

Solid-Phase Extraction Products

Introduction to Solid-Phase Extraction

Grace® Solid-Phase Extraction products provide fast and efficient pre-analysis sample clean-up and concentration. Obtaining optimal results from these products requires attention to four related factors:



The solubility of a compound in a particular solvent is an important consideration. An excellent wash solvent would dissolve interferences but not the analyte. Likewise, if the analyte is not soluble in a solvent, that solvent will not work as an elution solvent.

- Proper physical and chemical characterization of the sample.** Factors such as the analytes' polarity relative to the matrix, the presence of charged functional groups, solubility, molecular weight, etc. determine how strongly the analyte is retained by the packing bed.
- Selection of proper retention strategy.** Two approaches are possible: First, the analyte can be unretained by the packing bed while interfering compounds are retained, thus cleaning up the sample. Secondly, the analyte is retained by the packing bed while interferences are either unretained, or washed off of the packing bed prior to eluting the analyte. This second approach is commonly employed when concentration of the sample is desired.
- Selection of the proper packing type and bed size.** The different packing types offer various selectivities that should be exploited to maximize the structural differences between the analyte and sample interferences. Choosing the packing with the proper selectivity results in the cleanest extract with the highest recovery. Poor sample recovery often occurs when the packing bed size is not optimized. Too large a bed results in incomplete elution while too small a bed results in incomplete retention. Both situations result in poorer than expected recoveries.
- Selection of suitable conditioning, wash and elution solvents.** Attention should be paid to solvent strength relative to the packing material (**Table 1**). The final conditioning solvent should be a "weak solvent" which will not act as an elution solvent. Buffers should be used to control ionization of potentially charged compounds. Wash solvents should remove weakly retained interferences but should not be strong enough to elute the analyte. Elution solvents should be strong enough to completely elute an analyte in a small volume (1-2mL).

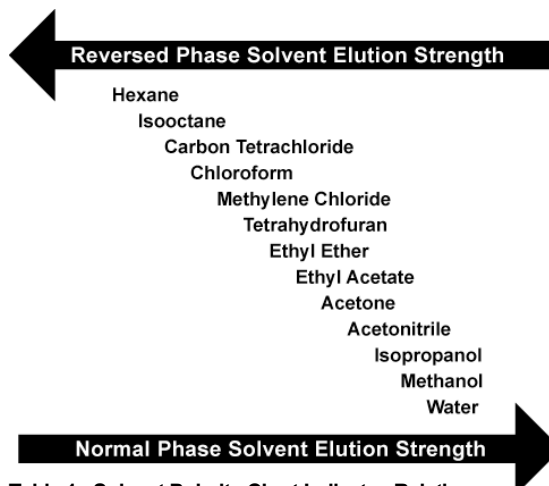


Table 1 - Solvent Polarity Chart Indicates Relative Elution Strength

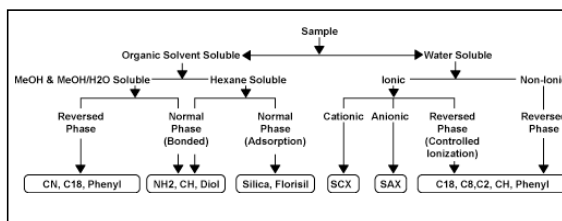


Table 2 – SPE Packing Selection Chart

Mode	Packing Type	Compounds Retained	Conditioning Solvents	Elution Solvents
Reversed-Phase	C18, C8, C4, C2, Cyclohexyl, Phenyl	Non-polar to moderately polar in a polar matrix	Organic solvent (i.e. ACN or MeOH) followed by water	MeOH or solvents which are more non-polar
Normal-Phase	Silica, Florisil, Amino, Cyano, Diol, Alumina	Polar compounds in a non-polar matrix	Non-polar solvent	Intermediate to polar solvents
Anion exchange	SAX	Anionic Compounds	DI water or low concentration buffer	Strong buffer or low pH buffer
Cation exchange	SCX	Cationic Compounds	DI water or low concentration buffer	Strong buffer or high pH buffer

How to Use Grace® Solid-Phase Extraction Products

SPE cartridges are designed for manual use with a luer-hub syringe. (Figure 1). SPE columns, when equipped with an adapter, can be used manually but are primarily designed for use with some type of vacuum system or automated equipment. 12 or 24 port vacuum manifolds are available and provide the ability to process multiple samples.

In addition to a vacuum manifold (Figure 2) SPE columns can be used with a syringe to pull (Figure 3) or push (Figure 4) a sample/solvent through the tube. Other methods for using SPE columns include placing the column into a centrifuge and spinning the sample/solvent down, (Figure 5) or modifying a rubber stopper for a vacuum flask by boring a hole in the stopper equal to the diameter of the tube (Figure 6).

SPE Cartridges



Figure 1 - by Syringe

SPE Columns



Figure 2 - by Vacuum Manifold

SPE Columns



Figure 3 - Pulling by Syringe



Figure 4 - Pushing by Syringe



Figure 5 - by Centrifuge



Figure 6 - by Filter Flask

General Extraction Protocols

Reversed-Phase:

A. Conditioning

- Rinse packing with 3-5mL methanol.
- Rinse packing with 3-5mL water or buffer. Don't let packing dry before adding sample.

B. Sample Application

- Apply sample to the top of the packing bed. Push or draw the sample through the bed at a flow rate of 1-5mL/minute. Collect sample for analysis if desired compound isn't retained.

C. Wash

- If the desired compound was retained, wash off any weakly retained interfering compounds with a polar solvent.

D. Elution

- Elute desired compound with 1-2mL of non-polar solvent and collect for analysis.

Normal-Phase:

A. Conditioning

- Rinse packing bed with 3-5mL of a non-polar solvent.

B. Sample Application

- Apply sample to the top of the packing bed. Push or draw the sample through the bed at a flow rate of 1-5mL/minute. Collect sample for analysis if desired compound isn't retained.

C. Wash

- If the desired compound was retained, wash off any weakly retained interfering compounds with a non-polar solvent.

D. Elution

- Elute desired compound with 1-2mL of a polar solvent and collect for analysis.

Ion Exchange:

A. Conditioning

- Rinse packing bed with 5mL of deionized water or low ionic strength buffer (e.g. 0.001M-0.01M).

B. Sample Application

- Apply sample to the top of the packing bed. Push or draw the sample through the bed at a flow rate of 1-2mL/minute. Collect sample for analysis if desired compound isn't retained.

C. Wash

- If the desired compound was retained, wash off any weakly retained interfering compounds with deionized water or low ionic strength buffer.

D. Elution

- Elute desired compound with 1-5mL of a high salt concentration solution (e.g. 0.1-0.5M) or change elution buffer pH such that the sample compound is no longer ionized and collect for analysis.



CAUTION

When using hazardous solvents, always wear appropriate personal protective equipment (eye protection, gloves, lab coat, etc.).



CAUTION

When using a centrifuge always follow the manufacturer's safety procedures.

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