Supel™-Select SPE

Supel-Select SPE is a series of hydrophilic modified styrene based polymer SPE phases ideal for extracting a broad range of compounds from aqueous samples. The retention mechanisms for the different phases range from reverse phase to a combination of reverse phase and ion exchange. However because the phase is hydrophilic modified, the phase is also selective for more polar compounds.

Specifications:

**Phase Chemistry:**
- **HLB:** Hydrophilic modified styrene
- **SAX:** Quaternary amine functionalized hydrophilic modified styrene
- **SCX:** Sulfonic acid functionalized hydrophilic modified styrene

**Particle size:** 50-70 µm
**Pore size:** 80-200 Å
**Pore volume:** 0.8-1.2 mL/g
**Surface area:** 160-420 m²/g
**MS Friendly:** Yes

**SPE Volume Guidelines**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>30 mg/1 mL</td>
<td>0.5 – 1 mL</td>
<td>0.3 – 1 mL</td>
</tr>
<tr>
<td>60 mg/3 mL</td>
<td>1 – 3 mL</td>
<td>0.5 – 3 mL</td>
</tr>
<tr>
<td>200 mg/6mL</td>
<td>3 – 6 mL</td>
<td>1 – 6 mL</td>
</tr>
<tr>
<td>500 mg/12mL</td>
<td>5 – 12 mL</td>
<td>2 – 12 mL</td>
</tr>
<tr>
<td>1 g/20 mL</td>
<td>8 – 20 mL</td>
<td>3 – 20 mL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Well plates:</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 mg/well</td>
</tr>
<tr>
<td>60 mg/well</td>
</tr>
<tr>
<td>0.5 – 2 mL</td>
</tr>
</tbody>
</table>

* The SPE volumes listed are general guidelines and are dependent on analyte and sample matrix relative to desired SPE speed, recovery, and selectivity.

**RECOMMENDED METHODS:**

**Sample Pre-treatment:**
- Filter or centrifuge, if organic solvent used for extraction, evaporate and reconstitute in aqueous buffer.
- Dilute sample 1:1 with buffer or DI water

**Condition with:** Methanol followed by water or buffer

**Load sample:** prepared from sample pre-treatment

**Wash:**
1. water/buffer
2. 10% methanol or 5% acetonitrile with pH modifiers

**Analyte elution:** methanol:acetonitrile (50:50 v/v) with pH modifiers

**HLB**
- **Basic** – adjust the sample pH to at least 2 pH units above analytes pKa
- **Acidic** – adjust sample pH to at least 2 pH units below analytes pKa

**SCX**
- For most compounds, pH 7 loading buffer can be used.
- For improved retention of basic compounds pH 3 buffer can be used as loading solvent

**SAX**
- To break the interactions of acidic compounds with proteins, acidify(pH 2-3) samples prior to loading

For strongly retained compounds adjust the pH of the elution solvent:
- **Basic compounds** – add 2% acetic acid
- **Acidic compounds** – add 2% ammonium hydroxide

During the second wash step, use acidic modifier (e.g. pH 3 phosphate) in order to minimize the risk of pre-mature elution for basic compounds

5% ammonium hydroxide in 50:50 methanol:acetonitrile elutes most basic analytes of interest

During the second wash step, use basic modifier (e.g. 0.5 M NH₄OH) in order to minimize the risk of pre-mature elution for acidic compounds

2% formic acid in methanol elutes most acidic analytes of interest

Evaporate and reconstitute SPE eluate as necessary prior to analysis
## Troubleshooting:

<table>
<thead>
<tr>
<th>ISSUE</th>
<th>RECOMMENDATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor absolute recovery</td>
<td>Poor analyte recovery is typically caused by one or more of the following: 1) poor analyte retention during sample load; 2) premature analyte elution during the wash step; 3) inadequate analyte elution; or 4) analyte loss during final evaporation / reconstitution. Prior to troubleshooting, it is important to determine what is the primary cause of low recovery. The use of standards (no matrix) is recommended to track and quantitate analyte breakthrough for each step of the SPE process (sample load, wash, and elution).</td>
</tr>
</tbody>
</table>

### Due to poor analyte retention
- Use the pH modification strategies as described in sample pre-treatment.
- Ensure that the SPE phase is wet or moist prior to sample load
- Increase SPE bed weight
- Reduce SPE sample load volume

### Due to premature analyte elution during the wash step
- Use the pH modification strategies as described in the wash step.
- Reduce % organic modifier during wash.
- Increase SPE bed weight
- Reduce SPE wash volume(s)

### Due to inadequate analyte elution
- Use the pH modification strategies as described in elution.
- Increase organic strength in elution solvent.
- Increase elution solvent volume.
- Elute in two separate fractions as opposed to 1.
- Soak the packed bed in elution solvent for 1-3 minutes.
- Use a stronger (greater % organic modifier) wash solvent.
- Decrease SPE bed weight.

### Due to analyte loss during final evaporation
- Eliminate the evaporation step AND elute with a smaller volume of elution solvent followed by dilution with appropriate buffer or solvent. Note that a smaller bed weight may be necessary to maintain efficient analyte elution and adequate recovery.
- Eliminate the evaporation step AND elute with a smaller and weaker elution solvent (organic modified buffer) amenable to direct LC analysis. Recovery of this step needs to be closely monitored to prevent insufficient elution. Note that a smaller bed weight may be required to maintain recovery. The use of a stronger wash solvent (greater % organic modifier) can additionally minimize the matrix interference during evaporation.

### Poor sample clean-up → ion-suppression
- Increase wash solvent strength by increasing % organic modifier in conjunction with pH modifications.
- Reduce bed weight to minimize co-extraction of endogenous sample interferences.
- Adjust chromatographic conditions to separate analyte(s) of interest from co-extracted interferences.

### Poor assay sensitivity
- Reduce elution volume. Note that a reduction in bed weight may be necessary to maintain adequate recovery.
- Reconstitute in smaller volume after eluent evaporation.
- Adjust chromatography efficiency (e.g., use smaller column particle size and dimension).

### Poor reproducibility
- Typically caused by one or more partially inadequate SPE steps. Use standards (no matrix) to track and quantitate analyte breakthrough or loss for each step of the SPE process (sample load, wash, and elution).
- Ensure consistent flow rate of each SPE step from sample load to elution.
- Ensure that the SPE phase is wet or moist prior to sample load
- Ensure reagents used in the SPE procedure are miscible with the reagent used in the preceding and subsequent step. If any are immiscible, adequately dry the phase by applying a strong vacuum for ~10 min. between the two immiscible steps.

<table>
<thead>
<tr>
<th>Description</th>
<th>Qty/Pk.</th>
<th>HLB</th>
<th>SAX</th>
<th>SCX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supel™-Select SPE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mg/1 mL</td>
<td>100</td>
<td>54181-U</td>
<td>54231-U</td>
<td>54240-U</td>
</tr>
<tr>
<td>60 mg/3 mL</td>
<td>50</td>
<td>54182-U</td>
<td>54233-U</td>
<td>54241-U</td>
</tr>
<tr>
<td>200 mg/6 mL</td>
<td>30</td>
<td>54183-U</td>
<td>54235-U</td>
<td>54242-U</td>
</tr>
<tr>
<td>500 mg/12 mL</td>
<td>20</td>
<td>54184-U</td>
<td>54236-U</td>
<td>54243-U</td>
</tr>
<tr>
<td>1 g/20 mL</td>
<td>20</td>
<td>54186-U</td>
<td>54237-U</td>
<td>54245-U</td>
</tr>
<tr>
<td>Supel™-Select 96-well SPE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/ well</td>
<td>1</td>
<td>Inquire</td>
<td>Inquire</td>
<td>Inquire</td>
</tr>
<tr>
<td>30 mg /well</td>
<td>1</td>
<td>575661-U</td>
<td>575660-U</td>
<td>575664-U</td>
</tr>
<tr>
<td>60 mg /well</td>
<td>1</td>
<td>575662-U</td>
<td>575663-U</td>
<td>575665-U</td>
</tr>
</tbody>
</table>

Supel is a trademark of Sigma-Aldrich Co. LLC