SPE Modes of Operation

This Chemistry Data Sheet discusses five typical ways of using SPE, Six Step SPE on page 1, Four Step SPE on page 4, Stacked Columns on page 5, Layered Columns on page 7 and Mixed-mode Columns on page 9. Each one offers a solution to different types of sample preparation challenges.

Mode	Sample Purification	Trace Enrichment	Ease of Automation
6-step	***	***	****
4-step	**	N/A	****
Stacked columns	****	****	N/A
Layered columns	****	****	****
Mixed-mode columns	****	***	****

N/A = not applicable

1. Six-Step SPE

Six-step SPE is the most commonly used mode (see **Figure 1** for illustration of six-step procedure). It can produce extracts having high purity, concentrated analytes, and is easily automated. This mode involves the following 6 steps:

1. Sample pre-treatment
2. SPE column solvation
3. SPE column equilibration
4. Sample loading
5. Interference elution
6. Analyte elution

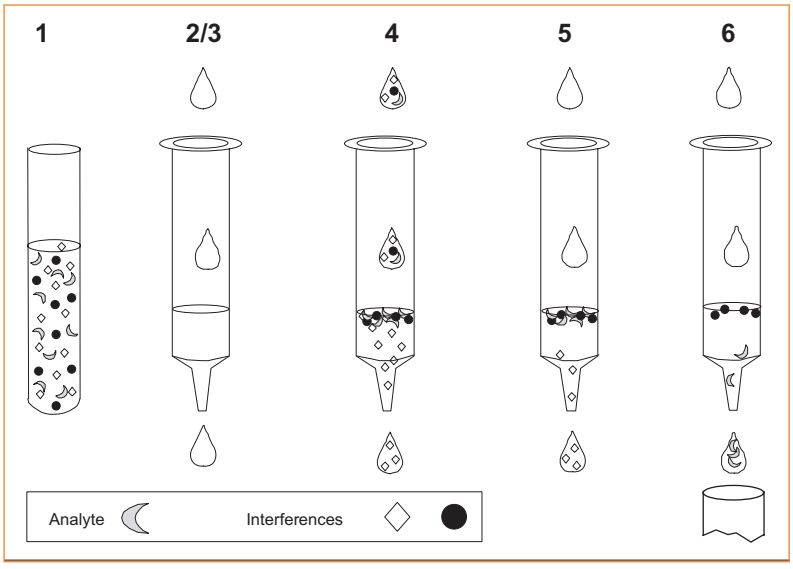

***Figure 1**. Six-Step SPE Procedure*

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Sample Pre-treatment

This step involves preparing the sample both physically and chemically for the SPE extraction in order to optimize conditions for the chosen retention mechanism. The type of sample pre-treatment will differ, depending on the stability of the analytes, type of matrix, and nature of the interactions between the analytes with the sorbent, and type of sorbent.

Aqueous Samples: Reagents may be added to stabilize the analyte. For aqueous samples containing analytes that are to be retained primarily by hydrophobic interactions, pH adjustment may be required to ensure that the surface and analyte (if ionizable) are not charged. It may be necessary to add 1 to 2% wetting agent (e.g. methanol) to large volume samples (> 100 mL) to maintain an active sorbent surface. Where the primary interaction for analyte retention is ion exchange, the pH should be controlled to ensure that total ionization of the analyte and surface of the sorbent has occurred. Ionic strength must also be controlled in order to facilitate maximum retention of analytes. The ionic strength of the sample should be reduced to < 0.05 M by dilution with deionized water or low ionic strength buffer. The selectivity of the buffer cation (for cation exchange) or anion (for anion exchange) should be considered. Buffers that contain ions of lower selectivity than the analyte itself facilitate analyte retention. The selectivity of some common cations is as follows (ions on the right will displace those on the left):

$$Li^+ < H^+ < Na^+ < NH_4^+, RNH_3^+ < K^+ < Mg^{2+} < Ca^{2+}$$

Selectivity of some common anions (ions on the right will displace those on the left):

$$OH^- < \text{acetate} < \text{formate} < HCO_3^- < Cl^- < HSO_3^- < CN^- < \text{citrate} < \text{benzene sulphonate}$$

Aqueous and non-aqueous samples: Dilution may be necessary to reduce sample viscosity, ensuring a free-flowing sample.

SPE Column Solvation

Solvation is necessary to prepare the SPE column for the extraction process. For retention of analytes to occur, the bonded phase must be able to interact with the sample matrix. A solvent is passed through the column to 'wet' the sorbent, and ensure interaction. The sorbent bed should not be allowed to dry out after solvation.

Aqueous samples: The sorbent is wetted with an organic solvent such as methanol.

Non-aqueous samples: The sorbent is wetted with the matrix solvent.

SPE Column Equilibration

To maximize retention of the analyte by the sorbent, the column is treated with a solvent that is as 'matrix-like' as possible. A typical volume of equilibration solvent is 0.5-2 mL /100 mg of sorbent. The sorbent bed should not be allowed to dry out between equilibration and sample loading.

Aqueous samples: The equilibration solvent should be similar to the sample matrix with respect to pH and ionic strength. If ion exchange is being used as the analyte retention mechanism, the ionic strength should be < 0.05 M. This step is often used to insure the presence of an appropriate counter ion on an ion exchange column. **See sample pre-treatment for counter ion selection.**

Non-aqueous samples: Not required, since the previous step is typically performed using the matrix solvent.

Sample Loading

Optimization of loading flow rates is an important part of method development. A good starting point is 1 mL/min for a 1 mL cartridge, 3 mL/min for a 3 mL cartridge and 7 mL/min for a 6 mL cartridge (wider diameter cartridges yield lower linear velocities). The flow rate can be increased after the method chemistry is optimized. Flow rate is increased until some sample breakthrough is seen (as indicated by a drop in recovery). A flow rate slightly lower than the upper limit should be used. The optimum flow rate should be controlled and recorded to ensure reproducibility.

Interference Elution

The purpose of interference elution is to selectively remove undesired compounds from the sorbent without eluting the analytes. Generally, a solvent is selected which is miscible with the sample matrix and in which the analytes are poorly soluble. A typical volume of interference elution solvent is 1-2 mL/100 mg of sorbent. The flow rate should be adjusted such that the solvent is in contact with the sorbent for 1-2 min.

Aqueous samples: Ionic strength and pH control should be maintained at this stage to prevent analyte loss. A good choice of solvent is often the equilibration buffer. A buffer containing 10-30% methanol or acetonitrile is often suitable for removing lipophillic interferences. Cartridge drying may be necessary to remove water if the elution solvent is water immiscible. Drying can be performed by vacuum aspiration, N_2 or CO_2 flow, or centrifugation (useful if the analytes are volatile). Drying times depend on factors such as sorbent type, bed dimensions, solvent to be selected for elution, and drying method. A typical range, depending on the degree of dryness required, is 30 seconds to 30 minutes. If a water miscible elution solvent is selected, the time required for cartridge drying can be reduced or eliminated. If the sample is to be concentrated to a smaller volume after elution, and the drying step was reduced or eliminated, care should be taken not to reduce the volume of solvent to where phase separation occurs, or the analytes precipitate.

Elution

The elution solvent should be one in which the analytes are soluble. It must often overcome primary and secondary retention mechanisms, and so a solvent or mixture of solvents offering multiple interactions is usually most effective. The elution solvent should be compatible with the final analysis technique. For example, for an HPLC analysis, a solvent similar to the mobile phase is a good choice of elution solvent. A volatile solvent is generally selected for subsequent GC analysis. Other factors to consider include whether there will be a derivatization step, as well as volatility of the solvent if further concentration is required. A minimum volume of elution solvent allows maximum concentration of the analytes. A typical minimum elution volume is 250 µL /100 mg of sorbent. Flow control is important to ensure reproducibility. The use of two small aliquots of solvent with a 1-4 minute soak step between elution volumes is often more efficient than one large aliquot. If a single elution is required, the flow rate of the elution solvent should be such that contact time between solvent and sorbent is 1-4 minutes.

Aqueous samples: A water miscible elution solvent may be used to elute analytes and minimize cartridge drying times (see interference elution in previous step). For analytes that are retained by ion exchange, high ionic strength (> 0.1 M) buffers can be used for elution. The high concentration of ions in the buffer will compete with an ionic analyte for ion exchange sites on the sorbent, thus causing elution of the analyte. For doubly charged analytes, buffers of > 0.2 M should be used. Buffers containing ions with a higher affinity for the sorbent than the analyte can be used for elution by displacement of the ionic analyte (**see selectivities in sample pre-treatment section**). An organic component in the elution solvent may be necessary to overcome secondary hydrophobic interactions.

Summary

Six-step SPE is the most common approach for solid phase sample preparation. In each of the steps, conditions must be optimized for interactions between the analyte, matrix and sorbent. These conditions include pH, ionic strength, solvent strength, solvent volume and flow rates.

2. Four-Step SPE

Four-step SPE is commonly used when trace enrichment is not required, but interferences must be removed (see **Figure 2** for illustration of a Four Step SPE Procedure). The interferences are retained on the column, while the analytes of interest pass through the sorbent bed and are collected with the sample solvent. A highly purified extract is produced. This mode is easily automated. The steps involved are:

1. Sample pre-treatment
2. Column solvation
3. Column equilibration
4. Sample loading

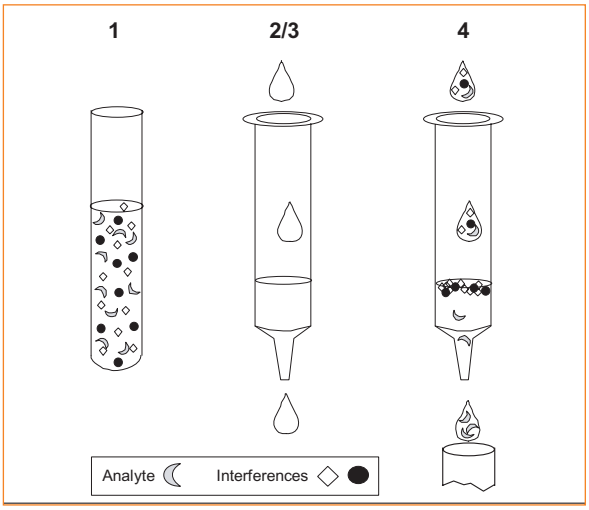

***Figure 2**. Four-Step SPE Procedure*

Sample Pre-treatment

As for the six-step procedure, except the conditions are selected in order to optimize retention of interferences, while minimizing interactions between the sorbent and analytes. As an example, for a non-polar analyte, to remove polar interferences using a polar sorbent phase, the sample environment should be made as non-polar as possible.

Column Solvation

As for the six-step procedure.

Column Equilibration

As for the six-step procedure, with conditions being chosen to maximize retention of interferences and minimize retention of analytes.

Sample Loading

As for the six-step procedure. Increase loading rate until breakthrough of interfering species is observed.

The eluent contains the analytes and should be collected. Further processing may be required.

Summary

The four-step mode should be selected when interferences are present, but analyte concentration is not required. It differs from the six-step procedure primarily in that the sample loading step is also the step in which the analytes are eluted.

3. Stacked Columns

Stacked columns are typically used when species are present that can be retained by both hydrophobic and ion exchange mechanisms. This mode can be used in either of two ways.

Case I: Samples containing analytes and interferences that can be retained by different mechanisms can be easily separated by using two different sorbents in separate columns. The interferences are retained on the top column, while the analytes are concentrated on the bottom column. The top column can then be discarded while the analytes are eluted from the bottom column. This mode of SPE combines principles of the previous two modes, but since it utilizes two columns stacked vertically, it is unsuitable for automation (see **Figure 3** below for illustration of column preocedure).

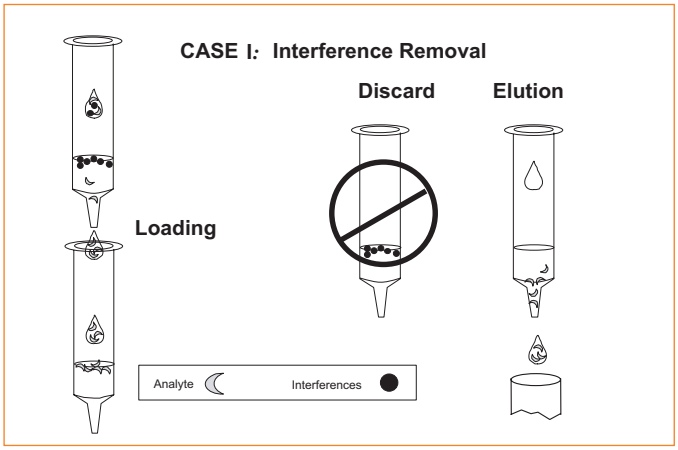

***Figure 3**. Stacked Column Procedure - Interference Removal*

Sample Pre-treatment

As for the six-step procedure. Conditions should be optimized for retention of analytes on the bottom column.

Column Solvation and Equilibration

As for the six-step procedure, with the columns stacked vertically using an ISOLUTE adaptor. Optimal conditions for analyte and interference retention should be considered on each phase.

Sample Loading

As for the six-step procedure.

Interference Elution

After loading, the top column (which retains interferences) should be discarded. Elution of additional interferences from the bottom column should be as in the six-step procedure.

Analyte Elution

As for the six-step procedure.

Case II: Samples containing analytes having a broad range of properties, or analytes requiring different analyte methods can be extracted using stacked phases. As an example, a cation exchanger can be stacked on top of a hydrophobic phase. Basic analytes will be retained on the anion exchanger, while hydrophobic species will be retained on the bottom column. The columns can then be separated and eluted separately. If different detection schemes are required (e.g., HPLC for the basic compounds and GC for the hydrophobic species) then the appropriate elution solvent for each detection method should be selected. The stacked column configuration is especially useful for method development, when a determination can be made at each step as to where the analyte(s) are retained. This is a very useful approach for method optimization (see **Figure 4** for illustration of stacked column procedure).

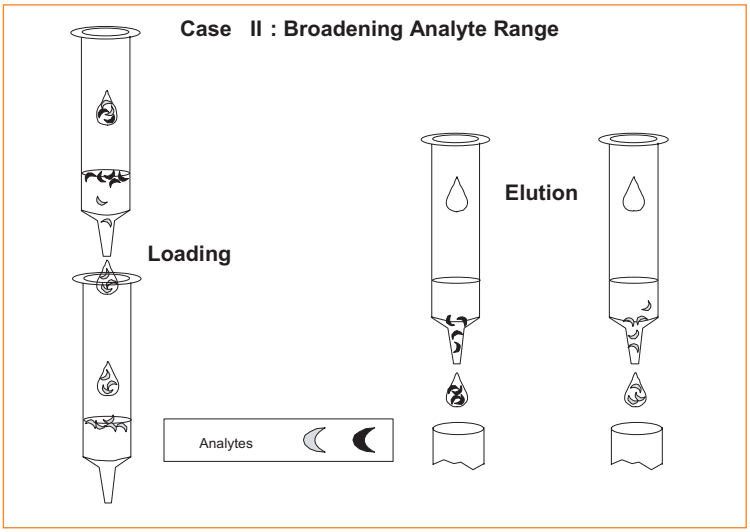

***Figure 4**. Stacked Column Procedure - Broadening Analyte Range*

Sample Pre-treatment

This should be as for the six-step method, with the matrix environment optimized for the efficient retention of analytes of interest on each column.

Column Solvation and Equilibration

As in the six-step procedure, with the columns stacked vertically using an ISOLUTE adaptor.

Sample Loading

As in the six-step procedure.

Interference Elution

Columns can be separated, and interferences eluted from each as in the six-step procedure.

Analyte Elution

If columns were not separated during interference elution, they should now be separated and analytes eluted from each column as in the six-step procedure.

Summary

The stacked column mode can improve interference removal as in Case I, or allow for different detection schemes, as in Case II. It has the added advantage of enhancing method development, by allowing optimization of bed mass, flow rates and solvent selection for each phase. This is particularly useful for developing methods where the final method will utilize mixed mode or layered phases.

4. Layered Columns

The use of layered columns is similar to stacked columns, in that they take advantage of species that can be retained by different types of interactions, or degree of interaction. In contrast to stacked columns, the two sorbents are contained in the same cartridge. Layered columns can be used in one of two ways.

Case I: Samples containing analytes and interferences with differing properties can be retained on different layered phases. The interferences are retained on the top layer, while the analytes are retained on the bottom layer. Elution conditions are selected such that the interferences continue to be retained while the analytes are eluted. This configuration has the advantage over stacked phases in that a method utilizing layered phases is amenable to automation (see **Figure 5** for illustration of Layered Column Procedure).

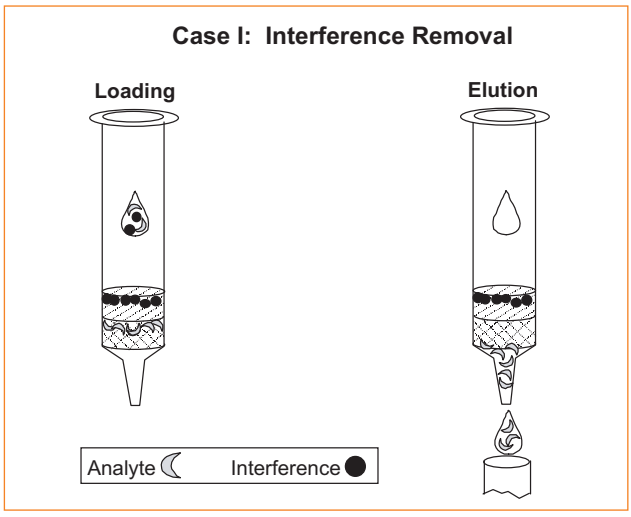

***Figure 5**. Layered Column Procedure - Interference Removal*

Sample Pre-treatment

This should be as for the six-step method, with the matrix optimized for the efficient retention of interferences on the top phase and analytes of interest on the bottom phase.

Column Solvation, Equilibration, Loading

As in the six-step procedure.

Interference Elution

Elute interferences that were not retained on the top layer as in the six-step procedure.

Analyte Elution

As for the six-step procedure. Elution conditions should be such that interferences are retained on top layer, while analytes are eluted from bottom layer.

Case II: Samples containing analytes with a broad range of properties, where different phases would be appropriate to optimize retention as well as elution, can be extracted using layered phases. For example, a sample may contain both high and low molecular weight analytes. A hydrophobic phase modified with a short chain hydrocarbon (e.g., C2) would be useful for retaining the high molecular weight compounds, but would be inadequate for retaining the low molecular weight species. Conversely, a hydrophobic phase such as C18 would retain both low and high molecular weight species, but elution of the latter from a C18 phase is often difficult. Application of a layered C2/C18 column accommodates the retention and elution of the full range of analytes (see **Figure 6** for illustration of Layered Column Procedure).

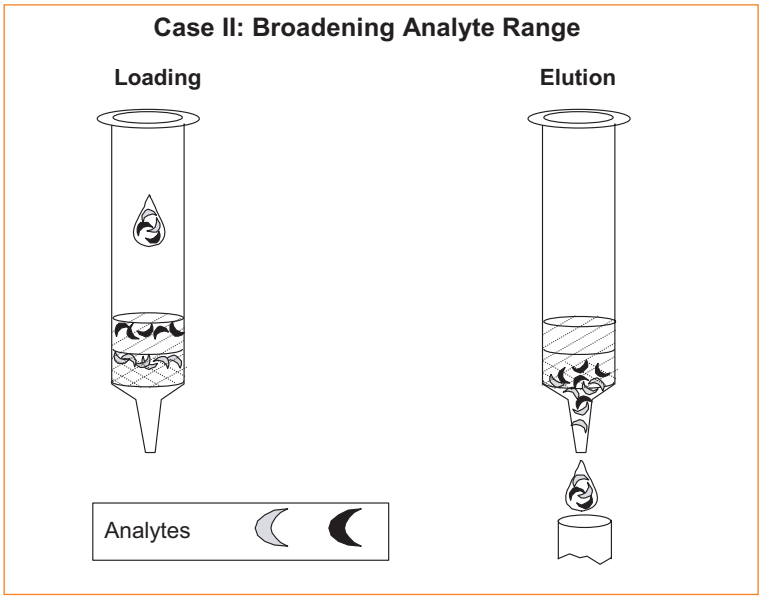

***Figure 6**. Layered Column Procedure - Broadening Analyte Range*

Sample pre-treatment, column solvation, equilibration, sample loading, and interference elution

As for the six-step procedure.

Analyte Elution

Analytes should be eluted as described in the six-step method. Elution conditions should be such that the elution solvent is strong enough to solvate analytes eluted from the top phase, so that they pass unretained through the bottom layer.

Summary

The layered column mode allows for an improvement in selectivity when interferences can be retained by a different mechanism than the analytes. It also broadens the analyte range when analytes differ in the degree to which they are retained on similar types of sorbents.

5. Mixed-mode Columns

ISOLUTE mixed-mode columns contain mixtures of C8 with either a strong anion (HAX) or strong cation exchanger (HCX) (See **Figure 7** for illustration). These columns are designed to utilize multiple interactions of the analyte or different types of interactions from more than one type of analyte. Mixed-mode columns allow the development of more robust procedures, which are less dependent on the matrix, making cleaner extracts possible. Mixed-mode columns can be used in either of two ways.

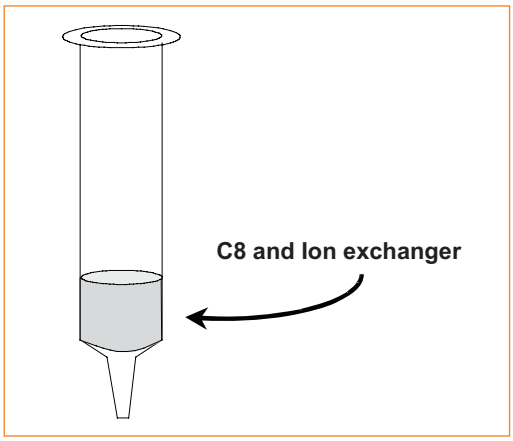

***Figure 7**. Mixed-mode Columns*

CASE I: One type of analyte is present and a very clean extract is required.

Sample Pre-treatment:

This should be as for the six-step method, with the matrix optimized for the efficient retention of the analyte based on one of the mechanisms that are available. For example, if the analyte is capable of being retained by either hydrophobic or ion exchange interactions, and is in a matrix having a high ionic strength, conditions should be optimized for the hydrophobic mechanism.

Column Solvation

As for the six-step procedure.

Column Equilibration

As for the six-step procedure, and consistent with mechanism selected for sample pre-treatment.

Sample Loading

As for the six-step procedure.

Interference Elution

The utility of mixed-mode columns is realized during the interference elution step. If the analyte is retained initially by hydrophobic interactions, then an initial rinse with an aqueous solvent of low ionic strength can be used to displace interfering ionic species. This can be followed with a rinse by an organic solvent to remove lipophilic interferences. Appropriate pH conditions should be maintained so that the analyte is charged during the organic rinse step, and transfer of the analyte to ion exchange sites is ensured.

Analyte Elution

As for the six-step procedure.

CASE II: Acidic, basic and/or neutral analytes are present. Analytes are to be recovered in separate elutions.

Sample Pre-treatment

The matrix is optimized for the efficient retention of the analytes based on both ion exchange and hydrophobic interactions. For example, the extraction of acidic, basic and neutral compounds can be performed on HCX (hydrophobic phase + strong cation exchanger). In this case, the pH is adjusted to neutralize the acidic compounds, and ionize the basic compounds. The acidic and neutral compounds will be retained by hydrophobic interactions, while the basic compounds will be retained primarily by ion exchange.

Column Solvation
As for the six-step procedure.

Column Equilibration
As for the six-step procedure, and consistent with mechanisms selected for sample pre-treatment.

Sample Loading
As for the six-step procedure.

Interference Elution
As for the six-step procedure.

Analyte Elution
Analytes can be selectively eluted by judicious choice of elution solvent. For the example given above, the acidic and neutral analytes can be eluted with an organic solvent. Conditions can be maintained such that the basic compounds continue to be retained during this first elution. Basic compounds can then be eluted as in the six-step procedure.

Summary
Mixed-mode columns are available for applications for both acidic and basic compounds. They are useful for producing very clean extracts, as well as for fractionating mixtures of compounds.

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