User Guide

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Agilent AdvanceBio Surfactant Profiling HPLC Columns

Analytical HPLC columns for surfactant degradation profiling

Introduction

Agilent AdvanceBio Surfactant Profiling HPLC columns are designed for high-resolution and high-throughput analysis of non-ionic surfactant degradation. The fully porous 3.5 µm silica particles allow robust analysis of samples with heavy matrices, such as formulation buffers.

Getting started

A column performance report, including a column-specific QC test chromatogram and a batch-specific oleic acid/polysorbate 80 separation, is enclosed with every AdvanceBio Surfactant Profiling HPLC column.

The Agilent QC test system may vary slightly from the system used in your lab. The Agilent system has been modified to minimize dead volume, which allows Agilent to evaluate column efficiency and ensure product consistency.

An optimized LC system will generate similar results to the chromatogram on your QC performance report.

Important safety considerations

- All points of connection in an LC system are potential leak sources. Always be aware of the potential toxicity or flammability of mobile phases.
- These columns are mechanically stable and have been tested to the recommended maximum operating pressure to ensure safe lab operation on many LC instruments.
- Because of the small particle size, dry column packings are respirable. Opening columns is strongly discouraged due to the safety risk and likelihood of reducing column performance.

Using your column

Installation

- Remove both end plugs and note the flow direction marked on the column.
- Use Agilent InfinityLab Quick Connect and Agilent Quick Turn LC fittings (part numbers 5067-5965 and 5067-5966, respectively) to quickly and easily connect your column to the LC instrument. Agilent also sells Quick Connect fittings with pre-fixed capillaries in different dimensions. Learn more at www.agilent.com/chem/infinitylabfittings.

Column conditioning

- Columns are shipped in a mix of water and acetonitrile.
- Columns should initially be flushed for 10 to 20 column volumes of the desired mobile phase, starting at a lower flow rate and gradually increasing to the desired flow rate.

Instructions for use

Operating parameters

Parameter	Output/Limit
Pressure Limit	Guard columns: 600 bar 2.1 × 50 mm and 4.6 × 50 mm: 400 bar All other dimensions: 600 bar
pH Range	1 to 8
Operating Temperature	Recommended: 25 to 30 °C Maximum: 80 °C
Compatible Solvents	Water and all common organic solvents; avoid tetrahydrofuran (THF)

Note: Working at extremes of the operating parameters may reduce column lifetime.

Recommended starting conditions for polysorbate analysis

Parameter	Value
Flow Rate	0.25 mL/min
Mobile Phase	A: 10 mM Ammonium acetate B: Methanol
Column Temperature	30 °C
Gradient	Time (min) %B 0 to 0.2 0 0.2 to 0.6 0 to 50 0.6 to 1.5 50 1.5 to 5.1 50 to 95 5.1 to 7 95 7 to 8 95 to 0 8 to 10 0
Needle Wash	20:80 methanol:water, flush for 10 seconds
Agilent 1290 Infinity II and Infinity III ELSD Conditions	Evaporator temperature: 30 °C Nebulizer temperature: 30 °C Gas flow rate: 1.20 SLM

Additional operating tips

- When using an evaporative light scattering detector (ELSD), consider using 10 mM ammonium acetate or lower in the aqueous phase, as higher concentrations of salts can cause the ELSD nebulizer to become dirtier and clog faster.
- If a noisy baseline or loss in sensitivity is experienced while using an ELSD, clean the nebulizer and evaporator tube. Refer to your ELSD manual for instructions.
- Using a needle wash (refer to the recommended starting conditions for polysorbate analysis) will increase consistency and reduce carryover. If fatty acids are causing carryover, consider increasing the needle wash to 70:30 methanol:water.

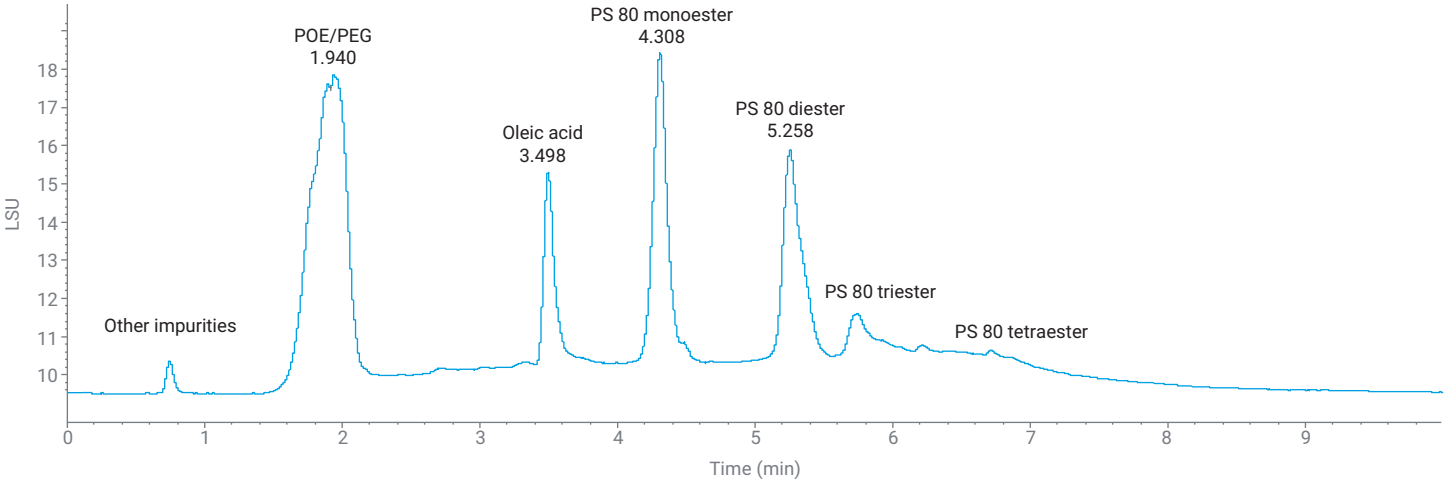

Figure 1. An example chromatogram of the batch-specific QC test. **Note**: the first two peaks (retention time < 3.0 minutes) will vary in abundance and shape based on LC instrument cleanliness, buffer freshness, and sample purity.

Column care and cleaning

Column care

- To extend the life of the column, consider using Agilent AdvanceBio Surfactant Profiling guard columns. For details on installing guard columns, see Section 2 of the Agilent Analytical and Semipreparative Guard User Guide.
- An increase in backpressure and decrease in performance may occur over time. If the pressure has increased, first identify whether this increase is due to a guard column that may need to be replaced. If the increase in pressure is in a system component, such as tubing or a filter, replace the component and retest.

Column cleaning

- Disconnect the column from the detector and run wash solvents into a beaker.
- Start your mobile phase without buffer salts (water/organic). Run 10 to 20 column volumes through.
- Next, use 100% organic solvent (methanol or acetonitrile).
- Check the pressure to see if it has returned to normal. If not, then:
  - Discard the column or consider stronger conditions, such as 75% acetonitrile/25% isopropanol.
  - Increase to 100% isopropanol, 100% methylene chloride, or 100% hexane (if using methylene chloride or hexane, you will need to flush the column with isopropanol prior to use and before returning to your mobile phase, as these solvents are not miscible with aqueous solutions).
  - This column can also be backflushed using these instructions.

Storage recommendations

- The columns may be safely stored for short periods in most mobile phases.
- Long-term storage should be in a pure organic solvent.
- If the column has previously been used with a buffered mobile phase, such as ammonium acetate, the buffer should first be removed by purging the column with 20 to 30 column volumes of a 50:50 mixture of methanol or acetonitrile and water, followed by 20 to 30 column volumes of the pure solvent.
- Before storing, end fittings should be tightly capped with end plugs to prevent the media from drying out.

Ordering details

Description	Part Number
AdvanceBio Surfactant Profiling, 2.1 × 50 mm	865750-907
AdvanceBio Surfactant Profiling, 2.1 × 100 mm	861775-907
AdvanceBio Surfactant Profiling, 2.1 × 150 mm	863750-907
AdvanceBio Surfactant Profiling, 2.1 mm guard (3 pack)	821126-927
AdvanceBio Surfactant Profiling, 4.6 × 50 mm	865973-907
AdvanceBio Surfactant Profiling, 4.6 mm guard (3 pack)	820951-927

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