APPLICATIONS

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Industry Marketing Manager
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Improved Analysis of EPA Method 625 Using Large-Particle, Large-Pore Solid Phase Extraction (SPE)

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Though historically performed using liquid-liquid extraction (LLE), solid phase extraction (SPE) is an attractive alternative sample preparation method that can be successfully implemented for EPA Method 625. This work explores the use of an optimized SPE protocol using a large-particle, large-pore polymeric sorbent; dramatic efficiency, reproducibility, and productivity improvements over the current methodology are demonstrated.

Introduction

EPA Method 625 is used to test for a wide range of semivolatile organic pollutants in water. The method specifies liquid-liquid extraction (LLE) followed by GC-MS analysis. Though sufficient to achieve method requirements, LLE presents limitations on recovery, accuracy, and procedural efficiency. Attempts have been made to transfer the method from LLE to solid phase extraction (SPE); in comparison to LLE, SPE has numerous benefits including faster extraction time, reduced solvent use, increased reproducibility, and increased recovery. When standard SPE methodology is applied to water samples containing particulates or sediment however, the SPE cartridges often become clogged and extraction data becomes unreliable.

With the increasing importance on productivity gains, successful implementation of the SPE technique has gained attention. This work presents an improved SPE methodology that incorporates large-particle polymeric SPE cartridges (Strata®-XL-C) and improvements in drying. The large-particle extraction method is effective for the entire range of EPA 625 analytes and is considerably faster and easier to use than LLE or standard SPE protocols. Following the optimized SPE protocol, the sample is analyzed by GC-MS using a Zebron™ ZB-SemiVolatiles GC column, resulting in a rapid 17 minute run time. By utilizing the large-particle SPE method and GC method outlined here, EPA Method 625 efficiency, reproducibility, and productivity are dramatically improved.

Experimental Conditions

Solid Phase Extraction

Multiple SPE tubes were processed simultaneously with a 12-position SPE manifold (Part No. AH0-6023). To accommodate large volumes, samples were continuously drawn through the Strata-XL-C SPE cartridge (Part No. 8B-S044-KEG) via an adapter cap (Part No. AH0-7379) connected to an empty 150 mL sample reservoir tube (Part No. AH0-7809). To prevent sample clogging due to particulates, a small amount of glass wool approximately the size of a quarter was positioned above the SPE cartridge frit. The glass wool was large enough to fully cover the top of the cartridge, but packed so densely that it would greatly restrict flow. A waste trap was inserted between the vacuum manifold and the pump. A Zymark TurboVap® LV Evaporator (Biotage, USA) was used for drying steps. The detailed SPE method can be seen in Table 1. An example of the setup can be seen in **Figure 2**.

GC/MS Analysis

GC/MS analysis was performed using a Zebron ZB-SemiVolatiles GC column on a Shimadzu® GC-2010 Plus GC system

Results and Discussion

Based on feedback from several laboratories, multiple factors for designing the optimized SPE protocol were considered. The final procedure should incorporate the ability to accommodate samples volumes of 500 mL to 1 L, improve flow for viscous matrices, retain and recover all analytes, withstand harsh solvent conditions, and ideally allow for processing in the lab or on-site at the sample source.

Initial development began with traditional silica-based SPE sorbents, including C18 and ion-exchange options. Several limitations were observed with silica however, including lack of versatility and durability, limited selectivity, and proclivity to deconditioning, and phase collapse (**Figure 1**).

The final SPE sorbent chosen was Strata-XL-C, a large-particle, large-pore polymeric sorbent. This particular sorbent displayed the widest selectivity for EPA 625, maximum flow with reduced clogging, large load volume capability, resistance to deconditioning, and was also amenable to varying instrumentation.

Figure 1.
Polymeric and silica-based SPE particles.

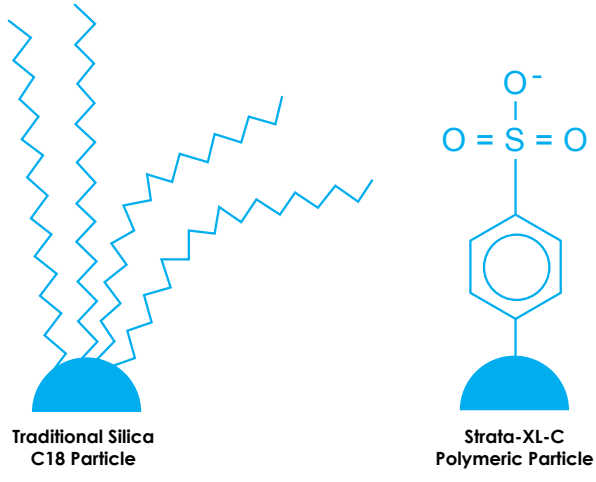
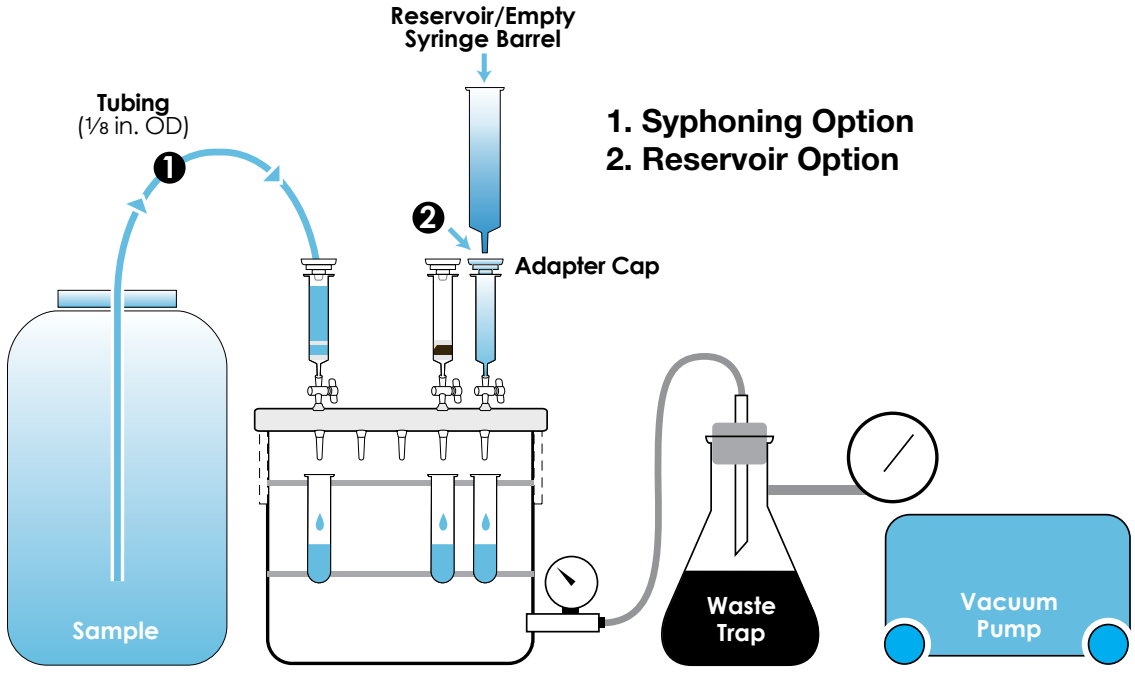


Table 1.
Optimized solid phase extraction protocol for EPA Method 625.

Sample Pre-Treatment	
2 mL concentrated HCl was spiked in 1 L of water matrix to target a pH between 1 and 3. 20 µL of each surrogate (acid and base) was spiked at 1000 µg/mL.	
Solid Phase Extraction	
Cartridge:	Strata®-XL-C, 2 g / 20 mL Giga™ Tube
Condition:	SPE sorbent was conditioned with 10 mL methanol followed by 10 mL DI water.
Load:	The pre-treated 1 L water sample was loaded at 10 – 12 mL/min.
Dry:	Reservoir tubes used for loading the sample were removed and the SPE cartridges were dried by applying vacuum (15-20" of Hg) for 4-5 minutes.
Elution 1:	• 2 aliquots of 2 mL acetone • 2 aliquots of 2 mL dichloromethane:acetone (3:1) • 3 aliquots of 2 mL dichloromethane
Elution 2:	2 aliquots of 4.5 mL ethyl acetate/methanol (1:1) in 1.5 % NH_4OH. To prepare the aliquots, 9.5 mL of ethyl acetate was combined with 9.5 mL of methanol and 1 mL of 30 % NH_4OH and vortexed for 30 seconds.
Water Removal:	Elution 1 and elution 2 fractions were passed through separate Strata Sodium Sulfate 10 g / 20 mL cartridges separately to remove water under gravity. Concentrated elution 1 and 2 fractions were collected in two separate test tubes. To collect residual amounts of sample from the Strata Sodium Sulfate cartridges, elution with an additional 4 mL of dichloromethane per cartridge was performed. After the addition of dichloromethane, two layers were formed. The stopcock was opened to collect the bottom organic layer.
Dry Down:	Samples were dried using a TurboVap® under nitrogen (no heat) until the volume of elution 1 and elution 2 was reduced to 0.5 mL. Samples were not evaporated to complete dryness to prevent analyte loss.
Reconstitute:	Elution 1 and elution 2 fractions were combined (total volume ~1 mL) and reconstituted to a total volume of 4 mL with dichloromethane. 50 µL of internal standard was spiked at 1000 µg/mL.

Figure 2.
SPE accessories and setup used for sample processing.



GC Conditions	
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
Sample:	1. N-Nitrosodimethylamine 2. Pyridine 3. Phenol 4. Bis(2-Chloroethyl)ether 5. 2-Chlorophenol 6. 1,3-Dichlorobenzene 7. 1,4-Dichlorobenzene-d4 (IS) 8. 1,4-Dichlorobenzene 9. Benzyl Alcohol 10. 1,2-Dichlorobenzene 11. 2-Methylphenol 12. Bis(2-chloroisopropyl)ether 13. 3-Methylphenol; 4-Methylphenol 14. N-Nitrosodi-n-propylamine 15. Hexachloroethane 16. Nitrobenzene 17. Isophorone 18. 2-Nitrophenol 19. 2,4-Dimethylphenol 20. Bis(2-chloroethoxy)methane 21. 2,4-Dichlorophenol 22. 1,2,4-Trichlorobenzene 23. Naphthalene-d8 (IS) 24. Naphthalene 25. 4-Chloroaniline 26. Hexachlorobutadiene 27. 4-Chloro-3-Methylphenol 28. 2-Methylnaphthalene 29. 1-Methylnaphthalene 30. Hexachlorocyclopentadiene 31. 2,4,6-Trichlorophenol 32. 2,4,5-Trichlorophenol 33. 2-Chloronaphthalene 34. 2-Nitroaniline 35. 1,4-Dinitrobenzene 36. Dimethyl phthalate 37. 1,3-Dinitrobenzene 38. 2,6-Dinitrotoluene 39. 1,2-Dinitrobenzene 40. Acenaphthylene 41. 3-Nitroaniline 42. Acenaphthene-d10 (IS) 43. Acenaphthene 44. 2,4-Dinitrophenol 45. 4-Nitrophenol 46. 2,4-Dinitrotoluene 47. Dibenzofuran 48. 2,3,5,6-Tetrachlorophenol 49. 2,3,4,6-Tetrachlorophenol 50. Diethyl phthalate 51. Fluorene 52. 4-Chlorophenyl phenyl ether 53. 4-Nitroaniline 54. 2-Methyl-4,6-Dinitrophenol 55. N-nitrosodiphenylamine 56. Azobenzene 57. 4-Bromophenyl phenyl ether 58. Hexachlorobenzene 59. Pentachlorophenol 60. Phenanthrene-d10 (IS) 61. Phenanthrene 62. Anthracene 63. Carbazole 64. Di-n-butylphthalate 65. Fluoranthene 66. Pyrene 67. Benzyl butyl phthalate 68. Bis(2-ethylhexyl) adipate 69. Benzo[a]anthracene 70. Chrysene-D12 (IS) 71. Chrysene 72. Bis(2-ethylhexyl)phthalate 73. Di-n-octylphthalate 74. Benzo[b]fluoranthene 75. Benzo[k]fluoranthene 76. Benzo[a]pyrene 77. Perylene-D12 (IS) 78. Indeno[1,2,3-cd]pyrene 79. Dibenz[a,h]anthracene 80. Benzo[g,h,i]perylene

Figure 3.
TCLP matrix extraction on ZB-SemiVolatiles.

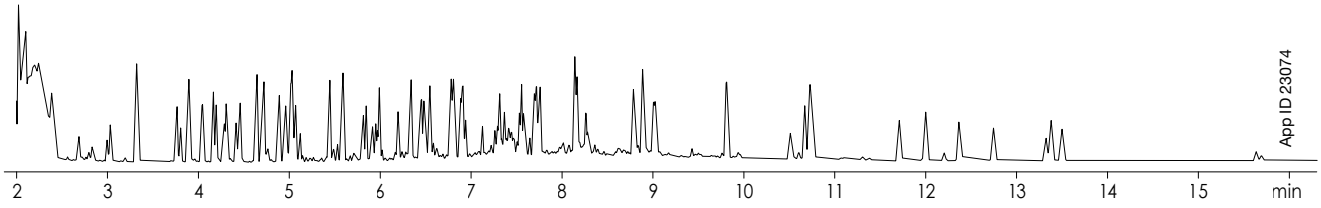


Figure 4.
Waste water matrix extraction on ZB-SemiVolatiles.

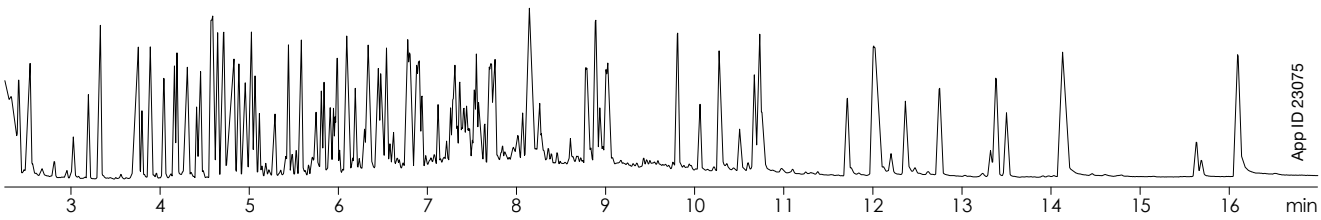
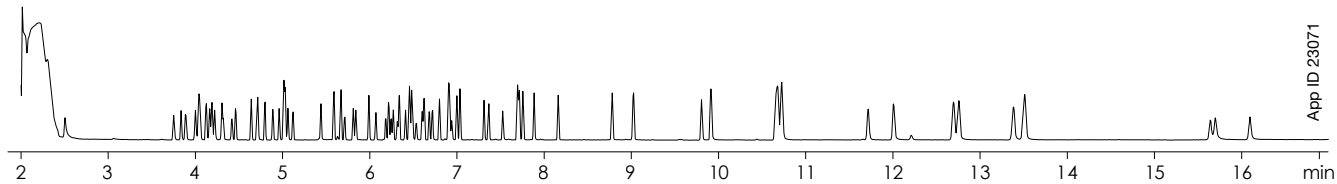


Figure 5.
EPA Method 625 standard curve at 25 µg/mL.



As shown in **Tables 2** and **3**, the outlined SPE protocol gives surrogate recovery values of 40 – 85 % for most compounds, with the exception of the very polar surrogate compound N-Nitroso-dimethylamine-D6 (recovery ~5 %). We suspect that the poor recovery for N-Nitrosodimethylamine is due to the compound not partitioning out of the aqueous phase during the sodium-sulfate-cartridge drying step.

Figures 3-5 exhibit the excellent chromatographic performance typical of the ZB-SemiVolatiles GC column. The specially deactivated GC column achieves good peak-shape for acids, bases, and neutrals. The excellent peak shape is extremely important when accurate quantification is required for dirty matrices such as TCLP or waste water, as shown in **Figures 3** and **4**. Additionally, the ZB-SemiVolatiles column achieves the high efficiency required to resolve the benzo[b] and benzo[k] fluoranthene isomers.

To confirm the robustness, recoveries, and results of the optimized method, an independent medium-sized environmental testing laboratory also performed the extraction and made several observations and comparisons between SPE and LLE as summarized in **Table 4**. During a typical heavy season, an average of 20 samples per day would be processed with two full-time employees (FTEs) when performing EPA 625 with LLE. After successful implementation of the optimized EPA method, the lab estimated that an improvement of 50 to 70 % would be generated with the same resources as their typical throughput, for an estimated 30 to 35 samples per day.

Table 2.
Surrogate recovery from water waste, water and TCLP matrix.

	Water			Waste Water				TCLP			
Surrogate	**S1**	**S2**	**% RSD**	**S1**	**S2**	**S3**	**% RSD**	**S1**	**S2**	**S3**	**% RSD**
N-Nitrosodimethylamine-D6	5.1	4.0	18.3	3.3	3	2.8	8.2	3.6	3.3	3.5	3.6
Phenol-D5	47.0	41.1	9.6	43.5	24.9	31.6	28.2	34.8	32.4	34.6	4
Bis(2-chloroethyl) ether-D8	65.3	55.9	10.9	61.4	39.5	33.7	32.6	49.9	49.8	57.9	8.8
2-Chlorophenol-D4	61.1	52.8	10.4	57	36.1	31.4	32.9	49.7	51	57.8	8.3
4-Methylphenol-D8	67.3	59.1	9.2	65.7	35.6	30.6	43.2	53.8	54.3	50.9	3.5
Nitrobenzene-D5	53.7	51.0	3.7	61.1	41.6	38.6	25.9	44.6	52.7	62.5	16.8
2-Nitrophenol-D4	63.5	57.4	7.0	65.1	46.4	49.3	18.8	55	58.2	68.8	11.9
2,4-Dichlorophenol-D3	51.9	46.4	7.8	50.8	35	47.9	18.9	46.1	51.2	49.7	5.4
4-Chloroaniline-D4	20.1	37.7	43.1	26.1	26.3	31	9.9	38.7	41	39.5	2.9
Dimethylphthalate-D6	84.6	73.1	10.3	73.6	51.5	68.1	17.9	72	77.9	75.7	4
Acenaphthylene-D8	55.3	48.1	9.8	52.4	45.9	53	7.8	51.8	54.2	57.2	5
4-Nitrophenol-D4	79.3	68.0	10.8	65.4	52.6	66.4	12.5	67.5	70.5	71	2.7
Fluorene-D10	65.5	61.6	4.3	62.4	53.9	60.3	7.5	66.8	68	66.2	1.4
4,6-Dinitro-2-methylphenol-D2	86.5	70.5	14.4	75	40.6	63.7	29.4	58	64.6	64.4	6
Anthracene-D10	52.3	55.0	3.6	50.2	41.1	50.8	11.4	51.8	54.5	52.3	2.7
Pyrene-D10	52.4	61.7	11.5	65.4	63.2	71.3	6.3	64.2	73.4	70.9	6.8
Benzo[a]pyrene-D12	64.4	68.8	4.7	41.3	36.9	45.8	10.8	63.2	72.1	68.3	6.6

Table 3.
Percent absolute recovery of analytes from extraction of D.I. water matrix.

Analyte	% Absolute Recovery
N-Nitrosodimethylamine	3.6
Pyridine	74.85
2-Fluorophenol	77.05
Phenol	72
Aniline	88.5
Bis(2-chloroethyl)ether	76
2-Chlorophenol	82.5
1,3-Dichlorobenzene	49.5
1,4-Dichlorobenzene	53
Benzyl Alcohol	67.5
1,2-Dichlorobenzene	57
2-Methylphenol	88
Bis(2-chloroisopropyl)ether	76
3-Methylphenol	85
4-Methylphenol	85
N-Nitrosodi-n-propylamine	85
Hexachloroethane	48
Nitrobenzene	80.5
Isophorone	85
2-Nitrophenol	83
2,4-Dimethylphenol	89
Bis(2-chloroethoxy)methane	86
2,4-Dichlorophenol	88.5
1,2,4-Trichlorobenzene	62.5
Naphthalene	81
4-Chloroaniline	81
Hexachlorobutadiene	39.5
4-Chloro-3-methylphenol	93.5
2-Methylnaphthalene	79.5
1-Methylnaphthalene	80
Hexachlorocyclopentadiene	42
2,4,6-Trichlorophenol	95.5
2,4,5-Trichlorophenol	96
2-Fluorobiphenyl	80.5
2-Chloronaphthalene	79.5
2-Nitroaniline	94
1,4-Dinitrobenzene	94.5
Dimethyl phthalate	93.5
2,6-Dinitrotoluene	92
Acenaphthylene	88.5

Analyte	% Absolute Recovery
3-Nitroaniline	78
1,2-Dinitrobenzene	94
Acenaphthene	83.5
2,4-Dinitrophenol	116
4-Nitrophenol	113
2,4-Dinitrotoluene	103
Dibenzofuran	83
2,3,5,6-Tetrachlorophenol	95.5
2,3,4,6-Tetrachlorophenol	98
Diethyl phthalate	96
4-Chlorophenyl phenyl ether	75
Fluorene	85
4-Nitroaniline	83.5
2-Methyl-4,6-Dinitrophenol	108.5
Azobenzene	85
2,4,6-Tribromophenol	95
4-Bromophenyl phenyl ether	69.5
Hexachlorobenzene	53
Pentachlorophenol	99.5
Phenanthrene	76.5
Anthracene	63.5
Di-n-butylphthalate	92.5
Fluoranthene	65
Benzidine	45
Pyrene	60.5
p-Terphenyl-d14	50
Benzyl butyl phthalate	79.5
Bis(2-ethylhexyl) adipate	47.5
3,3'-Dichlorobenzidine	48
Benzo[a]anthracene	49
Chrysene	48
Bis(2-ethylhexyl)phthalate	48
Di-n-octylphthalate	47.5
Benzo[b]fluoranthene	44.5
Benzo[k]fluoranthene	45
Benzo[a]pyrene	41.5
Indeno[1,2,3-cd]pyrene	39
Dibenz[a,h]anthracene	45
Benzo[g,h,i]perylene	44

Table 4.
Independent test lab observations and comparisons of optimized SPE method vs. LLE.

Protocol Components	Traditional LLE Method	Strata®-XL-C SPE Method	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**

In addition, when compared to LLE, the solvent usage with this method was reduced from greater than 360 mL per sample to only 41 mL per sample. This decrease in solvent usage provides cost savings, decreased evaporation time, and easier disposal. The optimized SPE method was also estimated to reduce daily glassware use from ~100 large pieces – which require careful handling, washing, and drying – to less than 100 small, easily-disposed test tubes.

Data quality also showed an estimated improvement due to a reduction in the number of steps required of an analyst to complete the procedure, as fewer steps mean less room for error. As for time savings, the lab reported that one sample could take hours to run while the optimized SPE method was performed in half the time. Finally, as written in EPA 625, the LLE method requires dozens of steps to perform, repeating the procedure in glass containers at least six times. The optimized SPE procedure required only six steps.

Overall, the optimized SPE method provided reductions in time, solvent use, glassware, manual labor, and procedural steps result in significant productivity gains while also allowing for improved throughput – all with the same resources or less.

Conclusion

EPA Method 625 is performed more effectively when using large-particle, large-pore SPE as an alternative to LLE. By switching from LLE to SPE, solvent usage is decreased, sample-throughput capability is increased and the extraction protocol becomes easier. Following extraction, GC-MS analysis using the highly-deactivated Zebron ZB-SemiVolatiles column gives rapid and accurately-quantifiable chromatography with an analysis time of less than 17 minutes.

Most Frequently Asked Questions (FAQ)

Why do we need to acidify the sample before loading onto the SPE tube?

Acidifying the sample maximizes the hydrophobic retention between the SPE sorbent and acidic compounds, which become uncharged, and promotes ionic interactions between basic compounds (that becomes positively charged) and the cation-exchange SPE sorbent (Strata®-XL-C).

Why is a large particle size SPE sorbent (Strata-XL-C) used in the extraction?

Strata-XL-C is designed to extract viscous samples, such as environmental sludge, without clogging.

Why is glass wool placed in the SPE tube?

Waste water samples may contain a large number of particulates. Placing a small amount of glass wool on top of the SPE sorbent bed can prevent these particulates from entering the pores of the SPE sorbent, which can obstruct the steady flow.

Why do you need to dry the SPE tubes before elution?

Polymeric phases can retain water during the load step which can be eluted during the elution step, resulting in a prolonged final dry down step. To prevent water from eluting during the elution step, a drying step prior to elution can remove any residual water that may be left in the sorbent.

Why do we need to dry the two eluted fractions (elution 1 & elution 2) separately?

The elution 2 fraction contains ammonium hydroxide, which can subject some analytes (that elute in elution) 1 to decomposition.

Why do we need to dry down the sample before injection on the GC-MS?

The injected samples may exhibit broad and/or tailing peaks due to the presence of the solvents used in the elution step. Solvent evaporation followed by reconstitution in methylene chloride allows partitioning of the analyte into the appropriate solvent of choice for GC injection.

Why are the samples not dried down to complete dryness?

The recovery of some analytes, particularly semi-volatiles, may be negatively affected.

Do we need to use sodium sulfate cartridges for drying the eluted samples?

Yes, the sodium sulfate cartridge will remove any residual water that may be present in the extracted samples, expediting the final dry down step.

What is the advantage of SPE over Liquid-Liquid Extraction (LLE)?

SPE is more user friendly with numerous benefits including faster extraction times, a significant reduction of solvent consumption, an increased reproducibility and recovery. LLE requires space and expensive glass or Teflon separatory funnels, can be prone to emulsions (which is difficult to mitigate), and produces significant amount of waste in the form of liquid or vapor.

What is the advantage of using Strata-XL-C instead of a traditional silica-based sorbent

- The large particles (100 µm vs. 50 µm) assures better flow which is critical for large environmental samples
- The larger particles result in less clogging which can be a concern when working with particulate laden environmental sample
- The polymeric Strata-XL-C sorbent is resistant to deconditioning which can prevent poor analytes retention and recovery (unlike a silica-based sorbent), particularly for more polar analytes.
- The N-vinyl pyrrolidine back-bone offers a unique selectivity which is not available in silica-based sorbents, providing enhanced retention of polar analytes.
- The strong cation-exchange stationary phase increases the retention of ionizable amines that have a wide range polarities which is not possible when using typical silica-based C18 phases.

What is the advantage of the SPE tube format?

- Tubes are a simple SPE format do not require significant instrumentation.
- A vacuum pump and a manifold is all that is required for SPE.
- Extraction can be done at a different location than the lab used for analytical run (which requires instrumentation), therefore, the transporting large volumes of samples is not required. Samples can be loaded and washed on a SPE cartridge followed by the dry step (before elution 1, as in SPE protocol). The dried cartridges (loaded with extracts) can be transported to the lab where the elution and subsequent steps can be performed to release target analytes for analysis.
- Very little sample extraction training required for technicians.

APPLICATIONS

Ordering Information

Zebron™ ZB-SemiVolatiles GC Columns

Length (m)	ID (mm)	df (µm)	Temperature Limits (°C)	Part No. Standard	Part No. with 5m Guardian™	Part No. with 10m Guardian
15	0.25	0.25	-60 to 325/350	7EG-G027-11	–	–
	0.25	0.50	-60 to 325/350	7EG-G027-17	–	–
20	0.18	0.18	-60 to 325/350	7FD-G027-08	–	–
	0.18	0.36	-60 to 325/350	7FD-G027-53	–	–
30	0.25	0.25	-60 to 325/350	7HG-G027-11	7HG-G027-11-GGA	7HG-G027-11-GGC
	0.25	0.50	-60 to 325/350	7HG-G027-17	7HG-G027-17-GGA	7HG-G027-17-GGC
60	0.25	0.25	-60 to 325/350	7KG-G027-11	–	–

Strata®-XL-C

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S044-TAK	1 mL (100/box)
	60 mg	8B-S044-UBJ	3 mL (50/box)
	100 mg	8B-S044-EBJ	3 mL (50/box)
	100 mg	8B-S044-ECH	6 mL (30/box)
	200 mg	8B-S044-FBJ	3 mL (50/box)
	200 mg	8B-S044-FCH**	6 mL (30/box)
	500 mg	8B-S044-HCH	6 mL (30/box)
Giga™ Tube			
	2 g	8B-S044-KEG	20 mL (20/box)
	5 g	8B-S044-LEG	20 mL (20/box)
	5 g	8B-S044-LFF	60 mL (16/box)
	10 g	8B-S044-MFF	60 mL (16/box)

**Tabless tubes available. Contact Phenomenex for details.

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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