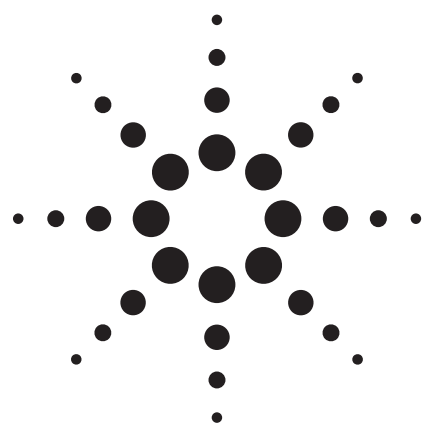


Agilent's New Octadecylsilane (ODS) (C18) Solid-Phase Extraction Cartridges: SampliQ C18



Technical Note

Agilent SampliQ C18 provides

- Excellent reproducibility
- High recoveries
- Applications for neutral, weakly acidic, and basic compounds
- Simple extraction protocol

General Description

Agilent is introducing the SampliQ line of solid-phase extraction (SPE) cartridges, replacing the AccuBOND[®] portfolio of products. This refreshed portfolio includes a variety of silica, Florisil PR, and alumina cartridges identical to the familiar AccuBOND[®] cartridges currently in use. In addition, Agilent is expanding the portfolio of SPE cartridges to include polymer phases for general purpose use and ion exchange extractions. A line of graphitized carbon cartridges is also now available. In this technical note, the performance of the new SampliQ C18 is demonstrated side-by-side with the AccuBOND[®] C18 SPE cartridge.

SPE is a cornerstone in the analytical workflow of complex samples, 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. SPE is a preferred sample preparation technique compared to liquid-liquid extraction because it offers greater flexibility, resulting in higher and more reproducible recoveries, lower

cost because less solvent is required, is more effective as a clean-up tool and is more easily automated. SPEs are used by researchers in the food safety, pharmaceutical, environmental, and forensic industries.

The Agilent SampliQ C18 sorbent is based on octadecylsilane-bonded, irregular silica gel (silica) particles. This nonpolar, nonendcapped sorbent provides reversed-phase binding of hydrophobic compounds. In addition, polar interactions are associated with the surface silanol groups. This functionality results in enhanced retention of basic compounds compared with the corresponding SampliQ C18 EC (endcapped) sorbent. Agilent SampliQ C18 is recommended as a general purpose SPE phase for both polar and nonpolar analytes.

Quality Controls

Through better process controls implemented during the production of our silica-bonded phase SampliQ SPE sorbents, we are able to manufacture a more consistent product. The data from our QA testing procedures reflect the lot-to-lot sorbent uniformity. Tighter acceptance ranges of critical parameters ensure reproducible retention and elution during sample processing. The SampliQ Certificate of Performance indicates the retention factor's stringent acceptance range and documents the performance of your lot within the range. As a result, SampliQ SPE phases are designed with an even higher degree of consistency and reproducibility, quality markers to provide more reproducible SPE extractions.



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Operational Guidelines

With SampliQ C18 cartridges, the extraction protocol is simple. There are typically four steps in a reverse-phase SPE procedure:

- 1) Conditioning
- 2) Loading
- 3) Washing
- 4) Elution

Figure 1 shows a recommended starting procedure for method development. In this example the volumes shown are for a 500-mg/6-mL cartridge. For other cartridge sizes, the volumes should be proportionally corrected. For many applications this simple protocol will be effective. The Agilent SampliQ C18 solid-phase extraction cartridges are compatible with water, aqueous buffers, and most organic solvents. Like other SPE cartridges, these are for single use only. The cartridges fit into the Agilent vacuum manifolds and any vacuum manifold that accepts Luer tip fittings.

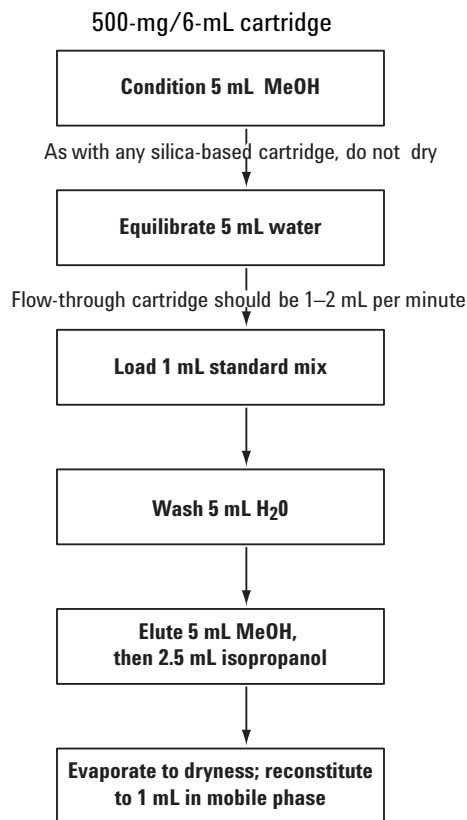


Figure1. SampliQ C18 SPE procedure.

Comparison of the New Agilent SampliQ C18 and the Current Agilent AccuBOND[®] C18 SPE Cartridges

The SampliQ C18 ODS sorbent 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, which include representatives from acidic, neutral, and basic classes of compounds.

The specific details of this experiment are described below.

Conditioning

Add 5 mL methanol to the cartridge. Apply vacuum and discard the eluent. Repeat condition with 5 mL H₂O. Do not allow the sorbent to go dry at any point during this step.

Loading

Add 1 mL of sample to cartridge. Apply vacuum and discard the eluent.

Washing

Add 5 mL H₂O to the cartridge. Apply vacuum and discard the eluent.

Elution

Place a collection tube beneath the cartridge. Add 5 mL of methanol to the cartridge. Apply vacuum and collect the eluent. Add 2.5 mL of isopropanol to the cartridge. Apply vacuum and collect the eluent in the same collection tube. Concentrate to dryness under a gentle stream of nitrogen. Dissolve the residue in 1 mL of mobile phase (85:15, see below).

HPLC Analysis Procedure

Column: ZORBAX Eclipse Plus C18, 4.6 x 150 mm, 3.5 µm

Catalog number: 959963-902

Mobile phase A: 0.1% formic acid in H₂O

Mobile phase B: 0.1% formic acid in acetonitrile

Gradient profile

| Time | %B |
|------|----|
| 0.1 | 15 |
| 5 | 21 |
| 18 | 30 |
| 30 | 67 |
| 30.1 | 15 |

Flow: 1 mL/min

Detector: Diode array at 254 nm

Run time 30 minutes, 5 minute equilibration

The six-component test mix is prepared at four calibration levels of 1, 10, 20, and 50 µg/mL concentrations. Recoveries of the compounds are quantitated using dexamethasone as an internal standard. The concentration of internal standard is 20 µg/mL. The internal standard was added to the sample during the loading step.

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

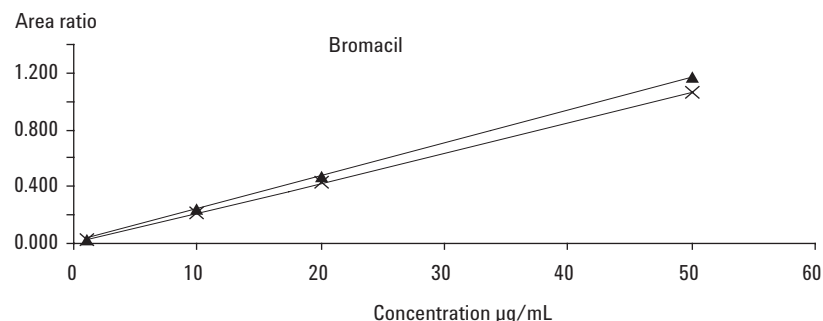
| Compound | Class | Log P | Comment |
|------------------|---------|---------------|----------------------------------|
| Caffeine | Base | -0.5 | Central nervous system stimulant |
| Sulfadimethoxine | Acid | 1.6 | Sulfa drug |
| Tebuthiuron | Neutral | Not available | Herbicide |
| Prednisone | Neutral | 0.6 | Steroid |
| Bromacil | Base | Not available | Pesticide – regulated in foods |
| Warfarin | Acid | 3 | Anticoagulant |
| Dexamethasone | Neutral | 1.1 | Synthetic steroid hormone |

Table 2 shows the exceptional reproducibility of the Agilent SampliQ C18 SPE cartridges. The relative standard deviations of the recoveries are < 5 percent at a concentration of 35 µg/mL solution using the simple generic protocol.

Figure 2 shows the linear regression analyses of the experiments for the compound bromacil, which is a regulated pesticide in foods. Notice that the calibration curves are virtually identical for AccuBOND^{II} C18 and SampliQ C18. The R² values are 0.9999. Figure 3 shows the recoveries on SampliQ C18 and AccuBOND^{II} C18 for the six different compounds. Six replicate samples spiked at a level of 35 µg/mL with an internal standard at 20 µg/mL concentration. Recoveries are calculated from the

Table 2. Comparison of SampliQ C18 and AccuBOND^{II} C18 Cartridges

| IS Added During Load Step | AccuBOND ^{II} C18 | AccuBOND ^{II} C18 | SampliQ C18 | SampliQ C18 |
|---------------------------|--------------------------------|----------------------------|--------------------------------|-------------|
| Compound | % Recovery IS during load step | % RSD n = 6 | % Recovery IS during load step | % RSD n = 6 |
| Caffeine | 98.3 | 6.0 | 107.9 | 4.4 |
| Sulfadimethoxine | 101.3 | 2.7 | 114.0 | 1.8 |
| Tebuthiuron | 100.3 | 4.1 | 97.4 | 2.6 |
| Prednisone | 100.2 | 1.4 | 108.7 | 0.7 |
| Bromacil | 100.1 | 3.5 | 99.5 | 1.7 |
| Warfarin | 102.2 | 3.4 | 98.4 | 1.9 |



Internal standard spiked following SPE extraction:

SampliQ C18 – x

AccuBOND^{II} C18 – ▲

Figure 2. Comparison of the performance of SampliQ C18 and AccuBOND^{II}. Calibration curves for bromacil, a regulated pesticide in foods.

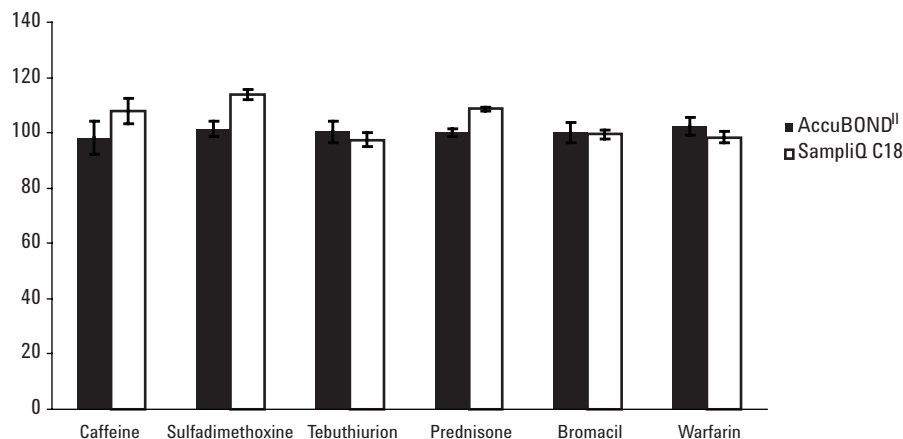


Figure 3 Comparison of the performance of SampliQ C18 and AccuBOND^{II} C18 on six different compounds that are representative of acidic, basic, and neutral compounds. Six replicate samples spiked at a level of 35 µg/mL using an internal standard at 20 µg/mL concentration.

individual calibration curves for each sample. Figure 3 shows the results for samples where the internal standard is spiked prior to the SPE extraction.

Summary

Agilent is introducing a new line of silica-based SPE cartridges. Agilent SampliQ C18 is a reversed-phase sorbent. A general protocol can be used to separate weakly basic, weakly acidic, and neutral compounds from complex mixtures. Applications using C18 silica SPE cartridges are found in the pharmaceutical, environmental, and forensic science industries. The precision and accuracy of the SampliQ C18 cartridges match the performance of the AccuBOND^{II} C18 cartridges. New quality control procedures have been established during the manufacturing process. The result is that the new SampliQ C18 has tighter performance specifications for lot-to-lot reproducibility. SampliQ C18 is a higher quality product with the same excellent performance as the AccuBOND^{II} C18 products.

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For More Information

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

Agilent SampliQ C18 general purpose cartridges and bulk materials.

| Part Number | Description |
|-------------|--------------------------------------|
| 5982-1111 | C18 ODS, 100 mg, 1 mL tube, 100/box |
| 5982-1132 | C18 ODS, 200 mg, 3 mL tube, 50/box |
| 5982-1135 | C18 ODS, 500 mg, 3 mL tube, 50/box |
| 5982-1160 | C18 ODS, 1000 mg, 6 mL tube, 30/box |
| 5982-1165 | C18 ODS, 500 mg, 6 mL tube, 30/box |
| 5982-1182 | C18 ODS SPE Bulk Sorbent, 25g bottle |

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