Agilent's New Polymer Solid-Phase Extraction Cartridges: SampliQ OPT

Technical Note

Optimized Polymer Technology (OPT) provides:

- High recoveries even when dried
- Excellent reproducibility
- Broad application for neutral and basic compounds
- Simple extraction protocol
- Controlled particle size

General Description

Solid-phase extraction (SPE) is a cornerstone in the analytical workflow of complex samples. Sample preparation remains an important part of the process, even with the adoption of highly specific detectors, such as LC/MS/MS, where ion suppression from coeluting impurities can adversely affect quantitative analyses. A cleaner extract can mean less complicated analysis conditions, longer HPLC column life, and more accurate results. Solid-phase extraction is a cost-effective alternative to liquid-liquid extractions because it uses less solvent, it is faster, and it produces less waste. SPE is an enhanced sample preparation technique compared to liquid-liquid extraction because it offers greater flexibility, resulting in higher and more reproducible recoveries, and is more effective as a cleanup tool. Solid-phase extractions are used by researchers in the food safety, pharmaceutical, environmental, and forensic industries.

The Optimized Polymer Technology (OPT) used in the Agilent SampliQ OPT utilizes a novel polyamide chemistry (patent pending). The result is a resin that exhibits retention for both polar and nonpolar compounds based on the balanced hydrophilic/lipophilic character. The behavior is reversed phase, which provides both ease of method development and compatibility with both gas chromatographic and liquid chromatographic separations of the extracts. As a general guideline, if the target compound is both hydrophilic (low log P) and strongly basic (pKa > 10) or strongly acidic (pKa < 3) the compounds will not be effectively retained on Agilent SampliQ OPT. The resin is inert to a wide variety of solvents, is stable in pH ranges from 0 to 14, and is water-wettable. Other SampliQ products are the SampliQ SCX and SampliQ SAX solid-phase extraction cartridges.

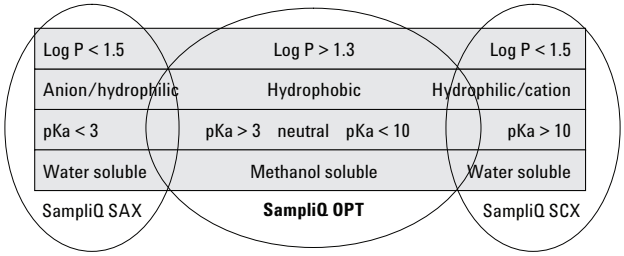

Figure 1. Agilent SampliQ polymer sorbent selection guide Agilent SampliQ OPT will retain compounds with a wide range of chemical properties, from moderately hydrophilic to organic soluble, as well as basic, neutral, or acidic.

Quality Controls

Quality control of the product provides a greater level of confidence, a crucial component in validated environments. The particle size distribution is strictly controlled. Particle size is measured by electrozone sensing analysis, particle shape is characterized by light microscopy, and surface

area and porosity are determined by nitrogen adsorption. The rigorous size controls result in excellent reproducibility and flow character. Additionally, every batch is tested for chromatographic performance and purity. Each lot of material is performance tested, and a certificate of performance is enclosed with each box.

Operational Guidelines

With Agilent SampliQ OPT cartridges the extraction protocol is simple. Figure 1 shows the basic starting procedure for method development. In this example, the volumes shown are for a 3 mL/60 mg cartridge. For many applications this simple protocol will be effective. For highly lipophillic, basic compounds which are found in the flow-through or wash, it is helpful to raise the pH of the load and wash solutions to a level at or above the pKa of the basic compounds which are not effectively retained. The addition of 0.1% formic acid to the methanol (MeOH) eluent has been found to enhance recoveries of some compounds. Like other SPE cartridges, these are for single-use only. The cartridges fit into the Agilent vacuum manifold or any other vacuum manifold which has the usual Luer fittings.

There are typically four steps in the SPE method development process:

1. Conditioning
2. Loading
3. Washing
4. Elution

It is critical that one understands the nature of each step and how to best optimize the solvent selection.

Conditioning Step

For a reversed-phase SPE product, the conditioning solvent is typically a water-miscible, organic solvent that prepares (wets) the surface to receive the sample. Methanol is typically used for this step. A typical flow rate would be 1 mL/min. Since most samples handled by reversed-phase SPE are loaded in aqueous solvent, the next step in the conditioning process is to remove the methanol with at least 5 bed volumes of water. Agilent SampliQ OPT cartridges are extremely robust and can actually run dry between the methanol conditioning and water equilibration steps. While drying the cartridge between the conditioning and equilibration step is not recommended, it has no effect on the reproducibility or accuracy of the results. Due to the strict particle size distribution, the flow character of the cartridge is excellent and these steps will require little or no vacuum to achieve good flow.

Loading Step

Samples in complex matricies such as urine, plasma, fruits, vegetables, sewage, tissue, and waste water may require additional preparation prior to loading. Preparations may include dilution, acidification, homogenization, centrifugation, and/or filtration. The prepared sample is generally spiked with an internal standard and loaded onto the cartridge as an aqueous solution. The flow through the cartridge should be no faster than 1 mL/minute for the loading step. Vacuum may be required depending on the viscosity of the sample. Loading volumes will be the same as those used by standard silica; however, the loading capacity of the resin is greater than a silica-based sorbent. A 60-mg bed of resin will perform comparably to a 200-mg bed of C18 silica sorbent.

Washing Step

The washing step should use the strongest (highest % organic) solvent that will not elute the target compounds. In the example shown in Figure 2, a very weak wash solvent (5% methanol in water) was used. A volume equivalent to a minimum of 5 times the bed volume should be used for the wash. The flow through the cartridge should be approximately 1 mL/min. A brief dry of the cartridge should be performed to remove as much residual water as possible at this step.

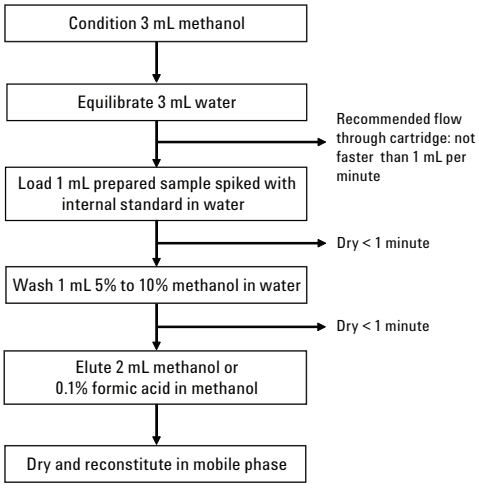

Figure 2. Agilent SampliQ OPT method development process for 3-mL cartridge.

Elution Step

The elution step should use the weakest (lowest % organic) solvent that will elute the target compounds. Occasionally, pH modification will help elute target compounds (0.1% formic is a recommended option). In the procedure shown in Figure 1, a moderately strong elution solvent (100% methanol) was used. Flow through the cartridge should not exceed 1 mL/minute, and a minimum of 5 times the bed volume should be used for elution The eluent is collected and the volume reduced. The sample should be brought to the desired volume in water or starting mobile phase solution.

The Agilent SampliQ OPT solid-phase extraction cartridges are compatible with water, acidic or basic solvents from pH 0 to 14 and most organic solvents. The cartridges are intended for single use and should not be reconditioned.

Performance

The polymer resin provides highly reproducible recoveries regardless of whether the cartridge runs dry at the conditioning stage. While drying the cartridge between is not the recommended procedure, this experiment was performed to demonstrate the ruggedness of the cartridge even under adverse conditions. This is very different from silica-based SPE, where the cartridge must not be allowed to run dry during the conditioning. Figure 3 shows the extraction recoveries performed under two different experimental conditions. In the "wet" experiment, the cartridge is conditioned with MeOH and the equilibration solvent (water) is added before the cartridge goes dry. In the "dry" experiment, the cartridge is conditioned with MeOH but the cartridge is dried under vacuum for 10 minutes before the addition of water for the equilibration step. Notice that no difference in recovery is observed when the cartridge is dried in the pre-extraction steps and when it is not. Table 1 shows the percent relative standard deviations (%RSDs) of the recoveries for each of the compounds (n = 5) for samples spiked at 10 ng/mL. For recovery calculations, caffeine was used as an internal standard and was spiked at 50 ng/mL. The area ratios were used to calculate the concentration from nonextracted standards [% recovery = (area ratio observed/area ratio standards)*100]. The recovery of caffeine was independently measured and found to be 100%,

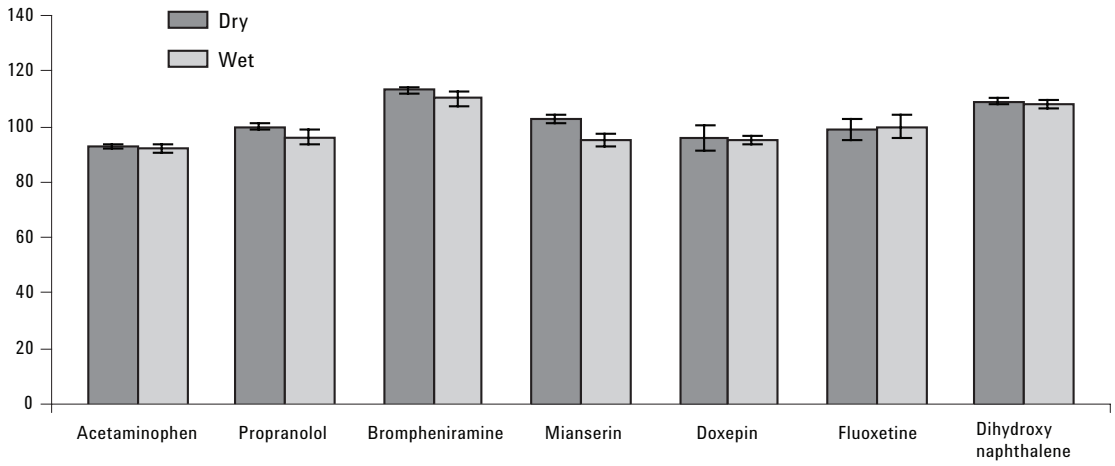

Figure 3. Recovery study for compounds extracted with Agilent SampliQ OPT.

Table 1. Recoveries of Compounds Extracted with Agilent SampliQ OPT Solid-Phase Extraction Cartridges for Solutions Concentrations of 10 ng/mL

Compound		Log P	% Recovery "wet" process	% RSD n = 5%	% Recovery "dry" process	% RSD n = 5
Acetaminophen	Neutral	0.9	92.4	1.4	92.6	0.7
Propranolol hydrochloride	Base	2.7	96.0	2.7	100.5	1.1
Brompheniramine	Base	3.7	110.1	2.5	113.1	1.4
Mianserin	Neutral	3.8	99.9	2.6	103	1.4
Doxepin hydrochloride	Base	4.2	94.7	1.7	96.4	5.4
Fluoxetine	Base	5.4	99.8	3.9	99.3	3.6
Dihydroxy naphthalene	Neutral	1.98	107.7	1.6	109.4	1.1

RSD 1.1%. The compounds evaluated range from very polar, basic compounds to hydrophobic, neutral compounds.

Summary

Agilent is introducing a complete line of polymeric resin based SPE cartridges. Agilent SampliQ OPT is a general purpose cartridge that can be used for compounds with a wide range of polarities, pKa's, and solubilities. The lipophilic and hydrophilic balance provides a mixed-mode retention mechanism. Compounds that are strongly basic or strongly acidic and also highly water soluble should be run on one of the complementary polymeric resin products: Agilent SampliQ SCX (cation exchange) or Agilent SampliQ SAX (anion exchange). No single SPE sorbent will provide maximum retention and clean-up capabilities for all compounds. Figure 1 provides some general guidelines that will help identify the most appropriate cartridge choice as a starting place for method development.

Agilent SampliQ OPT general purpose cartridges are for basic and neutral compound isolation.

Part Number	Description
5982-3013	30 mg, 1 mL cartridge, 100/pack
5982-3036	60 mg, 3 mL cartridge, 50/pack
5982-3067	150 mg, 6 mL cartridge, 30/pack
5982-3082	10 mg, 96 well plate

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