Improved EPA 625 Analysis
Using Large-Particle Polymeric Solid Phase Extraction

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Product Manager, GC
Our EPA 625 SPE Study Goals

- **Provide** an SPE alternative to traditional LLE
- **Develop** a procedure with equivalent or improved recoveries
- **Decrease** time, cost, and labor outputs
- **Improve** overall productivity without sacrificing mandatory requirements
Our Approach

What did labs want in a new SPE protocol?

- Faster
- Safer
- Greener
- Better Data
- Easier

- Simple procedure
- Minimize SPE material
- Versatile for multiple sample volumes
- Transfer without major equipment
- Amenable to GC
Our Approach

Provide an SPE procedure that can:

• Handle 500 mL – 1 L of waste water
• Improve flow for viscous samples
• Retain and recover all the analytes
• Withstand harsh solvent conditions
• Process in-lab or on-site
First Thoughts

Try traditional silica SPE media?

- C18 or silica SPE
- C18 followed by ion exchange silica SPE
- Tubes and loose media options

Limitations were observed with silica...

- Not as versatile and durable
- Limited selectivity
- Not resistant to deconditioning!
- Flow
We Found A Solution!

Large Particle, Large Pore Polymeric SPE Sorbent

- Versatile and Durable
  - Wide range of selectivities
  - Multiple particle sizes and formats
- Worry Free
  - Resistant to deconditioning
  - Superior lot-to-lot reproducibility
Versatility: strata™

- Small and large particle options
- 5 sorbent chemistries

For large volume, viscous samples or dirty samples for improved flow

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Mode</th>
<th>Analyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strata-XL</td>
<td>Large Particle Reversed Phase</td>
<td>Polar and Non-Polar</td>
</tr>
<tr>
<td>Strata-XL-C</td>
<td>Large Particle Reversed Phase and Strong Cation-Exchange</td>
<td>Bases</td>
</tr>
<tr>
<td>Strata-XL-CW</td>
<td>Large Particle Reversed Phase and Weak Cation-Exchange</td>
<td>Bases (including Quaternary Amines)</td>
</tr>
<tr>
<td>Strata-XL-A</td>
<td>Large Particle Reversed Phase and Strong Anion-Exchange</td>
<td>Acids</td>
</tr>
<tr>
<td>Strata-XL-AW</td>
<td>Large Particle Reversed Phase and Weak Anion-Exchange</td>
<td>Acids (including Sulfonic acids)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>33 μm, 85Å</td>
<td>100 μm, 300Å</td>
</tr>
</tbody>
</table>
**Versatility:** strataX

**Variety of Formats**
- 1, 3, 6, 12, 20, & 60 mL volume
- Tabless tubes
- Online cartridges

**Sample Processing**
- Several samples at one time
- Multiple port manifold
- Automation using robot

Versatile for a range of sample volumes and workflows
Durability: **strataX**

Reduce limitations on preparation

- Traditional silica-based SPE sorbents cannot perform under extremely high or low pH

**Improve pH resistance**

- Strata-X sorbents have a polymeric backbone
Worry Free: strataX

• Strata-X polymeric SPE sorbents are resistant to deconditioning
  ➢ Silica-based media may lose phase structure
• Will never experience poor analyte-sorbent interactions due to phase collapse when media dries
Why Strata™-XL-C?

- Widest selectivity for EPA 625
- Maximum flow with reduced clogging
- Large load volume capability
- Amenable to varying instrumentation
- Resistant to deconditioning

Large Particle Reversed Phase And Strong Cation-Exchange
Sample Pre-Treatment

• Target sample pH 1 - 3
  ➢ Spike 2 mL concentrated HCl in 1 L water
• Spike surrogate at 100 µg/L

Notes
• 150 mL sample reservoir tube to load 1 L sample
• Use glass wool above SPE tube frit to prevent clogging with dirty samples
SPE Manifold Set-Up

1. Syphoning Option
2. Reservoir Option
## SPE Protocol

**Strata™-XL-C 2 g / 20 mL Giga Tube**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>10 mL methanol followed by 10 mL D.I. water</td>
</tr>
<tr>
<td>Load</td>
<td>Pre-treated 1 L water sample at 10-12 mL/min</td>
</tr>
<tr>
<td>Dry</td>
<td>SPE cartridges at 15-20” of Hg for 4-5 minutes</td>
</tr>
<tr>
<td>Elution 1</td>
<td>2 x 2 mL Acetone&lt;br&gt;2 x 2 mL Dichloromethane / Acetone (3:1)&lt;br&gt;3 x 2 mL Dichloromethane</td>
</tr>
<tr>
<td>Elution 2</td>
<td>2 x 4.5 mL Ethyl Acetate: Methanol (1:1) in 1.5 % NH₄OH</td>
</tr>
<tr>
<td>Water Removal</td>
<td>Pass Elution 1 &amp; Elution 2 samples through Na₂SO₄ (10 g / 20 mL) cartridges followed by 2 x 2 mL DCM rinse</td>
</tr>
</tbody>
</table>
SPE Protocol

Sample Dry Down

- Dry each elution fraction in a turboVap, under nitrogen (no heat) until the volume is reduced to ~1 mL for each

Reconstitute

- Combine fractions (~2 mL total) and bring to final volume of ~4 mL with DCM
- Spike 50 µL of IS (1000 µg/mL)
- Inject into GC/MS
# Test Lab – Observations

<table>
<thead>
<tr>
<th>Protocol Components</th>
<th>Traditional LLE</th>
<th>Strata™-XL-C SPE</th>
<th>SPE Improvements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throughput (samples / day)</td>
<td>20</td>
<td>30-35</td>
<td>50-75 % Increase</td>
</tr>
<tr>
<td>Solvent Usage (mL / sample)</td>
<td>&gt; 360</td>
<td>41</td>
<td>Significant Decrease</td>
</tr>
<tr>
<td>Glassware</td>
<td>~ 100 pieces (large)</td>
<td>&lt; 100 test tubes (disposable)</td>
<td>Significant Decrease</td>
</tr>
<tr>
<td>Data Quality</td>
<td>Sufficient</td>
<td>Improved</td>
<td>Increase</td>
</tr>
<tr>
<td>Manual Labor</td>
<td>High</td>
<td>Very Low</td>
<td>Significant Decrease</td>
</tr>
<tr>
<td>Procedural Steps</td>
<td>Dozens</td>
<td>6</td>
<td>Significant Decrease</td>
</tr>
</tbody>
</table>
GC/MS Analysis
Optimized GC/MS Parameters

**Column:** Zebron™ ZB-SemiVolatiles

**Dimensions:** 30 meter x 0.25 mm x 0.25 μm

**Part No.:** 7HG-G027-11

**Injection:** Splitless @ 250 °C, 1 μL

**Carrier Gas:** Helium @ 1.6 mL/min (constant flow)

**Oven Program:** 40 °C for 0.66 min to 260°C @ 30 °C/min to 295 °C @ 6 °C/min to 325 °C @ 25 °C/min for 2 min

**Detector:** MSD @ 300 °C, 40-500 amu
EPA 625 Curves @ 25 µg/mL

Standard Curve, 2.0 – 5.7 min

Standard Curve, 7.5 – 17 min

Surrogate Curve
Matrix Extraction Results

Results for unknown spike solution

App ID 23075
Matrix Extraction Recovery

<table>
<thead>
<tr>
<th>Surrogate</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water S1</td>
<td>Water S2</td>
</tr>
<tr>
<td>Phenol-D5</td>
<td>47.0</td>
<td>41.1</td>
</tr>
<tr>
<td>2-Chlorophenol-D4</td>
<td>61.1</td>
<td>52.8</td>
</tr>
<tr>
<td>4-Methylphenol-D8</td>
<td>67.3</td>
<td>59.1</td>
</tr>
<tr>
<td>bis(2-chloroethyl) ether-D8</td>
<td>65.3</td>
<td>55.9</td>
</tr>
<tr>
<td>Nitrobenzene-D5</td>
<td>53.7</td>
<td>51.0</td>
</tr>
<tr>
<td>2-Nitrophenol-D4</td>
<td>63.5</td>
<td>57.4</td>
</tr>
<tr>
<td>2,4-Dichlorophenol-D3</td>
<td>51.9</td>
<td>46.4</td>
</tr>
<tr>
<td>4-Chloroaniline-D4</td>
<td>20.1</td>
<td>37.7</td>
</tr>
<tr>
<td>Dimethylphthalate-D6</td>
<td>84.6</td>
<td>73.1</td>
</tr>
<tr>
<td>Acenaphthylene-D8</td>
<td>55.3</td>
<td>48.1</td>
</tr>
<tr>
<td>4-Nitrophenol-D4</td>
<td>79.3</td>
<td>68.0</td>
</tr>
<tr>
<td>Fluorore-D10</td>
<td>65.5</td>
<td>61.6</td>
</tr>
<tr>
<td>4,6-Dinitro-2-methylphenol-D2</td>
<td>86.5</td>
<td>70.5</td>
</tr>
<tr>
<td>Anthracene-D10</td>
<td>52.3</td>
<td>55.0</td>
</tr>
<tr>
<td>Pyrene-D10</td>
<td>52.4</td>
<td>61.7</td>
</tr>
<tr>
<td>Benzo(a)pyrene-D12</td>
<td>64.4</td>
<td>68.8</td>
</tr>
</tbody>
</table>
Did We Achieve the Goal?

- Faster
- Safer
- Greener
- Better Data
- Easier

YES
Considerations

• Acidify the sample before loading to maximize acidic and basic analyte retention
• Accommodate viscous samples with a large particle sorbent
• Capture particulates by using glass wool on top of the sorbent bed
• Dry SPE cartridges before elution to remove water
  ➢ May prolong evaporation time
Considerations

• Dry down both elution fractions separately
  ➢ Elution 2 contains ammonium hydroxide
  ➢ Reduces GC solvent effect
• Limit analyte loss by preventing complete dry down
• Use sodium sulfate to remove residual water in the sample extract
  ➢ May need to bake out sodium sulfate prior to use
  ➢ Decreases the dry down time
Conclusion

Recommended EPA 625 SPE Protocol

- Simple, easy procedure with large particle, large pore sorbent (Strata™-XL-C)

Procedure Advantages

- Cleaner
- Safer
- Similar results
- Easier!

Converting from LLE to SPE results in massive productivity and cost improvements.
Environmental Support
www.phenomenex.com/Environmental

- SPE Method Development Tool
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- On-Site Lab Demos
- Environmental Edge Newsletter
- Technical Notes
- Digital Learning Tutorials

Thank You! Questions?