Improved EPA 625 Analysis

Using Large-Particle Polymeric Solid Phase Extraction

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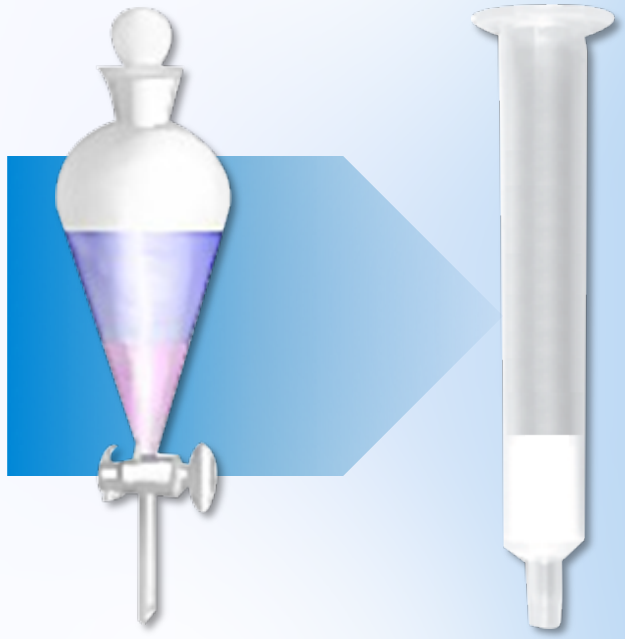
GC

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

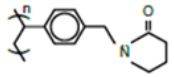
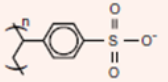
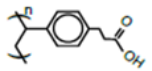
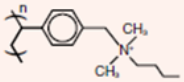
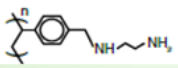
We Found A Solution!

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™-X

- Small and large particle options
- 5 sorbent chemistries

	Functional Group	Mode	Analyte
Strata-XL		Large Particle Reversed Phase	Polar and Non-Polar
Strata-XL-C		Large Particle Reversed Phase and Strong Cation-Exchange	Bases
Strata-XL-CW		Large Particle Reversed Phase and Weak Cation-Exchange	Bases (including Quaternary Amines)
Strata-XL-A		Large Particle Reversed Phase and Strong Anion-Exchange	Acids
Strata-XL-AW		Large Particle Reversed Phase and Weak Anion-Exchange	Acids (including Sulfonic acids)

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

Strata-X, X-C, X-A, X-CW, X-AW	Strata-XL, XL-C, XL-A, XL-CW, XL-AW
33 µm, 85 Å	100 µm, 300 Å

Versatility: strata™-X

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

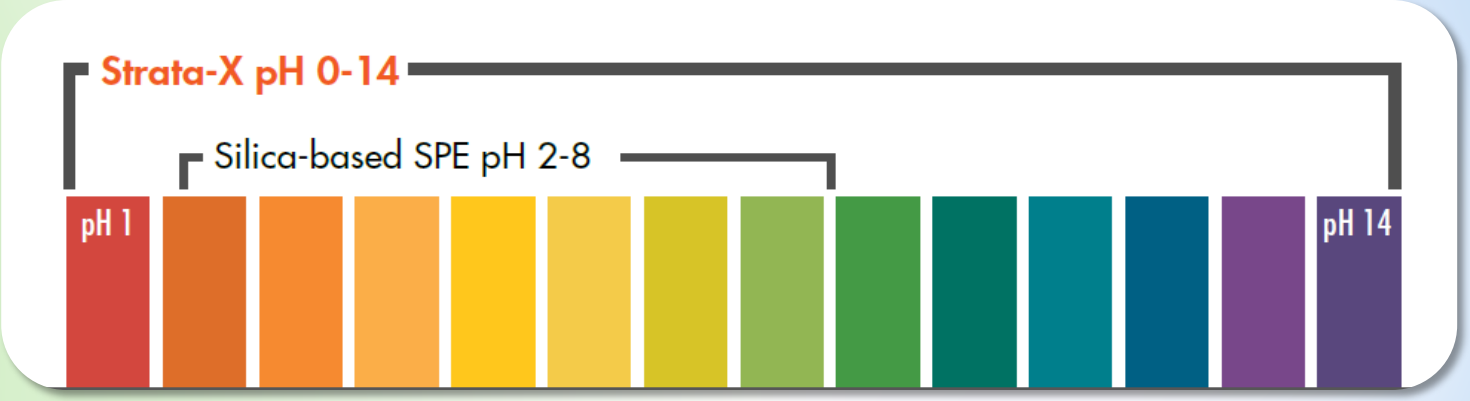
Durability: strata™X

Reduce limitations on preparation

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

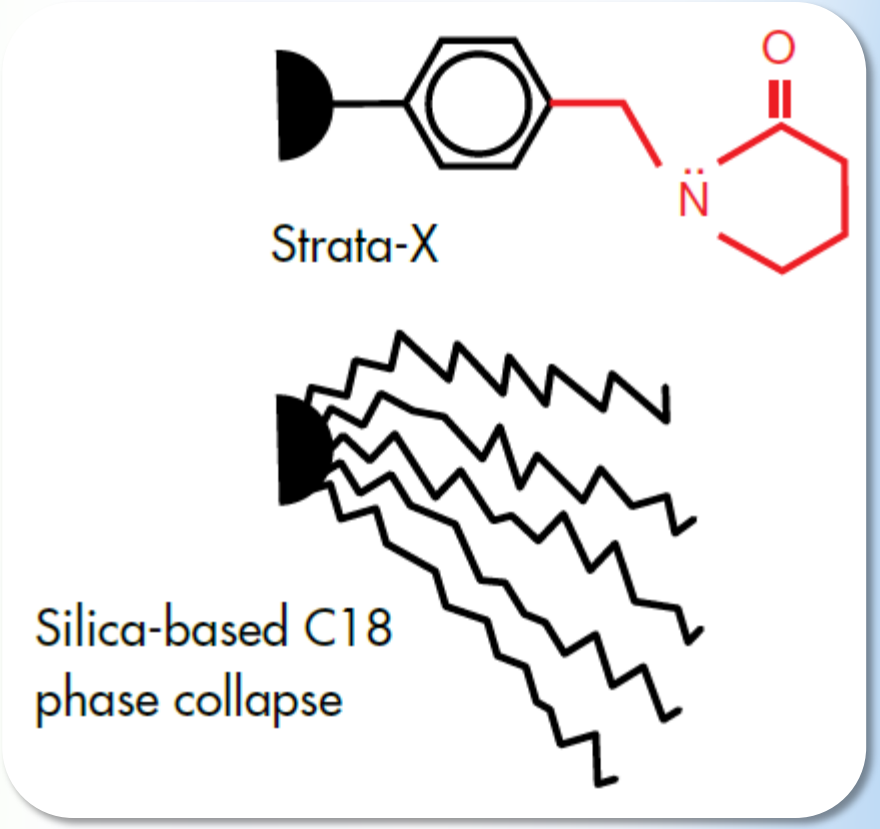
Improve pH resistance

- Strata-X sorbents have a polymeric backbone

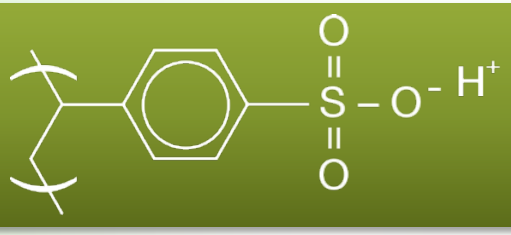


Worry Free: strata-X

- 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?



Large Particle Reversed Phase And Strong Cation-Exchange

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

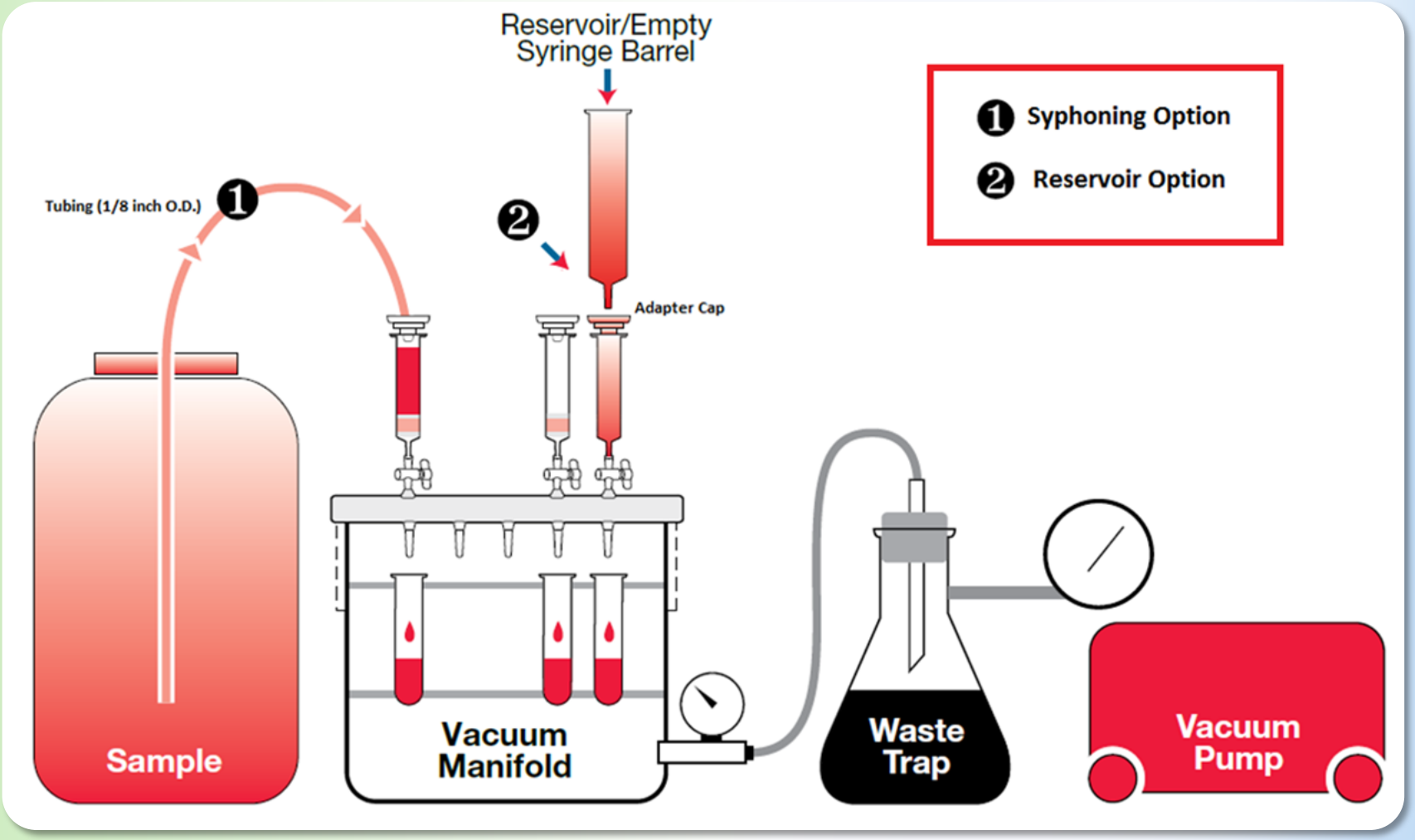
Sample Pre-Treatment

- 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



SPE Protocol

Strata™-XL-C 2 g / 20 mL Giga Tube

Step	Description
Condition	10 mL methanol followed by 10 mL D.I. water
Load	Pre-treated 1 L water sample at 10-12 mL/min
Dry	SPE cartridges at 15-20" of Hg for 4-5 minutes
Elution 1	2 x 2 mL Acetone 2 x 2 mL Dichloromethane / Acetone (3:1) 3 x 2 mL Dichloromethane
Elution 2	2 x 4.5 mL Ethyl Acetate: Methanol (1:1) in 1.5 % NH_4OH
Water Removal	Pass Elution 1 & Elution 2 samples through Na_2SO_4 (10 g / 20 mL) cartridges followed by 2 x 2 mL DCM rinse

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

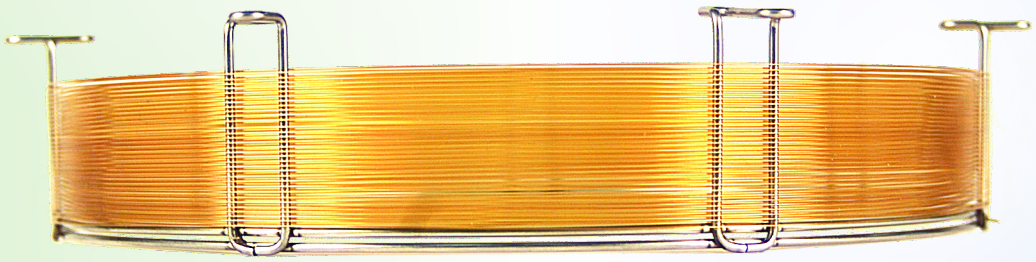
Test Lab – Observations

Protocol Components	Traditional LLE	Strata™-XL-C SPE	SPE Improvements
Throughput (samples / day)	20	30-35	↑ **50-75 % Increase**
Solvent Usage (mL / sample)	> 360	41	↓ **Significant Decrease**
Glassware	~ 100 pieces (large)	< 100 test tubes (disposable)	↓ **Significant Decrease**
Data Quality	Sufficient	Improved	↑ **Increase**
Manual Labor	High	Very Low	↓ **Significant Decrease**
Procedural Steps	Dozens	6	↓ **Significant Decrease**

GC/MS Analysis

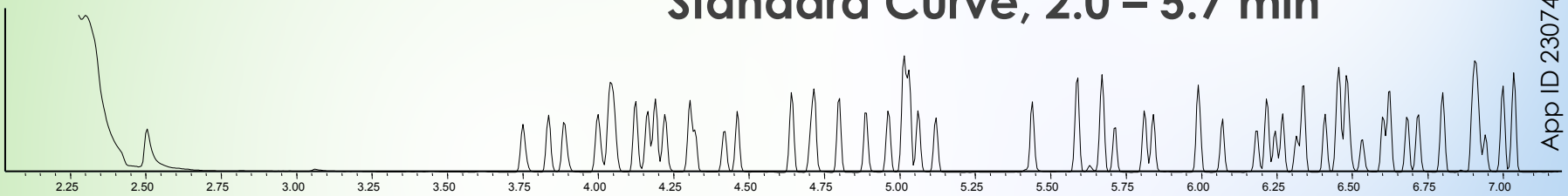
Optimized GC/MS Parameters

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



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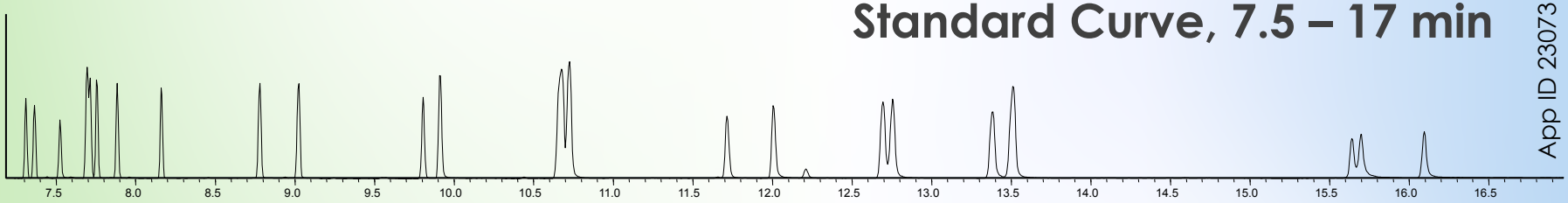
Standard Curve, 7.5 – 17 min



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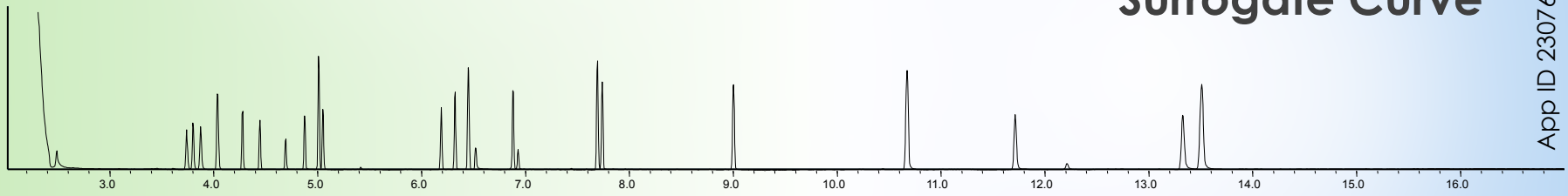
Surrogate Curve



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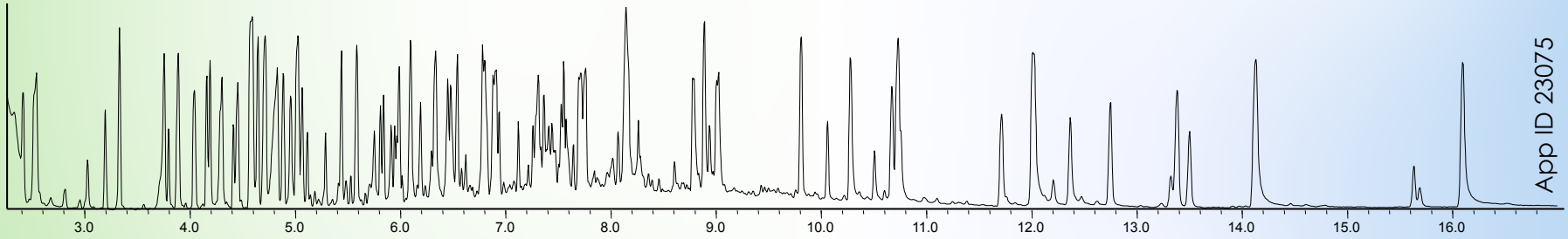
Matrix Extraction Results

Results for unknown spike solution



Matrix Extraction Recovery

Surrogate	% Recovery		% RSD
	Water S1	Water S2	
Phenol-D5	47.0	41.1	9.6
2-Chlorophenol-D4	61.1	52.8	10.4
4-Methylphenol-D8	67.3	59.1	9.2
bis(2-chloroethyl) ether-D8	65.3	55.9	10.9
Nitrobenzene-D5	53.7	51.0	3.7
2-Nitrophenol-D4	63.5	57.4	7.0
2,4-Dichlorophenol-D3	51.9	46.4	7.8
4-Chloroaniline-D4	20.1	37.7	43.1
Dimethylphthalate-D6	84.6	73.1	10.3
Acenaphthylene-D8	55.3	48.1	9.8
4-Nitrophenol-D4	79.3	68.0	10.8
Fluorore-D10	65.5	61.6	4.3
4,6-Dinitro-2-methylphenol-D2	86.5	70.5	14.4
Anthracene-D10	52.3	55.0	3.6
Pyrene-D10	52.4	61.7	11.5
Benzo(a)pyrene-D12	64.4	68.8	4.7

Did We Achieve the Goal?

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)

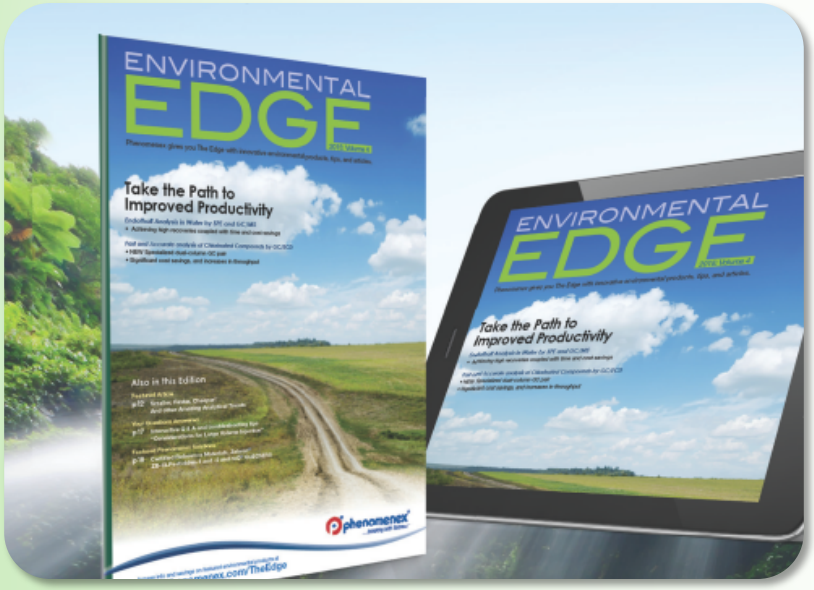
Procedure Advantages

- 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
- 1,000s of Application Notes
- On-Site Lab Demos
- Environmental Edge Newsletter
- Technical Notes
- Digital Learning Tutorials

Thank You! Questions?