Agilent's New Mixed-Mode Anion Exchange Polymer Solid-Phase Extraction Cartridges: SampliQ SAX

Technical Note

Agilent SampliQ SAX Provides:

- Excellent reproducibility
- Applications for acidic 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 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 clean-up tool; and is more easily automated. SPEs are used by researchers in food safety, pharmaceutical, environmental, and forensic industries.

The Agilent SampliQ SAX resin is tertiary amine modified divinyl benzene polymer (Figure 1). The result is a resin that exhibits retention for both acidic and neutral compounds over a wide range of hydrophobicity (log P). The cartridge exhibits both anion 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.

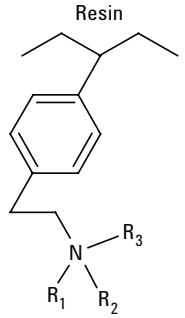


Figure 1. SAX resin.

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 SAX cartridges the extraction protocol is simple. Figure 2 shows the 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:

1. Conditioning
2. Loading
3. Washing with high percentage aqueous solvent
4. Organic solvent wash for neutral compound fractionation
5. 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. 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/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 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 acids and neutrals together, the pH of the loading solution should be between 5 and 7. For selective retention of acids the pH should be high (above 7).

Washing Step (Aqueous)

The washing step should use the strongest (highest % organic) solvent that will not elute the target compounds. The wash solution should be modified with buffer to a pH > 7 for preferential retention of acids. In the example shown in Figure 2, a very weak wash solvent (5% methanol) in aqueous 50 mM sodium acetate 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.

Washing Step (Organic)

In many applications the clean-up of the sample is aided by the selective removal of neutral and basic compounds. However, the methanol eluent can be collected to isolate the neutral molecules if desired. This mixed-mode (anion exchange/reversed phase) behavior provides greater flexibility in method design, the ability to fractionate neutral and acidic compounds, and cleaner extracts. In Figure 3 the neutral and basic compounds (secobarbital and nortriptyline) elute exclusively in the methanol (MeOH) step and the acidic compounds elute quantitatively in the acidified methanol step.

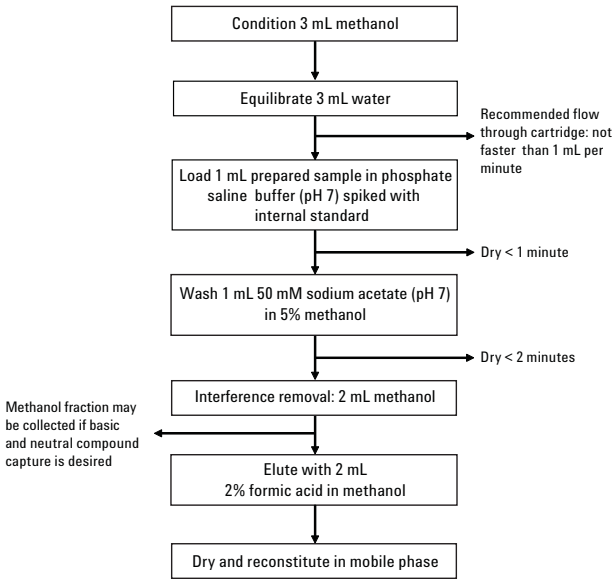


Figure 2. Agilent SampliQ SAX method development process for 3-mL cartridge.

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; a pH 2 units below the pKa of the target compounds will result in the cleanest extracts. In this example, the acidic compounds (salicylic acid, ketoprofen, naproxen, and ibuprofen) elute exclusively in the 2% formic acid in methanol elution. 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 SAX 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 SAX anionic 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 and to accommodate the analytical steps that will follow. For example, if only retention of acids is desired, the equilibration, loading, and washing solutions should be aqueous sodium or ammonium acetate (pH 2 units above the pKa of the target acids). If the downstream analyses will involve electrospray HPLC/MS, any potassium or sodium salts should be exchanged for the ammonium salt at the same pH whenever possible. The ammonium ions are more compatible with the electrospray ionization technique, which is highly sensitive to ion suppression from salts and ion pairing reagents. Table 1 shows the compounds used in this study. The compounds range from strong acids (salicylic acid) to weak bases (nortriptyline). The hydrophobicity of the compounds runs from highly water soluble compounds (ketoprofen) to hydrophobic compounds (nortriptyline).

Figure 4 shows the exceptional reproducibility of the Agilent SampliQ SAX cartridges. The relative standard deviations are < 2% (Table 2) at a concentration of 2.5 µg/mL spiked solution standards using the simple generic protocol.

Summary

Agilent SampliQ SAX is a mixed-mode sorbent with both ion exchange and reverse-phase retention mechanisms. A general protocol can be used to remove basic and neutral interferences and recover acidic compounds. The same protocol can be used to recover neutral compounds in one eluent and the acidic compounds in the second eluent. By changing the pH of the load and wash solutions, high selectivity for acid compounds is achieved. In addition to Agilent SampliQ SAX, there are two other mixed-mode polymeric resin cartridges, one for cation exchange (Agilent SampliQ SCX) and a general purpose polymeric resin

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

Compound		Elutes in	Log P	pKa
Salicylic acid	Acid	Formic*	2.23	2.97
Ibuprofen	Acid	Formic	3.60	4.40
Naproxen	Acid	Formic	3.18	4.53
Ketoprofen	Neutral	Methanol	0.97	5.94
Secobarbital	Neutral	Methanol	1.97	7.90
Nortriptyline	Base	Formic	4.28	9.70

* Formic is the 2% formic acid in methanol eluent

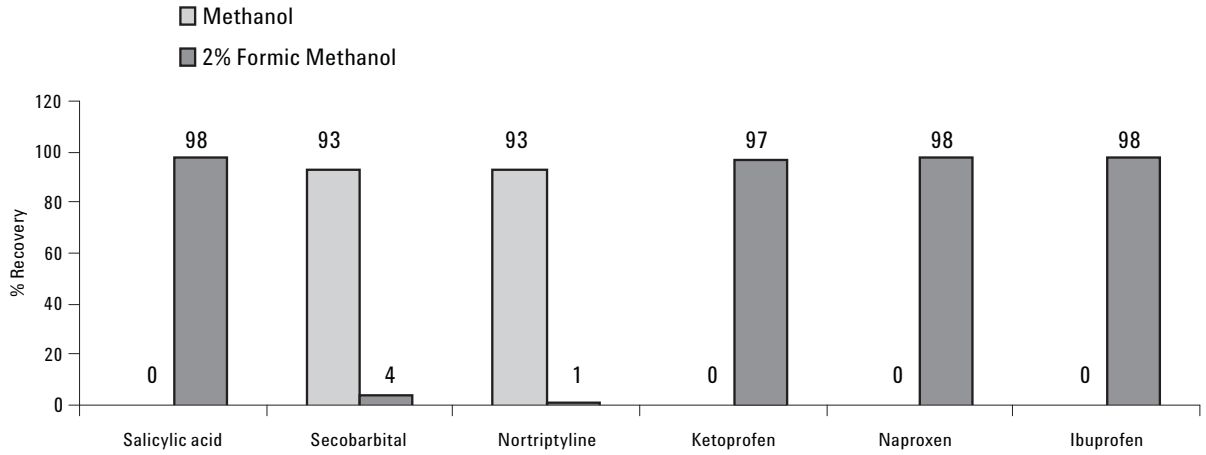


Figure 3. Recovery by eluent fraction. Neutral and basic compounds are recovered in the methanol eluent and acidic compounds are recovered in the acidified methanol fraction (2% formic acid).

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

Compound	% Recovery 2.5 µg/mL	% RSD
Salicylic acid	98	0.7
Ibuprofen	91	1.3
Naproxen	94	1.0
Ketoprofen	97	1.0
Secobarbital	98	0.6
Nortriptyline	98	1.3

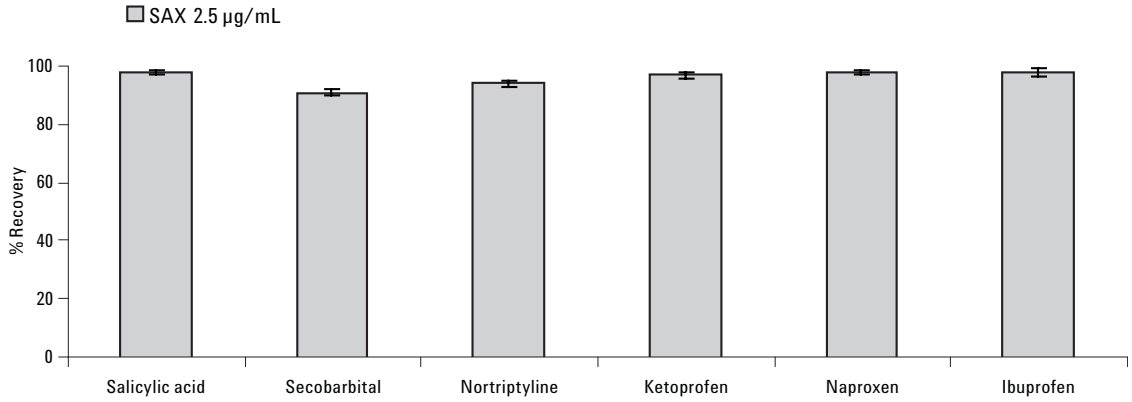
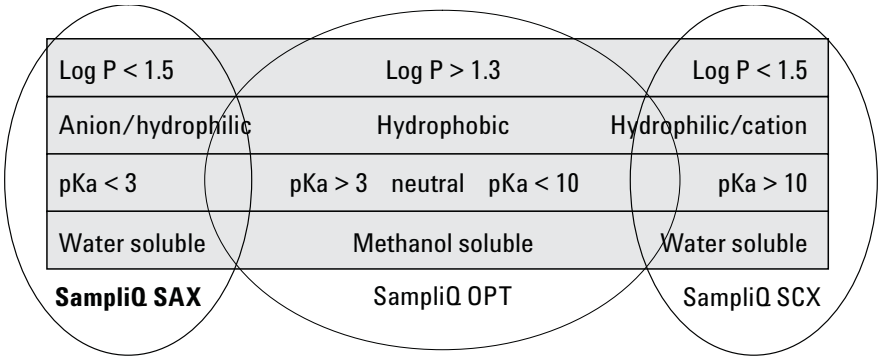


Figure 4. Recovery and reproducibility of acid, base, and neutral compounds on Agilent SampliQ SAX on a scale from 80 to 100% to show reproducibility.



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

Figure 5. Agilent SampliQ polymer sorbent selection guide.

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cartridge (Agilent SampliQ OPT). Figure 5 is a chart which provides guidance on which cartridge type to choose.

Agilent SampliQ SAX general purpose cartridges for acidic and neutral compound isolation.

Part Number	Description
5982-3313	30 mg, 1 mL cartridge, 100/pack
5982-3336	60 mg, 3 mL cartridge, 50/pack
5982-3367	150 mg, 6 mL cartridge, 30/pack

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For more information on our products and services, visit our Web site at www.agilent.com/chem/sampliq.

Printed in the USA
August 14, 2008
5989-8944EN

Agilent Technologies