安捷伦新型弱阴离子交换（WAX）固相萃取柱：SampliQ WAX

Technical Note

Agilent's SampliQ WAX provides

- Applications for strongly acidic, acidic and neutral compounds
- Excellent reproducibility
- High recoveries
- Spherically controlled particles

General Description

固相萃取（SPE）是复杂样品分析流程中的整体样品制备技术。即使采用了高选择性的检测器、LC/MS/MS 或 GC/MS/MS，共洗脱杂质产生的离子抑制效应仍会给定量分析带来负面影响。纯净的萃取物对于简化复杂的分析条件、延长色谱柱寿命以及获取更为精确的结果具有直接的影响。相比于液–液萃取（LLE）、蛋白质沉淀（PPT）或固相微萃取（SPME），SPE 是更为理想的样品制备技术，它能提供更好的灵活性、更高的回收率重现性、较低的溶剂消耗及更有效的纯化，还能实现自动化。SPE 样品制备已为制药业、法医学、食品安全和环境领域中的研究人员广泛采用。

The Agilent SampliQ WAX polymeric resin is a neutral primary amine modified divinyl benzene polymer (Figure 1). The resin exhibits retention for acidic, neutral and more importantly strongly acidic compounds (for example, sulfonates) that are irreversibly retained on strong anion exchange resins. The pKa (~6) of the SampliQ-WAX is more versatile than a strong anion exchange resin, which is always charged (pKa > 16). This offers more flexibility since SampliQ-WAX charge or neutrality is based on pH. SampliQ-WAX exhibits both anion exchange and reversed-phase behavior, which equates to easy method development. The weak anion exchange resin is inert to a wide variety of solvents, stable in the pH range 0 to 14, and water-wettable.

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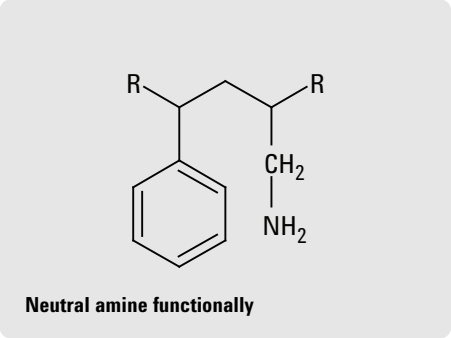

Figure 1. WAX resin.

Quality Controls

The data from our QA testing procedures reflect lot-to-lot resin uniformity. 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. The particle shape is characterized by light microscopy, and the surface area and porosity are determined by nitrogen adsorption. The rigorous size controls provide excellent reproducibility and flow characteristics. Every batch is tested for chromatographic performance and purity. The SampliQ Certificate of Performance (enclosed with each box) indicates the stringent acceptance range and documents each lot within the range.

Operational Guidelines

With SampliQ-WAX cartridges, the extraction procedure is simple. There are typically five steps in a mixed-mode SPE procedure:

1. Conditioning
2. Load pH ~ 1–2
3. Aqueous solvent wash, pH 4.5–5.0
4. Organic solvent wash
5. Elution with alkaline organic

It is imperative to understand the nature of each step in order to best optimize the solvent selection.

Figure 2 shows a recommended starting procedure for method development. In this example the volumes shown are for a 60 mg/3 mL cartridge. For various amounts of SPE material, proportionally correcting the volumes should be effective for many applications. The Agilent SampliQ-WAX weak anion polymeric solid phase extraction cartridges are compatible with the aqueous and organic solvents associated with mixed-mode SPE. The cartridges fit into the Agilent vacuum manifolds and any vacuum manifold that accepts Luer tip fittings.

Conditioning Step

Almost all SPE material uses a water-miscible organic solvent in its first conditioning step to "prepare" (wet) the resin to receive the sample. Methanol is the most popular conditioning solvent. The next step in the conditioning process is equilibration, which is removal of the methanol with at least five bed volumes of an aqueous solution.

Loading Step

Samples in complex matrices will require additional preparation prior to loading. Common preparation methods are dilution, pH adjustment, homogenization, centrifugation, and filtration. The prepared sample is usually spiked with an internal standard and loaded onto the cartridge as an aqueous solution. The flow through the cartridge is crucial and should not exceed 1 mL/min. If the flow is too fast, channeling will occur resulting in low recoveries. Vacuum will probably be required, depending on the volume and viscosity of the sample. For the strongest retention of the acids and neutrals, the pH of the loading solution should be approximately 1 to 2. An acidic loading solution maintains a charge on the weak anion exchange resin. Loading volumes for a mixed-mode polymeric SPE resin are synonymous with standard silica bonded material. However, the loading capacity of all polymeric SPE material is greater than silica-based sorbent. A 60-mg bed of polymeric material is therefore comparable to a 200-mg bed of C18 silica sorbent. In general, polymeric SPE material can retain approximately 10% to 15% of its bed volume, whereas silica based sorbents are approximately 5% of its bed volume.

Washing (Aqueous)

The wash solution should be modified with buffer to a pH of approximately 4.5 for the preferential retention of acids. A minimum volume of five times the bed volume should be used for the wash. The flow through the cartridge should be approximately 1 mL/min. The cartridge should dry for 1 minute at 10 inches Hg to remove as much residual water as possible.

Wash (Organic)

In many applications, the cleanup 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. The mixed-mode (weak anion exchange/reversed phased) behavior provides greater flexibility in method design, the ability to fractionate neutral compounds, and cleaner extracts. The cartridge should dry for 1 minute at 10 inches Hg.

Elution Step

A pH modification is required to elute the target compounds; two pH units (pH > 8) above the pKa of the weak anion resin (pKa~6) will result in the cleanest extracts. Flow through the cartridge should not exceed 1 mL/min, and a minimum of five times the bed volume should be used for elution. The eluent is collected, evaporated and reconstituted in the initial starting mobile phase.

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

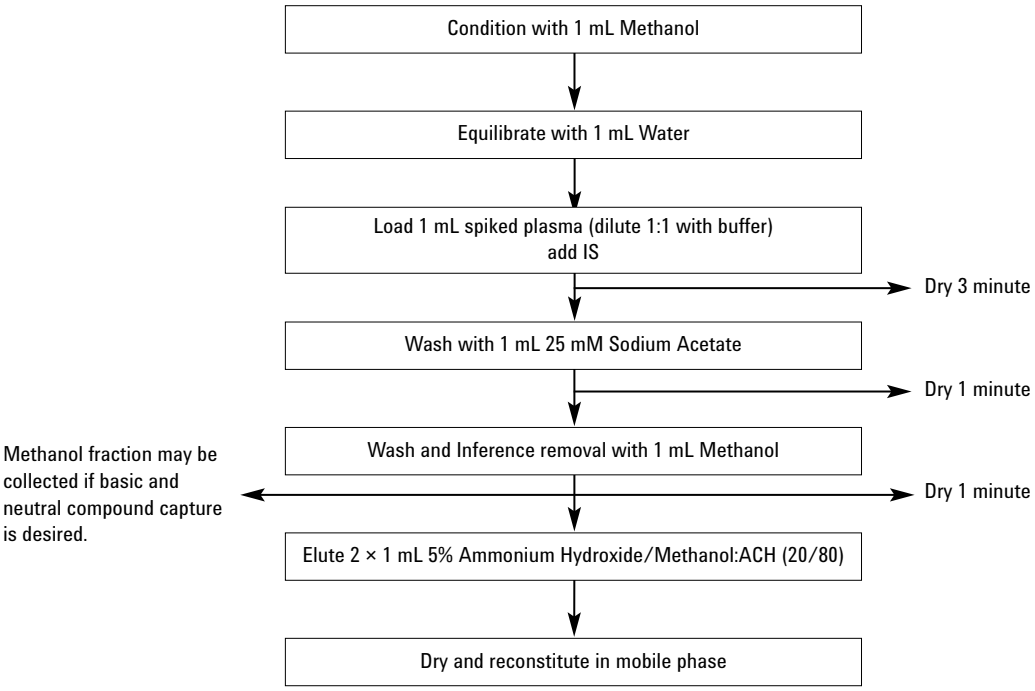

Figure 2. Agilent SampliQ WAX method development process for 60 mg/3 mL cartridge.

Performance

The SampliQ WAX weak anion polymeric resin provides highly reproducible recoveries for a wide range of acidic 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 to follow.

HPLC Analysis

Column:	Agilent Zorbax Rapid Resolution Eclipse Plus C18, 4.6 × 150 mm, 5.0 µm (p/n: 959993-906)
Mobile phase:	A: 20 mM ammonium acetate/water B: 10:90 water: acetonitrile 20 mM ammonium acetate
Gradient profile:	Time %B 0 2 18 75 18.1 2
Flow rate:	0.8 mL/min
Injection volume:	2 µL
HPLC:	Agilent 1200 HPLC DAD, 230 and 254 nm

Table 1 show the compounds used in this study. The compounds are a series of acids (weak to strong) with a range of hydrophobicity.

Compound	Log P	pKa
Indoprofen	2.77	5.45
Naproxen	4.15	3.18
Diflunisal	4.44	2.9
Indomethacin	4.27	4.5
Ketoprofen	0.97	5.94
2-naphthalenesulfonic acid	0.63	1.29

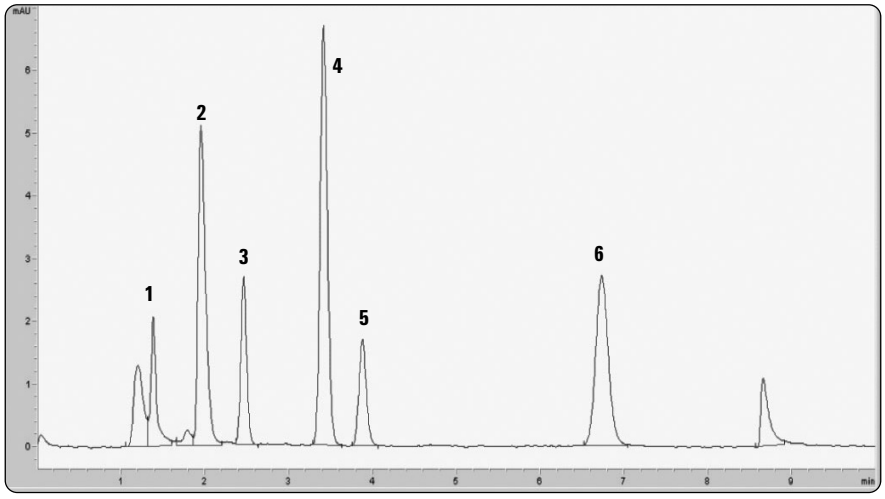

Figure 3. Chromatographic representation of the acidic and neutral compounds extracted from human plasma by SampliQ WAX: Peak 1. Indoprofen, 2. Naproxen, 3. Diflunisal, 4. Indomethacin, 5. Ketoprofen, 6. 2-napthalenesulfonic acid, 1 ug/mL spiked sample.

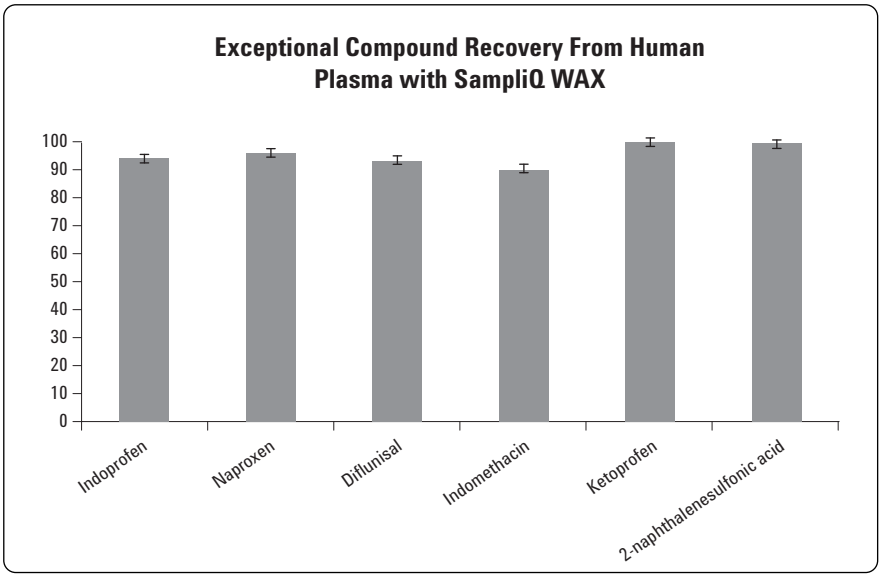

Figure 4. Exceptional recovery of acidic and neutral compounds from human plasma via SampliQ-WAX, > 90% recoveries and <2.0% RSD, for a 1 ug/mL spiked sample.

Conclusion

Agilent SampliQ WAX is a weak anion exchange polymeric resin designed for the extraction and concentration of strongly acidic, acidic and neutral compounds. A general protocol can be used to extract strongly acidic, acidic and neutral compounds that range in hydrophobicity. Applications employing WAX polymeric resin can be found in pharmaceutical, forensics, food/flavors and environmental sectors.

Description	Unit	30 micron part no.	6 micron part no.
30 mg, 1 mL	100/pk	5982-3613	5982-6613
60 mg, 3 mL	50/pk	5982-3636	5982-6636
150 mg, 6 mL	30/pk	5982-3667	5982-6667
96-well plate, WAX, 30 mg, 2 mL		5982-3696	5982-6696

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Printed in the USA
April 6, 2010
5990-5394EN

Agilent Technologies