

# Supel™-Select HLB SPE

Supelco Supel-Select HLB SPE is a hydrophilic modified styrene based polymer developed for the solid phase extraction of a highly broad range of compounds from aqueous samples. The retention mechanism is predominately based on reversed-phase interaction. However, because the phase is hydrophilic modified, the phase is also selective for more polar compounds. Examples of more polar compounds that are retained and recovered on Supel-Select HLB include (but not limited to): pyridoxine (logPo/w -0.56), riboflavin (logPo/w -2.02), biotin (logPo/w 0.11).

## Specifications:

<b>Phase chemistry:</b>	Hydrophilic modified styrene polymer
<b>Particle Size:</b>	55-60 µm
<b>MS friendly:</b>	Yes
<b>Surface area:</b>	400-410 m <sup>2</sup> /g
<b>Pore volume:</b>	0.88 mg/g
<b>Pore size:</b>	87 Å

## SPE Volume Guidelines:\*

Configuration	SPE Step(s) Vol.	Elution Vol.
30 mg/1 mL	0.5 – 1.0 mL	0.3 – 1 mL
60 mg/3 mL	1 – 3 mL	0.5 – 3 mL
200 mg/6 mL	3 – 6 mL	1 – 6 mL
500 mg/12 mL	5 – 12 mL	2 – 12 mL
1 g/20 mL	8 – 20 mL	3 – 20 mL
30 mg /well	0.5 – 1.0 mL	0.3 – 2 mL
60 mg/ well	1 – 3 mL	0.5 – 2 mL

\* The SPE volumes (condition, load, wash, condition) listed are general guidelines and are dependent on analyte and sample matrix relative to desired SPE speed, recovery, and selectivity. Volume optimization studies should be conducted for each application.

## Recommended Method:

SPE STEP	USEFUL TIPS
<p><b>1. Sample Pre-Treatment:</b></p> <ul style="list-style-type: none"> <li>◆ Filter or centrifuge to remove particulate matter</li> <li>◆ Dilute aqueous sample 1:1 (v/v) with buffer or DI Water</li> </ul>	<p><b>For tissue and solid samples</b>, homogenize/extract sample with buffer or solvent followed by filtration and/or centrifugation to remove particulate matter. If solvent is used for extraction, evaporate and reconstitute in aqueous buffer prior to SPE.</p> <p><b>For more polar compounds</b> (LogPo/w ≤ 0.5) that are difficult to retain, adjust sample pH to increase hydrophobic character of analyte(s).</p> <ul style="list-style-type: none"> <li>◆ For polar basic compounds (e.g., R-NH<sub>4</sub><sup>+</sup> → R-NH<sub>3</sub>), adjust sample pH to at least 2 pH units above analytes' pKa (e.g., dilute sample w/ 10-25 mM ammonium acetate or ammonium formate, pH 9-11)</li> <li>◆ For polar acidic compounds (e.g., R-COO<sup>-</sup> → R-COOH), adjust sample pH to at least 2 pH units below analytes' pKa (e.g., dilute sample w/ 10-25 mM ammonium acetate or ammonium formate, pH 1-3)</li> </ul>
<p><b>2. Condition &amp; Equilibrate</b> SPE phase with methanol followed by DI water or buffer</p>	<p>Supel-Select HLB SPE is "water wettable" as it is hydrophilic modified and contains no alkyl functional groups (e.g., C18); therefore, the phase can be allowed to dry during conditioning without greatly affecting recovery. Nevertheless, maintaining phase "wetness" is still recommended for optimal performance.</p> <p>Note that the reagent used to dilute or reconstitute the sample in step 1 should also be used to equilibrate the phase after methanol conditioning.</p>
<p><b>3. Load Sample</b> prepared from sample pre-treatment (step 1).</p>	<p>A slower flow rate (e.g., 1-2 drops per second) is recommended to ensure analyte retention.</p>
<p><b>4. Wash</b> off or elute co-retained endogenous sample interferences with 10% methanol OR 5% acetonitrile**.</p> <p>If a final evaporation / reconstitution step is desired after analyte elution, apply a strong vacuum through the SPE bed for 5-10 min. to remove residual moisture</p>	<p>An aqueous wash step (to remove co-retained polar interferences) can be applied prior to 10% methanol or 5% acetonitrile step.</p> <p>For complex matrices (e.g., biological fluids), water or buffer alone is an inadequate wash reagent. An organic modifier is necessary to maximize selectivity/clean-up.</p> <p>** To minimize risk of premature elution of polar basic/acidic compounds, reduce organic modifier percentage and/or dilute methanol/acetonitrile wash solution in pH modified buffer (For more polar compounds) recommended in sample pre-treatment (step 1).</p>
<p><b>5. Elute</b> analyte(s) with methanol:acetonitrile*** (50:50, v/v)</p> <p>Evaporate and reconstitute SPE eluate as necessary prior to analysis.</p> <p>*** Hydrophobic retention on Supel-Select is somewhat stronger than other commercially available polymer SPE phases. The addition of acetonitrile is recommended during elution.</p>	<p>A slower flow rate (e.g., 1-2 drops per second) is recommended to ensure analyte elution.</p> <p>For strongly retained compounds, pH adjustment is recommended to reduce hydrophobic character of the analyte(s).</p> <ul style="list-style-type: none"> <li>◆ For strongly retained basic compounds, elute with 2% acetic acid diluted in methanol:acetonitrile (50:50)</li> <li>◆ For strongly retained acidic compounds, elute with 2% ammonium hydroxide diluted in methanol:acetonitrile (50:50)</li> </ul>

**Troubleshooting:**

ISSUE	RECOMMENDATION
POOR ABSOLUTE RECOVERY	<p>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.</p> <p>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 break through for each step of the SPE process (sample load, wash, and elution).</p>
Due to poor analyte retention	<ul style="list-style-type: none"> <li>◆ Use the pH modification strategies as described in sample pre-treatment (step 1).</li> <li>◆ Ensure that the SPE phase is wet or moist prior to sample load</li> <li>◆ Increase SPE bed weight</li> <li>◆ Reduce SPE sample load volume</li> </ul>
Due to premature analyte elution during the wash step	<ul style="list-style-type: none"> <li>◆ Use the pH modification strategies as described in the wash step (step 4).</li> <li>◆ Reduce % organic modifier during wash.</li> <li>◆ Increase SPE bed weight</li> <li>◆ Reduce SPE wash volume(s)</li> </ul>
Due to inadequate analyte elution	<ul style="list-style-type: none"> <li>◆ Use the pH modification strategies as described in elution (step 5).</li> <li>◆ Increase acetonitrile percentage in elution solvent.</li> <li>◆ Increase elution solvent volume.</li> <li>◆ Elute in two separate fractions as opposed to 1.</li> <li>◆ Soak the packed bed in elution solvent for 1-3 minutes.</li> <li>◆ Use a stronger (greater % organic modifier) wash solvent (step 4).</li> <li>◆ Decrease SPE bed weight.</li> </ul>
Due to analyte loss during final evaporation	<ul style="list-style-type: none"> <li>◆ 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.</li> <li>◆ 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.</li> </ul>
POOR SAMPLE CLEAN-UP / ION-SUPPRESSION	<ul style="list-style-type: none"> <li>◆ Increase wash solvent strength by increasing % organic modifier (e.g., 20-40% methanol:acetonitrile) in conjunction with pH modification strategies as described in step 4</li> <li>◆ Reduce bed weight to minimize co-extraction of endogenous sample interferences.</li> <li>◆ Adjust chromatographic conditions to separate analyte(s) of interest from co-extracted interferences.</li> </ul>
POOR ASSAY SENSITIVITY	<ul style="list-style-type: none"> <li>◆ Improve analyte recovery</li> <li>◆ Reduce elution volume. Note that a reduction in bed weight may be necessary to maintain adequate recovery.</li> <li>◆ Reconstitute in smaller volume after eluent evaporation.</li> <li>◆ Improve sample clean-up by using troubleshooting strategies listed in POOR SAMPLE CLEAN-UP / ION-SUPPRESSION.</li> <li>◆ Adjust chromatography (e.g., use smaller column particle size and dimension).</li> </ul>
POOR REPRODUCIBILITY	<ul style="list-style-type: none"> <li>◆ Typically caused by one or more partially inadequate SPE steps. Use standards (no matrix) to track and quantitate analyte break through for each step of the SPE process (sample load, wash, and elution).</li> <li>◆ Ensure consistent flow rate of each SPE step from sample load to elution.</li> <li>◆ Ensure that the SPE phase is wet or moist prior to sample load</li> <li>◆ 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.</li> </ul>

Description	Qty/Pk.	Cat. No.
<b>Supel™-Select HLB SPE</b>		
30 mg/1 mL	100	54181-U
60 mg/3 mL	50	54182-U
200 mg/6 mL	30	54183-U
500 mg/12 mL	20	54184-U
1 g/20 mL	20	54186-U
<b>Supel™-Select HLB 96-well SPE</b>		
10 mg/ well	1	Inquire
30 mg /well	1	575661-U
60 mg/ well	1	575662-U