Simplify Your SPE

SPE Method Solution Guide

SOLID PHASE EXTRACTION

phenomenex®
. . . breaking with tradition℠

Polymeric Sorbents

A Simplified Approach to Solid Phase Extraction (SPE)

Most traditional solid phase extraction sorbents on the market are just variations of the same technology, and often result in poor analyte recoveries, insufficient cleanup, or irreproducibility from extraction to extraction. With this in mind, Phenomenex set out to develop a SPE sorbent that exceeded the limitations of traditional SPE sorbents and offered increased speed, simplicity, and reproducibility for sample preparation. Utilizing a controlled manufacturing process and rigorous performance specifications we are pleased to bring you strata-X polymeric sorbents. A line of polymeric SPE sorbents that allows for a simplified approach to solid phase extraction.

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guarantee

If Strata SPE products do not perform as well or better than your current SPE product of similar phase, mass and size, send in your comparative data within 45 days and keep the Strata SPE products for FREE!

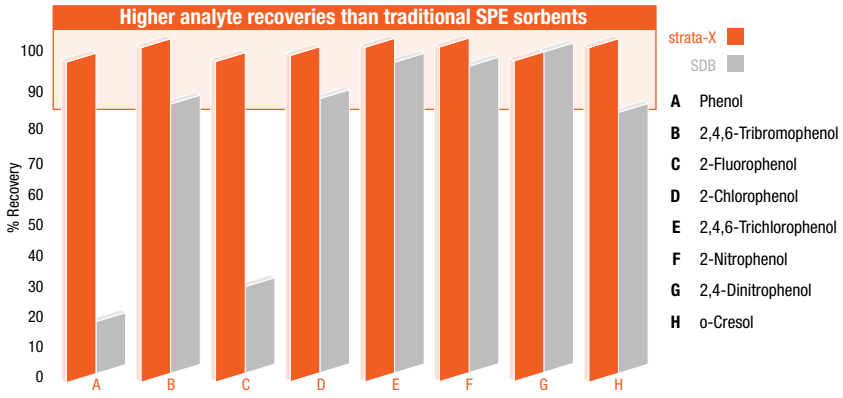
STRATA™-X

Polymeric Based Particles

Benefits to your SPE Methods

- Provides reproducible recoveries from extraction-to-extraction
- Allows for simple method development and sample processing
- Removes contaminants and eliminates matrix effects
- Offers controlled selectivity for target analytes
- Results in streamlined sample preparation

TN-001: Phenols from Water



SPE Conditions

Sorbent: strata-X 500 mg/6 mL;
Strata SDB-L 500 mg/6 mL

Part No.: 8B-S100-HCH
8B-S014-HCH

Condition: 5 mL methanol

Equilibrate: 5 mL water

Load: 500 mL of sample
(loaded in a continuous fashion)

Elute 5 mL acetone, followed by 5 mL
methylene chloride

Drying: Extracts were dried using anhydrous sodium
sulfate concentrated to 0.5 mL

GC Column: Zebron™ ZB-5

Dimension: 30 m x 0.25 mm x 0.25 µm

Part No.: 7HG-G002-11

Injection: 1µL at 250 ˚C in 11:1 split ratio

Carrier Gas: Helium

Oven Program: 40 ˚C to 325 ˚C, at 9 ˚C/min

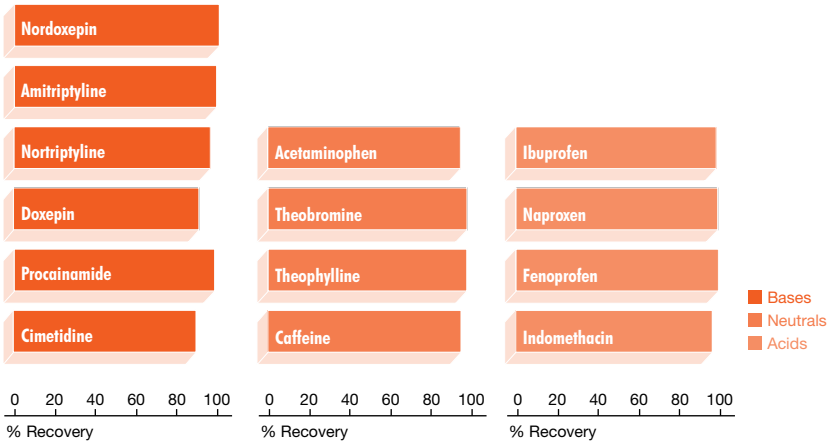
Detention: MS in selective ion mode

Polymeric Based Particles

Benefits to your SPE Methods

- De-conditioning resistant surface resists dry out and de-activation and provides worry-free manual and automated processing
- pH stability from 1-14 for flexible method development
- 800 m^2/g surface area and high analyte capacity results in the ability to load up to 66 % more in comparison to silica-based sorbents per gram
- Grafted polymeric surface offers even distribution of functional groups ensuring superior lot-to-lot reproducibility

High Recoveries of Acidic, Basic, and Neutral Compounds



Conditions

Method: Analytes spiked in porcine serum at concentration of 0.5 or 1.0 µg/mL. The strata-X method was used. Contact Phenomenex for more complete method details.

Selectivity

The strata™-X Phases

Polymeric Selectivities Designed to Meet your Separation Needs

With strata-X polymeric sorbents the guesswork that leads to lengthy method development and complications of screening multiple SPE sorbents is eliminated. A suite of unique selectivities have been developed to cover a diverse spectrum of analytes and simplify the method development process for fast and efficient sample preparation.

Selectivity

The strata™-X Phases

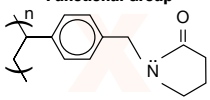
Polymeric Selectivities Designed to Meet your Separation Needs

strata-X

Mode: Reversed Phase

Analyte: Polar and Non-polar

Functional Group

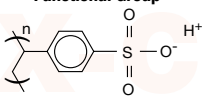


strata-X-C

Mode: Strong Cation Exchange and Reversed Phase

Analyte: Bases

Functional Group

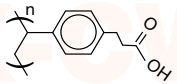


strata-X-CW

Mode: Weak Cation Exchange and Reversed Phase

Analyte: Bases *(including Quaternary Amines)*

Functional Group

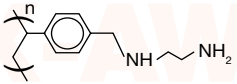


strata-X-AW

Mode: Weak Anion Exchange and Reversed Phase

Analyte: Acids

Functional Group

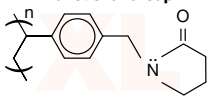


strata-XL

Mode: Reversed Phase

Analyte: Polar and Non-polar

Functional Group

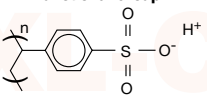


strata-XL-C

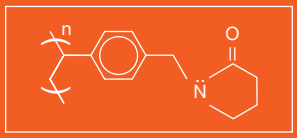
Mode: Strong Cation Exchange and Reversed Phase

Analyte: Bases

Functional Group



Selectivity: strata™-X
Reversed Phase



Reversed Phase Selectivity Without Limitations

Standard reversed phase SPE sorbents such as C18 and C8 retain analytes strictly by hydrophobic interactions. The strata-X reversed phase sorbent retains analytes by hydrophobic interaction and has H- and π-π bonding capabilities for enhanced retention of polar and aromatic analytes. The enhanced retention mechanism of strata-X can be very powerful for applications that require simultaneous extraction of polar analytes (log P<3) and non-polar parent compounds (log P>3). The strong retention mechanisms of strata-X allow for aggressive washing with organic solvents to remove impurities without breakthrough of analyte. strata-X offers reversed phase selectivity without the limitations such as improper cleanup, breakthrough, pH considerations, and method development concerns.

Material Characteristic

Particle size (µm)	33
Pore Size (Å)	85
Surface Area (m^2/g)	800
pH Stability	1-14

Selectivity: strata™-X

Reversed Phase

- Enhanced retention for polar and aromatic compounds
- Strong π-π retention mechanism allows for aggressive organic wash (>5 % organic) without breakthrough of analyte
- Effectively retains hydrophobic contaminants

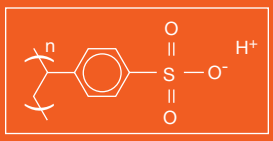
Polar and Non-Polar

Condition:	with methanol
Equilibrate:	with water
Load Sample:	Phosphoric acid (2 % of total volume) can be used to disrupt drug and protein interaction
Wash:	with 5-60 % methanol in water (organic concentration used in wash can vary based on nature of analyte)
Dry:	1 minute
Elute Analyte:	with methanol/acetonitrile (50:50)

See page 37 for recommended solvent volumes

Selectivity: strata™-X-C

Strong Cation Exchange and Reversed Phase



Cleaner Reproducible Extractions

Unlike traditional silica-based mixed mode sorbents that are blended, strata-X-C has a strong cation exchange group uniformly bonded on the polymeric surface completely eliminating recovery or reproducibility problems. The strong cation exchange mechanism gives consistent and extremely clean extracts from biological matrices such as plasma and urine since hydrophobic contaminants can be completely removed by using 100 % organic wash solvents.

Material Characteristic

Particle size (µm)	33
Pore Size (Å)	85
Surface Area (m^2/g)	800
pH Stability	1-14
Ionic Capacity	1 meq/g

Selectivity: strata™-X-C

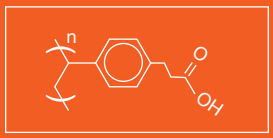
Strong Cation Exchange and Reversed Phase

- Strong cation exchange mechanism results in strong retention for basic compounds
- Complete elimination of matrix contaminants resulting in better LODs/LOQs
- Multimode retention consists of strong cation exchange (primary interaction) and π-π retention for reversed phase (secondary interaction)
- Fractionation of acidic, basic and neutral analytes in a mix

Bases

Condition:	with methanol
Equilibrate:	with water
Load Sample:	Acidified with 2 % Phosphoric acid
Wash 1:	with 0.1 N HCl
Wash 2:	*with methanol (elutes acidic and neutral analytes).
Elute Analyte:	with 5 % ammonium hydroxide/methanol† or acetonitrile

† For very hydrophobic compounds methanol can be substituted with methylene chloride and/or isopropanol.
* This helps in removing endogenous contaminants from biological sample matrices and eliminates ion suppression in LC/MS under ESI mode.



Selectivity: strata™-X-CW

Weak Cation Exchange and Reversed Phase

Cleanup of Bases (including Quaternary Amines)

Weak cation exchange functionality of strata-X-CW allows for cleanup of strong bases such as quaternary amines that are irreversibly retained on strong cation exchangers. The pK_a of the strata-X-CW sorbent is more versatile in comparison to strong cation exchangers, giving a wider range of flexibility for elution conditions. Also strata-X-CW allows for elution under acidic conditions with LC/MS compatible buffer. This eliminates the dry down step resulting in faster analysis.

Material Characteristic

Particle size (µm)	33
Pore Size (Å)	85
Surface Area (m^2/g)	800
pH Stability	1-14
Ionic Capacity	0.76 meq/g

Selectivity: strata™-X-CW

Weak Cation Exchange and Reversed Phase

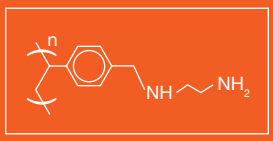
- Weak cation exchange mechanism gives selectivity for weak and strong bases
- Complete elimination of matrix contaminants resulting in better LODs/LOQs
- Weak cation exchange mechanism allows for elution at high and low pH
- Elution with LC/MS compatible buffer eliminates the dry down step resulting in faster analysis

Bases (including Quaternary Amines)

Condition:	with methanol
Equilibrate:	with water
Load Sample:	pH of sample should be between 4.5-7.0**
Wash 1:	with 25 mM ammonium acetate buffer (pH 6.5)
Wash 2:	with methanol (elutes acidic and neutral analytes). *This serves to remove endogenous contaminants from biological sample matrices and eliminates ion suppression.*
Dry:	1 minute
Elute Analyte:	with 2 % formic acid in methanol/acetonitrile (20:80)‡

** Samples may need to be acidified to disrupt drug-protein interaction. Also sample should be diluted 1:1 with buffer or water ensuring that sample is two pH units below pK_a of analyte and two pH units above the pK_a of the sorbent.

‡Depending on analyte solubility and hydrophobicity isopropanol/methylene chloride (20:80) can be substituted for methanol/acetonitrile



Selectivity: strata™-X-AW

Weak Anion Exchange and Reversed Phase

Selective Elution of Acidic Analytes

The weak anion exchange functionality of strata-X-AW allows for cleanup of strong acids such as sulfonates that are irreversibly retained on strong anion exchangers. The pK_a of the strata-X-AW sorbent is more versatile in comparison to strong anion exchangers, giving a wider range of flexibility. Also, strata-X-AW sorbent allows for the selective elution of acidic compounds under acidic conditions or basic conditions for direct inject analysis onto a MS.

Material Characteristic

Particle size (µm)	33
Pore Size (Å)	85
Surface Area (m^2/g)	800
pH Stability	1-14
Ionic Capacity	0.60 meq/g

Selectivity: strata™-X-AW

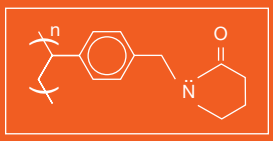
Weak Anion Exchange and Reversed Phase

- Weak anion exchange mechanism gives selectivity for weak and strong acids
- Complete elimination of matrix contaminants resulting in better LODs/LOQs
- Weak anion exchange mechanism allows for elution at high and low pH
- Elution with LC/MS compatible buffer eliminates the dry down step resulting in faster analysis

Acids

Condition:	with methanol
Equilibrate:	with water
Load Sample:	pH of sample should be two pH units above the pK_a of the acidic analyte to ensure that the analyte is fully deprotonated
Wash 1:	with water
Wash 2:	with methanol (elutes basic and neutral analytes)
Elute Analyte:	with 2 % NH_4OH in methanol/acetonitrile

Modified conditioning step(s) such as acidifying the methanol (2 % formic) may be needed to improve selectivity.



Selectivity: strata™-XL

Large Particle Reversed Phase Extraction

Large Particle for Viscous Samples with the Selectivity of strata-X

strata-XL is a high capacity large particle designed to be used for viscous samples which tend to clog on standard particle SPE sorbents. This SPE sorbent is ideal for applications in which cleanup is required from difficult sample matrices such as grains, fruits, vegetables, meats, and tissues. In addition strata-XL also has a 300 Å pore size. This large pore size can be especially useful for applications that require the cleanup or concentration of large biomolecules such as proteins.

Material Characteristic

Particle size (µm)	100
Pore Size (Å)	300
Surface Area (m^2/g)	520
pH Stability	1-14

Selectivity: strata™-XL

Large Particle Reversed Phase Extraction

- Provides excellent flow for viscous biological fluids such as horse urine and plasma*
- Suited for applications that require cleanup from tissue and food matrices
- Consistent tube-to-tube flow

Polar and Non-Polar

Condition: with methanol

Equilibrate: with water

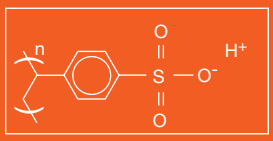
Load Sample: Phosphoric acid (2 % of total volume) can be used to disrupt drug and protein interaction

Wash: with 5-60 % methanol in water (organic concentration used in wash can vary based on nature of analyte)

Dry: 1 minute

Elute Analyte: with methanol/acetonitrile (50:50)

*To control flow rate with strata-XL, use a stopcock (AH0-6048) when processing samples with a vacuum manifold.



Selectivity: strata™-XL-C

Large Particle Strong Cation Exchange Extraction

Large Particle for Viscous Samples with the Selectivity of strata-X-C

strata-XL-C is a high capacity large particle designed to be used for viscous samples which tend to clog on standard particle SPE sorbents. This SPE sorbent is ideal for applications in which basic drugs need to be cleaned up from difficult sample matrices such as tissue, whole blood, or horse urine. In addition, strata-XL-C also has a 300 Å pore size. This large pore size can be especially useful for applications that require the cleanup or concentration of large biomolecules such as proteins.

Material Characteristic

Particle size (µm)	100
Pore Size (Å)	300
Surface Area (m^2/g)	520
pH Stability	1-14
Ionic Capacity	1 meq/g

Selectivity: strata™-XL-C

Large Particle Strong Cation Exchange Extraction

- Provides excellent flow for viscous biological fluids such as horse urine and plasma
- Suited for applications that require cleanup from tissue and food matrices
- High recoveries for basic drugs

Bases

Condition:	with methanol
Equilibrate:	with water
Load Sample:	Acidified with 2 % Phosphoric acid
Wash 1:	with 0.1 N HCl
Wash 2:	*with methanol (elutes acidic and neutral analytes).
Elute Analyte:	with 5 % ammonium hydroxide/methanol† or acetonitrile

† For very hydrophobic compounds methanol can be substituted with methylene chloride and/or isopropanol.
* This helps in removing endogenous contaminants from biological sample matrices and eliminates ion suppression in LC/MS under ESI mode.

Formats:
Traditional Screening
Ordering Information*

1 mL (100/box)

Phase	30 mg
strata-X	8B-S100-TAK
strata-X-C	8B-S029-TAK
strata-X-CW	8B-S035-TAK
strata-X-AW	8B-S038-TAK

3 mL (50/box)

Phase	60 mg	200 mg	500 mg
strata-X	8B-S100-UBJ	8B-S100-FBJ	8B-S100-HBJ
strata-X-C	8B-S029-UBJ	8B-S029-FBJ	8B-S029-HBJ
strata-X-CW	8B-S035-UBJ	8B-S035-FBJ	8B-S035-HBJ
strata-X-AW	8B-S038-UBJ	8B-S038-FBJ	8B-S038-HBJ

6 mL (30/box)

Phase	100 mg	200 mg	500 mg
strata-X	8B-S100-ECH	8B-S100-FCH	8B-S100-HCH
strata-X-C	8B-S029-ECH	8B-S029-FCH	8B-S029-HCH
strata-X-CW	8B-S035-ECH	8B-S035-FCH	8B-S035-HCH
strata-X-AW	8B-S038-ECH	8B-S038-FCH	8B-S038-HCH

*Contact Phenomenex about tabless or Teflon® coated tubes

Formats:

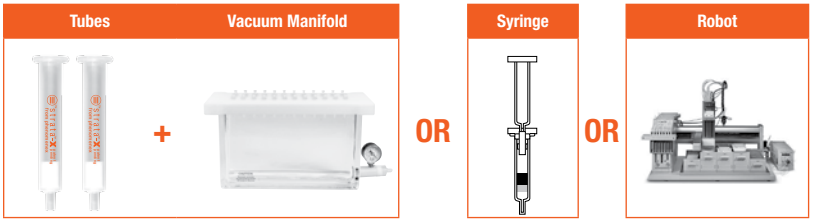
1, 3, and 6 mL Polypropylene Tubes

Traditional Screening

- Compatible with automated liquid handling systems, 12- and 24-position vacuum manifolds, and syringes

Tubes

Requires 12- or 24-position vacuum manifold, or syringe and adaptor cap, or robot



Formats:
High-Throughput Screening
Ordering Information

High-Throughput 96-Well Plates (2/box)

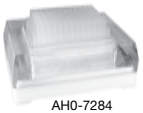
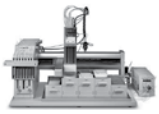
Phase	10 mg	30 mg	60 mg
strata-X	8E-S100-AGB	8E-S100-TGB	8E-S100-UGB
strata-X-C	8E-S029-AGB	8E-S029-TGB	8E-S029-UGB
strata-X-CW	8E-S035-AGB	8E-S035-TGB	8E-S035-UGB
strata-X-AW	8E-S038-AGB	8E-S038-TGB	8E-S038-UGB

Formats:
96-Well Plates
High-Throughput Screening

- Compatible with standard 96-well manifolds and automated liquid handling systems
- Up to 2 mL sample volume per well
- Consistent well-to-well flow
- Inert polypropylene housing

96-Well Plates

Requires 96-well plate manifold or robot

96-Well Plate	96-Well Plate Manifold		Robot
	 AH0-7284	OR	

Formats:
Rapid Screening
Ordering Information

On-Line SPE

Part No.	Description	Unit/Box
00M-S033-B0-CB	strata-X on-line extraction cartridge, 20 x 2.0 mm	ea
00M-S036-B0-CB	strata-X-CW on-line extraction cartridge, 20 x 2.0 mm	ea
CH0-5845	Cartridge holder, 20 mm	ea

Formats:
On-Line SPE
Rapid Screening

- 20 x 2.0 mm easy to use cartridge and holder
- Concentrate and cleanup target analytes in minutes
- Compatible with standard HPLC systems and HPLC switching valves
- Removes protein contaminants

On-Line Screening

Requires HPLC system and switch valve

On-Line SPE	2-position, 10-port*		2-position, 6-port*
	+ 	OR	

*Fluid Processors available in PEEK and stainless steel. 10-position also available.

Formats:
Large Volume/Flash Analysis
Ordering Information

strata-X Giga Tubes

	12 mL (20/box)		20 mL (20/box)
Phase	**500 mg**	**1 g**	**1g**
strata-X	8B-S100-HDG	8B-S100-JDG	8B-S100-JEG
strata-X-C	8B-S029-HDG	8B-S029-JDG	—
strata-X-CW	8B-S035-HDG	8B-S035-JDG	8B-S035-JEG
strata-X-AW	8B-S038-HDG	8B-S038-JDG	—

strata-XL Giga Tubes

	20/box			16/box	
Phase	**2 g/12 mL**	**2 g/20 mL**	**5 g/20 mL**	**5 g/60 mL**	**10 g/60 mL**
strata-XL	8B-S043-KDG	8B-S043-KEG	8B-S043-LEG	8B-S043-LFF	8B-S043-MFF
strata-XL-C	8B-S044-KDG	8B-S044-KEG	8B-S044-LEG	8B-S044-LFF	8B-S044-MFF

Formats:
Strata® Giga™ Tubes
Large Volume/Flash Analysis

- 12, 20, 60 and 150 mL* polypropylene tubes
- Luer slip ensures compatibility with 10-position and all standard vacuum manifolds.

Giga Tubes

Requires Giga Tube Manifold

Giga Tubes	Giga Tube Manifold
 +	 AH0-7502

*Contact Phenomenex or your local Phenomenex distributor about availability of 150 mL tubes.

SPE Accessories:
Ordering Information

Vacuum Manifolds*

Part No.	Description	Unit/Box
10-Position Vacuum Manifold		
AH0-7502	Tall-Boy Vacuum Manifold Complete Assembly for Giga Tubes	ea
12-Position Vacuum Manifold		
AH0-6023	Complete Assembly for 1, 3, 6 mL Tubes	ea
24-Position Vacuum Manifold		
AH0-6024	Complete Assembly for 1, 3, 6 mL Tubes	ea

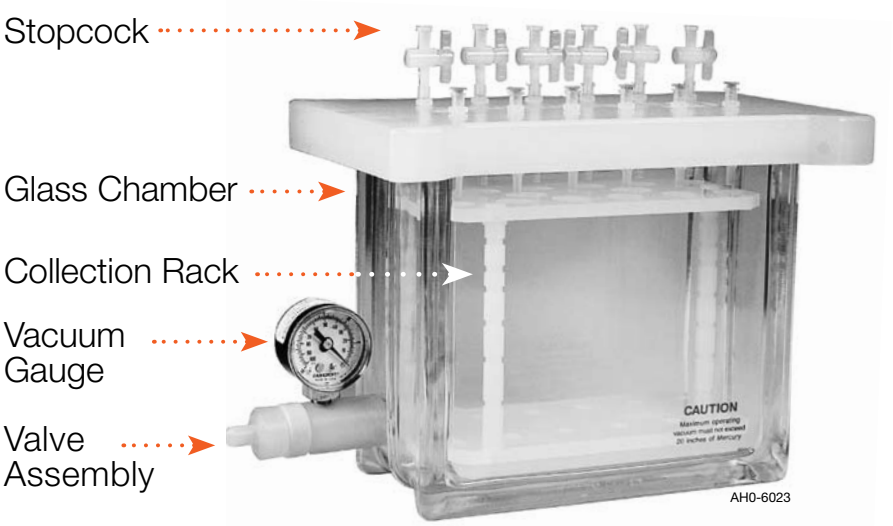
*Replacement parts available, contact your local Phenomenex technical consultant or distributor.

Adaptor Caps

Part No.	Description	Unit/Box
Adaptor Caps for 1, 3 and 6 mL		
AH0-7191	SPE tubes, polyethylene, with Luer tip	15/pk
Adaptor Caps for 12 and 20 mL		
AH0-7378	SPE tubes, polyethylene, with Luer tip	5/pk
Adaptor Caps for 60 mL		
AH0-7379	SPE tubes, polyethylene, with Luer tip	5/pk
Syringe and Adaptor Kit		
AH0-8278	Strata Syringe and Adaptor Kit	ea

SPE Accessories:

10-, 12- and 24-Position Vacuum Manifolds



SPE Accessories:
Ordering Information

96-Well Plate Accessories

Part No.	Description	Unit/Box
Manifold		
AH0-7284	96-Well Plate Manifold, Acrylic	ea
Sealing Mats		
AH0-7195	Pierceable Sealing Mats, 96 Square Well	50/pk
AH0-7362	Sealing Tape Pad	10/pk
Collection Plates		
AH0-7192	0.35 mL/well 96 Square Well Conical V-bottom Collection Plate	50/pk
AH0-7193	1 mL/well 96 Square Well Conical V-bottom Collection Plate	50/pk
AH0-7194	2 mL/well 96 Square Well Conical V-bottom Collection Plate	50/pk
AH0-7279	1 mL/well 96 Square Well Round Bottom Collection Plate	50/pk
AH0-7408	Solvent Waste Reservoir Tray	50/pk

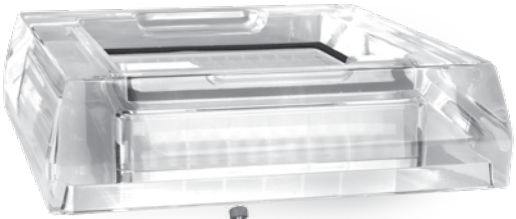
SPE Accessories:

96-Well Plate Accessories

Well Plate

Glass Chamber

Manifold Base Plate

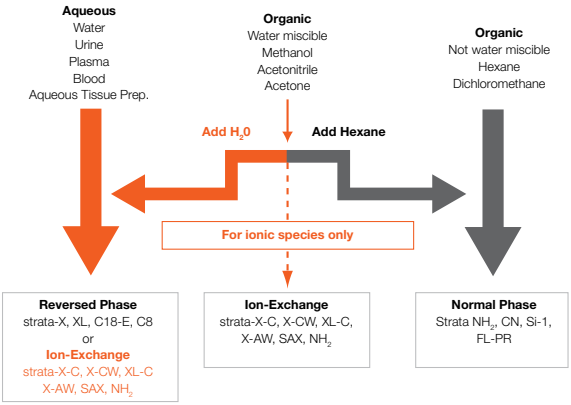


SPE Method Development Tips

Sorbent Selection

Identify the possible SPE retention mechanism:

- Reversed Phase (RP), Ion-Exchange (IEX), or Normal Phase (NP)



Once the general mechanism is identified it will be necessary to identify the most specific strata-X sorbent by matching the analyte functional groups to the sorbent functional groups.

SPE mechanism	Analyte functional group	strata-X sorbent*
Reversed Phase	Hydrocarbon, aromatic	strata-X strata-XL
Ion-Exchange	**Bases** Quaternary amines 1˚,2˚,3˚ amines	 strata-X-CW strata-X-C strata-XL-C
	Acids* Carboxylic acids Sulfonates	 strata-X-AW

* For analytes that contain a carboxylic acid functional group, Strata SAX can be used as an alternative to strata-X-AW.

SPE Method Development Tips

Sample Pre-Treatment

Reproducible, high efficiency solid phase extraction requires that the sample be made liquid prior to loading onto a SPE device. The SPE sample should meet the following conditions:

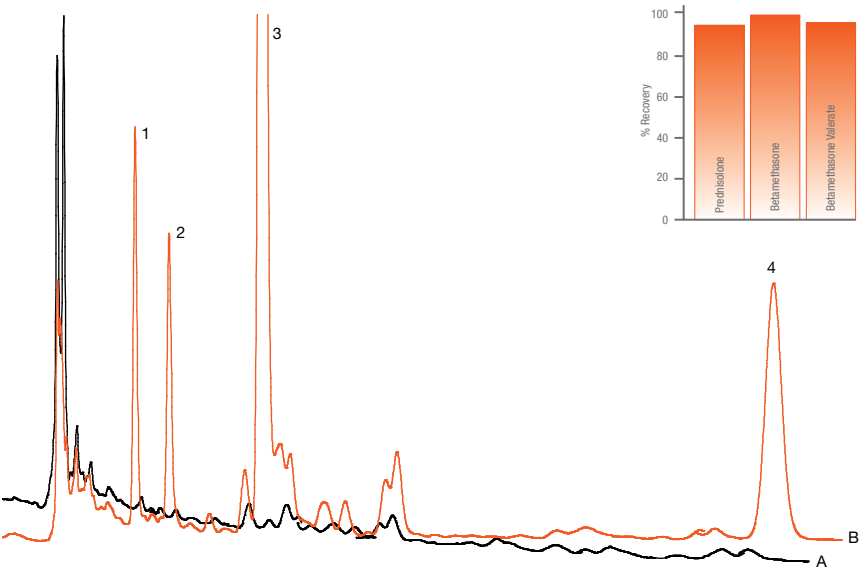
1. Liquid of low viscosity (to pass through the cartridge).
2. Low solids or particulate contaminants (to prevent clogging).
3. Solvent composition that is suitable for retention (each mechanism has different matrix solvent composition requirements for proper retention).

Recommendations

Biological Samples (Liquid)	
Urine, whole blood, serum, plasma, bile, etc.	Dilute sample 1:1 with appropriate buffer, precipitate proteins if proteinaceous ($ZnSO_4$ or ACN), hydrolyze urinary glucuronides, disruption of protein binding (sonication, enzymatic, acids/bases).
Biological Samples (Solid)	
Organ tissues, feces, GI contents	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.
Sample Matrix	
Water (waste, river, etc.)	Buffer to appropriate pH and filter particulates from sample.
Soil, sludge	Homogenize with organic or aqueous solvent depending upon analyte solubility. Settle, decant, and filter supernatant; perform Soxhlet extraction.
Ointments, creams	Oil based: Dissolve in non-polar organic (hexane) and extract via polar SPE. Water based: Dissolve in water or water miscible organic (methanol) and extract via non-polar SPE.
Fruit, vegetable, herbs	Homogenize with organic or aqueous solvent depending upon analyte solubility and filter supernatant. Use appropriate SPE mechanism for the dissolution solvent (hexane = polar mechanism; aqueous = non-polar mechanism; methanol/ACN = either non-polar or polar after proper dilution).

Extraction of Polar Steroids from Plasma

CN-008



SPE Conditions

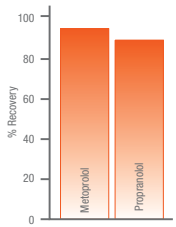
Sorbent: strata-X 30 mg/1 mL
Part No.: 8B-S100-TAK
Condition: 1 mL methanol
Equilibrate: 1 mL water
Load: 1 mL porcine spiked with analyte
Wash: 1 mL 5 % methanol in water
Elute: 1 mL methanol
Evaporate: add 25 µL of 0.1 mg/mL butyl paraben (external standard) dry down under slow stream of N_2 and reconstitute in 200 µL acetonitrile

HPLC Column: Synergi™ Max-RP 4 µm 150 x 4.6 mm SecurityGuard™ C18 4 x 3.0 mm
Part No.: 00F-4337-E0 and AJ0-4287
Sample: 50 µL of reconstituted extract
Mobile Phase: A: 20 mM KH_2PO_4 (pH 7.0); B: methanol
Isocratic: A/B (35:65) for 20 min
Temperature: 25 °C
Detector: UV @ 254 nm
Peaks: 1. Prednisolone (1.0 µg/mL)
2. Betamethasone (1.0 µg/mL)
3. Butyl Paraben (external std)
4. Betamethasone Valerate (4.0 µg/mL)

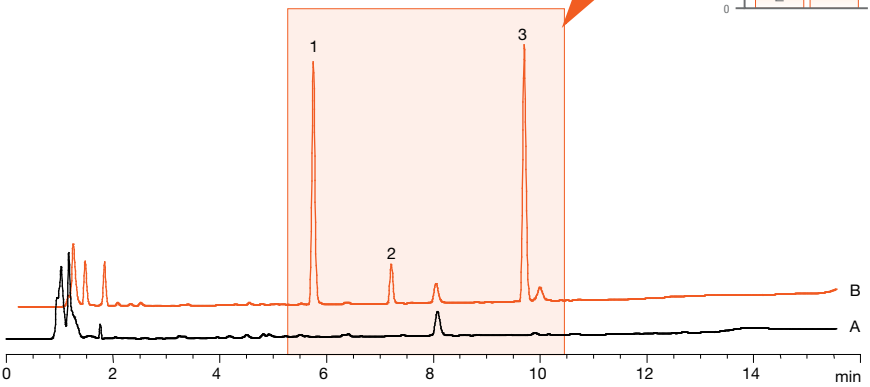
Chromatogram of Extracts:
A) Blank B) Spiked Sample

Extraction of Beta Blockers from Urine

CN-015



100 % organic wash results in clean extracts



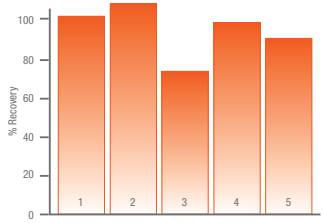
SPE Conditions

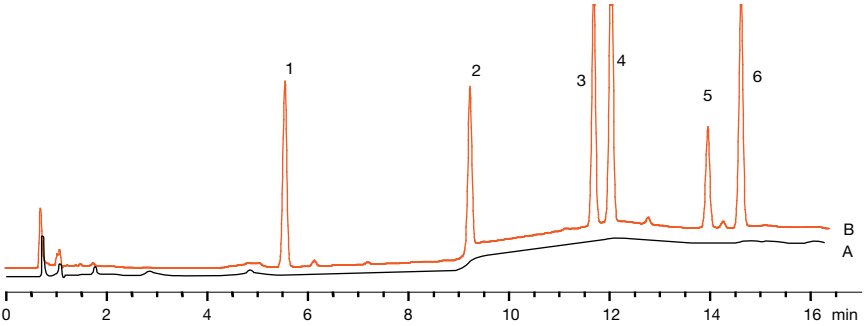
Sorbent: strata-X-C 30 mg/1 mL
Part No.: 8B-S029-TAK
Condition: 1 mL methanol
Equilibrate: 1 mL water
Load: 1 mL urine spiked with analytes; dilute 1:1 with phosphate buffer saline solution pH 7; acidified with 2 % phosphoric acid
Wash 1: 1 mL 0.1 N HCl
Wash 2: 1 mL methanol, Dry for 1 min
Wash 3: 1 mL NH_4OH/methanol/water (2:50:48)
Elute: 1 mL 5 % NH_4OH/methanol
Evaporate: Spike extract with external standard (1 µg/mL); dry down under nitrogen and reconstitute with 200 µL 20 mM KH_2PO_4 (pH 2.5)

HPLC Column: Luna® 5 µm C18(2), 150 x 4.6 mm
Part No.: 00F-4252-E0
Sample: 50 µL of reconstituted extract
Mobile Phase: A: 20 mM KH_2PO_4 (pH 2.5); B: acetonitrile
Gradient: A/B (95:5) to (50:50) in 15 minutes
Flow Rate: 1.5 mL/min
Temperature: 25 °C
Detector: UV @ 210 nm
Peaks: 1. Pindolol (ext. std.),
2. Metoprolol (1.0 µg/mL)
3. Propranolol (1.0 µg/mL)

Chromatogram of Extracts:
A) Blank B) Spiked Sample

Extraction Urea Pesticides from Water
CN-022





SPE Conditions

Sorbent: strata-X 60 mg/3 mL
Part No.: 8B-S100-UBJ
Condition: 2 mL dichloromethane
Equilibrate: 2 mL acetonitrile; 2 mL water
Load: 2 mL spiked tap water
Wash 1: 2 mL water; Dry for 2 min
Wash 2: 1 mL methanol, Dry for 1 min
Wash 3: 1 mL NH_4OH/methanol/water (2:50:48)
Elute: 2 mL of acetonitrile/dichloromethane (1:1) add 20 µg/mL of Linuron (external standard, E.S)
Evaporate: under nitrogen and reconstitute with 200 µL water

HPLC Column: Luna® 5 µm C18(2) 150 x 4.6 mm
Part No.: 00F-4252-E0
Sample: 50 µL of reconstituted water extract
Mobile Phase: A: water; B: acetonitrile
Gradient: A/B (85:15) hold for 2 min to A/B (42:58) in 13 min hold for 2 min
Flow Rate: 2 mL/min
Temperature: 25 °C
Detector: UV @ 214 nm
Peaks: 1. Fenuron (0.5 µg/mL)
2. Monuron (0.5 µg/mL)
3. Forchlorfenuron (0.5 µg/mL)
4. Diuron (0.5 µg/mL)
5. Siduron (0.5 µg/mL)
6. Linuron (E.S.)

Chromatogram of Extracts:
A) Blank B) Spiked Sample

*Method can be scaled up for 1-2 L sample volumes.

SPE Method Development Tips

Sorbent Wash and Elution Volumes

Method and Sorbent Volume Selection

The volume of solvent needed for SPE processing is directly related to the mass of sorbent in the SPE tube and more specifically the “bed volume” of the SPE device. Intuitively we know more sorbent requires more solvent, less sorbent = less solvent. Typically 4 - 16 bed volumes are used in SPE methods.

Polymer- Based Sorbent Mass*	Practical Minimum Wash and Elution Volume 4 bed volumes	Recommended Wash and Elution Volume 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
150 mg	1.5 mL	3 mL
200 mg	2 mL	4 mL
500 mg	5 mL	10 mL
1 g	10 mL	20 mL

* Strata polymeric resins have a larger surface area than Strata silica-based material, hence requiring slightly more solvent per gram for processing. The elution volumes are specific to the chemical nature of the analyte being extracted, its concentration in the sample, the chemical nature of the eluting solvent and the bed mass used. The above is a guideline; an elution study should be conducted to determine the appropriate volume to use.

Suggested flow rate should be 1-3 mL/min or 1-3 drops/sec

SPE Method Development Tips

Sorbent Mass Selection

To select the proper sorbent mass, it is first necessary to determine the volume of sample needed for extraction in order to meet the method detection limits (not including buffer). The following table lists the proper masses that can be used with strata-X polymeric sorbents.

Bio Samples

Plasma/Serum	Urine	Recommended Sorbent Mass
100 µL	200 µL	10 mg
250 µL	500 µL	30 mg
500 µL	1 mL	60 mg
1 mL	2 mL	100 mg

Environmental Samples

Water (particulate free)	Water (particulate-laden)*	Recommended Sorbent Mass
50 mL	25 mL	200 mg
100 mL	50 mL	500 mg
500 mL	100 mL	1 g
1 L	200 mL	2 g

*strata-XL sorbents are recommended for particulate laden samples

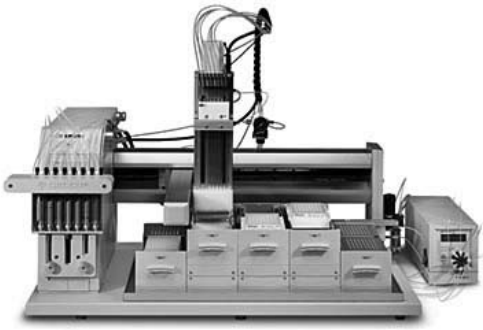
SPE Method Development Tips

Liquid Handling System

1. When performing SPE extraction at the elution step, the height of the collection plate under the SPE plate should be adjusted to be as close as possible to the SPE plate without interfering with the vacuum application, so when the vacuum is applied, the funnels under the SPE plate are inside the collection plate wells without the collection plate coming in direct contact with the SPE plate. The purpose of this is to minimize cross-well-contamination that can easily occur at this step.

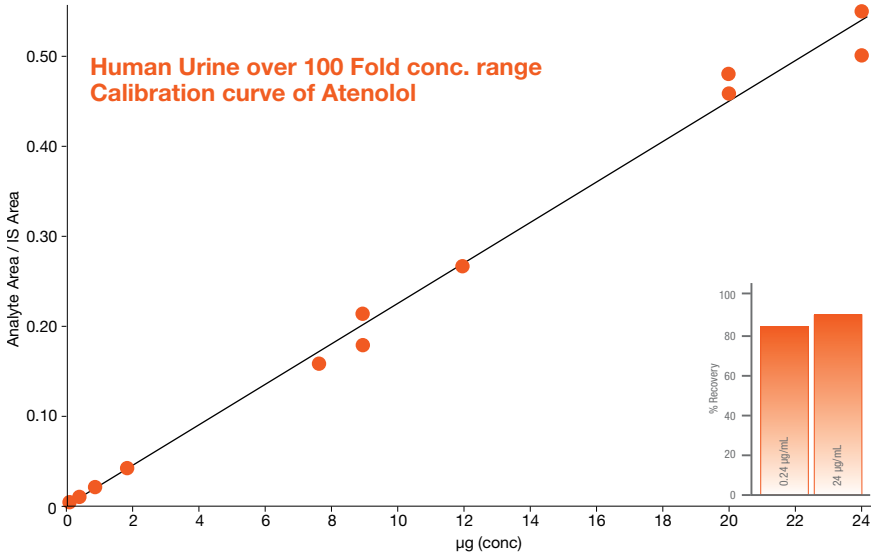
2. Before capping the 96-well plate with a sealing mat, check for any liquid residue near the top of the plate (often observed after an SPE extraction). Use a lab wipe to remove the residue by gently pressing it against the plate surface. This will prevent cross-contamination that may occur by capillary effect between the sealing mat and well plate. The residue itself should not contribute to cross-contamination since it is a wash solution and does not contain any analyte.

3. When using any liquid handler with liquids that may contribute to cross-contamination, it is recommended that the dispense step be programmed so it ends with the tips in contact with the surface of the liquid. This will efficiently wipe the last drop from the disposable tip and prevent the formation of beads or drops on the tip after the liquid has been dispensed. This method is more effective than a tip touch-off on the side of the wall or an air blowout.



strata-X 96-Well Plates

Automated SPE Extraction



SPE Conditions

Sorbent: 30 mg strata-X 96-well plate
Part No.: 8E-S100-TGB
Processing station: PerkinElmer MultiPROBE® II
Condition: 40 µL Methanol
Equilibrate: 400 µL Water
Load: 500 µL urine diluted with 1mL water
Wash 1: 1 mL 5 % methanol in water
Wash 2: 800 µL 30 % Methanol/Water
Elute: 800 µL water
Elution: 400 µL methanol (in two aliquots)

HPLC Column: Gemini® 3 µm C18
Dimension: 50 x 2.0 mm
Part No.: 00B-4439-B0
Mobile Phase: 0.1 % Formic Acid in Water/ 0.1 % Formic Acid in Acetonitrile (70:30)
Flow Rate: 1 mL/min
Temperature: Ambient
Detector: ESI+

Sample Processing Guidelines

Vacuum Manifolds

- Maximize throughput with simple, cost effective batch processing of up to 96 samples per hour. (24 samples per 15 minutes)
- Fits 13 mm and 16 mm test tubes up to 125 mm in height
- “Slow is Safe” for loading and elution. A flow rate: 1 – 3 drops per second (1 – 3 mL /min) is recommended during the loading and elution steps for typical small volume samples (< 5 mL). At these critical steps the analytes are chemically interacting with the sorbent.
- Large volume samples (> 100 mL) in large cartridges (>1 gram) may be processed at flow rates between 5 – 10 mL/minute
- Conditioning and Wash steps are generally not flow critical
- Flow rate is easily adjusted via master vacuum controller or individual stopcocks if necessary
- Individual stopcocks are typically not needed when using the strata-X family of sorbents (strata-X, X-C, X-CW, X-AW, XL, XL-C). They are very forgiving of improper flow rates and are truly resistant to deconditioning effects caused by excessive drying during the method.
- Reversed phase methods are more forgiving of fast flow rates than ion-exchange or normal phase

METHOD DEVELOPMENT KITS

Method Development
Tubes

- Contains 200 mg/3 mL (5 tubes each) of strata-X, X-C, X-CW and X-AW. Allows for traditional screening and method development

KS0-7908

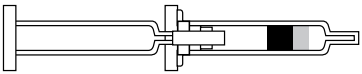
Method Development
96-Well Plate

- Contains 3 rows each of strata-X, X-C, X-CW and X-AW. Allows for high-throughput screening and method development

KS0-8209

* 10 mg/well also available, Part No. KS0-8241

Method Development
Syringe and Adaptor Kit

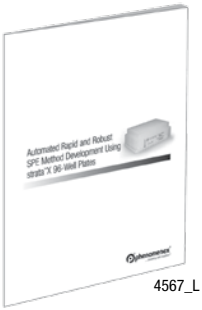


AH0-8278

* To request a kit or plate contact Phenomenex or your Phenomenex distributor.

SELECTED TECHNICAL NOTES

- TN-001 Phenols from Water with strata-X
- TN-002 Strata Method Development 96-Well Plate
- TN-003 Nitroanilines from Water with strata-X
- TN-004 Acidic, Basic, and Neutral Drugs on strata-X
- TN-006 TCA from Serum with strata-X
- TN-007 Acrylamide from French Fries using strata-X-C
- TN-008 Basic Drugs from Biological Matrices on strata-X-C
- TN-009 On-line Extraction of Acyclovir using strata-X
- TN-010 Sulfa Drugs on strata-X-C
- TN-012 Extraction and Analysis of Sulfonamides from Honey
- strata-X 96-Well Plate Method Development White Paper



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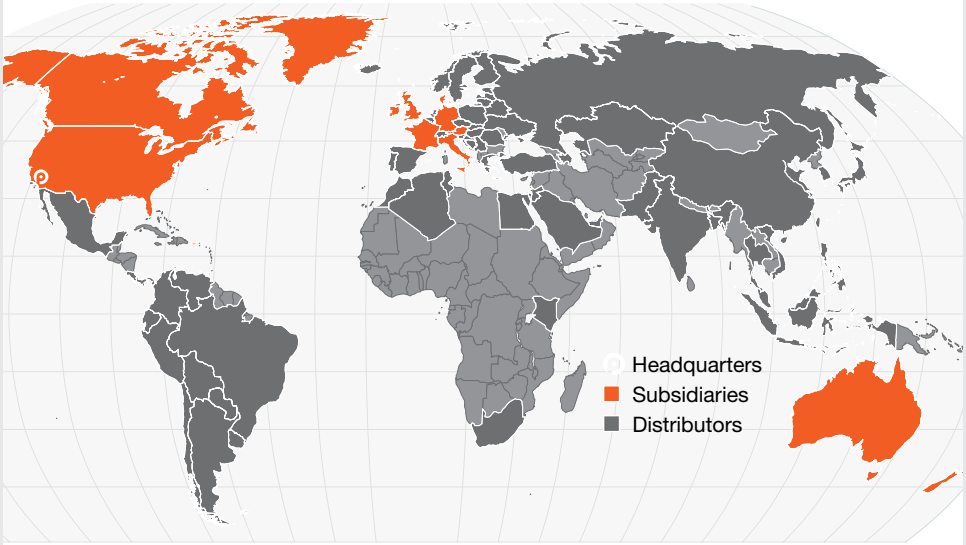
Please visit **www.StrataSPE.com** for a full listing of application notes

AVAILABLE WORLDWIDE



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