QuickStart Guide to SPE

Biotage®

QuickStart Guide to SPE

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Introduction

Solid Phase Extraction (SPE) is a powerful technique for rapid, selective sample preparation. Sample preparation prior to analysis is as important to the success of an assay as the analytical technique itself. This is especially true when low levels of detection are required, or for analytes in complex matrices, such as biological fluids, tissue, foodstuffs, agricultural products and environmental samples.

The versatility of SPE allows it to be used for a variety of purposes, such as:

- Purification
- Trace enrichment
- Solvent exchange (analytes are transferred from one matrix to another e.g. from aqueous to organic solvent)
- Desalting
- Derivatization (analytes are retained on a sorbent, derivatized, then eluted)
- Class fractionation (sample is separated into different compound groups that share common properties)

SPE offers many benefits and advantages over other sample preparation techniques such as liquid-liquid extraction, including:

- High, reproducible analyte recovery
- Concentration of analytes
- Highly purified extracts
- Ease of automation
- Compatibility with instrumental analysis
- Productivity enhancement
- Reduction of organic solvent consumption

SPE is a very simple technique to use, employing disposable extraction columns or microplates (see **Figure 1**) which are available in a wide range of reservoir volumes, formats and sorbents.

In principle, SPE is analogous to liquid-liquid extraction (LLE). As a liquid sample passes through the SPE column, compounds are 'extracted' from the sample and adsorbed onto the support or sorbent material in the column. Interferences can then be selectively removed from the column using the correct choice of wash or interference elution solvents. Finally, the desired analytes may be selectively recovered from the column by an elution solvent, resulting in a highly purified extract. The analyte concentration in this extract is often higher than in the original sample.

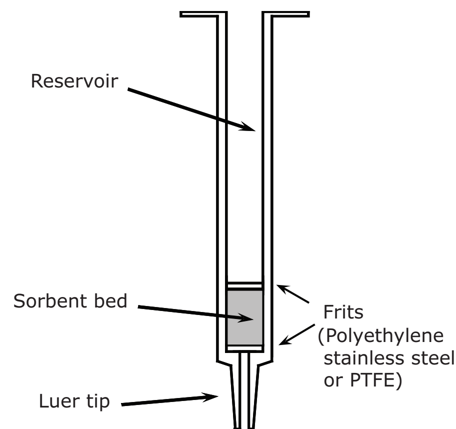


Figure 1. Components of an SPE column or well (microplate format)

Alternatively, an extraction column may be selected that retains the interferences present in the sample, but allows the analytes to pass through un-retained, providing clean-up but not analyte trace enrichment.

SPE sorbents have a typical mean particle size of 30-50 µm. Many organic solvents can flow through SPE columns or plates under gravity, but for aqueous samples and more viscous solvents, liquids must be passed through the sorbent bed using vacuum applied to the column outlet, positive pressure applied to the column inlet, or centrifugation (see **Figure 2**).

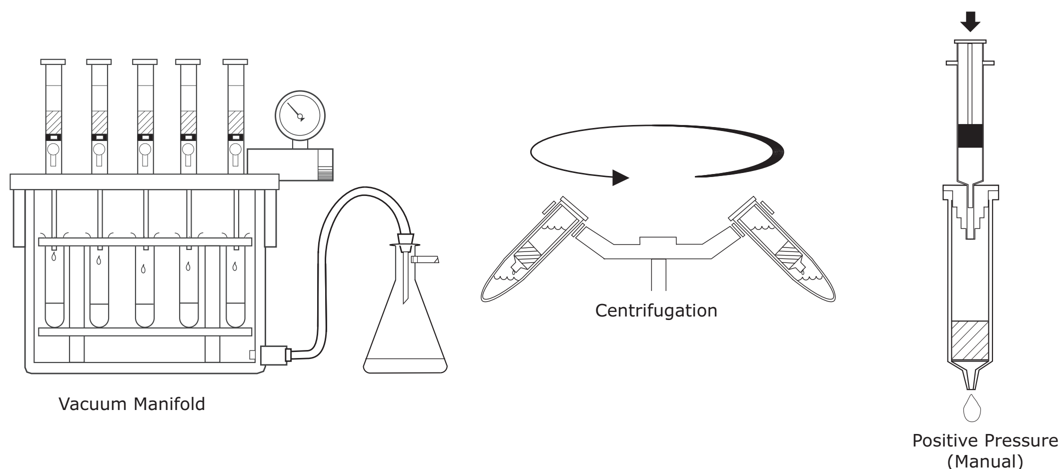


Figure 2. Techniques for processing SPE columns

SPE columns are available in a wide range of sorbent chemistries. Each exhibits unique properties for retention of analytes through a variety of molecular interactions (referred to as retention mechanisms).

The most common retention mechanisms in SPE are:

- Non-polar (based on Van der Waals forces)
- Polar (based on hydrogen bonding, dipole-dipole or π-π interactions)
- Ion exchange (interactions between cations (positively charged species) and anions (negatively charged species))
- Mixed-mode (combination of non-polar and ion exchange interactions)

SPE sorbents are classified by retention mechanism. Each sorbent offers a unique combination of these properties which can be applied to a wide variety of specific extraction problems.

Guide to SPE Sorbent Selection

Proper choice of SPE sorbent is critical to the success of the SPE procedure. There are many aspects of the application that should be considered as part of sorbent selection, including:

- Nature of the analytes (functionality, etc.)
- Nature of the sample matrix
- Degree of purity required
- Nature of major contaminants in the sample
- Analytical procedure

The first factor affecting the choice of a sorbent type or retention mechanism is based on the properties of the analyte (i.e. functional groups of the analyte which can be used for retention on the sorbent). There may be a number of different possibilities depending on the analyte characteristics (see **Table 1**).

Selectivity and Capacity in SPE

Selectivity

The extensive range of sorbent chemistries available provides one of the most powerful aspects of SPE, high selectivity. Selectivity is the degree to which an extraction technique can separate the analyte of interest from the interferences in the original sample. The highly selective nature of SPE is due to two factors. First, each extraction sorbent chemistry offers unique and distinctive retention properties, to address a wide range of analyte characteristics. The second factor is best understood by comparison with liquid-liquid extraction. In liquid-liquid extraction, the two liquids (phases) must be immiscible with each other. Clearly, an aqueous sample cannot be extracted with methanol. In SPE however, one phase is a solid support or sorbent, and is therefore by definition immiscible with any extraction solvent used. This results in a huge variety of possible sorbent/solvent combinations, with potential to achieve highly selective extractions.

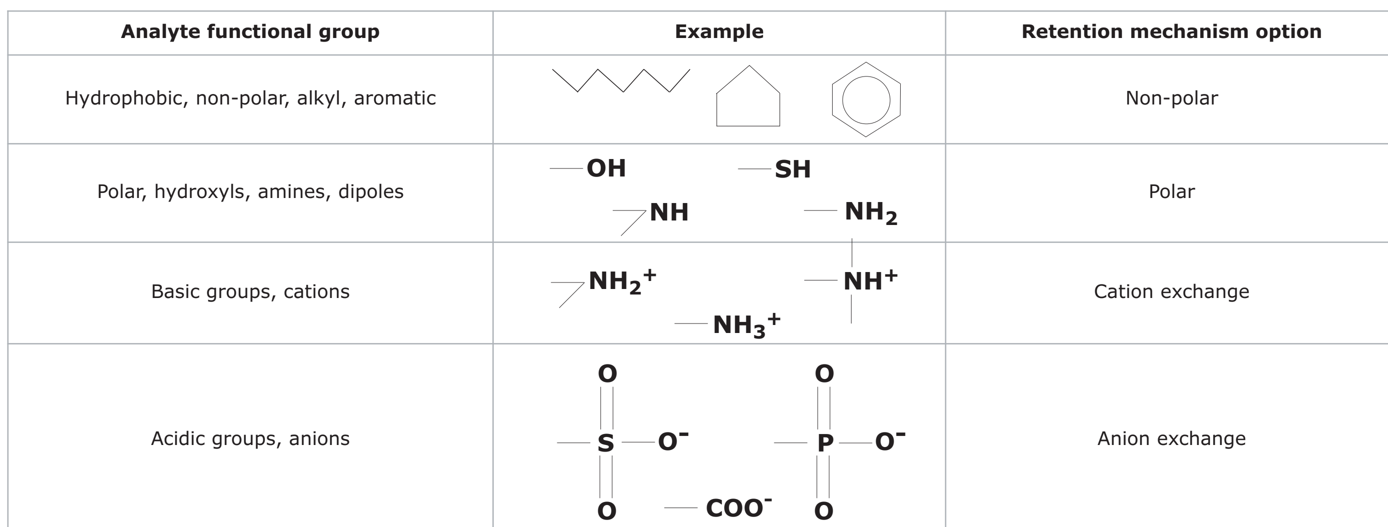
Capacity

The capacity of a sorbent is defined as the total mass of strongly retained analyte that can be retained by a given sorbent mass under optimum conditions. When determining the amount of sorbent required for an extraction, it is essential to consider not only the capacity requirement for the analyte, but also for any undesired sample components (interferences) which may be co-extracted with the analyte using the same retention mechanism. Choosing a more selective retention mechanism will increase the relative capacity of the SPE bed, in effect reducing the amount of sorbent required for a given extraction. This has benefits in reducing the amounts of solvent used, and increasing the final concentration of analyte in the extract. Maximum selectivity (and therefore capacity) is achieved when the selected sorbent interacts with functional groups present on the analyte, but not other sample components.

Typically, non-polar and polar SPE sorbents have a capacity of between 1 and 5% of the sorbent mass (i.e. 100 mg of sorbent can retain up to 5 mg of strongly retained analyte under optimum conditions).

Ion exchange sorbent capacity is measured in milli-equivalents per gram of sorbent (meq/g), based on the number of available ionic groups on the sorbent. For example, ISOLUTE SAX has an exchange capacity of 0.6 meq/g. This means a 1 g ISOLUTE SAX column can retain up to 0.6 mmol of an anionic (acidic) compound.

Table 1. Retention mechanism options for various analyte functional groups



Analyte functional group	Example	Retention mechanism option
Hydrophobic, non-polar, alkyl, aromatic		Non-polar
Polar, hydroxyls, amines, dipoles	—OH —SH —NH —NH_2	Polar
Basic groups, cations	NH_2^+ NH^+ NH_3^+	Cation exchange
Acidic groups, anions	SO_3^- COO^- PO_3^-	Anion exchange

The choice of sorbent is also influenced by the nature of the sample, since certain sample matrices promote analyte retention better by one retention mechanism than another. (**see Table 2**)

Table 2. Retention mechanism options for various sample types

Sample matrix type	Example	Retention mechanism option
Aqueous, low ionic strength	River water, diluted biological fluids	Non-polar, ion exchange
Aqueous, high ionic strength	Sea water, biological fluid*	Non-polar
Non-polar	Hexane or olive oil	Polar

* For extraction of basic or acidic compounds from complex biological fluid samples such as plasma or urine, mixed-mode SPE columns can provide additional clean-up.

Practical Aspects of SPE Method Development

Once an appropriate sorbent type/retention mechanism has been selected, this guide provides a summary of the more important points to consider during method development. The table on pages 9–11 of this guide summarizes this information for non-polar, polar, ion exchange and mixed-mode retention mechanisms.

An SPE procedure typically consists of 6 steps: (see **Figure 3**).

1. Sample pre-treatment
2. SPE column solvation
3. SPE column equilibration
4. Sample application
5. SPE column washing (interference elution step)
6. Analyte elution from the SPE column

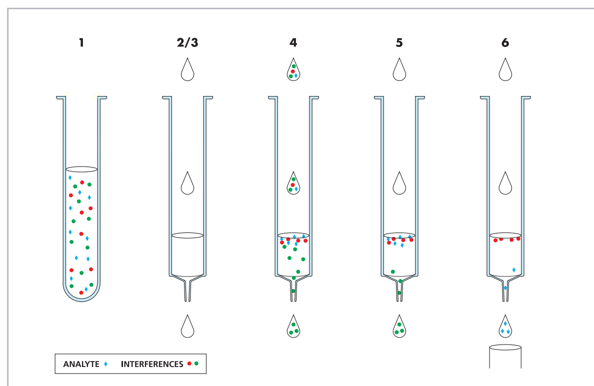


Figure 3. Typical SPE procedure

If using the SPE column to retain interferences while allowing analytes to pass straight through, follow the same guidelines, but use conditions to promote retention of interferences, rather than analytes, during steps 1 - 4. Collect the sample once it has passed through the SPE column, as this will contain the analytes.

Flow rate control

Flow rate control during sample loading, interference and analyte elution steps is important. Too fast a flow rate can result in low recoveries due to breakthrough during the analyte retention (sample loading) step, or inadequate elution during the elution step. Too high a flow rate during the interference elution step can result in dirty extracts. Flow rates should be specified in all protocols to ensure trouble free transfer from one analyst or laboratory to another.

See **Table 4** for typical flow rates used during method development.

Step 1:

Sample pre-treatment

Sample pre-treatment may simply be a dilution of the sample with an appropriate solvent in order to reduce viscosity, or it could involve addition of a buffer to control the pH of the sample prior to retention by non-polar, mixed-mode or ion exchange sorbents.

a) Ensure analytes are in solution and available for interaction with the sorbent. If the sample contains particulate matter to which the analytes absorb, it is essential to first desorb the analytes from the particulates before applying the sample to the SPE column. Similarly, if analytes are bound to large molecules in the sample (e.g. drugs bound to proteins present in biological fluids), this binding must be disrupted to achieve high extraction efficiencies. This can usually be achieved by the addition of a small percentage of organic solvent, or pH adjustment of the sample.

b) Filter or otherwise remove (e.g. centrifuge) sample particulates to prevent column blockage.

c) Non-polar sorbents:

(i) Enhance retention of ionizable analytes by adjusting sample pH to suppress ionization of the analyte.
For acidic analytes: adjust pH to 2 pH units below the pK of the analyte
For basic analytes: adjust pH to 2 pH units above the pK of the analyte

(ii) Use of non-polar, non-endcapped silica based sorbents (ISOLUTE® C18, C8, C6, C4, C2, PH, CN) for extraction of basic analytes:
The retention of basic analytes by non-polar sorbents can often be enhanced using the secondary interaction with silanol ($Si\text{-}O^-$) groups present on the surface of the silica particle. To use this approach, the pH of the sample should be adjusted to ionize the silanol groups ($Si\text{-}O^-$) and protonate the analyte. A pH range between 3 and 8 is normally evaluated.

d) For ion exchange interactions, pH must be adjusted to ensure complete ionization of the analyte, and in the case of weak ion exchange sorbents (e.g. ISOLUTE CBA, NH2 and PSA), the sorbent functional group.

Analyte ionization conditions

i) For basic analytes: adjust pH to 2 pH units below the pK of the analyte
ii) For acidic analytes: adjust pH to 2 pH units above the pK of the analyte

Sorbent ionization conditions

(i) For ISOLUTE CBA, sample pH must be greater than 6.8
(ii) For ISOLUTE NH2, sample pH must be less than 7.8 (or 8.1 for ISOLUTE PSA)

It is important to ensure that the ionic strength of the sample is not so high as to weaken the ionic interactions. To overcome this, dilute with low ionic strength buffer. Failure to deal with high and variable ionic strength samples can result in low or variable recoveries when using ion exchange sorbents.

e) For extraction of large volume aqueous samples (>25 mL) using non-polar silica based sorbents, it is necessary to add 0.5-5 % methanol or isopropanol to the sample in order to maintain solvation of the sorbent bed during sample loading, and ensure high analyte recovery.

Step 2:
Column solvation

In order for a proper phase interface to exist between the sorbent and the sample, most ISOLUTE SPE columns should be solvated prior to sample loading. Typical solvents are listed in **Table 3**. A typical volume of solvation solvent is 1 mL/100 mg sorbent. For EVOLUTE® and other wettable SPE sorbents, this step may not be necessary.

Step 3:
Column equilibration

Prior to sample loading, the SPE column should be 'normalized' to match the conditions of the pre-treated sample. This will ensure maximum analyte recovery.

For example, if the sample is pH adjusted to suppress/ensure ionization of the analyte, equilibrate the column with a buffer of the same pH and ionic strength.

For weak ion exchange sorbents, use buffer pH to also ensure ionization of sorbent for maximum capacity (see **Table 3** for guidelines for specific sorbents). For EVOLUTE and other wettable SPE sorbents, this step may not be necessary.

Step 4:
Sample loading

Extraction efficiency is flow rate dependent, so evaluation of the extraction efficiency vs. flow rate is a useful part of the method development process. See **Table 4** for suggested flow rates.

Step 5:
Interference elution

Table 3 provides useful guidelines on the choice of interference elution solvents. N.B. for maximum analyte recovery, analyte retention conditions (e.g. pH and ionic strength) should be maintained during this step.

Step 6:

Analyte Elution

A good elution solvent should elute the analyte in as low a volume as possible. Choose a solvent or solvent mixture in which the analyte is highly soluble. Elution solvents should overcome both primary and any secondary interactions by which the analyte is retained.

Table 3 provides some useful guidelines on the choice of analyte elution solvents.

During method development, it is important to evaluate the effect of different flow rates on the efficiency of analyte elution, and specify the optimum flow rate range in the protocol. Lower flow rates are often required for ion exchange sorbents. It is sometimes useful to elute the analyte with two aliquots of X/2 mL of solvent, rather than a single elution step with X mL solvent.

Where basic analytes have been extracted using a non-polar, non-endcapped sorbent, the elution solvent should be optimized to also overcome secondary interactions between surface silanol groups and the positively charged (or protonated) analyte. This can be achieved by acidifying the elution solvent to suppress silanol ionization or increasing the pH to eliminate the positive charge on the basic analyte. Alternatively, elute with solvent containing 1% 1M ammonium acetate.

Table 3

Suggestions for the use of EVOLUTE and ISOLUTE Non-polar, Polar, Ion Exchange and Mixed-mode Sorbents

Sorbent	Conditioning	Equilibration	Sample pH	Interference Elution	Analyte Elution
Non-polar polymer-based sorbents					
EVOLUTE ABN	Methanol Optional.**	Aqueous formic acid (0.1%, v/v). Optional.**	Dilute with aqueous formic acid (1%, 1:3, v/v). pH control may be required to suppress ionization of acidic and basic analytes.	Water:methanol (95:5, v/v).	Methanol
ISOLUTE ENV+ Hydroxylated polystyrene-divinyl-benzene (water-wettable sorbent).	Methanol or acetonitrile. Optional.**	Water and/or 20-50 mM buffer, the same pH as the sample. Optional.**	pH control required to suppress ionization of acidic and basic compounds.	Water or buffer; add up to 40 % methanol or acetonitrile to remove more polar interfer-ences; ensure no analyte breakthrough. Maintain pH control.	Methanol. Evaluate other solvents (e.g. hexane*, ethyl acetate* etc.) if additional selectivity is required. Addition of acid or base may be necessary to break ionic secondary interactions.
ISOLUTE 101 Unmodified polystyrene-divinyl-benzene (non-wettable sorbent).	Methanol or acetonitrile.	Water and/or 20-50 mM buffer, the same pH as the sample.	pH control required to suppress ionization of acidic and basic compounds.	Water or buffer; add up to 40% methanol or acetonitrile to remove more polar interfer-ences, ensure no analyte breakthrough. Maintain pH control.	Methanol. Evaluate other solvents (e.g. hexane*, acetone, ethyl acetate* etc.) if additional selectivity is required.

* If using a water immiscible elution solvent, the column should first be thoroughly dried using centrifugation or aspirating air, N_2 or CO_2 for at least 15-20 mins using vacuum or positive pressure.

**Application dependent. Evaluate with and without conditioning and equilibration steps.

Sorbent	Conditioning	Equilibration	Sample pH	Interference Elution	Analyte Elution
Mixed-mode polymer-based sorbents					
EVOLUTE CX	Methanol Optional**	Ammonium acetate (0.05M, pH 6) Optional**	Dilute with ammonium acetate (0.05M, pH 6, 1:3, v/v).	1. Ammonium acetate (0.05M, pH 6) followed by 2. Methanol.	Methanol: ammonium hydroxide (95:5, v/v).
EVOLUTE WCX (A) (Quats and strong bases only).	Methanol Optional**	Ammonium hydroxide (5%, v/v) Optional.**	Dilute with ammonium hydroxide (5%, 1:3, v/v).	1. Ammonium hydroxide (5%, v/v) followed by 2. Methanol.	Methanol: formic acid (98:2, v/v).
EVOLUTE WCX (B) (combined weak and strong bases).	Methanol Optional**	Ammonium acetate (0.05M, pH 7) Optional.**	Dilute with ammonium acetate (0.05M, pH 7, 1:3, v/v).	1. Ammonium acetate (0.05M, pH 7) followed by 2. Methanol.	Methanol: formic acid (98:2, v/v).
EVOLUTE AX (A) (plasma samples).	Methanol Optional**	Ammonium hydroxide (2%, v/v) Optional.**	Plasma. Dilute with ammonium hydroxide (2%, 1:3, v/v).	1. Ammonium acetate (0.05M, pH 7): methanol (95:5, v/v) followed by 2. Methanol.	Methanol: formic acid (98:2, v/v).
EVOLUTE AX (B) (urine or other high ionic strength matrix).	Methanol Optional**	Water Optional.**	Urine. Dilute with ammonium acetate (0.05M, pH 7, 1:3, v/v).	1. Ammonium acetate (0.05M, pH 7): methanol (95:5, v/v) followed by 2. Methanol.	Methanol: formic acid (98:2, v/v).
EVOLUTE WAX	Methanol Optional**	Formic acid (2%, v/v) Optional.**	Dilute with formic acid (2%, 1:3, v/v).	1. Formic acid (2%, v/v) followed by 2. Methanol.	Methanol: ammonium hydroxide (95:5, v/v).
Mixed-mode silica-based sorbents					
ISOLUTE HAX	Methanol	Formic acid (pH 2, 2% v/v).	Dilute with formic acid (pH 2, 2%, 1:1, v/v).	1. Ammonium acetate (0.1M, pH 7.0) followed by 2. methanol: water (1:1, v/v).	Methanol: acetic acid (98:2, v/v).
ISOLUTE HCX, HCX-3, HCX-5.	Methanol	Ammonium acetate (0.05M, pH 6).	Dilute with ammonium acetate (0.05M, pH 6, 1:1, v/v).	1. Ammonium acetate (0.05M, pH 6) followed by 2. Acetic acid (1 M) followed by 3. Methanol.	Methanol: ammonium hydroxide (95:5, v/v).
ISOLUTE HCX-Q	Methanol	Ammonium acetate (0.05M, pH 7).	Dilute with ammonium acetate (0.05M, pH 7, 1:4, v/v).	Methanol: ammonium acetate (0.05M, pH7.0) (20:80, v/v).	0.2M monochloroacetic acid in methanol.
Non-polar silica-based sorbents					
ISOLUTE C18 C18(EC), MFC18, C8, C8(EC), C6, C4, C2, C2(EC), PH, PH(EC), CH(EC) CN, CN(EC).	Methanol or Acetonitrile.	Water and/or 20-50 mM buffer, the same pH as the sample.	pH control required to suppress ionization of acidic and basic compounds. See previous section of Guide on extraction of basic compounds.	Water or buffer; add up to 40% methanol or acetonitrile to remove more polar interferences. Check for analyte breakthrough. Maintain pH control.	Methanol. Evaluate other solvents (e.g. hexane*, ethyl acetate* etc.) if additional selectivity is required. See previous section for additional information for elution of basic compounds using non-polar SPE.

* If using a water immiscible elution solvent, the column should first be thoroughly dried using centrifugation or aspirating air, N_2 or CO_2 for at least 15-20 mins using vacuum or positive pressure.
**Application dependent. Evaluate with and without conditioning and equilibration steps.

Sorbent	Conditioning	Equilibration	Sample pH	Interference Elution	Analyte Elution
Polar silica-based sorbents					
ISOLUTE SI, NH2, CN, DIOL, PSA.	Non-polar solvent e.g. hexane (preferably the same solvent as the sample).	N/A	N/A	Non-polar solvent which may contain a low concentration of a polar modifier that will not elute the analyte (e.g. hexane and ethyl acetate or isopropanol).	Semi-polar solvent mixture e.g. hexane:ethyl acetate. For a non-selective elution use a polar solvent such as methanol.
Ion exchange silica-based sorbents					
ISOLUTE SCX-2 Strong cation exchange.	Methanol or Acetonitrile.	20-50 mM buffer, the same pH as the sample.	At least 2 pH units below the pK of the analyte.	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 breakthrough.	Elute with buffer at least 2 pH units above the pK of the basic analyte. Alternatively use high ionic strength buffer (>0.1 M). Elution with methanol containing 2-5 % ammonia or other volatile base is common.
ISOLUTE SCX-3 Strong cation exchange with significant non-polar secondary interactions.	Methanol or Acetonitrile.	20-50 mM buffer, the same pH as the sample.	At least 2 pH units below the pK of the analyte.	Maintain the same pH as the sample and evaluate the addition of 20-70 % methanol to elute non-polar interferences. % methanol can be increased for additional extract cleanliness. Check for analyte breakthrough.	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.
ISOLUTE CBA Weak cation exchange (sorbent pK 4.8).	Methanol or Acetonitrile.	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.	Buffer at pH <2.8 will to eliminate the charge on the sorbent. Alternatively use high ionic strength buffer (>0.1M), or acidified solvent.
ISOLUTE PE-AX Strong anion exchange (acetate counter ion).	Methanol or Acetonitrile.	20-50 mM buffer, the same pH as the sam-ple. Acetate, formate or hydroxide buffers are recommended.	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.	Elute with buffer at least 2 pH units below the pK of the acidic analyte. Alternatively use high ionic strength buffer (>0.1M), or acidified methanol.
ISOLUTE SAX Strong anion exchange (chloride counter ion).	Methanol or Acetonitrile.	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.	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 ionic strength buffer (>0.1M), or acidified methanol.
ISOLUTE NH2 Weak anion exchange (sorbent pK 9.8).	Methanol or Acetonitrile.	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.	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.

Table 4
Recommended flow rates for method development

Column size	1 mL (A)	3 mL (B)	6 mL (C)
Flow rate	1 mL/min	3 mL/min	7 mL/min

Once the optimum chemistry has been established, optimize flow rate to maximize productivity. Increase flow until breakthrough is observed. The final flow rate should be set at 10 - 20 % lower than the breakthrough limit.

Table 5
Recommended volumes for method development

Step	Volume/100 mg sorbent
Column solvation	250–1 mL
Column equilibration	250–1 mL
Sample application	Application specific, based on sorbent capacity and analyte concentration in sample
Interference elution	250–1 mL
Analyte elution	250 µL–1 mL – dependent on choice of elution solvent. To minimize elution volume, apply 2 x X/2 mL aliquots, including a soak step, rather than 1 x X mL aliquot.

Note: 1 bed volume is approximately 120 µL/100 mg sorbent

Method Optimization Process

In order to optimize an SPE method, the following steps should be followed.

1. Select desired retention mechanism
    - Non-polar
    - Polar
    - Ion exchange
    - Mixed-mode
2. Using the above guidelines, screen sorbents with the selected retention mechanism for retention of standards from a 'clean' matrix similar to the sample type. For example, if assay is to be developed for extraction of drugs from an aqueous biological fluid, first evaluate retention of standards from aqueous buffer.
3. Screen a range of elution solvents for elution of standards. Using the guidelines above, identify the elution solvent giving highest analyte recovery. Note that full recovery should be achieved using 2-10 bed volumes. If larger volumes are necessary, it is likely that a less retentive sorbent or alternative solvent is required.
4. Evaluate the initial procedure established in steps 1 - 3 for a spiked sample of 'real' sample matrix. For example, if assay is to be developed for a biological fluid matrix, and good recovery of analytes has been achieved from spiked buffer samples, move to 'blank' biological fluid, spiked with analytes.
5. Optimize interference elution solvents to achieve cleanest possible extract without loss of analyte.
6. Optimize flow rates for each step to maximize productivity.
7. Optimize sorbent bed mass. If this can be reduced without loss of analyte, benefits in reduction of solvent usage will result. This can be particularly important when working with low volume biological fluid samples.
8. Validate final method.

Troubleshooting SPE Methods

The information in this guide provides a starting point for SPE method development. However, if low or variable analyte recoveries are still a problem once a method has been developed using these guidelines, this section outlines some simple strategies for identifying the nature of the problem.

The most common reasons for low analyte recovery in SPE are:

- Poor analyte retention, or
- Poor analyte elution

To identify inadequate retention, use one of the following methods.

a) Stack two pre-conditioned and equilibrated columns together using an adaptor and load the sample. Separate the columns, and elute analyte from the lower column.

b) Alternatively, collect the eluent during sample load from a single SPE column, and re-extract using a known method (e.g. liquid-liquid extraction).

If analyte is present on the second SPE column (a) or in the liquid-liquid extraction solvent (b), the analyte is not sufficiently retained on the SPE column.

To identify inadequate elution

a) Elute analyte with up to 10 bed volumes of chosen elution solvent. Repeat. If a significant amount of analyte is present in the second elution, the analyte is not being eluted efficiently from the column.

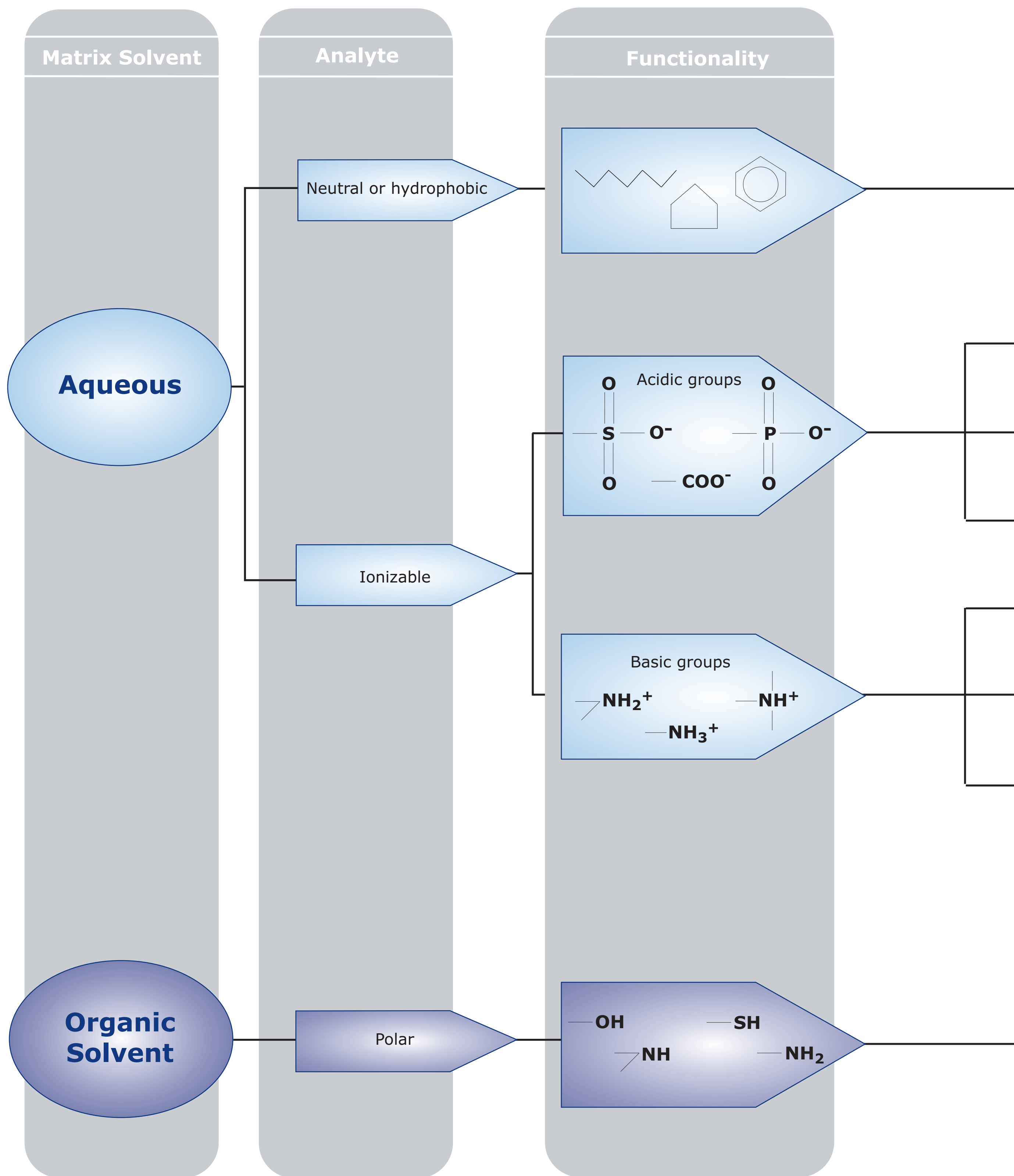
Please do not hesitate to contact 1-Point Support for further assistance in SPE method development or troubleshooting.

Detailed Chemistry Data Sheets for all the sorbents mentioned in this QuickStart Guide are available – visit **www.biotage.com** for details.

Sorbent Selection Guide

Select a SPE Sorbent

Analyte and Matrix Considerations



Retention Mechanism	Comments
Non-polar	
Non-polar	Adjust pH to neutralize analyte 2 pH units < pK
Mixed-mode (Non-polar with Anion Exchange)	Dual retention mechanism provides very clean extracts from biological fluids
Anion Exchange	Adjust pH to ionize analyte (2 pH units above pK). If ionic-strength matrix, dilute or consider non-polar or mixed-mode SPE
Non-polar	Adjust pH to neutralize analyte 2 pH units > pK
Mixed-mode (Non-polar with Cation Exchange)	Dual retention mechanism provides very clean extracts from biological fluids
Cation Exchange	Adjust pH to ionize analyte (2 pH units below pK). If high ionic strength matrix, dilute or consider non-polar or mixed-mode SPE
Polar	

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