New Method US EPA 625 with Solid Phase Extraction for Challenging Wastewaters

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Key Words
Wastewater, US EPA method 625, solid phase extraction

Introduction

US EPA method 625\(^1\) is used to determine acidic, basic, and neutral semi-volatile organic compounds (SVOC) in municipal and industrial wastewater using GC/MS, in many cases for National Pollution Discharge Elimination System (NPDES) compliance. In method 625 revision A, the method is applied to a total possible list of 364 compounds that include; polynuclear aromatic hydrocarbons, chlorinated hydrocarbons, pesticides, phthalate esters, organophosphate esters, nitrosamines, haloethers, aldehydes, ethers, ketones, anilines, pyridines, quinolones, aromatic nitro compounds, and phenols.

Liquid-liquid extraction (LLE) or continuous liquid-liquid extraction (CLLE) followed by sodium sulfate drying are the prescribed sample preparation procedures in method 625, one of the few US EPA methods that does not specifically mention solid phase extraction (SPE) as an alternative extraction procedure. Laboratories currently need to apply to the EPA for an Alternate Test Procedure (ATP) if SPE is to be used for sample preparation. The Resource Conservation and Recovery program requires the extraction of similar matrices for a similar analyte list, SPE method 3535A is used for sample preparation and for analysis method 8270D for semi-volatile organic analysis by GC/MS is followed. Data collected for the RCRA program using SPE indicates that most of the compound classes can be extracted using SPE successfully. This experience has led to the development of Horizon Technology methodology that can be readily applied to wastewater extraction of many of the required compounds.\(^2,3,4\)

Last year the Independent Laboratories Institute (ILI), a non-profit workgroup within the American Council of Independent Laboratories (ACIL), met with representatives from the US EPA, vendors, and the laboratory community to develop a plan to collect SPE data from wastewater samples to better understand how performance using SPE compares to the liquid-liquid extraction techniques employed at this time. A mini-round robin study was conducted using an ASTM synthetic wastewater matrix and spike mixtures of undisclosed composition amongst multiple participating laboratories of which Horizon Technology, Inc. and several independent laboratories using Horizon Technology equipment participated.\(^5\) This paper will discuss the results of work done using synthetic wastewater in a single laboratory to refine methodology used in the subsequent round robin study.
**Experimental**

Although SPE has been used for many years to automate the traditional acid-base-neutral liquid-liquid type of extraction, in this case, a modern approach passing the sample through the SPE material once, rather than twice was used. In addition, the SPE process was automated, reducing the labor component required.

**Instrumentation**

<table>
<thead>
<tr>
<th>Method 1: 100-mL Sample Size</th>
<th>Method 2: 1000–mL Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Horizon Technology</strong></td>
<td>Uses the same instrumentation as listed in Method 1 but the standard 47-mm Disk Holder is replaced by the following</td>
</tr>
<tr>
<td>• SPE-DEX® 4790 Automated Extraction Systems</td>
<td>• EZ Flow Disk Holder</td>
</tr>
<tr>
<td>• Envision Platform Controller</td>
<td>• 8-Second Elution Magnet</td>
</tr>
<tr>
<td>• DryVap® Concentrator System</td>
<td>• Fine Mesh Screen</td>
</tr>
<tr>
<td>• Reclaimer Solvent Recovery System</td>
<td>• Atlantic 100 mm, Fine Prefilter</td>
</tr>
<tr>
<td>• Atlantic® 8270 One Pass SPE Disks (47 mm)</td>
<td>• Atlantic 100 mm, Coarse Prefilter</td>
</tr>
<tr>
<td>• 8270 Max Detect Carbon Cartridges</td>
<td></td>
</tr>
<tr>
<td>• DryDisk® Separation Membranes (65 mm)</td>
<td></td>
</tr>
<tr>
<td>• 8270 One-Pass Hardware Kit</td>
<td></td>
</tr>
<tr>
<td>• Standard 47-mm Disk Holder</td>
<td></td>
</tr>
</tbody>
</table>

**Agilent**

• 6890 Gas Chromatograph
• 5973 Mass Selective Detector

**Outline of the SPE Procedure**

The sample is acidified to pH 2 and placed on the SPE-DEX 4790 automated extractor that has been set up with an Atlantic 8270 One Pass disk and an 8270 Max Detect Carbon Cartridge, as shown in Figure 1.

**Acid & Neutral (A&N) Disk Extract**: The A&N method is started on the SPE-DEX 4790 where the disk is preconditioned with solvents, the sample is passed through the disk and carbon cartridge and the disk (only) is eluted with acetone and methylene chloride for acidic and neutral semi-volatile organics (SVO). The collected extract is removed and covered until needed for the Carbon Cartridge Extract.

**Ion-Exchange Disk Extract**: A new collection flask is placed on the SPE-DEX 4790 and the ion-exchange method is started. Here the remaining organic bases are eluted off the disk with acetone, 1% ammonium hydroxide, and methylene chloride. The collected extract is removed and covered until the DryDisk drying step is performed.
**Carbon Cartridge Extract:** The A&N extract or a clean container is placed back on the SPE-DEX 4790, the disk holder is removed and the Carbon Cartridge is attached in its place with a reservoir adapter. The carbon method is started and the light-end SVOs are eluted with acetone and methylene chloride. The combined A&N plus light-end SVO extract is removed and is now ready for extract drying.

**DryDisk Drying:** The A&N plus light-end SVO extract is poured into the DryDisk apparatus and pulled through the membrane with vacuum. The remaining water on top of the membrane is rinsed with methylene chloride and the solvent collected. The water is discarded before pouring in the ion-exchange extract. Pull the solvent through and rinse with solvent. The combined extracts are directly transferred into the DryVap EV tube for concentration.

**DryVap Concentration:** The extract is concentrated down to 0.9 mL. The tube is rinsed with methylene chloride to bring the volume to 1.0 mL and transferred to a GC vial for analysis.

For detailed instructions and instrumental methods refer to Appendix 1 for the 100-mL Sample Method and Appendix 2 for the 1-L Sample Method.

**Results and Discussion**

**Limitations of Manual LLE Techniques**

Continuous liquid-liquid extraction (CLLE) generates 300 to 500 mL of extract and requires a double extraction process. In the first extraction, the sample is adjusted to pH 2 and a second extraction with the same sample adjusted to pH 11. Each of the two pH extractions requires 18 to 24 hours to complete. This means that the sample preparation time can take from 1.5 to 2 days.

Liquid-liquid extraction (LLE) is a manually-intensive sample preparation technique. Like CLLE, the sample is extracted into a large volume of organic solvent (360 mL), and the extraction procedure is performed twice under alkaline pH 11 and acidic pH 2 conditions. This technique is prone to emulsions from all the shaking of the aqueous/organic solvent mixture and takes nearly 4 hours from start to finish.

**Advantages of SPE Disks and Automated Sample Preparation**

Solid phase extraction disks provide a larger surface area and filtering capability that is suitable for sedimentary wastewater. The risk of creating an emulsion is eliminated as the aqueous sample is passed through the disk out to waste. Any remaining water is removed from the disk when it is rinsed with acetone. Since the water and the eluting non-polar solvent (methylene chloride) are not physically in contact with each other or mixed, the emulsion cannot form. In addition, the sorbent(s) used in the disk can be optimized for maximum recovery of the large and disparate classes of SVOCs in method 625.

**Figure 2. SPE-DEX 4790 Extractor and DryVap In-line Drying and Evaporation System**
Shown in Figure 2 is the SPE-DEX 4790 disk/cartridge extractor and the DryVap system. The DryVap system combines membrane sample extract drying and extract concentration and was used to reduce the extract, in this case, to a 1-mL volume automatically. The SPE-DEX 4790 processes one sample at a time and up to eight modules can be connected and operated with one controller, the DryVap system can handle from one to six samples independently or in batch mode.

**Horizon Technology Sorbents and Drying Membrane**

Important consumables for this method are shown in Figure 3. The Atlantic 8270 One-pass Disk is a 47-mm multi-modal media disk. It contains a proprietary HLB (hydrophilic, lipophilic balanced resin) plus ion exchange sites for organic bases. The sample is acidified to pH 2 and the sample is passed through the disk only once out to waste. No basification is done to the sample as the ion exchange sites capture the organic bases in their ionic form. This saves time and eliminates metal hydroxide precipitation which can slow the sample flow through the disk.

The 8270 carbon cartridge is placed on the effluent side of the disk. The carbon cartridge recovers poorly retained light-end organics like NDMA, benzyl alcohol and methyl methanesulfonate.

The DryDisk separation membrane efficiently removes water from the extract, has unlimited capacity for water removal and eliminates sodium sulfate drying.

Shown in Table 1 is a list of 108 SVO compounds plus 6 surrogates used in method 8270 analysis that were spiked into 1 liter of reagent grade water and adjusted to pH 2. The majority of the compounds in this list are also found in method 625. The compounds listed in black are extracted by the HLB portion of the disk, the blue compounds are extracted by the same disk using the ion exchange mechanism and the compounds in bold text are extracted by the carbon cartridge.

The recovery chart for the 50 µg spike reagent grade water prepared for 8270 analysis is shown in Figure 4; the compounds are shown by order of elution from the GC/MS chromatogram and the same order in the previous table (top to bottom and left to right). The acids and neutrals are depicted by the red bars, the compounds captured by the carbon cartridge are shown in black while the blue bars are the organic bases. To generate this chart, the acid extract, ion exchange extract and carbon extracts were analyzed individually so that the contribution of each solid phase media is visualized and quantitated. The black bars clearly illustrate the benefit of the carbon cartridge recovery of light-end SVOCs.

The recoveries are excellent and the utility of the disk chemistry and carbon cartridge are illustrated. Method performance on a variety of compounds in a simple matrix was a good initial demonstration of performance indicating that this method was suitable for further testing with a wastewater matrix.

The spike mixture supplied in the ILI round-robin study was comprised of up to 82 compounds, up to 47 base and neutrals, up to 15 acids and up to 20 organochlorine pesticides. Except for benzidine, the upper limit spike level concentration was 200 µg/L acids, bases and neutrals while the organochlorine pesticides upper limit was an order of magnitude lower at 20 µg/L. Table 2 lists the target compounds.
Table 1. Test Compounds for Candidate Method

<table>
<thead>
<tr>
<th>Compound</th>
<th>Compound</th>
<th>Compound</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Nitrosodimethylamine (NDMA)</td>
<td>4-Chloroaniline</td>
<td>2,4,6-Tribromophenol (surr)</td>
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<tr>
<td>Pyridine</td>
<td>Hexachloropropene</td>
<td>1,3,5-Trinitrobenzene</td>
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<tr>
<td>2-Methylpyridine</td>
<td>Hexachlorobutadiene</td>
<td>Phenacetin</td>
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<tr>
<td>N-Nitrosomethylmethylenimine</td>
<td>N-Nitroso-di-n-butylamine</td>
<td>4-Bromophenyl phenyl ether</td>
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<tr>
<td>Methylanethanesulphonate</td>
<td>4-Chloro-3-methylphenol</td>
<td>Hexachlorobenzene</td>
<td></td>
</tr>
<tr>
<td>2-Fluorophenol (surr)</td>
<td>cis-Isosafrole</td>
<td>Pentachlorophenol</td>
<td></td>
</tr>
<tr>
<td>N-Nitrosodimethylenimine</td>
<td>2-Methylnaphthalene</td>
<td>Pentachloronitrobenzene</td>
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<tr>
<td>Ethyl methanesulphonate</td>
<td>Hexachlorocyclopentadiene</td>
<td>4-Aminobiphenyl</td>
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</tr>
<tr>
<td>Phenol-6 (surr)</td>
<td>1,2,4,5-Tetrachlorobenzene</td>
<td>Dinoseb</td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>trans-Isosafrole</td>
<td>Phenanthrene</td>
<td></td>
</tr>
<tr>
<td>Aniline</td>
<td>2,4, 6-Trichlorophenol</td>
<td>Anthracene</td>
<td></td>
</tr>
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<td>Bis(2-chloroethyl) ether</td>
<td>2,4,5-Trichlorophenol</td>
<td>Carbazole</td>
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<tr>
<td>Pentachloroethane</td>
<td>2-Fluorobiphenyl (surr)</td>
<td>Di-n-butyl phthalate</td>
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<tr>
<td>2-Chlorophenol</td>
<td>Safrole</td>
<td>4-Nitroquinoline-1-oxide</td>
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<tr>
<td>1,3-Dichlorobenzene</td>
<td>2-Chloronaphthalene</td>
<td>Methapyriline</td>
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<tr>
<td>1,4-Dichlorobenzene</td>
<td></td>
<td>Fluorantheno</td>
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</tr>
<tr>
<td>1,2-Dichlorobenzene</td>
<td>2-Nitroaniline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzy! alcohol</td>
<td>1,4-Naphthyquinone</td>
<td>Benzidine</td>
<td></td>
</tr>
<tr>
<td>2-Methylphenol</td>
<td>Dimethyl phthalate</td>
<td>Pyrene</td>
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<tr>
<td>Bis(2-chloroisopropyl) ether</td>
<td>1,3-Dinitrobenzene</td>
<td>p-Terphenyl-d14 (surr)</td>
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<tr>
<td>N-Nitrosopyridine</td>
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<td>p-Dimethylaminazoobenzene</td>
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<td>N-Nitrosomorpholine</td>
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<td>3,3'-Dimethylbenzidine</td>
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<tr>
<td>3-Methylphenol &amp; 4-Methylphenol</td>
<td>Acenaphthene</td>
<td>Butyl benzyl phthalate</td>
<td></td>
</tr>
<tr>
<td>N-Nitrosodimethylamine</td>
<td>3-Nitroaniline</td>
<td>2-Acetylaminofluorene</td>
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<tr>
<td>Hexachloroethane</td>
<td>Acenaphthene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o-Toluidine</td>
<td>Acenaphthene</td>
<td></td>
<td></td>
</tr>
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<td>Nitrobenzene-d5 (surr)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>Acenaphthene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Nitrosopiperidine</td>
<td>Acenaphthene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isophorone</td>
<td>Acenaphthene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Nitrophenol</td>
<td>Acenaphthene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Methylphenol</td>
<td>Acenaphthene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bis(2-chloroethoxy)methane</td>
<td>Acenaphthene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>Acenaphthene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.4-Dichlorophenol</td>
<td>Acenaphthene</td>
<td></td>
<td></td>
</tr>
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<td>1.2.4-Trichlorobenzene</td>
<td>2-Acetylaminofluorene</td>
<td></td>
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</tr>
<tr>
<td>Naphthaleine</td>
<td>2-Acetylaminofluorene</td>
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<td></td>
</tr>
<tr>
<td>2,6-Dichlorophenol</td>
<td>2-Acetylaminofluorene</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. Recovery of a 50 ug Spike into 1L Reagent Grade Water Extracted and Concentrated to 1 mL.
<table>
<thead>
<tr>
<th>Base/Neutrals</th>
<th>µg/L</th>
<th>Acids</th>
<th>µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthene</td>
<td>10 to 200</td>
<td>4-Chloro-3-methylphenol</td>
<td>30 to 200</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>10 to 200</td>
<td>2-Chlorophenol</td>
<td>30 to 200</td>
</tr>
<tr>
<td>Anthracene</td>
<td>10 to 200</td>
<td>2,4-Dichlorophenol</td>
<td>30 to 200</td>
</tr>
<tr>
<td>Benzidine</td>
<td>200 to 1000</td>
<td>2,6-Dichlorophenol</td>
<td>30 to 200</td>
</tr>
<tr>
<td>Benzo(a)anthracene</td>
<td>10 to 200</td>
<td>2,4-Dimethylphenol</td>
<td>40 to 200</td>
</tr>
<tr>
<td>Benzyl butyl phthalate</td>
<td>50 to 200</td>
<td>2,4-Dinitrophenol</td>
<td>100 to 200</td>
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<tr>
<td>Benzo(b)fluoranthene</td>
<td>20 to 200</td>
<td>2-Methyl-4,6-Dinitrophenol</td>
<td>40 to 200</td>
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<tr>
<td>Benzo(k)fluoranthene</td>
<td>20 to 200</td>
<td>2-Methylphenol (o-Cresol)</td>
<td>40 to 200</td>
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<tr>
<td>Benzo(g,h,i)perylene</td>
<td>10 to 200</td>
<td>4-Methylphenol (p-Cresol)</td>
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<tr>
<td>Benzo(a)pyrene</td>
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<tr>
<td>4-Bromophenyl-phenylether</td>
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<tr>
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<td>Phenol</td>
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<tr>
<td>bis(2-Chloroethyl)ether</td>
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<tr>
<td>bis(2-Chloroisopropyl) ether</td>
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<td>2,4,5-Trichlorophenol</td>
<td>30 to 200</td>
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<tr>
<td>Bis(2-ethylhexyl) phthalate</td>
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<td>2,4,6-Trichlorophenol</td>
<td>30 to 200</td>
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<tr>
<td>4-Chlorophenyl-phenylether</td>
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<td>2-Chloronaphthalene</td>
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<td>Chrysene</td>
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<td>Dibenzo(a,h)anthracene</td>
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<td>Dibenzofuran</td>
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</tr>
<tr>
<td>3,3′-Dichlorobenzidine</td>
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<td>Hexachloroethane</td>
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<tr>
<td>Indeno(1,2,3,cd)pyrene</td>
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<td>Naphthalene</td>
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<td>Phenanthrene</td>
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<td>Pyrene</td>
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<tr>
<td>1,2,4-Trichlorobenzene</td>
<td>20 to 200</td>
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</table>

**Organochlorine Pesticides**

<table>
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<th>Pesticides</th>
<th>µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>1.0 to 15</td>
</tr>
<tr>
<td>alpha-BHC</td>
<td>2.0 to 20</td>
</tr>
<tr>
<td>beta-BHC</td>
<td>2.0 to 20</td>
</tr>
<tr>
<td>delta-BHC</td>
<td>2.0 to 20</td>
</tr>
<tr>
<td>gamma-BHC (lindane)</td>
<td>2.0 to 20</td>
</tr>
<tr>
<td>alpha-Chlordane</td>
<td>1.0 to 10</td>
</tr>
<tr>
<td>gamma-Chlordane</td>
<td>1.0 to 10</td>
</tr>
<tr>
<td>4,4'-DDD</td>
<td>2.0 to 10</td>
</tr>
<tr>
<td>4,4'-DDE</td>
<td>1.0 to 10</td>
</tr>
<tr>
<td>4,4'-DDT</td>
<td>1.0 to 10</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>1.0 to 15</td>
</tr>
<tr>
<td>Endosulfan I</td>
<td>4.0 to 20</td>
</tr>
<tr>
<td>Endosulfan II</td>
<td>4.0 to 20</td>
</tr>
<tr>
<td>Endosulfan sulfate</td>
<td>4.0 to 20</td>
</tr>
<tr>
<td>Endrin</td>
<td>2.0 to 20</td>
</tr>
<tr>
<td>Endrin aldehyde</td>
<td>4.0 to 20</td>
</tr>
<tr>
<td>Endrin ketone</td>
<td>4.0 to 20</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>1.0 to 10</td>
</tr>
<tr>
<td>Heptachlor Epoxide (beta)</td>
<td>1.0 to 10</td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>2.0 to 20</td>
</tr>
</tbody>
</table>
In the mini-round robin study conducted by the ILI, a standard wastewater matrix was selected for the participants to analyze. In this case it was the substitute wastewater matrix from ASTM consisting of flour, ocean salts, kaolin, Triton™ X-100 and beer. The developed method was tested with two different sample sizes, reflecting the current typical sample volume of 1 L and a smaller volume of 100 mL, which may reflect future market needs. The performance of the two sample sizes was compared and the overall performance was compared to the requirements of method 625.

A one-liter sample was extracted using an EZ Flow Disk Holder, it provides 6x more filtering surface for the 47-mm SPE disk and uses fine and coarse 100-mm prefilters (both 1 and 5 µm filters combined). The extraction volume is approximately 200 mL and the time to extract, dry and concentrate the sample is 3 hrs.

In addition to the larger sample volume (method 2) a second volume was processed in parallel (method 1). Here a 100-mL sample was extracted with the 47 mm disk using our standard disk holder. To keep mass loading on the GC equivalent to the 1-liter sample, the injection mode on the GC was splitless. The benefits of a smaller sample volume was a smaller extraction volume of 80 mL of solvent and the processing time to extract, dry and concentrate was reduced to 1.5 hrs.

**DATA REVIEW**

Shown in Figure 5 are the recoveries for bases and neutrals in reagent-grade water spiked with the test mix. Figure 6 shows the corresponding compounds in the wastewater matrix. The 100-mL and 1-L sample recoveries are shown in a bar chart side by side. The compounds from different elution fractions are shown in separate charts for illustrative purposes. The recoveries obtained with the 100-mL sample volume were slightly better than with the conventional 1-L volume.
Shown in Figure 7 are the recoveries for acids in the reagent water spiked with the test mix. The 100 mL and 1 L sample recoveries are shown side by side.
Shown in Figure 8 are the recoveries for acids in the synthetic wastewater spiked with the test mix. The 100 mL and 1 L sample recoveries are shown side-by-side. Here we find benzoic acid recoveries to be high. Benzoic acid recoveries are GC-MS dependent and generally not a reflection of the SPE process. To best analyze these acids is through esterification prior to analysis.

![Figure 8. Spiked Synthetic Wastewater: Acid Fraction](image)

Shown in Figure 9 are the recoveries for pesticides in reagent grade water spiked with the test mix. Pesticides in the test mix were about an order of magnitude lower than the acid/neutral and basic SVOCs.

![Figure 9. Spiked Reagent-Grade Water: Pesticide Fraction](image)
Shown in Figure 10 are the recoveries for pesticides in synthetic wastewater spiked with the test mix. The 100 mL and 1 L sample recoveries are shown side by side. A slight improvement in recoveries was found with the 100-mL sample method.

Table 3. Method Results Summary Spiked Synthetic Wastewater

<table>
<thead>
<tr>
<th>Method</th>
<th>Bases &amp; Neutrals Avg % Recovery</th>
<th>Acids Avg % Recovery</th>
<th>Pesticides Avg % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mL</td>
<td>80.0</td>
<td>89.2</td>
<td>88.6</td>
</tr>
<tr>
<td>1000 mL</td>
<td>56.8</td>
<td>76.7</td>
<td>61.2</td>
</tr>
<tr>
<td>LLE</td>
<td>69.2</td>
<td>69.2</td>
<td>70.9</td>
</tr>
</tbody>
</table>

Table 4. Method Results Summary Spiked Reagent Water

<table>
<thead>
<tr>
<th>Method</th>
<th>Bases &amp; Neutrals Avg % Recovery</th>
<th>Acids Avg % Recovery</th>
<th>Pesticides Avg % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mL</td>
<td>80.5</td>
<td>80.8</td>
<td>75.9</td>
</tr>
<tr>
<td>1000 mL</td>
<td>61.9</td>
<td>74.8</td>
<td>69.0</td>
</tr>
<tr>
<td>LLE</td>
<td>69.2</td>
<td>69.2</td>
<td>70.9</td>
</tr>
</tbody>
</table>
Tables 3 and 4 summarize the data for the spiked synthetic wastewater and reagent water. The synthetic wastewater is compared to replicate LLE data to demonstrate comparability. Although the smaller sample volume provided improved recoveries, the recoveries for both sample sizes were within the acceptable range for the method and for some compounds were much better than LLE.

**Conclusion**

In conclusion, the SPE results were comparable, if not better, than LLE method results. Recoveries were found to be well within the acceptance parameters of method 625. The smaller sample volume method has been shown to have a slight recovery improvement over the traditional 1-liter volume which is advantageous because smaller extraction volumes and faster processing times are also provided. These same Horizon Technology test methods were performed by four independent laboratories, using both the small and large sample volumes, to further validate the performance of solid phase extraction disks with wastewater.

**References**

1. US EPA Method 625, available from the EPA site at:
Appendix 1: Method 1 Summary, 100 mL Sample Size

System Setup

1. Prepare a 1% solution of Ammonium Hydroxide in DI water to be used as a wash solvent.
   - Sigma-Aldrich ACS reagent, NH3 content 28-30%, PN 221228-500ML-A.
   - Prep. 34 mL soln. to 1 L volumetric with reagent grade water.
2. Install the 8270 One-Pass Hardware Kit:
   a. Mount the carbon cartridge perch to the side of the extractor shelf by tightening the thumbscrew.
   b. Disconnect the Water-to-Waste line on the back of the extractor.
   c. Connect one end of the yellow line to the extractor port labeled water waste.
   d. Connect one end of the green line to the Water-to-Waste line and the other end to the yellow line connected in step 2 c.

Prepare Samples & Notes

1. Sample volume recommended is 100 mL, however, if necessary a maximum of 200 mL can be used with the 47 mm holder. (Actual amount of sample will depend on laboratory preference and regulatory requirements).
2. IMPORTANT: Recalibrate the GC/MS based on the amount of sample used. e.g., reduce the range of standards to 1/10 the concentration range if using 100 mL as 1 L is the reference volume.
3. Although it is mentioned in the startup procedure, check that the solvent pressure is set to 15 psi.

Sample Processing and Acid and Neutral Extraction

1. Adjust 100-L aqueous sample to pH 2 with HCl, cap the bottle and mix. (approx. 4 drops of conc. HCl.)
2. Place an EZ-SEAL or a piece of aluminum foil over the opening of the bottle and screw on the bottle cap adaptor.
3. Press the cap into the top of an 8270 Carbon Cartridge.
4. Insert the Carbon Cartridge with the cap into the perch.
5. Attach the Water Waste line with the tube clamp on it to the cap on the Carbon Cartridge.
6. Attach the other Water Waste line from the extractor to the tip of the Carbon Cartridge.
7. Load the disk holder with the Atlantic 8270 One-Pass 47 mm disk.
8. Place a clean 125-mL Erlenmeyer flask or equivalent receiver onto the extractor.
9. Load the pH 2 sample onto the SPE-DEX 4790.
10. Start the extraction method in Table 1 at high vacuum (~25 in. Hg).
11. Cap and label extract as the acid/neutral fraction.

Ion Exchange Extraction

1. Place a clean 40-mL VOA vial or equivalent receiver onto the extractor.
2. Load and start the extraction method in Table 2.
3. Cap and label the extract as the ion exchange fraction.

**Cartridge Extraction**

1. Remove the disk holder with the Atlantic 8270 One-Pass disk from the SPE-DEX 4790.
2. Disconnect the lines from the Carbon Cartridge and remove it from the perch.
3. Reconnect the waste lines removed from the Carbon Cartridge.
4. Remove the cap from the Carbon Cartridge and install the funnel in its place.
5. Install the Carbon Cartridge/funnel assembly onto the SPE-DEX 4790 for elution.
6. Re-attach the 125 mL flask containing the acidic fraction onto the extractor.
7. Elute the Carbon Cartridge using the Carbon Extraction Method given in Table 3 into the 125 mL flask containing the acidic fraction.
8. Alter the flask label to indicate that it contains the carbon fraction also.

**Concentration**

1. Assemble the DryDisk reservoir with a DryDisk Separation Membrane.
2. Load the DryDisk reservoir onto the DryVap and set the conditions as shown in Table 4.
3. Start the concentration process by adding the acid/carbon fraction into the DryDisk reservoir.
4. Once the solvent filters through the DryDisk, manually rinse the DryDisk reservoir with approximately 10 mL of methylene chloride. Repeat this rinse two additional times.
5. Press stop when all the solvent has been processed.
6. Empty the acidic water from the DryDisk reservoir into the correct waste container.
7. Load the DryDisk reservoir back onto the DryVap Concentration System.
8. Repeat Steps 3 and 4 for the ion exchange fraction.
9. The DryVap will concentrate the extract to 0.9 mL.
10. Rinse the sides and heater of the concentrator tube with methylene chloride and bring the extract up to a 1.0-mL final volume.
11. Transfer the extract to a GC vial.
12. Analyze by GC/MS using the conditions in the section labeled GC/MS Method.
### Table 1: Acid and Neutral Extraction Method

<table>
<thead>
<tr>
<th>Step</th>
<th>Solvent</th>
<th>Soak Time</th>
<th>Dry Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prewet 1</td>
<td>Acetone</td>
<td>0:30 min</td>
<td>0:15 min</td>
</tr>
<tr>
<td>Prewet 2</td>
<td>Acetone</td>
<td>0:30 min</td>
<td>0:15 min</td>
</tr>
<tr>
<td>Prewet 3</td>
<td>Reagent Water</td>
<td>0:10 min</td>
<td>0:02 min</td>
</tr>
<tr>
<td>Prewet 4</td>
<td>Reagent Water</td>
<td>0:10 min</td>
<td>0:02 min</td>
</tr>
</tbody>
</table>

**Sample Process**

Air Dry 0:30 min

| Rinse 1  | Acetone | 3:00 min  | 0:20 min |
| Rinse 2  | MeCl    | 3:00 min  | 0:20 min |
| Rinse 3  | MeCl    | 1:00 min  | 0:20 min |
| Rinse 4  | MeCl    | 1:00 min  | 0:20 min |
| Rinse    | MeCl    | 1:00 min  | 1:00 min |

### Table 2: Ion Exchange Extraction Method

<table>
<thead>
<tr>
<th>Step</th>
<th>Solvent</th>
<th>Soak Time</th>
<th>Dry Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Dry</td>
<td></td>
<td>0:00 min</td>
<td></td>
</tr>
<tr>
<td>Rinse 1</td>
<td>Acetone</td>
<td>0:30 min</td>
<td>0:30 min</td>
</tr>
<tr>
<td>Rinse 2</td>
<td>1% NH₄OH</td>
<td>1:00 min</td>
<td>0:20 min</td>
</tr>
<tr>
<td>Rinse 3</td>
<td>Acetone</td>
<td>3:00 min</td>
<td>0:20 min</td>
</tr>
<tr>
<td>Rinse 4</td>
<td>MeCl</td>
<td>3:00 min</td>
<td>0:20 min</td>
</tr>
<tr>
<td>Rinse 5</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>0:20 min</td>
</tr>
<tr>
<td>Rinse 6</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>0:20 min</td>
</tr>
<tr>
<td>Rinse 7</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>1:00 min</td>
</tr>
</tbody>
</table>
Table 3: Carbon Extraction Method

<table>
<thead>
<tr>
<th>Step</th>
<th>Solvent</th>
<th>Soak Time</th>
<th>Dry Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinse 1</td>
<td>Acetone</td>
<td>1:00 min</td>
<td>0:00 min</td>
</tr>
<tr>
<td>Rinse 2</td>
<td>Acetone</td>
<td>1:00 min</td>
<td>1:00 min</td>
</tr>
<tr>
<td>Rinse 3</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>0:03 min</td>
</tr>
<tr>
<td>Rinse 4</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>0:03 min</td>
</tr>
<tr>
<td>Rinse 5</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>0:03 min</td>
</tr>
<tr>
<td>Rinse 6</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>0:03 min</td>
</tr>
<tr>
<td>Rinse 7</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>0:03 min</td>
</tr>
<tr>
<td>Rinse 8</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>0:03 min</td>
</tr>
<tr>
<td>Rinse 9</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>0:03 min</td>
</tr>
<tr>
<td>Rinse 10</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>1:00 min</td>
</tr>
</tbody>
</table>

Air Dry 2:00 min

Table 4: DryVap Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Volume</td>
<td>100 mL</td>
</tr>
<tr>
<td>Heat Power</td>
<td>5</td>
</tr>
<tr>
<td>Heat Timer</td>
<td>OFF</td>
</tr>
<tr>
<td>Auto Rinse Mode</td>
<td>OFF</td>
</tr>
<tr>
<td>Nitrogen Sparge</td>
<td>20 psi</td>
</tr>
<tr>
<td>Vacuum</td>
<td>-7 in. Hg</td>
</tr>
</tbody>
</table>

GC/MS Method

System: Agilent 6890 GC with an Agilent 5973 MS Detector
Column: Phenomenex ZB Semi-volatiles, 30 m x 0.25 mm ID, 0.25 µm
Flow Rate: 9 psig helium which is ramped up with the oven temp to maintain a constant flow
Temp Ramp:

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Rate (°C/min)</th>
<th>Hold (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>270</td>
<td>15</td>
<td>0.00</td>
</tr>
<tr>
<td>320</td>
<td>6.0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Total Run Time: 24.33 min.
Injection Method: 1.0 µL injected, Temp 280°C, Pulsed splitless
- Inlet pulse pressure 50.0 psi for 0.3 min
- Purge flow to split vent 50 mL/min for @0.60 min
Appendix 2: Method 2 Summary, 1L Sample Size

System Setup

1. Prepare a 1% solution of Ammonium Hydroxide in DI water to be used as a wash solvent.
   • Sigma-Aldrich ACS reagent, NH3 content 28-30%, PN 221228-500ML-A.
   • Prep. 34 mL soln. to 1 L volumetric with reagent grade water.
2. Install the 8270 One-Pass Hardware Kit:
   a. Mount the carbon cartridge perch to the side of the extractor shelf by tightening the thumbscrew.
   b. Disconnect the Water-to-Waste line on the back of the extractor.
   c. Connect one end of the yellow line to the extractor port labeled water waste.
   d. Connect one end of the green line to the Water-to-Waste line and the other end to the yellow line connected in step 2 c.
3. Install the 8-Second Elution Magnet over the IR sensor on the SPE-DEX 4790.
4. Install the Tefzel® Plug into the SPE-DEX 4790 platform adjustment hole to set the platform to a lower height.

Prepare Samples & Notes

Sample Processing and Acid and Neutral Extraction

1. Adjust 1000-mL aqueous sample to pH 2 with HCl, cap the bottle and mix.
2. Place an EZ-SEAL or a piece of aluminum foil over the opening of the bottle and screw on the bottle cap adaptor.
3. Press the cap into the top of an 8270 Carbon Cartridge.
4. Insert the Carbon Cartridge with the cap into the perch.
5. Attach the Water Waste line with the tube clamp on it to the cap on the Carbon Cartridge.
6. Attach the other Water Waste line from the extractor to the tip of the Carbon Cartridge.
7. Load the EZ Flow Disk Holder with the Atlantic 8270 One Pass 47 mm disk and Atlantic Prefilters onto the extractor.
8. First place the 100-mm fine filter into the holder.
9. Second place the 100-mm coarse filter into the holder.
10. Third place the fine mesh screen into the holder.
11. Place a clean 125-mL Erlenmeyer flask or equivalent receiver onto the extractor.
12. Load the pH 2 sample onto the SPE-DEX 4790.
13. Start the extraction method in Table 1 at high vacuum (-25 in. Hg).
14. Cap and label extract as the acid/neutral fraction.

Ion Exchange Extraction

1. Place a clean 125 mL Erlenmeyer flask or equivalent receiver onto the extractor.
2. Load and start the extraction method in Table 2.
3. Cap and label the extract as the ion exchange fraction.
Cartridge Extraction

1. Remove the EZ Flow Disk Holder from the SPE-DEX 4790.
2. Disconnect the lines from the Carbon Cartridge and remove it from the perch.
3. Reconnect the waste lines removed from the Carbon Cartridge.
4. Remove the cap from the Carbon Cartridge and install the funnel in its place.
5. Install the Carbon Cartridge/funnel assembly onto the SPE-DEX 4790 for elution.
6. Re-attach the 125 mL flask containing the acidic fraction, or a clean flask if the acidic fraction flask is too full, onto the extractor.
7. Elute the Carbon Cartridge using the Carbon Extraction Method given in Table 3 into the 125 mL flask containing the acidic fraction or a clean flask.
8. Alter the flask label to indicate that it also contains the carbon fraction.

Concentration

1. Assemble the DryDisk reservoir with a DryDisk Separation Membrane.
2. Load the DryDisk reservoir onto the DryVap and set the conditions as shown in Table 4.
3. Start the concentration process by adding the Acid and Neutral fraction into the DryDisk tube.
4. Allow the extract to filter through the DryDisk into the Concentrator tube.
5. Manually rinse the Erlenmeyer flask with methylene chloride adding this to the DryDisk reservoir, allowing the rinse solvent to process through the DryDisk. Do this three times.
6. Follow steps 4 and 5 for the carbon fraction.
7. Once the A&N and carbon fractions filter through the DryDisk, manually rinse the DryDisk reservoir with methylene chloride.
8. When the methylene chloride has filtered into the concentration tube, allow the station to transition to the heat stage.
9. Once the volume has concentrated to a point where there is enough volume within the concentrator tube for the ion exchange fraction, press stop on the DryVap.
10. Clean the DryDisk reservoir by emptying out the acidic water and then rinsing it out with methylene chloride.
11. Load the DryDisk reservoir back onto the DryVap Concentration System.
12. Use the conditions given in Table 4, follow steps 4 and 5 to process the ion exchange fraction.
13. Once the ion exchange fraction and rinses filter completely through the DryDisk, rinse the DryDisk reservoir with methylene chloride.
14. Concentrate the extract to less than 1.0 mL.
15. Rinse the sides and heater of the concentrator tube with methylene chloride and bring the extract up to a 1.0-mL final volume.
16. Transfer the extract to a GC vial.
17. Analyze by GC/MS using the conditions in the section labeled GC/MS Method.
### Table 1: Acid and Neutral Extraction Method

<table>
<thead>
<tr>
<th>Step</th>
<th>Solvent</th>
<th>Soak Time</th>
<th>Dry Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prewet 1</td>
<td>Acetone</td>
<td>1.00 min</td>
<td>1:00 min</td>
</tr>
<tr>
<td>Prewet 2</td>
<td>Reagent Water</td>
<td>1:00 min</td>
<td>1:00 min</td>
</tr>
</tbody>
</table>

**Sample Process**

<table>
<thead>
<tr>
<th>Step</th>
<th>Solvent</th>
<th>Soak Time</th>
<th>Dry Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Dry</td>
<td></td>
<td>3:00 min</td>
<td></td>
</tr>
<tr>
<td>Rinse 1</td>
<td>Acetone</td>
<td>3:00 min</td>
<td>3:00 min</td>
</tr>
<tr>
<td>Rinse 2</td>
<td>MeCl</td>
<td>3:00 min</td>
<td>3:00 min</td>
</tr>
<tr>
<td>Rinse 3</td>
<td>MeCl</td>
<td>2:00 min</td>
<td>2:00 min</td>
</tr>
<tr>
<td>Rinse 4</td>
<td>MeCl</td>
<td>2:00 min</td>
<td>2:00 min</td>
</tr>
<tr>
<td>Rinse 5</td>
<td>MeCl</td>
<td>2:00 min</td>
<td>3:00 min</td>
</tr>
<tr>
<td>Rinse 6</td>
<td>MeCl</td>
<td>2:00 min</td>
<td>3:00 min</td>
</tr>
<tr>
<td>Rinse 7</td>
<td>MeCl</td>
<td>2:00 min</td>
<td>3:00 min</td>
</tr>
</tbody>
</table>

### Table 2: Ion Exchange Extraction Method

<table>
<thead>
<tr>
<th>Step</th>
<th>Solvent</th>
<th>Soak Time</th>
<th>Dry Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Dry</td>
<td></td>
<td>0:00 min</td>
<td></td>
</tr>
<tr>
<td>Rinse 1</td>
<td>Acetone</td>
<td>0:00 min</td>
<td>3:00 min</td>
</tr>
<tr>
<td>Rinse 2</td>
<td>1% NH₄OH</td>
<td>2:00 min</td>
<td>2:00 min</td>
</tr>
<tr>
<td>Rinse 3</td>
<td>Acetone</td>
<td>3:00 min</td>
<td>2:00 min</td>
</tr>
<tr>
<td>Rinse 4</td>
<td>MeCl</td>
<td>3:00 min</td>
<td>3:00 min</td>
</tr>
<tr>
<td>Rinse 5</td>
<td>MeCl</td>
<td>2:00 min</td>
<td>3:00 min</td>
</tr>
<tr>
<td>Rinse 6</td>
<td>MeCl</td>
<td>2:00 min</td>
<td>3:00 min</td>
</tr>
<tr>
<td>Rinse 7</td>
<td>MeCl</td>
<td>2:00 min</td>
<td>3:00 min</td>
</tr>
</tbody>
</table>
**Table 3: Carbon Extraction Method**

<table>
<thead>
<tr>
<th>Step</th>
<th>Solvent</th>
<th>Soak Time</th>
<th>Dry Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0:30 min</td>
<td></td>
</tr>
<tr>
<td>Air Dry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rinse 1</td>
<td>Acetone</td>
<td>1:00 min</td>
<td>1:00 min</td>
</tr>
<tr>
<td>Rinse 2</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>0:04 min</td>
</tr>
<tr>
<td>Rinse 3</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>0:04 min</td>
</tr>
<tr>
<td>Rinse 4</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>0:04 min</td>
</tr>
<tr>
<td>Rinse 5</td>
<td>MeCl</td>
<td>1:00 min</td>
<td>1:00 min</td>
</tr>
</tbody>
</table>

**Table 4: DryVap Conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Volume</td>
<td>200 mL</td>
</tr>
<tr>
<td>Heat Power</td>
<td>5</td>
</tr>
<tr>
<td>Heat Timer</td>
<td>OFF</td>
</tr>
<tr>
<td>Auto Rinse Mode</td>
<td>OFF</td>
</tr>
<tr>
<td>Nitrogen Sparge</td>
<td>20 psi</td>
</tr>
<tr>
<td>Vacuum</td>
<td>-7 in. Hg</td>
</tr>
</tbody>
</table>

**GC/MS Method**

System: Agilent 6890 GC with an Agilent 5973 MS Detector
Column: Phenomenex ZB Semi-volatiles, 30 m x 0.25 mm ID, 0.25 µm
Flow Rate: 9 psig helium which is ramped up with the oven temp to maintain a constant flow

<table>
<thead>
<tr>
<th>Temp Ramp:</th>
<th>Temp (°C)</th>
<th>Rate (°C/min)</th>
<th>Hold (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>270</td>
<td>15</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>6.0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Total Run Time: 24.33 min.
Injection Method: Split, Ratio 1:10, 1.0 µL injected