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for choosing Strata-X PRO Solid Phase Extraction (SPE) Products

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STRATA-X PRO

A Rapid Solid Phase Extraction Solution

User Guide

phenomenex®
breaking with tradition™

Revision: 1

PHEN-RUO-00082

STRATA-X PRO
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1 Prepare Your Sample

Plasma/Serum

If the analyte of interest is an acid, 2 % phosphoric acid can be used (20 µL 85 % H_3PO_4 to 1 mL of plasma (or serum) to disrupt the drug-protein interaction. If the analyte of interest is basic, 0.1 M 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 g $ZnSO_4$ 7 • H_2O. After the solution is clear and the salt crystals have dissolved, add 100 % methanol. Refrigerate the solution at 2-8 °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, pH4.0): Dissolve 3.0 g of glacial acetic acid and 4.1 g of sodium acetate in a 1 L 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.

2 Find the Correct Volumes

Sample Matrix	Sorbent Mass	Strata-X PRO
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	**Sorbent Mass**	**Strata-X PRO**
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

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Recommended Sorbent Wash and Elution Volumes

Strata-X PRO Sorbent Mass	Practical Minimum Wash and Elution Volume 4 bed volumes	Recommended Wash and Elution 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

3 Determine Your Method

2-Step Protocol

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

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.

Elute
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 mg/1 mL tubes, adjust based on sorbent size.

3-Step Protocol

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

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 the analyte is basic, dilute with a base.

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.

Accessories

Part No.	Description	Unit
Collection Plates (deep well, polypropylene)		
AH0-7192	96-Well Collection Plate, 350 µL/well	50/pk
AH0-7193	96-Well Collection Plate, 1 mL/well	50/pk
AH0-7279	96-Well Collection Plate, 1 mL/well Round, 7 mm	50/pk
AH0-7194	96-Well Collection Plate, 2 mL/well	50/pk
AH0-8635	96-Well Collection Plate, 2 mL Square/Round-Conical	50/pk
AH0-8636	96-Well Collection Plate, 2 mL Round/Round, 8 mm	50/pk
AH1-7036	96-Well Collection Plate, 2 mL Low Protein Binding	120/pk

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