Solid Phase Extraction Selection Guide and Procedures

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Introduction to Solid Phase Extraction

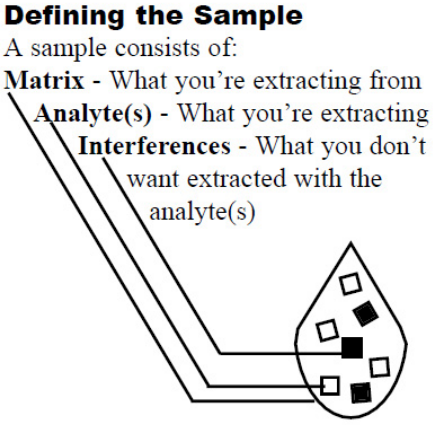
Sample preparation is important to the success of many analytical procedures. Solid phase extraction is required to concentrate dilute analytes and for cleanup of interfering compounds in complex matrices, such as, biological, tissue, or agricultural products and environmental samples.

SPE offers many benefits over other sample preparation techniques including:
• Reduced organic solvent consumption
• Purified extracts
• Reproducible analyte recovery
• Concentration of dilute analytes
• Rapid sample preparation
Fractionation of sample compounds by class

Defining the Sample

A sample is composed of a matrix, analyte, and interferences. The matrix is the material that dissolves the analyte and interferences. The analyte is a compound that is dissolved in the matrix and it is the compound that you want to analyze. Interferences are compounds that are also dissolved in the matrix that interfere with the analysis of the analyte. Interferences are compounds that you want to separate from the analyte.

A sample consists of: Matrix - What you're extracting from
Analyte(s) - What you're extracting
Interferences - What you don't want extracted with the analyte(s)

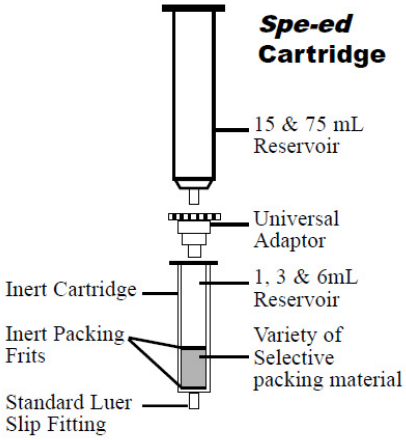


Solid Phase Extraction is a sample preparation technique that uses solid chromatographic material packed in a plastic cartridge to separate different dissolved compounds contained in a liquid sample.

As liquid sample is passed through a SPE column, some compounds are removed from the sample matrix and are adsorbed onto the sorbent material in the column. Absorbed Interfering compounds can then be selectively washed from the column using the appropriate wash solution. Finally, analytes may be selectively washed from the SPE column by an appropriate elution solvent. The analyte may be concentrated in this technique if the elution solvent contains less volume than the sample volume passed through the column.

Processing *Spe-ed*

Spe-ed is best processed with the *Spe-ed* Mate™ manifolds. Maintain a 5-8 mL/min. flow rate through the cartridge. Silica based chromatographic sorbents have a particle size of 40 microns and a pore size of 60 angstroms. Aqueous samples and more viscous solvents are typically processed using vacuum manifold system or positive pressure to force the sample through the cartridge.



1050	*Spe-ed* Mate 12-Glass Vacuum Manifold	1/pk
1060	*Spe-ed* Mate 16-Glass Vacuum Manifold	1/pk
1070	*Spe-ed* Mate 24-Glass Vacuum Manifold	1/pk

Solvent/Solution Compatibility

Spe-ed is chemically compatible with most of the common solvents and solutions used in Gas and Liquid Chromatography. Avoid sustained pH conditions below pH 2 and above pH 10. Do not use DMSO, sulfuric acid, hydrofluoric acid, or sodium hydroxide solutions with *Spe-ed* cartridges.

Sorbent Retention Mechanisms

The wide range of sorbent chemistries exhibit unique properties for the retention of analytes by molecular interactions between the functional groups of the chromatographic sorbent and analytes.

- Non-polar (based on Van der Waals forces)
- Polar (based on hydrogen bonding, dipole-dipole or pi-pi interactions)
- Ion exchange (interactions between cations (positively charged species) and anions (negatively charged species))

SPE sorbents are typically classified by their unique retention mechanism.

Spe-ed Sorbent Selection Guide

Choosing the correct sorbent based upon the sample characteristics and retention mechanisms is essential for solid phase extraction success.

First identify the analyte of interest. Is the analyte non-polar, polar, cationic, or anionic. Next, identify the sample matrix. Is it aqueous, or a nonpolar liquid? The chart below can be used to easily select an appropriate cartridge based upon characterization of the analyte (compound type) and the sample solution or matrix. The weight of the SPE cartridge sorbent and size of the SPE cartridge is based on the extractable capacity of the sorbent as detailed below.

Sample Characterization Recommended Cartridges						Extractables (<10,000MW) Capacity	Cat No.
Compound Type	**Sample Solution**	**Sample Volume**	**Packing**	**Weight**	**Size**		
Nonpolar, Hydrophobic	Polar, Aqueous	<5mL 5-25mL 5-50mL	Octadecyl (C18)	100mg 200mg 500mg	1mL 3mL 3mL	12mg 25mg 60mg	2001 2002 2003
		5-500mL 5mL-1L+		500mg 1000mg	6mL 6mL	60mg 120mg	2006 2007
Moderately Nonpolar Hydrophobic	Polar, Aqueous	1-25mL 5-50mL 5-500mL	Octyl (C8)	200mg 500mg 500mg	3mL 3mL 6mL	15mg 40mg 40mg	2012 2013 2016
Polar, Strong Cation	Less Polar, Aqueous	<5mL 5-250mL	Cyano (CN)	100mg 500mg	1mL 3mL	5mg 25mg	2201 2203
Moderately Polar	Less Polar, Nonaqueous	1-25mL 5-250mL 1-25mL 5-250mL 1-25mL 5-250mL	Silica Gel Florisil* Alumina (Neutral)	500mg 1000mg 500mg 1000mg 500mg 1000mg	3mL 6mL 3mL 6mL 3mL 6mL	40mg 80mg 35mg 70mg 35mg 70mg	2103 2107 2113 2117 2123 2127
Polar, Acidic Strong Anion	Aqueous, Nonaqueous	1-250mL	Amino (NH2)	500mg	3mL	0.45meq	2213
Acidic, Weak Anion	Aqueous, Nonaqueous	1-250mL	Quaternary Amino (N+)	500mg	3mL	0.45meq	2303
Basic, Strong Cation	Aqueous, Nonaqueous	1-250mL	Carboxylic Acid (COOH)	500mg	3mL	0.45meq	2313
Basic, Weak Cation	Aqueous, Nonaqueous	1-250mL	Sulfonic Acid (SO3H)	500mg	3mL	0.45meq	2323

An SPE procedure typically consists of 6 steps:

1. Sample preparation
2. SPE column conditioning
3. Sample application
4. SPE column washing (interference elution step)
5. Air dry cartridge
6. Analyte elution

Sample Preparation – Step 1

Spe-ed will prepare only sample solutions. Solids must be dissolved or analytes of interest released into solution. Gross particulates should be filtered from the sample solution. *Spe-ed* will filter particulates down to 20 µm.

Conditioning – Step 2

Spe-ed must be conditioned and remain wet with appropriate solvents/solutions. The last conditioning solvent/solutions must be similar to the sample solution in polarity, buffer strength and pH.

Note: Flow Rate Variation It is normal for flow rates to vary slightly from cartridge to cartridge. When batch processing under vacuum, stop solvent/solution level above the sorbent bed on the fastest flowing cartridge. With the vacuum off, equalize the level of the other cartridges by positive pressure with a pipet bulb. The *Spe-ed* Stopcock (Cat. # 2403) can be used to adjust flow variations between cartridges. Conditioning solvents/solutions are easily applied with a squeeze-bottle. Care should be taken that the solvents/solutions used do not leach any interferences from their container. Aspirate through the cartridge one to two reservoir volumes of each recommended solvent/solution. After each addition, leave approximately 2mm of solvent/solution above the packing bed to ensure sorbent wetness.

Sample Application - Step 3

Capacity

Aspirate through the cartridge volume of the sample required. Cartridge capacity is dependent on the amount (concentration) of extractables present in the solution and not the sample volume (refer to *Spe-ed* Selection Guide for capacities).

Sample Addition

Sample solutions may be added to the cartridge sequentially as long as approximately 5mm is left above the packing bed between additions. This ensures that the sorbent will remain wet.

Large Sample Volumes

Larger sample volumes may be added directly through a *Spe-ed* 15mL (Cat. #2400) or 75mL (Cat. #2401) Reservoir. Fill the cartridge reservoir two thirds full with the last conditioning solvent/solution or sample solution. Snap on the *Spe-ed* Universal Adaptor (Cat. #2402) and fit the 15mL or 75mL Reservoir into the adaptor. Add the remaining sample solution to the reservoir and aspirate through the cartridge. This ensures that the sorbent will remain wet.

Completely aspirate the sample solution through the cartridge and then air dry under vacuum for approximately two minutes.

Washing -Step 4
The wash step is required to remove residual sample matrix in the packing bed and coextracted interferences that differ chemically from the retained analytes. Interferences differing in functional groups, solubility, and pK can be selectively removed.

Note: Solubility Changes
Many ionic organic compounds may lose their solubility in the wash solvent/solution after the charge is neutralized. Add sufficient amounts of a miscible solvent/solution that will solubilize the analytes.
Recommended Wash Volumes:

Cartridge Size	Packing Weight	Wash	Wash Volume
1mL	100mg	1-2	100µL aliquots
3mL	200mg	1-2	200µL aliquots
3mL	500mg	1- 2	500µL aliquots
6mL	500mg	1-2	500µL aliquots
6mL	1000mg	1-2	1.0mL aliquots

Air Drying Cartridge- Step 5
After washing, air dry cartridge under vacuum for 2-5 minutes. Residual water may also be removed by centrifuging the cartridge for 2-3 minutes at 2000 - 4000 rpm, or purging the packing bed with 1-2 50µL aliquots of hexane.

Elution- Step 6
The elution step will remove the extracted and concentrated analytes from the packing bed. Solubility is the governing factor in elution, therefore select an elution solvent that easily dissolves the analytes of interest.
Recommended Elution Volumes:

Cartridge Size	Packing Weight	Wash	Wash Volume
1mL	100mg	2	100µL aliquots
3mL	200mg	2	200µL aliquots
3mL	500mg	2	500µL aliquots
6mL	500mg	2	500µL aliquots
6mL	1000mg	2	1.0mL aliquots

Maximum analyte recovery is achieved with two or more successive aliquots of elution solvent/solution. Add the first aliquot of elution solvent/solution to the cartridge and allow it to percolate through the packing bed for 10-15 seconds before aspirating completely through. Turn vacuum off between additions. Collect and combine eluates in a test tube, volumetric, or vial positioned under the Spe-ed Cartridge. Eluates are analyzed or evaporated and redissolved in a suitable solvent solution.

Analytical Compatibility
To eliminate eluate reconstitution, elute analytes with solvent/solutions compatible with the final means of analysis.

Using Nonpolar Sorbents (Silica Based)

These *Spe-ed* packings are used to extract nonpolar, hydrophobic, and slightly acidic or basic compounds from aqueous or polar solvents/ solutions. If necessary, the following adjustments must be made to the sample to ensure retention of the analytes.

Sorbent	Conditioning	Sample	Wash	Air Dry	Elution
Spe-ed Non-Polar C18/22 C18/18 C18/14 C18/0H C8 C4 C2 Phenyl Cyclohexyl	1. Methanol or acetonitrile 2. Water or buffer at the sample pH	Control pH to suppress ionization of acidic and basic compounds	Water or buffer increasing to 40% methanol or acetonitrile, maintain pH control	Air dry cartridge under vacuum for 2-5 minutes	Methanol or other solvents that will selectively elute the analyte of interest. Add acid or base to suppress secondary interactions.

Sample Modification

1. Reduce analyte's solubility in solution as much as possible. Dilute with lesser solubilizing miscible solvent/ solution such as water or salt solution with sodium chloride or lower the temperature.
2. Remove high level fat or lipid interferences in solution. Dissolve in ethyl acetate, then precipitate with methanol. Let stand and dilute supernatant to 90% aqueous solution.
3. Neutralize slightly acidic or basic analytes. Adjust pH below pK or acidic analytes and above pK of basic analytes.

Conditioning

Condition with methanol followed by distilled-deionized water or appropriately pH/buffered water.

Extraction / Sample Addition

Aspirate through the cartridge volume of the sample required. Cartridge capacity is dependent on the amount (concentration) of extractables present in the solution and not the sample volume (refer to Spe-ed Selection Guide for capacities).

Washing

An effective means of selectively washing off interferences is to use different solvents/solutions of various solvent strength. Mixtures of different miscible solvents/solutions (refer to Table I) with varying polarity (refer to Table II) and solubilizing ability are also effective.

Air Drying Cartridge
After washing, air dry cartridge under vacuum for 2-5 minutes. Residual water may also be removed by centrifuging the cartridge for 2-3 minutes at 2000 - 4000 rpm, or purging the packing bed with 1-2 50µL aliquots of hexane.

Elution Solvent Strength
Elute analytes with solvent(s)/solution(s) or mixtures or miscible solvents/solutions having appropriate polarity, solvent strength, and solubilizing ability. The more soluble the analytes are in the elution solvent/solution, the greater the recovery will be (refer to Tables I & II).

Using Silica Gel, Florisil, and Alumina (Neutral)

These *Spe-ed* packings are used to extract polar compounds from less polar solvents/solutions. Do not use these packings with aqueous solutions. If necessary, the following adjustments must be made to the sample to ensure retention of the analytes:

Sorbent	Conditioning	Sample	Wash	Air Dry	Elution
Spe-ed Polar Adsorbents Silica Florisil Alumina N	hexane, ethyl acetate, or ethyl ether	Aspirate sample through cartridge	Non-polar solvent with a low concentration of a polar modifier that does not elute the analyte (e.g. hexane and ethyl acetate or iso- propanol).	Air dry cartridge under vacuum for 2-5 minutes	NonPolar/polar solvent mixture e.g. hexane:ethyl acetate mix. use a polar solvent such as methanol for a non selective elution

Sample Modification

Reduce analyte's solubility in solution as much as possible. Required Adjustment :Dilute with lesser solubilizing miscible solvent/solution or lower the temperature or adjust the pH

Conditioning

Condition with solvents/solutions similar to the sample solution - typically nonpolar to moderately polar solvents such as hexane, methylene chloride, ethyl ether, or ethyl acetate.

Extraction

Aspirate through the cartridge volume of the sample required. Cartridge capacity is dependent on the amount (concentration) of extractables present in the solution and not the sample volume (refer to Spe-ed Selection Guide for capacities).

Washing

An effective means of selectively washing off interferences is to use different solvents/solutions of various solvent strength. Mixtures of different miscible solvents/solutions (refer to Table I) with varying polarity (refer to Table II) and solubilizing ability are also effective

Air Drying Cartridge

After washing, air dry cartridge under vacuum for 2-5 minutes. Residual water may also be removed by centrifuging the cartridge for 2-3 minutes at 2000 - 4000 rpm, or purging the packing bed with 1-2 50µL aliquots of hexane.

Elution

Solvent Strength

Elute analytes with solvent(s)/solution(s) or mixtures or miscible solvents/solutions having appropriate polarity, solvent strength, and solubilizing ability. The more soluble the analytes are in the elution solvent/solution, the greater the recovery will be (refer to Tables I & II).

Cation Exchangers

Using Carboxylic Acid (COOH), and Sulfonic Acid (SO3H): These *Spe-ed* packings are used to extract ionic compounds from aqueous or nonaqueous solvents/solutions. If necessary, the following adjustments must be made to the sample to ensure retention of the analytes:

Sorbent Cation Exchangers	Conditioning	Sample	Wash	Air Dry	Elution
SCX (Benzene sulfonic acid)	1.Methanol 2. Water or 20-50 mM buffer, the same pH as the sample	Adjust pH at least 2 pH units below analyte's pK , Reduce counterion concentration Dilute or dialyze .	Maintain the same pH as the sample and evaluate the addition of 20-40 % methanol to elute non-polar interferences. % methanol can be increased for additional extract cleanliness. Check for analyte breakthough.	Air dry cartridge under vacuum for 2-5 minutes	Elute with buffer at least 2 pH units above the pK of the basic analyte. This should contain at least 25-50 % organic solvent to overcome secondary interactions. Alternatively use high ionic strength buffer (>0.1 M). Elution with methanol containing 2-5 % ammonia or other volatile base is common.
WCX Carboxylic Acid Weak cation exchange (sorbent pK 4.8)	1. Methanol or Acetonitrile. 2. 20-50 mM buffer, the same pH as the sample.	pH≥6.8 and 2 pH units below the analyte pK.	Maintain the same pH as the sample and evaluate the addition of 20-50 % methanol to elute non-polar interferences.	Air dry cartridge under vacuum for 2-5 minutes	1. Buffer at pH <2.8 will suppress the charge on the carboxylic acid sorbent 2. Use a high ionic strength buffer (>0.1M), 3. Acidified solvent.

Sample Modification: Ionize basic or cationic analytes

Required Adjustment:
Adjust 1-2 pH units below analyte's pK
Reduce counterion concentration
Dilute or dialyze

Conditioning
Using Carboxylic Acid (COOH), and Sulfonic Acid (SO3H)
Aqueous Samples: Condition with methanol followed by distilled-deionized water.
Nonaqueous Samples: Condition with the same solvent as the sample solution

Extraction
Aspirate through the cartridge volume of the sample required. Cartridge capacity is dependent on the amount (concentration) of extractables present in the solution and not the sample volume (refer to *Spe-ed* Selection Guide for capacities).

Washing
Nonionic Interferences
An effective means of selectively washing off interferences is to use different solvents/solutions of various solvent strength. Mixtures of different miscible solvents/solutions (refer to Table I) with varying polarity (refer to Table II) and solubilizing ability are also effective.

Ionic Interferences
Ionic interferences are removed with solvents/solutions of varying pH or buffer strength, that will neutralize and solubilize the interferences and not the analytes.

Air Drying Cartridge
After washing, air dry cartridge under vacuum for 2-5 minutes. Residual water may also be removed by centrifuging the cartridge for 2-3 minutes at 2000 - 4000 rpm, or purging the packing bed with 1-2 50µL aliquots of hexane.

Using Carboxylic Acid (COOH), and Sulfonic Acid (SO3H) Elute analytes with solvents/solutions having appropriate pH or buffer strength that will neutralize and solubilize the analytes.

Elution
Using Carboxylic Acid (COOH), and Sulfonic Acid (SO3H) Elute analytes with solvents/solutions having appropriate pH or buffer strength that will neutralize and solubilize the analytes.

Anion Exchangers

Using Amino (NH2), Quaternary Amine (N+), These *Spe-ed* packings are used to extract ionic compounds from aqueous or nonaqueous solvents/solutions.

Sorbent Anion Exchangers	Conditioning	Sample	Wash	Air Dry	Elution
SAX (chloride counter ion)	1.Methanol 2.20-50 mM buffer, the same pH as the sample	At least 2 pH units above the pK of the analyte.	Maintain the same pH as the sample and evaluate the addition of 20-50 % methanol to elute non-polar interferences.	Air dry cartridge under vacuum for 2-5 minutes	Elute with buffer at least 2 pH units below the pK of the acidic analyte. Evaluate addition of 10 % methanol to reduce elution volume. Alternatively use high onic strength buffer (>0.1M), or acidified methanol.
NH2	1.Methanol 2.20-50 mM buffer, the same pH as the sample	≤7.8 and 2 pH units above the analyte pK.	Maintain the same pH as the sample and evaluate the addition of 20-50 % methanol to elute non-polar interferences.	Air dry cartridge under vacuum for 2-5 minutes	Elute with buffer or methanol at pH>11.8 to eliminate the charge on the sorbent. Alternatively use high ionic strength buffer (>0.1M), or acidified methanol.

Sample Modification:
Ionize acidic or anionic analytes

Required Adjustment:
Adjust 1-2pH units above analyte's pK and Reduce counterion concentration : Dilute or dialyze

Conditioning

Using Amino (NH2), Quaternary Amine (N+)
Aqueous Samples: Condition with methanol followed by distilled-deionized water.
Nonaqueous Samples: Condition with the same solvent as the sample solution.

Extraction

Aspirate through the cartridge volume of the sample required. Cartridge capacity is dependent on the amount (concentration) of extractables present in the solution and not the sample volume (refer to *Spe-ed* Selection Guide for capacities).

Washing

Nonionic Interferences
An effective means of selectively washing off interferences is to use different solvents/solutions of various solvent strength. Mixtures of different miscible solvents/solutions (refer to Table I) with varying polarity (refer to Table II) and solubilizing ability are also effective.

Ionic Interferences
Ionic interferences are removed with solvents/solutions of varying pH or buffer strength, that will neutralize and solubilize the interferences and not the analytes.

Air Drying Cartridge

After washing, air dry cartridge under vacuum for 2-5 minutes. Residual water may also be removed by centrifuging the cartridge for 2-3 minutes at 2000 - 4000 rpm, or purging the packing bed with 1-2 50µL aliquots of hexane.

Elution

Using Amino (NH2), Quaternary Amino (N+), Elute analytes with solvents/solutions having appropriate pH or buffer strength that will neutralize and solubilize the analytes.

Method optimization Process

Follow these steps to optimize your SPE procedure:

1. First identify the compound to be analyzed and the sample matrix. Is the analyte nonpolar, polar or ionic? Is the matrix aqueous or non aqueous? Next. Using the Sorbent Selection Chart select the appropriate retention mechanism and sorbent
2. Test the selected sorbents for retention of standards from a "simple" matrix similar to the complex sample matrix. For example, if the method is for a biological fluid like blood, serum or urine, then evaluate retention of standards from aqueous buffer similar to the complex matrix.
3. Screen elution solvents for complete elution of standards using 2 to 10 bed volumes of solvent. If larger volumes are required, use a stronger solvent or less retentive sorbent.
4. Evaluate the above preliminary procedure using a real sample matrix with spiked standards.
5. Optimize the wash step. Test various strength wash solvents to remove interfering compounds and not remove the analyte of interest.
6. Optimize sorbent bed mass. Reduce sorbent mass until analyte loss is experienced.
7. Validate the optimized method.

Solvent Miscibility

Table I

	Hexane	Carbon Tetrachloride	Chloroform	Methylene Chloride	Ethyl Ether	Ethyl Acetate	Acetone	Acetonitrile	Isopropanol	Methanol	Water	Acetic Acid
Hexane								●			●	
Carbon Tetrachloride											●	
Chloroform											●	
Methylene Chloride											●	
Ethyl Ether											●	
Ethyl Acetate											●	
Acetone												
Acetonitrile	●											
Isopropanol												
Methanol												
Water	●	●	●	●	●	●						
Acetic Acid												

● = Immiscible

Relative Solvent Strength on Silica Gel

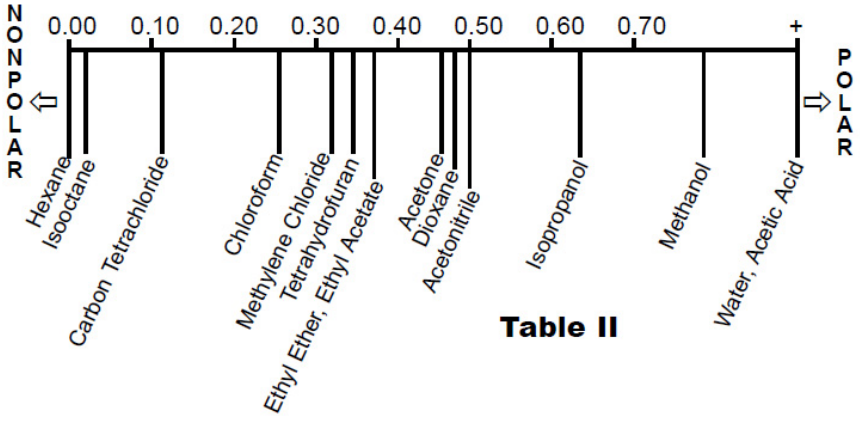


Table II