Agilent's New Mixed-Mode Cation Exchange Polymer Solid-Phase Extraction Cartridges: SampliQ SCX

Technical Note

Agilent SampliQ SCX provides:

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

General Description

Solid phase extraction (SPE) is a cornerstone in the analytical workflow of complex samples and 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 results. A cleaner extract can mean less complicated analysis conditions, longer HPLC column life and more accurate results. SPE is a cost-effective alternative to liquid-liquid extractions because it uses less solvent, it is faster, and it produces less waste. SPE is a preferred sample preparation technique compared to liquid-liquid extraction because it offers greater flexibility, resulting in higher and more reproducible recoveries; is more effective as a clean-up tool; and is more easily automated. SPEs are used by researchers in food safety, pharmaceutical, and forensic industries.

The Agilent SampliQ SCX resin is sulfonic acid modified divinyl benzene polymer (Figure 1). The result is a resin that exhibits retention for both basic and neutral compounds over a wide range of pKa's and hydrophobicity (log P). The cartridge exhibits both cation exchange and reversed phase behavior, which provides ease of method development. The resin is inert to a wide variety of solvents, is stable in pH ranges 0 to 14, and is water-wettable. Other SampliQ products are the SampliQ SAX and SampliQ OPT solid-phase extraction cartridges.

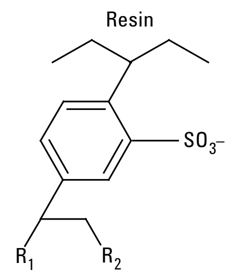

Figure 1. SCX resin.

Quality Controls

Quality control of the product provides a higher level of confidence in the results, a crucial component in validated environments. The particle characteristics are carefully controlled and monitored. Spherical polymeric particles are used to ensure homogeneous and reproducible packing. Particle size and distribution are 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. The performance of every lot of material is tested, and a certificate of performance is enclosed with each box.

Operational Guidelines

With SampliQ SCX cartridges the extraction protocol is simple. Figure 2 shows a recommended starting procedure for method development. In this example the volumes shown are for a 3 mL/ 60 mg cartridge. For other cartridge sizes, the volumes should be proportionally corrected. For many applications this simple protocol will be effective. Like other SPE cartridges, these are for single-use only. The cartridges fit into the Agilent vacuum manifolds as well as any vacuum manifold that has the usual Luer fittings.

There are typically five steps in a mixed-mode SPE procedure:

- Conditioning
- Loading
- Aqueous wash
- Organic wash for neutral compound fractionation
- Elution

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

Conditioning Step

For virtually all SPE products, the conditioning solvent is typically a water-miscible, organic solvent that prepares (wets) the surface to receive the sample. Methanol is the most popular solvent used for this step. A typical flow rate would be 1 mL/min; slower is acceptable but faster is not recommended. The next step in the conditioning process is to remove the methanol with at least five bed volumes of an aqueous solution. For Agilent SampliQ SCX, 2% formic acid in water provides the best performance. With a mixed retention mechanism (ion exchange and reversed phase), the SPE cartridge is loaded in aqueous solvent. Due to the strict particle size distribution, the flow through the cartridge will require little or no vacuum to achieve acceptable flow.

Loading Step

Samples in complex matrices may require additional preparation prior to loading. Preparations may include dilution, pH adjustment, homogenization, centrifugation, and/or filtration. The prepared sample is generally spiked with an internal standard and loaded onto the cartridge as an aqueous solution. Again, the flow through the cartridge should be no faster than 1 mL/min 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 that of a silica-based sorbent. A 60-mg bed of resin will perform comparably to a 200-mg bed of C18 silica sorbent. For the strongest retention of basic and neutral compounds in a single run, the pH of the loading solution should be between 5 and 7.

Washing Step (Aqueous)

The washing step should use the strongest (highest % organic) solvent in water at low pH that will not elute the target compounds. In the example shown in Figure 2, a very weak wash solvent (2% formic acid in water) was used. A volume equivalent to a minimum of five 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.

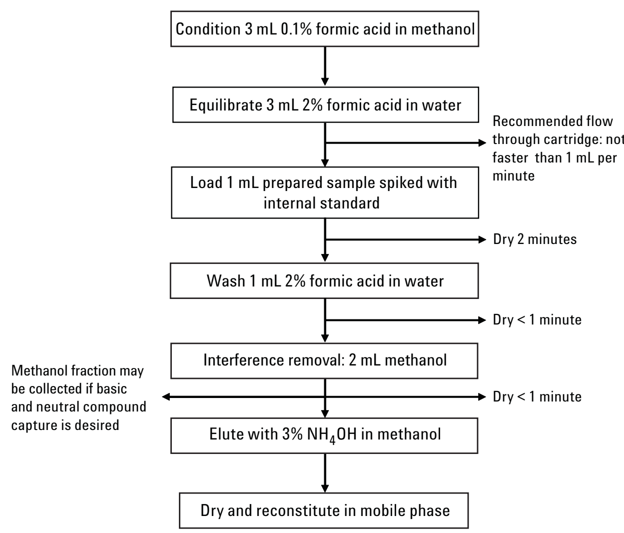

Figure 2. Agilent SampliQ SCX method development process for 3-mL cartridge.

Washing Step (organic)

In many applications the clean-up of the sample is aided by the selective removal of neutral and hydrophobic compounds. The methanol elution shown in Figure 2 removes these interferences. However, the methanol eluent can be collected to isolate the neutral molecules if desired. This is particularly important if there is more than one target compound and they include both neutral and basic compounds. This mixed-mode behavior provides greater flexibility in method design, the ability to fractionate neutral compounds and basic compounds, and cleaner extracts. In Figure 3 the neutral compounds (dihydroxy-naphthalene, and acetaminophen) elute exclusively in the methanol (MeOH) eluent. The basic compounds elute exclusively in the high pH methanol eluent.

Elution Step

The elution step should use the weakest (lowest % organic) solvent that will elute the target compounds. In the procedure shown in Figure 2, a strong solvent (3% ammonium hydroxide in methanol) was used. The eight basic compounds are recovered exclusively in the 3% ammonium hydroxide in methanol eluent (Figure 3). Flow through the cartridge should not exceed 1 mL/minute, and a minimum of five times the bed volume should be used for elution. The eluent is collected and the volume reduced by evaporation. The sample should be brought to the desired volume in water or starting mobile phase solution.

The Agilent SampliQ SCX solid-phase extraction cartridges are compatible with water, acid, or basic solvents from pH 0 to 14 and most organic solvents. The cartridges are intended for single use; reconditioning is not recommended.

Performance

The SampliQ SCX cationic polymer resin provides highly reproducible recoveries for a wide range of compounds following a simple protocol. Optimization of the method may be required to enhance the specificity of the separation. Table 1 shows the compounds used in this study. The compounds range from strong bases (propranolol and atenolol) to hydrophobic and neutral compounds (acetaminophen, dihydroxy-napththalene). The hydrophobicity of the compounds runs from highly water soluble compounds (acetaminophen and atenolol) to hydrophobic compounds (dihydroxy-naphthalene and fluoxetine).

Table 1. Compounds Used in the Evaluations and Their Physical Characteristics

Recoveries		Elutes In	Log P	pKa
Atenolol	Base	NH_4OH*	1.26	9.6
Quinidine	Base	NH_4OH	2.53	8.56
Holoperidol	Base	NH_4OH	4.36	8.66
Mianserin	Base	NH_4OH	3.8	7.05
Dihydroxy naphthalene	Neutral	Methanol	1.98	5.74
Brompheniramine	Base	NH_4OH	3.74	3.59/9.12
Fluoxetine	Base	NH_4OH	5.37	8.7
Acetaminophen	Neutral	Methanol	0.92	4.38/9.38
Propranolol	Base	NH_4OH	3.58	9.5
Doxepin	Base	NH_4OH	4.19	8.0

*NH_4OH is the 3% ammonium hydroxide in methanol eluent

Table 2 shows the exceptional reproducibility of the Agilent SampliQ SCX cartridges. The relative standard deviations are < 5% (with the exception of brompheniramine in the dried cartridge experiment) at a concentration of 10 µg/mL as solution standards using the simple generic protocol. In this study replicate samples were analyzed where no drying occurs between the conditioning and equilibration steps and with drying for 10 minutes between these steps. While drying the cartridge between the conditioning and equilibration step is not recommended, this experiment was performed to demonstrate the ruggedness of the cartridge even under adverse conditions. The results show that the performance of the SPE extraction is reproducible with high recoveries under conditions which would negatively affect performance with silica-based SPE products.

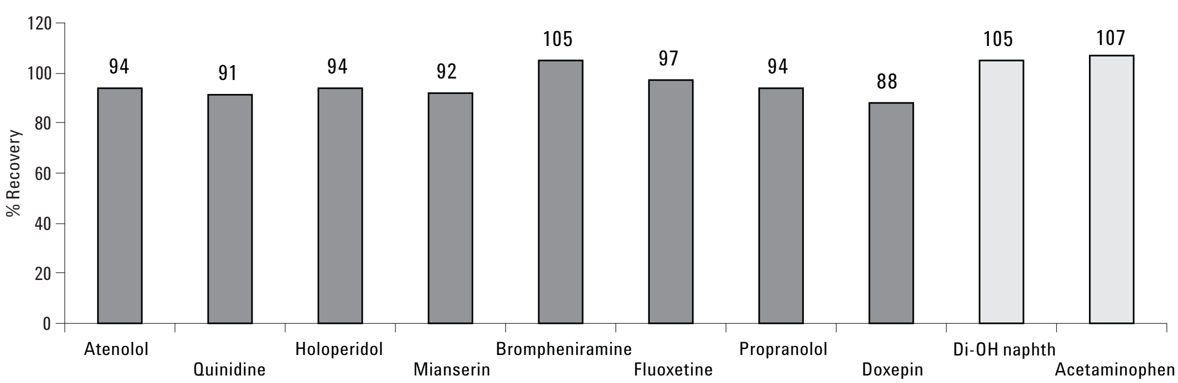

Figure 3 **Compound fractionation on the Agilent SampliQ SCX cartridge. Neutrals (light grey bars) elute exclusively in the methanol step. Capture of the methanol eluent provides a means of characterizing neutral compounds separately from the basic compounds. Likewise, basic compounds (dark gray bars) are exclusively isolated in the methanolic ammonium hydroxide eluent.**

Table 2. Recovery and Reproducibility Results for Base and Neutral Compounds on the Agilent SampliQ SCX Cartridge

Recoveries	SCX wet	SCX dry	SCX wet %RSD	SCX dry %RSD
Atenolol	94	98	3.7	3.1
Quinidine	91	97	2.9	3.1
Holoperidol	94	99	3.2	2.1
Mianserin	92	95	3.4	1.5
Dihydroxy naphthalene	105	92	1.6	4.1
Brompheniramine	105	90	2.1	6.7
Fluoxetine	97	84	1.2	5.0
Acetaminophen	107	103	3.1	2.5
Propranolol	94	99	1.4	2.1
Doxepin	88	96	2.8	3.2

Summary

Agilent SampliQ SCX is a mixed-mode sorbent with both ion exchange and reverse-phase retention mechanisms. A general protocol can be used to remove acidic and neutral interferences and for recovery of basic compounds. The same protocol can be used to recover neutral compounds in one fraction (methanol) and the basic compounds in a second fraction (ammonium hydroxide in methanol). In addition to Agilent SampliQ SCX, there are complementary polymeric resin cartridges for anion exchange (Agilent SampliQ SAX) and a general purpose polymeric resin cartridge (Agilent SampliQ OPT) for acids, bases, and neutrals. Figure 4 is a chart which provides guidance on which cartridge type to choose.

Agilent SampliQ SCX cation exchange cartridges for basic and neutral compound isolation.

Part Number	Description
5982-3213	30 mg, 1 mL cartridge, 100 / pack
5982-3236	60 mg, 3 mL cartridge, 50 / pack
5982-3267	150 mg, 6 mL cartridge, 30 / pack

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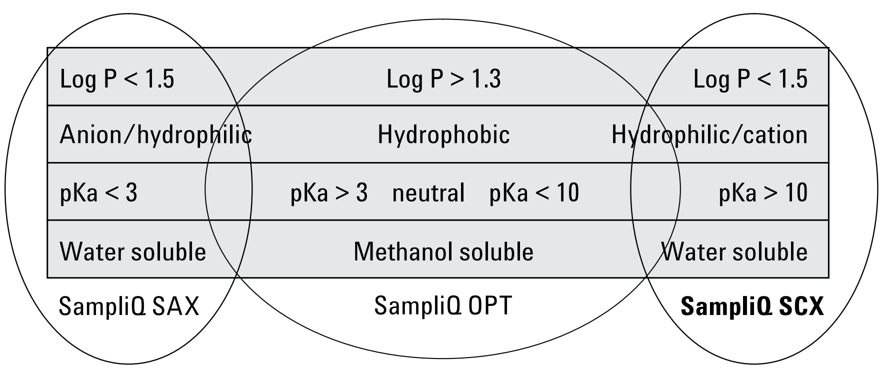

All of Agilent's polymer phases exhibit mixed-mode behavior. For SampliQ SCX this characteristic results in one cartridge which is useful for both neutral and basic compound separation.

Figure 4. Agilent SampliQ polymer sorbent selection guide.

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