Resprep SPE Products

General

Solid-phase extraction (SPE) is a sample preparation technique that can be used for purification, enrichment/concentration, fractionation, or solvent exchange. Strategies are based on either analyte retention/cleanup/elution (matrix is washed through) or analyte pass-through (matrix is retained). The procedure described here uses analyte retention/cleanup/elution. SPE typically involves sample pretreatment, conditioning/equilibration of the SPE sorbent (required for reversed-phase extraction), sample loading, washing, and elution. However, not all steps are always necessary, so the exact procedures for a given analyte and matrix should be established in the lab prior to processing analytical samples.

Resprep products are available in cartridges for manual extraction and in 96-well plates for automated methods. A comparison of extraction methods based on different sorbent chemistries is provided in Table I. All cartridges and plates may be processed by positive pressure, vacuum (manifold or sidearm flask), or centrifugation.

Table I: Extraction Method Overview

Extraction Method	Sorbent Packing Material	Analytes	Sample Matrix	Conditioning Solutions (Weak Solvents)	Elution Solution (Strong Solvent)**
Reversed phase	C8, C18, Carbon, Diol, NH_2,* PSA*, Styrene-divinylbenzene (SDVB)	Nonpolar or hydrophobic (low to moderate polarity)	Polar (often aqueous)	(1) Methanol, then water or buffer (same as sample), or (2) final extraction solvent, then water-miscible solvent, then water or buffer.	Nonpolar solvent or mixed solvent solution (e.g., acetonitrile, methanol, or aqueous/organic mixture). If using multiple elution solutions, increase the moderate to high polarity organic content as you progress.
Normal phase	Alumina (A, B, and N), Carbon, Diol, Florisil, NH_2,* PSA,* Silica	Polar/uncharged (moderate to high polarity)	Nonpolar (often organic solvent)	Fresh, nonpolar organic solvent (e.g., hexane, toluene).	Fresh solvent (e.g., methylene chloride, methanol). If using multiple elution solutions, increase the moderate to high polarity organic content as you progress.
Ion exchange	• Weak cation exchange (WCX) • Strong anion exchange (SAX)	Charged or ionized	Aqueous/low ionic strength	• WCX: Low ionic strength buffer, pH >4 • SAX: Low ionic strength buffer, pH <8	• WCX: High ionic strength buffer, or pH <2 • SAX: High ionic strength buffer, or pH >10

* In addition to their primary use mechanisms (reversed-phase or normal phase), NH_2 and PSA may also be used as weak anion exchange (WAX) sorbents. PSA has a higher ion-exchange capacity than NH_2.
**Strong solvents are also used in the initial wetting step (if performed) that occurs prior to conditioning.

If using cartridges, choose the proper size by (1) closely matching the sample volume to the cartridge volume and bed weight, and (2) closely matching the analyte load to the bed weight or exchange capacity of the packing material. Table II gives approximations for guidance purposes; volume and load should be adjusted to specific sample and analyte characteristics.

Table II: Guidelines for Cartridge Size Selection

Sample Volume	Analyte Load	Cartridge Size	Bed Weight
1-10 mL	2-6 mg	1 mL	30-300 mg
10-100 mL	6-1000 mg	3 and 6 mL	300-1000 mg
100-1000 mL	>1000 mg	6 mL and higher	500+ mg

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Sample Pretreatment

- **Solid Samples**

  Solid samples must be either dissolved or extracted prior to loading. For polar analytes, use a polar organic solvent, such as methanol or acetonitrile. For nonpolar analytes (and multiresidue samples), use an organic solvent, such as dichloromethane or acetone), and a drying agent.

  - Ensure the sample is properly homogenized and initially extracted into either an aqueous or organic extract using conditions that have been optimized to provide the maximum initial extraction efficiency with minimal coextraction of unwanted matrix components. This step may benefit from the addition of buffers, dispersive salts, or the use of cosolvents to influence extraction efficiency.
  - Initial extracts of solid samples may require pH adjustment, solvent composition adjustment, or even evaporation and reconstitution using a different solvent in order to optimize SPE sorbent performance. Filtration or centrifugation may also be needed to remove particulates prior to extraction.

- **Aqueous Samples**

  If the sample contains suspended matter, filtration or centrifugation may be needed prior to extraction.

- **Nonaqueous Liquid Samples**

  It may be possible to dilute a nonaqueous liquid sample using buffered water and organic cosolvents, and then treat it as an aqueous sample in the following procedures. Nonaqueous liquid samples that are soluble in hexane should be diluted or exchanged into hexane.

Extraction Steps

The following are general extraction steps that are applicable for using Resprep SPE cartridges and well plates in reversed-phase, normal phase, ion-exchange, and mixed modes. These steps can be optimized through method development for specific analyte/matrix combinations.

Note that Resprep sodium sulfate cartridges are typically used just to remove residual water from extracts as a final step in extractions using other cartridges, but they may also be used independently if water removal is the only sample preparation requirement.

1. **Setup**

   Mount Resprep SPE cartridges/plates to a vacuum manifold along with a waste collection vessel of sufficient size. Alternately, positive pressure or centrifugation setups may be used.

2. **Conditioning**

   The following steps are recommended to prepare the sorbent for interaction with the analyte:

   a. All SPE cartridges/plates should be conditioned to wet and settle the bed, activate the sorbent, ensure maximum surface area for sorbent-analyte interactions, and remove any residual process materials (e.g., fines).

   b. Use 1 to 2 cartridge or well volumes of the conditioning solution(s) recommended in Table I (strong solvent to wet the bed, followed by equilibration with a weak solvent).

   c. Use a high flow rate.

   d. Do not allow the sorbent bed to dry before adding sample.

3. **Sample Load**

   If necessary, adjust pH and dilute sample according to the Sample Pretreatment section above before loading the sample. See also the Preventing Breakthrough section at the end for examples of when dilution and pH adjustment may be beneficial.

   a. Load the diluted sample.

   b. Slowly apply vacuum, starting at the lowest possible setting, to pull the entire sample volume into the SPE sorbent bed. Gradually increase vacuum as necessary.

   c. Pass sample completely through the sorbent packing using a dropwise flow rate to ensure adequate interaction time between the analytes and the sorbent.

4. **Wash**

   Washing is performed to remove matrix interferences while retaining analytes of interest. Use a weak solvent to remove weakly retained matrix components.

   a. Add the appropriate volume of wash solvent.

   b. Apply vacuum to the cartridge/plate.

      i. Set vacuum initially to 5" Hg and adjust as necessary to fully elute the wash solvent.

      ii. To ensure sufficient interaction, establish a flow that results in the formation of discrete drops. For aqueous samples, you may need to increase the vacuum in order to obtain an appropriate flow.

   c. Continue to apply vacuum for an additional 30-60 seconds to ensure the elimination of any residual wash solvent.

5. **Discard Waste**
    a. Turn off the vacuum, making sure to first reduce it to its lowest setting, so that it will be at the proper level for the elution step.
    b. Release the vacuum from the manifold and discard the waste liquid.
    c. Insert new, clean collection vessel(s) and replace the manifold cover.

6. **Drying (only when necessary)**
    a. Use an analytical-grade, inert gas (e.g., nitrogen) or draw vacuum through until the packing appears dry.
    b. Use caution when drying semivolatiles; always dry for the minimum suggested length of time (i.e., 1-10 minutes).

7. **Elution**

    Use a strong solvent or buffer solution following the recommendations in Table I to elute the analytes (strongly bound interferences will be retained in the sorbent bed). Elution solvent volume and flow rate should be precisely controlled to ensure reproducible results.
    a. Add the appropriate volume of the elution solvent.
    b. Allow the elution solvent to flow by gravity before applying vacuum.
    c. Apply vacuum at the lowest setting and gradually increase the vacuum as necessary. Establish a flow that produces discrete drops to ensure sufficient interaction.
    d. Continue to apply vacuum for an additional 30-60 seconds to collect all of the elution solvent.
    e. Turn off the vacuum and release it from the manifold.
    f. Remove the collection vessel(s).

8. **Prepare Extract for Analysis**

    Transfer the extracts from the collection vessel to an appropriate analysis vessel. If using plates, be sure to cover the well plate with a sealing mat.

    Note: If needed, evaporation and reconstitution may be performed prior to transfer to concentrate the sample extract. If initial analysis indicates cleaner extracts are needed to lower background noise and improve sensitivity and/or selectivity, the extraction process can be optimized by varying pH and solvent concentration in the wash and elution steps.

Storage

To ensure best performance, do not open packaging until ready for use. To store open packages, squeeze out air, tape shut to reseal, and place in desiccator.

Preventing Breakthrough

Breakthrough occurs when the target analytes are not fully retained on the sorbent and pass through prior to the elution step. This is undesirable and leads to poor recovery for all methods (except for pass-through cleanup procedures, which are based on it). Breakthrough can be minimized using the troubleshooting tips in Table III.

Table III: Breakthrough Mitigation Strategies

Cause	Solution
Sample loading solution is too highly organic for polar analytes.	Dilute sample 1:1 (or more) with water or buffer before loading.
Analytes are bound to proteins that travel through the sorbent packing.	Treat sample with acid or base to free analytes prior to loading.
Analyte concentration exceeds sorbent capacity.	Review Table II and select an SPE product with enough sorbent mass to prevent overloading.
Inadequate contact time between analyte and sorbent.	Reduce flow to a slower dropwise rate (it should not be a continuous stream).

Questions about this or any other Restek product?
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#800-00-001 Rev. date: 02/21

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