protocol

Non-retentive SPE method to help achieve the fastest extraction.



3-Step Protocol

Rapid protocol to reduce matrix effects and increase recovery of polar analytes.



Load

1 mL Pre-treated sample/0.1 % Formic acid in Acetonitrile (1:4) Apply 5" Hg vacuum until all tubes or wells have cleared



75 µL Water/0.1 % Formic acid in Acetonitrile (1:4)

Apply 5" Hg vacuum until all tubes or wells have cleared

Protocols are written for $30\,\text{mg}/1\,\text{mL}$ tubes, adjust based on sorbent size.



Load

500 µL Pre-treated sample/buffer* (1:1)

Apply 2-5" Hg vacuum until liquid is no longer visible above top frit

Wash

600 µL 5 % Methanol in Water

Elute

600 µL 0.1 % Formic acid in Acetonitrile/Methanol (90:10) Apply 2-5" Hg vacuum for 1 minute

Protocols are written for 30 mg/1 mL tubes, adjust based on sorbent size.

Select a buffer that maximizes the hydrophobicity of the analytes. For example, if an analyte is basic, dilute with a base.



Cleaner Extractions

Strata-X PRO uses cutting-edge technology to reduce traditional SPE protocol time and still results in high recoveries of the analytes. Its broad range of target applications makes it a great selection for labs that need a clean, fast, and efficient sample preparation method.

SPE Protocol

96-Well Plate: Strata-X PRO, 30 mg/well

Part No.: 8E-S536-TGA

Load: 400 µL Plasma/0.1 % Formic acid in Water (1:1)

Wash: 1 mL 5 % Methanol in Water

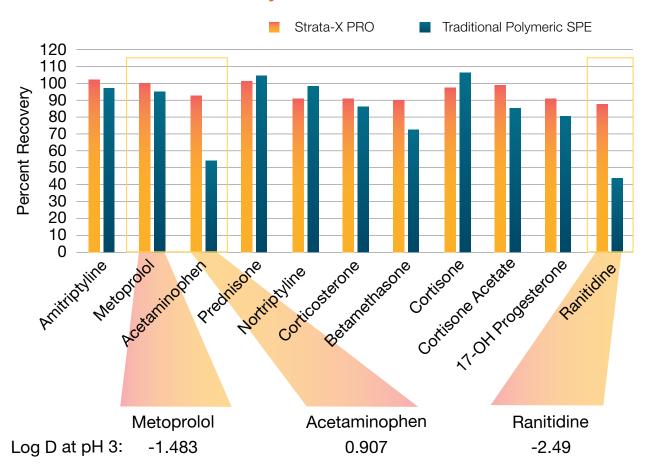
Dry: 1 minute at 5" Hg

Elute: 1 mL 0.1 % Formic acid in Acetonitrile/Methanol (90:10)

Dry Down: 1 minute at 5" Hg

Reconstitute: 200 µL 5 % Methanol in Water

Recovery from Human Plasma



For extremely polar analytes, Strata-X PRO provides higher recoveries!



Ultra-Quick Clean-Up

Strata-X PRO accomplishes what was unimaginable. Its 3- and 2- rapid clean-up system reduces protocol time by 40 %. When working with milk as a matrix, phospholipids from milk fat must be removed to reduce any ion suppression that could occur during LC-MS/MS analysis for veterinary drugs. This ultra-quick SPE works for complex matrices and applications from a variety of industries: Forensics/Toxicology, Environmental, Clinical, Bio/Pharma, and Food Safety and Quality.

SPE Protocol

Pre-treatment

To 1 mL of milk add 3 mL of 0.2% Formic acid in Acetonitrile/Methanol (90:10) and mix or vortex for 15-20 seconds. Centrifuge for 5 minutes at 10,000 RPM and collect supernatant.

Cartridge: Strata-X PRO, 60 mg/3 mL

Part No.: 8B-S536-UBJ

Load: Pass the pre-treated sample through the SPE cartridge and collect

Dry: Evaporate the extract to dryness under a gentle stream of nitrogen at room temperature **Reconstitute:** The dried sample in 1 mL of initial mobile phase (0.1 % Formic acid in Water/0.1 %

Formic acid in Methanol (95:5)) spiked with deuterated internal standard.

% Recovery and % CVs for Veterinary Drugs from Milk Using Strata-X PRO

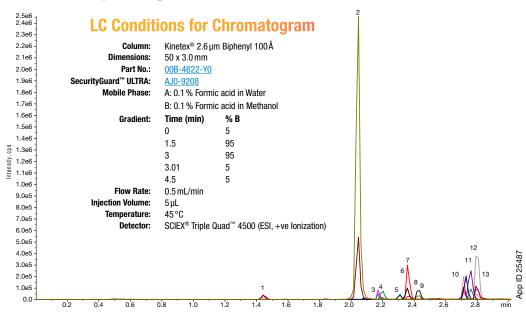
Peak No.	Analyte Name	Retention Time (min)	% Recovery	% CV	Q1	Q3
1	Sulfaguanidine	1.48	46	5	215	156.1
2	Lincomycin	2.07	92	5	407.1	126
3	Sulfadiazine	2.19	38	7	251	156
4	Cephapirin	2.22	76	7	424	292.1
5	Sulfamerazine	2.32	44	5	265.1	155.8
6	Sulfamethoxazole	2.36	53	13	254.1	156.1
7	Sulfamethizole	2.36	45	8	271.1	92
8	Cefalexin	2.39	66	4	348.2	174.2
9	Sulfamethazine	2.44	59	13	279.1	186.1
10	Cortisone	2.72	83	8	361.2	163.2
11	Cortisol	2.73	95	6	363.4	120.9
12	β-methasone	2.76	97	3	393.4	355.2
13	Prednisolone	2.81	92	10	361.2	147.2

So good you'll think it's made up.



Ultra-Quick Clean-Up (cont'd)

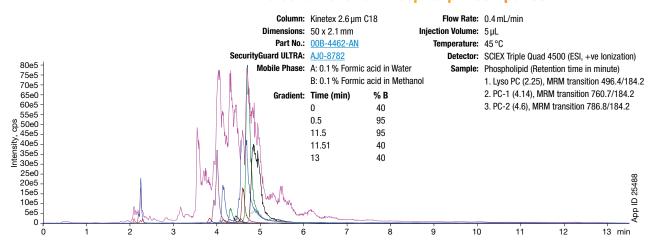
Veterinary Drugs from Milk



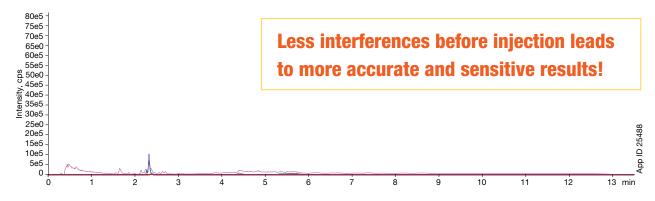
Comparative Phospholipid Trace of Clean-Up Methods

Phospholipid Trace After Protein Preciptation

LC Conditions for Phospholipid Comparison



Phospholipid Trace After Strata®-X PRO Extraction





Additional Applications

Strata-X Pro



Strata-X PRO Solid Phase Extraction User Guide

<u>Utilizing a Rapid, Two-Step Method for the Clean-Up of Veterinary Drugs in Milk Using Strata®-X PRO Solid Phase Extraction (SPE)</u>

<u>Comparison of Tradition Solid Phase Extraction (SPE) and Strata®-X PRO SPE for the Extraction of Acids, Neutrals, and Bases from Plasma</u>

A Faster SPE Method for Pain Relievers Using Strata-X PRO from Plasma

High Recovery of Peptides Using Strata-X PRO Solid Phase Extraction

Fast Extraction of Barbiturates from Serum Using Strata-X PRO Solid Phase Extraction

SPE Guides and Resources



Solid Phase Extraction for Clinical Research

Solid Phase Extraction for Food Samples

Clinical Resources for Toxicology Analysis

SPE Applications



A Fast and Effective Quantitation Method for Uracil, 5,6-Dihydrouracil, and 5-Fluorouracil from Human Serum by LC-MS/MS

A Rapid and Robust Sample Preparation Method for Quantitation of Nicotine from Oral Fluid

Download Applications at phenomenex.com/StrataXPRO



Ordering Information

Strata®-X PRO SPE

Format	Sorbent Mass	Part Number	
Tube			
	10 mg	8B-S536-AAK	1 mL (100/box)
STRATAN 2	30 mg	8B-S536-TAK	1 mL (100/box)
	30 mg	8L-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
SITAL STATE OF THE	60 mg/well	8E-S536-UGA	ea
96-Well Microelution	ı Plate		
strate manimum	2 mg/well	8M-S536-4GA	ea

Round Well Collection Plates (polypropylene)

Part No.	Well Bottom	Well Volume	Unit	Suggested Sealing Mats
AH1-7025	Round	1 mL	50/pk	AH0-8631 AH0-8632
<u>AH0-8636</u>	Round	2 mL	50/pk	AH0-8633 AH0-8634

Square Well Collection Plates (nolypropylene)

•	oquare wen concentral rates (polypropyrene)					
	Part No.	Well Bottom	Well Volume	Unit	Suggested Sealing Mats	
	AH0-7192	Conical	350 µL	50/pk	AH0-8597 AH0-8598 AH0-8199 AH0-7195	
	AH0-7193	Conical	1 mL	50/pk	AH0-8597 AH0-8598 AH0-8199 AH0-7195	
	AH0-7194	Conical	2 mL	50/pk	AH0-8597 AH0-8598 AH0-8199 AH0-7195	



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www.phenomenex.com/behappy

Round Well Collection Plate (Low Bind)

Part No.	Well Bottom			Suggested Sealing Mats
<u>AH1-7036</u>	Conical	2 mL	120/pk	<u>AH0-8633</u> <u>AH0-8634</u>

Round Well Sealing Mats

Part No.			
AH0-8631	Pierceable, 7 mm diameter	Silicone	50/pk
AH0-8632	Pre-Slit, 7 mm diameter	Silicone	50/pk
AH0-8633	Pierceable, 8 mm diameter	Silicone	50/pk
AH0-8634	Pre-Slit, 8 mm diameter	Silicone	50/pk
AH0-7362	Sealing Tap Pad	_	10/pk

Square Well Sealing Mats

	Part No.			
	AH0-8597	Pierceable	Silicone	50/pk
	AH0-8598	Pre-Slit	Silicone	50/pk
	AH0-8199	Pierceable	Santoprene™	100/pk
	AH0-7195	Pierceable	Ethylene Vinyl Acetate (EVA)	50/pk
	AH0-7362	Sealing Tap Pad	_	10/pk

Redefine Your Solid Phase Extraction with Strata-X PRO!



Ordering Information (cont'd)

Sample Processing

Streamline your 96-well plate processing for easier sample preparation with a pneumatic positive pressure manifold.

Pneumatic Handling

Consistent Flow Rates

Safe and Easy-to-Use



Do More with Presston™ 1000

Safer Lab Environment

Use both hands to move manifold shield, ensuring enhanced safety.

Determine Your Operating Pressure

Conveniently monitor and maintain a consistent pressure.

3 Easily Load Samples

Moveable locator plate makes sample loading and cleaning easy.

Never Lose Pressure

Always maintain a tight seal between the manifold and 96-well plate.

Sleek, Low Profile Design

Width: 11", Depth: 15", Height: 14.8"

Know Your Step

Simply move the "SPE Procedure Indicator" to the correct step to stay on track of your extraction.

Presston™ 1000 Positive Pressure Manifold

AH1-7033

Presston 1000 Positive Pressure Manifold, 96-Well Plate



Phenomenex warrants the Presston 1000 Positive Pressure Manifold against 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

Your solution to increased productivity.

Learn more at www.phenomenex.com/Presston

Expand Your Solid Phase Extraction Portfolio

3 Unique Sorbent Platforms



Underline reversed phase polymer with matrix removal technology offers a faster, cleaner way to perform SP.







Polymeric sorbent available in reversed phase and ion-exchange capabilities for wide range of applications.

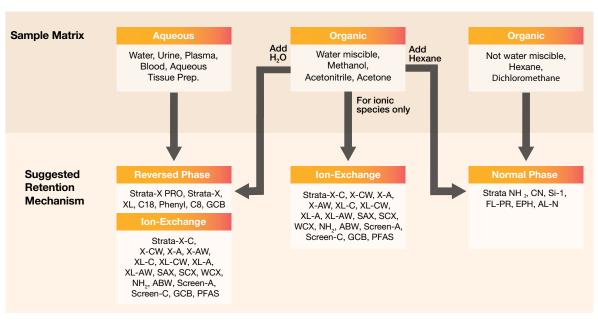




Silica-based SPE sorbent provides a reliable and clean extracts with high recoveries for target analytes across all sample matrices.



Identify Your SPE Retention Mechanism





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