A Simple Approach to Fast and Practical Solid Phase Extraction (SPE) Method Development

Michael Rummel, Terrell Mathews
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Solid phase extraction is an effective technique for cleaning up and concentrating samples. In the following communication we outline a simple approach for solid phase extraction method development using Strata® SPE sorbents.

STEP 1. 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:

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

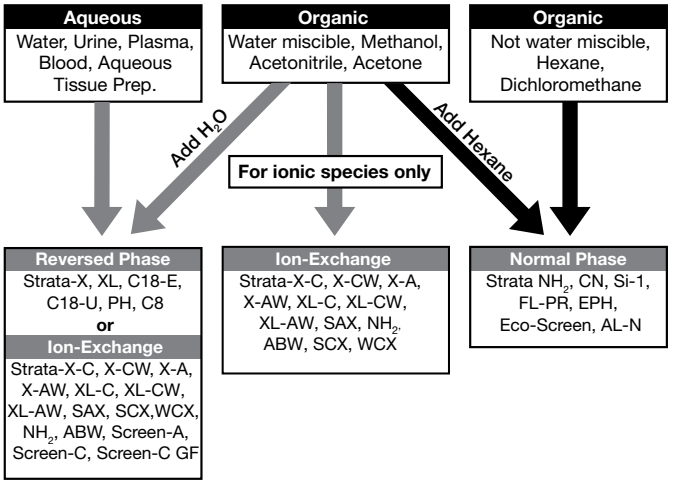
Sample Pre-treatment Recommendations

Biological Samples (liquid)	
Urine, Whole blood, Serum, Plasma, Bile, etc.	Dilute sample 1:2 with appropriate buffer, precipitate proteins if proteinaceous ($ZnSO_4$, 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).

STEP 2. Selecting Strata Sorbents

Identify the possible SPE retention mechanism: Reversed Phase (RP), Ion-Exchange (IEX) or Normal Phase (NP):

The sample solvent composition will guide you towards an appropriate SPE mechanism.



Once the general mechanism is identified, it will be necessary to identify the most specific Strata sorbent by matching the analyte functional groups to the sorbent functional group.

SPE Mechanism	Analyte Functional Group	Sorbent Functional Group	Strata Sorbent
Reversed Phase	R ~ hydrocarbon	R ~ hydrocarbon	C18-E, C18-U, C8 C18-T, X, XL
	aromatic	aromatic	X, PH, SDBL, XL
Normal Phase	R - OH hydroxyl	CN polar	CN, NH_2
	R - NH_2 amino	OH polar	Si-1, CN, EPH
Ion-Exchange	Pos: NR_4^+ strong	$-O_2C$—weak	X-CW, XL-CW, WCX
	Pos: RNH_3^+ weak	$-O_3S$—strong	X-C, SCX, XL-C
	Neg: RSO_3^- strong	^+H_3N—weak	X-AW, XL-AW, NH_2
	Neg: RCO_2^- weak	^+R_3N—strong	SAX, X-A

STEP 3. Sorbent Mass Selection

To select the proper sorbent mass, it is first necessary to determine the volume of sample needed to be extracted in order to meet method detection limits (not including buffer). Two tables are included below: Polymer-based and silica-based. This is necessary because the large surface area of polymeric sorbents such as Strata-X have a higher analyte capacity per gram than silica-based sorbents.

Suggested Loading Capacity

Table 1.

Polymer-Based Sorbents (Strata-X, X-C, X-A, X-AW, X-CW, XL, XL-C, XL-CW, XL-AW and SDB-L)

Sample Matrix	Sorbent Mass
Blood, serum, plasma	30 mg sorbent per 250 µL
Urine	30 mg sorbent per 1 mL
Filtered tissue homogenates	60 mg sorbent per 100 mg tissue

Environmental Samples	Sorbent Mass
Water (particulate-free) drinking	200 mg/100 mL - 400 mL sample
Water (particulate-laden) rivers, runoff, etc.	500 mg/100 mL - 400 mL sample
Soil Extracts	500 mg/100 g of soil extract

Table. 2

Silica-Based Sorbents (Strata C18-E, C8, SCX, SAX, WCX, NH_2, etc.)

Sample Matrix	Sorbent Mass
Blood, serum, plasma	50 mg sorbent per 250 µL
Urine	50 mg sorbent per 500 µL
Filtered tissue homogenates	100 mg sorbent per 100 mg tissue

Environmental Samples	Sorbent Mass
Water (particulate-free) drinking	500 mg/100 mL - 500 mL sample
Water (particulate-laden) rivers, runoff, etc.	1 g/100 mL - 500 mL sample
Soil Extracts	1 g/100 g of soil extract

Generic Method

Each SPE mechanism / phase has a general set of solvent conditions under which SPE may be performed. Use the solvents/pH conditions listed below, volumes as determined in Method and Sorbent Volume Selection.

STEP 4. 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.

Sorbent Wash and Elution Volumes*

Silica-Based Sorbent Mass	Practical Minimum Wash and Elution Volume 4 bed volumes	Recommended Wash and Elution Volume 8 bed volumes	Polymer-Based Sorbent Mass*	Practical Minimum Wash and Elution Volume 4 bed volumes	Recommended Wash and Elution Volume 8 bed volumes
10 mg	60 µL	120 µL	10 mg	100 µL	200 µL
—	—	—	30 mg	300 µL	600 µL
50 mg	300 µL	600 µL	—	—	—
—	—	—	60 mg	600 µL	1.2 mL
100 mg	600 µL	1.2 mL	100 mg	1 mL	2 mL
150 mg	900 µL	1.8 mL	150 mg	1.5 mL	3 mL
200 mg	1.2 mL	2.4 mL	200 mg	2 mL	4 mL
500 mg	3 mL	6 mL	500 mg	5 mL	10 mL
1 g	6 mL	12 mL	1 g	10 mL	20 mL
2 g	12 mL	24 mL	—	—	—
5 g	30 mL	60 mL	—	—	—
10 g	60 mL	120 mL	—	—	—

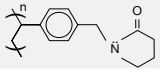
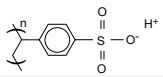
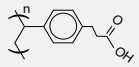
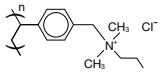
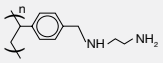
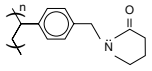
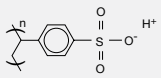
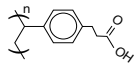
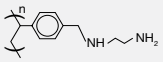
*Strata polymeric resins have a larger surface area than Strata silica-based material, hence requiring slightly more solvent per gram for processing. The elution volumes are specific to the chemical nature of the analyte being extracted, its concentration in the sample, the chemical nature of the eluting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

	Reversed Phase SPE Method		Normal Phase SPE Method		Strong Ion-Exchange SPE Method	
Sorbent	**X, SDB-L, C18, C8, PH, CN, XL**		**Silica, Florisil®, NH_2, CN**		**Anion exchange: SAX, X-A, X-AW, XL-AW Cation exchange: SCX, X-C, X-CW, XL-C, XL-CW**	
Analyte Properties	Low to moderate polarity (or non-polar) Hydrophobic Neutralized/uncharged	Pharmaceuticals Pesticides, herbicides	Moderate to high polarity compounds (neutralized/uncharged)	Pesticides	Ionized/charged compounds	Anion exchange: Acidic analytes Cation exchange: Basic drugs
Sample/Matrix	Aqueous, diluted with buffer	Biological fluids Water	Non-polar organic solvents or moderately polar organic solvents	Hexane, chloroform, petroleum ether, toluene or methylene chloride	Aqueous; Low ionic strength buffers (<30 mM), pH adjusted	Biological fluids plus buffer
Conditioning Step	1. Solvation – polar organic solvents 2. Equilibration – aqueous, buffers	1. Methanol 2. Water or buffer	1. Solvation – polar organic solvents (optional) 2. Equilibration – sample/matrix solvent	1. Methanol (optional) 2. Hexane or chloroform	1. Conditioning – polar organic solvents 2. Equilibration – low ionic strength buffers, pH adjusted	1. Methanol 2. 25 mM Tris-OAc, pH 7
Wash Step	Aqueous buffers with 5 to 50 % polar organic solvent	Methanol: Water (5:95)	Non-polar organic solvents with a low concentration (1 to 5 %) of moderate to low polarity organic solvents	Hexane with 1 % THF, ethyl acetate, acetone, acetonitrile or IPA	Aqueous buffers of low salt concentrations with or without organic solvent	Anion exchange: Buffer pH 7: Methanol (50:50) Cation exchange: 1. Buffer pH 6 2. 1 M acetic acid 3. Methanol
Elution Step	Polar or non-polar organic solvent(s) with or without water, buffer and/or strong acid or base	Methanol: Acetonitrile (50:50)	Non-polar organic solvents containing higher concentrations (5 to 50 %) of moderate to high polarity organic solvents	Hexane with 10 % THF, ethyl acetate, acetone, acetonitrile or IPA	• Neutralize the charge on the weak anion or cation • Increase the ionic strength and counter ion concentration • Add a strong counter ion displacer	Anion exchange: Hexane: ethyl acetate (75:25) +1 % glacial acetic acid Cation exchange: Methanol + 5 % NH_3

TN-013
APPLICATIONS

Strata™-X Polymeric SPE Sorbents

- Clean extracts from biological sample matrices
- Streamlined method development and simple processing

Sorbent	Functional Group	Mode	Analyte
Strata-X		Reversed Phase	Polar and Non-Polar
Strata-X-C		Reversed Phase and Strong Cation Exchange	Bases
Strata-X-CW		Reversed Phase and Weak Cation Exchange	Bases *(including Quaternary Amines)*
Strata-X-A		Reversed Phase and Strong Anion Exchange	Acids
Strata-X-AW		Reversed Phase and Weak Anion Exchange	Acids
Strata-XL		Reversed Phase	Polar and Non-Polar
Strata-XL-C		Reversed Phase and Strong Cation Exchange	Bases
Strata-XL-CW		Reversed Phase and Weak Cation Exchange	Bases *(including Quaternary Amines)*
Strata-XL-AW		Reversed Phase and Weak Anion Exchange	Acids

guarantee

If Strata SPE products do not perform as well or better than your current SPE product of similar phase, mass and size, return the product with comparative data within 45 days for a FULL REFUND.

Strata® SPE Sorbents

- Extremely reproducible from batch-to-batch
- Formats for large and small volume samples

Reversed Phase

C18-E
- Extraction of hydrophobic molecules from aqueous and biological samples

C18-U
- Increased extraction efficiency and enhanced clean up of hydrophobic compounds that contain hydroxy or amine functional groups from water or biological fluids

C18-T (wide pore)
- Extracting large hydrophobic molecules (up to 75 kD) from water or biological matrices

C8
- Extracting hydrophobic compounds from water or biological fluids that are retained too strongly on Strata C18-E or Strata-X

Phenyl
- Extracting aromatic hydrophobic compounds

CN
- Extracting non-polar, compounds that are retained too strongly on Strata C18-E or C8

SDB-L (styrene-divinylbenzene)
- Extraction of non-polar and polar molecules

Normal Phase

CN
- Normal phase sorbent that can effectively extract polar compounds from non-polar solvents

NH_2
- Extraction of strong anions from aqueous samples

EPH (Extractable Petroleum Hydrocarbon)
- Fractionation of aliphatic and aromatic extractable hydrocarbons from soil and water samples

Silica
- Extraction of polar compounds that are similar in structure

Florisil®
- Extraction of pesticides from environmental samples

Alumina-N
- Extraction of polar compounds from food and environmental samples

Eco-Screen
- Extraction of naphthalene from environmental samples

Cation Exchange

WCX (weak cation exchange)
- Extraction of quaternary amines

SCX (strong cation exchange)
- Extraction of 1˚, 2˚ and 3˚ amines from biological fluids

Screen-C (mixed-mode cation exchange)
- Extraction of basic drugs from biological matrices such as blood, serum and urine

Anion Exchange

WAX (weak anion exchange)
- Extraction of strong ions from aqueous solvent

SAX (strong anion exchange)
- Extraction of organic acids

Screen-A (mixed-mode anion exchange)
- Extraction of acidic drugs from biological matrices such as blood, serum and urine

ABW (specialty phase)
- Fractionation of neutral compounds such as amides from acidic and basic analytes

TN-013
APPLICATIONS

PRODUCT INFORMATION

Strata Tubes	1 mL (100/box)		3 mL (50/box)			6 mL (30/box)		
Silica-based sorbents								
Phase	**50 mg**	**100 mg**	**100 mg**	**200 mg**	**500 mg**	**200 mg**	**500 mg**	**1 g**
C18-E	8B-S001-DAK	8B-S001-EAK	8B-S001-EBJ	8B-S001-FBJ	8B-S001-HBJ	8B-S001-FCH	8B-S001-HCH	8B-S001-JCH
C18-U	—	8B-S002-EAK	—	8B-S002-FBJ	8B-S002-HBJ	—	8B-S002-HCH	8B-S002-JCH
C18-T	—	8B-S004-EAK	—	8B-S004-FBJ	8B-S004-HBJ	—	8B-S004-HCH	8B-S004-JCH
C8	—	8B-S005-EAK	—	8B-S005-FBJ	8B-S005-HBJ	—	8B-S005-HCH	8B-S005-JCH
Phenyl	—	8B-S006-EAK	—	8B-S006-FBJ	8B-S006-HBJ	—	8B-S006-HCH	8B-S006-JCH
SCX	—	8B-S010-EAK	8B-S010-EBJ	8B-S010-FBJ	8B-S010-HBJ	—	8B-S010-HCH	8B-S010-JCH
WCX	—	8B-S027-EAK	—	8B-S027-FBJ	8B-S027-HBJ	—	8B-S027-HCH	8B-S027-JCH
SAX	—	8B-S008-EAK	8B-S008-EBJ	8B-S008-FBJ	8B-S008-HBJ	—	8B-S008-HCH	8B-S008-JCH
NH_2	—	8B-S009-EAK	—	8B-S009-FBJ	8B-S009-HBJ	—	8B-S009-HCH	8B-S009-JCH
CN	—	8B-S007-EAK	—	8B-S007-FBJ	8B-S007-HBJ	—	8B-S007-HCH	8B-S007-JCH
Si-1	—	8B-S012-EAK	—	8B-S012-FBJ	8B-S012-HBJ	—	8B-S012-HCH	8B-S012-JCH
Florisil®	—	—	—	—	8B-S013-HBJ	—	8B-S013-HCH	8B-S013-JCH
EPH	—	—	—	—	8B-S031-HBJ	—	—	—
AL-N	—	—	—	—	—	—	—	8B-S313-JCH
Mixed-mode sorbents (for drugs of abuse)								
Phase	**—**	**100 mg**	**100 mg**	**150 mg**	**200 mg**	**—**	**200 mg**	**500 mg**	**—**
Screen-C	—	8B-S016-EAK	8B-S016-EBJ	8B-S016-SBJ	8B-S016-FBJ	—	8B-S016-FCH	8B-S016-HCH	—
Screen-A	—	8B-S019-EAK	—	—	8B-S019-FBJ	—	8B-S019-FCH	8B-S019-HCH	—
Polymeric sorbents								
Phase	**50 mg**	**100 mg**	**—**	**200 mg**	**500 mg**	**200 mg**	**500 mg**	**1 g**
SDB-L	8B-S014-DAK	8B-S014-EAK	—	8B-S014-FBJ	8B-S014-HBJ	8B-S014-FCH	8B-S014-HCH	8B-S014-JCH
Strata-X Tubes								
Phase	**30 mg**	**60 mg**	**60 mg**	**200 mg**	**500 mg**	**100 mg**	**200 mg**	**500 mg**
Strata-X	8B-S100-TAK	8B-S100-UAK	8B-S100-UBJ	8B-S100-FBJ	8B-S100-HBJ	8B-S100-ECH	8B-S100-FCH	8B-S100-HCH
Strata-X-C	8B-S029-TAK	—	8B-S029-UBJ	8B-S029-FBJ	8B-S029-HBJ	8B-S029-ECH	8B-S029-FCH	8B-S029-HCH
Strata-X-CW	8B-S035-TAK	—	8B-S035-UBJ	8B-S035-FBJ	8B-S035-HBJ	8B-S035-ECH	8B-S035-FCH	8B-S035-HCH
Strata-X-A	8B-S123-TAK	—	8B-S123-UBJ	8B-S123-FBJ	8B-S123-HBJ	8B-S123-ECH	8B-S123-FCH	8B-S123-HCH
Strata-X-AW	8B-S038-TAK	—	8B-S038-UBJ	8B-S038-FBJ	8B-S038-HBJ	8B-S038-ECH	8B-S038-FCH	8B-S038-HCH
Strata-XL	8B-S043-TAK	—	8B-S043-UBJ	8B-S043-FBJ	8B-S043-HBJ	—	8B-S043-FCH	8B-S043-HCH
Strata-XL-C	8B-S044-TAK	—	8B-S044-UBJ	8B-S044-FBJ	8B-S044-HBJ	8B-S044-ECH	8B-S044-FCH	8B-S044-HCH
Strata-XL-CW	8B-S052-TAK	—	8B-S052-UBJ	8B-S052-FBJ	8B-S052-HBJ	8B-S052-ECH	8B-S052-FCH	8B-S052-HCH
Strata-XL-AW	8B-S051-TAK	—	8B-S051-UBJ	8B-S051-FBJ	8B-S051-HBJ	8B-S051-ECH	8B-S051-FCH	8B-S051-HCH

Terms and Conditions
Subject to Phenomenex Standard Terms & Conditions, which may be viewed at www.phenomenex.com/TermsAndConditions

Trademarks
Strata is a registered trademark of Phenomenex, Inc. Strata-X is a trademark of Phenomenex, Inc. Florisil is a registered trademark of U.S. Silica Co.

Strata-X is patented by Phenomenex, Inc. U.S. Patent No. 7,119,145

Copying or re-use of this information is not allowed without written permission from Phenomenex.

Contact Phenomenex for more information on product offerings and FREE samples.

© 2010 Phenomenex, Inc. All rights reserved.

www.phenomenex.com

Phenomenex products are available worldwide. Email us at international@phenomenex.com

Australia
t: 02-9428-6444
f: 02-9428-6445
auinfo@phenomenex.com

Austria
01-319-1301
01-319-1300
anfrage@phenomenex.com

Belgium
+31 (0)30-2418700
+31 (0)30-2383749
beinfo@phenomenex.com

Canada
(800) 543-3681
(310) 328-7768
info@phenomenex.com

Denmark
4824 8048
4810 6265
nordicinfo@phenomenex.com

Finland
(09)4789 0063
+45 4810 6265
nordicinfo@phenomenex.com

France
t:01 30 09 21 10
f:01 30 09 21 11
franceinfo@phenomenex.com

Germany
06021-58830-0
06021-58830-11
anfrage@phenomenex.com

Ireland
01 247 5405
+44 1625-501796
eireinfo@phenomenex.com

Italy
051 6327511
051 6327555
italiainfo@phenomenex.com

Luxembourg
+31 (0)30-2418700
+31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
(55) 5018 3791
(310) 328-7768
tecnicomx@phenomenex.com

Netherlands
t:030-2418700
f:030-2383749
nlinfo@phenomenex.com

New Zealand
09-4780951
09-4780952
nzinfo@phenomenex.com

Norway
81 00 20 05
+45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
800) 541-HPLC
(310) 328-7768
info@phenomenex.com.com

United Kingdom
01625-501367
01625-501796
ukinfo@phenomenex.com

All other countries: Corporate Office USA
(310) 212-0555
(310) 328-7768
info@phenomenex.com

TN56000410_L